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Pressure Sensitivity of Streptococcal Growth in Relation to Catabolism

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The sensitivity of *Streptococcus faecalis* growth to hydrostatic pressures ranging up to 550 atm was found to depend on the source of adenosine triphosphate for growth. Barotolerance of cultures growing in a complex medium with ribose as major catabolite appeared to be determined primarily by the pressure sensitivity of ribose-degrading enzymes. Apparent activation volumes for growth were nearly identical to those for lactate production from ribose, and yield coefficients per mole of ribose degraded were relatively independent of pressure. In contrast, cultures with glucose as main catabolite were less sensitive to pressure; glycolysis was less severely restricted under high pressure than was growth, and yield coefficients declined with pressure, especially above 400 atm. Thus, two distinct types of barotolerance could be defined—one dominated by catabolic reactions and one dominated by noncatabolic reactions. The results of experiments with a series of other catabolites further supported the view that catabolic reactions can determine streptococcal barotolerance. We also found that growing, glucose-degrading cultures increased in volume under pressure in the same manner that they do at 1 atm. Thus, it appeared that the bacterium has no alternative means of carrying out glycolysis under pressure without dilatation. Also, the observation that cultures grown under pressure did not contain abnormally large or morphologically deformed cells suggested that pressure did not inhibit cell division more than cell growth.

Hydrostatic pressure is a major ecological factor influencing the distribution of marine organisms and limiting penetration of the oceans' depths by organisms, including man, with limited capacities to adapt to compression. Although pressure adversely affects certain specialized structures in higher organisms, its most universal and basic effects are on cell functions, and the most useful subjects for study of these latter effects are unicellular organisms. However, even with relatively simple organisms, there is still a major difficulty in identifying just which processes are the major determinants of cellular barotolerance.

Both growth and division of cells are inhibited by pressure. Generally, the division process is the more sensitive, and pressurized cultures often contain abnormally large cells. Inhibition of cell division has been related to interference with gelation during cytokinesis (13), to depressed microtubule formation and disorganization of the mitotic apparatus (17, 21), and to decreased synthesis of "division proteins" (19). [Much of the work on cellular barophysiology has been reviewed recently in a book edited by A. M. Zimmerman (20).] Pressure inhibits division of both eukaryotic and prokaryotic cells, even though the latter do not contain microtubules and are not known to undergo sol-gel transitions during division. For example, *Escherichia coli*, *Serratia marinorubra* (*marcescens*), and *Flavobacterium okeanokoites* have all been observed to grow under pressure as long filaments with a sparsity of cross-walls (23, 24).

ZoBell and Cobet (23) found that three different strains of *E. coli* grown under pressure of up to 450 atm formed giant cells that were relatively poor in deoxyribonucleic acid (DNA), relatively rich in ribonucleic acid (RNA), and nearly equivalent to control cells in their protein contents per gram of dried cells. Later studies of the capacities of *E. coli* cells to incorporate radioactively labeled precursors of DNA, RNA, and protein (1, 10, 16, 18) indicated that amino acid incorporation is much more sensitive to pressures below 450 to 550 atm than is nucleotide incorporation, and that pressure inhibition of DNA synthesis is probably a consequence of inhibited syn-

thesis of initiator proteins needed to start new rounds of DNA replication. Thus, pressure appears to inhibit bacterial DNA synthesis in much the same manner that amino acid analogues do—not by directly inhibiting polymerization but by inhibiting initiation. However, it is well known that only certain organisms respond to amino acid analogues by becoming relatively poor in DNA and producing grossly enlarged cells (11). In this paper, we present evidence to indicate that giant cells are not prevalent in pressurized *Streptococcus faecalis* cultures, and that pressure inhibition of catabolic reactions can play a major role in determining sensitivity of growth to pressure.

MATERIALS AND METHODS

Bacterium and growth conditions. *S. faecalis* ATCC strain 9790 was grown in a medium (TGM medium) prepared by dissolving 30 g of tryptone (Oxo Ltd., London, England), 10 g of glucose, 1 g of Marmite (a commercial yeast extract from Marmite Ltd., London, England), and 0.5 mmole of H_3PO_4 in 1 liter of distilled water. A number of variants of TGM medium were used also, in particular, one buffered with 0.1 M phosphate, one buffered with 0.1 M imidazole, and others with glucose replaced weight for weight with ribose (TRM medium), maltose, lactose, gluconic acid, or pyruvic acid. The *p*H of each medium was adjusted to 7 after autoclaving with concentrated NaOH or HCl solutions.

Growth under pressure. For most experiments, cultures were inoculated with ca. 10^9 cells per 100 ml and were then placed in plastic syringes, with care being taken to remove all gas bubbles. The tips of the syringes had been previously tapped so that a metal screw with a rubber gasket could be used to seal tightly the tapered end of the syringe barrel. When the filled syringes were placed in a pressure bomb and compressed, the plungers moved into the barrels to compress the cultures. Care was taken to avoid contaminating the cultures, and microscopic observations plus streak-plating of samples only rarely revealed contaminants. Pressure bombs were placed in a thermostated water bath for incubation, and the cultures were decompressed and sampled at intervals. Control cultures were incubated in the same water bath.

Figure 1 shows examples of the growth curves obtained and the rates of lactate production in control and compressed cultures. Here, optical density was measured by use of a Beckman model DU spectrophotometer with light of 700-nm wavelength and a 1-cm optical path length. Lactic acid was assayed enzymatically with lactic dehydrogenase and nicotinamide adenine dinucleotide (NAD) by the methods described by Bergmeyer (3). The enzyme preparation was obtained from Boehringer Mannheim Corp., New York, N.Y. Glucose was assayed by use of glucose oxidase obtained from Worthington Biochemical Corp., Freehold, N.J. Ribose was assayed by means of the orcinol reaction (4).

Dry weights were determined by centrifuging cells from 30- to 50-ml culture samples in the cold, washing the cells once with cold distilled water, resuspending them in cold distilled water, and then drying the suspension to constant weight at 100 C. In all cases, there was a linear relationship between optical density and dry weight of cells per milliliter. Cell numbers were determined by direct counting with a Petroff-Hausser counting chamber with the use of procedures described previously (6).

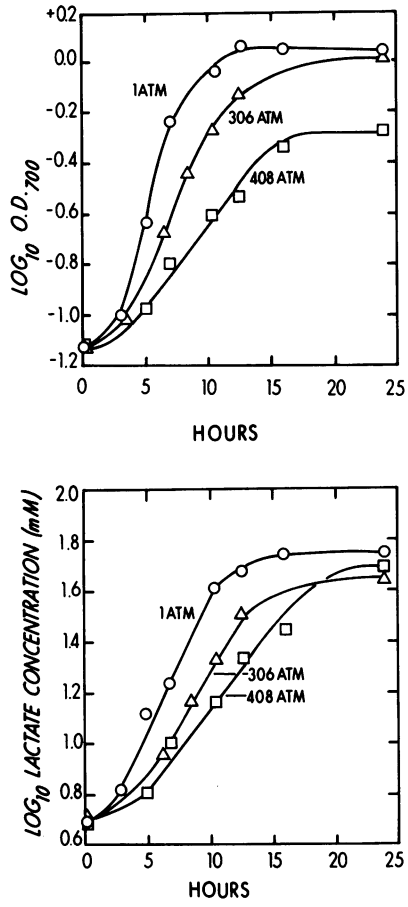

FIG. 1. *Growth and glycolysis of Streptococcus faecalis under pressure in TGM medium at 24 C.*

Ribose uptake. The amount of ribose within control and pressurized cells was determined by centrifuging 20-ml culture samples in the cold, washing the pellets once with cold distilled water, resuspending the cells in water to make 10 g of suspension, heating the suspensions at 80 C for 15 min in sealed tubes, and then measuring the ribose contents of supernatant fluids. The cells were chilled for the washing procedure to prevent leakage of pooled materials (9).

Pycnometric and dilatometric measurements. Two methods were used to measure changes in the volume of compressed cultures during growth. The first method measured the change in weight of a fixed volume before and after growth under pressure by use of standard pycnometers of 100-ml capacity. The second method measured the change in volume of a given weight of culture by use of the apparatus shown in Fig. 2. The

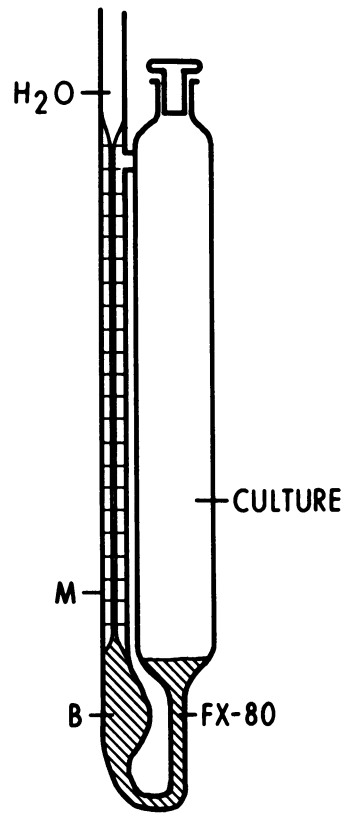

FIG. 2. *Volumeter for measuring changes in volume during growth under pressure. The apparatus is designed to fit into the lumen of a pressure chamber which measures 3.7 cm in diameter and 27 cm in depth. The meniscus, M, was formed by a water-FX-80 interface; oil can be used in place of water. The capillary holds 3.17 mm³/cm, and the main chamber holds about 50 ml of culture. The stopper is held in by elastic bands not shown.*

bottom of the U tube was filled with fluorocarbon FX-80, which is immiscible with water and heavier than water. Because it is nontoxic to *S. faecalis*, it serves better than mercury for this purpose.

We also tried filling the capillary with oil and reading an oil-culture meniscus in the capillary, but the oil spread out into small drops during the rise and fall in pressure, and accurate readings became impossible. (In subsequent experiments with another organism, we used a light kerosene oil stained red for greater visibility and were successful.) The FX-80 meniscus in the capillary was adjusted to a suitable height when the culture was first introduced by slight warming and cooling of the mixture with addition or subtraction of FX-80 as needed. Then the volumeter was placed in a pressure vessel which was pressurized and left in a water bath overnight. When the pressure was released slowly, the volumeter was returned to the water bath before the meniscus was read. The bulb, B, was included in the apparatus to avoid pushing water past the FX-80 into the culture when the pressure was raised. The culture was, of course, introduced without inclusion of any gas bubbles under the stopper.

RESULTS

Pressure effects on TGM-medium cultures. The curves presented in Fig. 1 show clearly that hydrostatic pressures of 306 and 408 atm slowed both growth and glycolysis of *S. faecalis*. A pressure of 408 atm also had a marked diminishing effect on the extent of growth but relatively little effect on yields of lactic acid. Thus, growth in TGM medium under high pressure appeared to be inefficient in terms of cell yields per mole of glucose utilized. Values for yield coefficients, Y (lactate), expressed as grams (dry weight) of cells per mole of lactate produced in a series of experiments, are presented in Fig. 3A. Average Y (lactate) at 306 atm was somewhat below the control value, and that at 408 atm was markedly below the control value. In effect, high pressure appeared to produce an uncoupling of glycolysis from growth of the type described by Forrest (8) for *S. faecalis* cultivated at suboptimal temperatures.

S. faecalis 9790 is a homofermentative, lactic acid bacterium, and glycolysis is essentially the sole source of adenosine triphosphate (ATP) for growth in media such as TGM medium with excess glucose. Cultures stopped growing in our experiments when only about one-half of the available glucose had been used, and the medium *p*H had dropped to ca. 4.7. Neutralization of the acid permitted additional growth. High pressures seemed to have little or no effect on the products of glycolysis. Average molar ratios of lactate produced to glucose consumed were 1.92 at 1 atm and 1.98 at 408 atm. These results contrast with those of Chumak and Blokhina (5), who found that glucose was fermented differently under pressure than at 1 atm by *Pseudomonas desmolyticum* with alteration in the types of acids produced anaerobically. This contrast in results is probably related to differences in the catabolic versatilities of the test organisms.

The most commonly used index for characterizing pressure sensitivities of reactions or processes is the apparent activation volume (ΔV^*) which can be calculated by means of the equation,

$$\Delta V^* = \frac{2.303\ RT \log_{10}(k_{p1}/k_{p2})}{p2 - p1}$$

where R is the gas constant, T is the Kelvin temperature, $p1$ and $p2$ are pressures, and k_{p1} and k_{p2} are reaction rate constants at $p1$ and $p2$. ΔV^* values for growth and glycolysis in TGM medium are presented in Fig. 3A. Here, exponential

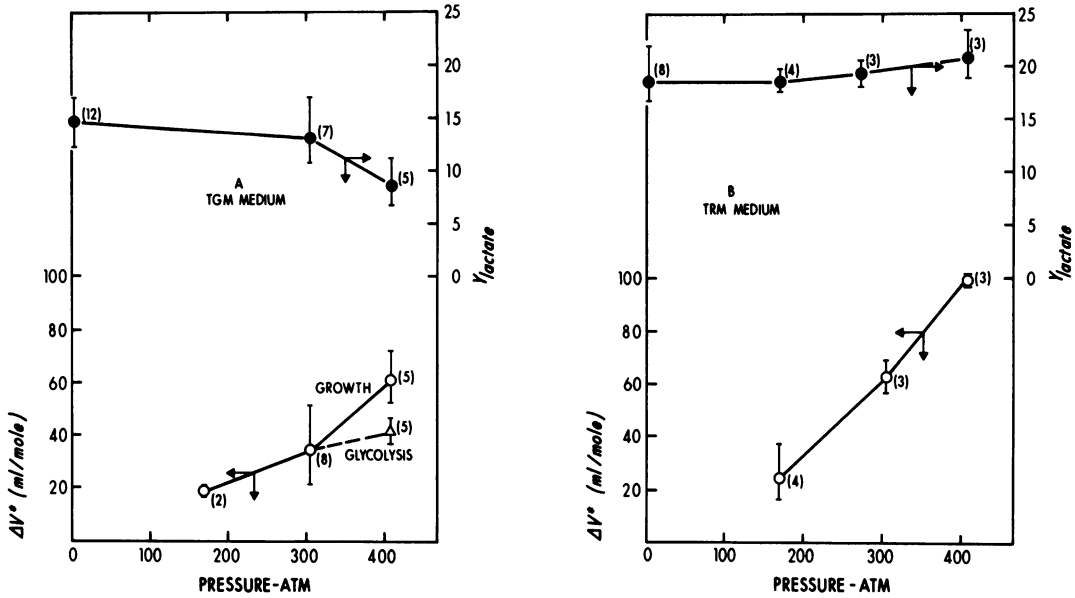

FIG. 3. *Apparent activation volumes and cell yields per mole of lactate produced for Streptococcus faecalis cultures growing in TGM medium or TRM medium at 24 C. Arrows on the curves point to the pertinent axes. Numbers in parentheses give the numbers of individual values averaged to obtain the points shown. The vertical lines indicate ranges of experimental values.*

growth rate constants or exponential acid production rate constants for compressed and uncompressed cultures were used for calculations. The constants were estimated graphically from plots of time versus $\log_{10}$ (optical density) or $\log_{10}$ (mmoles of lactate per ml of culture). At pressures of 306 atm or less, differences between ΔV^* for growth and ΔV^* for glycolysis were minor. For example, the average ΔV^* for growth at 306 atm was 34.8 ml/mole, and that for glycolysis was 35.7 ml/mole. However, it is apparent from Fig. 3A that, at 408 atm, ΔV^* for growth was significantly greater than that for glycolysis. Thus, it appears that growth was not limited by the rate of glycolysis and ATP supply at 408 atm. In fact, the bacterium produced excess ATP under pressure and so must have disposed of it in nonuseful ways. The net effect is that growth at 408 atm was energetically inefficient.

We found that the amino acid analogue *p*-fluorophenylalanine had a similar effect on *S. faecalis*; 37.5 mM *p*-fluorophenylalanine added to TGM medium slowed growth more severely than it slowed glycolysis, so that Y (lactate) dropped to very low values.

In all, it appeared that the pressure sensitivity of *S. faecalis* growth in TGM medium with glucose as major catabolite was determined primarily by noncatabolic reactions.

Pressure effects on TRM-medium cultures. *S. faecalis* can degrade ribose to produce lactate, acetate, and ATP for growth. Hydrostatic pressure slowed both growth and ribose degradation in tryptone-Marmite medium with glucose replaced by ribose. However, pressure did not uncouple catabolism from growth in TRM medium even at 408 atm (Fig. 3B). In fact, growth appeared to be slightly more efficient under high pressure. The bacterium had significantly lower barotolerance when growing in TRM medium compared with TGM medium. This lower tolerance is reflected by higher ΔV^* values for growth in TRM medium (Fig. 3B). ΔV^* values for ribose breakdown were nearly identical to those for growth at all pressures tested. For example, the average ΔV^* for growth at 408 atm was 99.5 ml/mole; that for lactate production from ribose was 97.3 ml/mole.

The entry of ribose into cells did not appear to be affected by pressure. Samples taken from control and pressurized (408 atm) cultures in the mid-exponential phase of growth were assayed for intracellular ribose. Control cells contained 142 μmoles of ribose per g (dry weight); the comparable value for pressurized cells was 162. These values indicate intracellular ribose concentrations of 40 and 46 μmoles per ml of cell water. Since the extracellular ribose concentration was initially ca. 66 μmoles per ml and declined to about half that amount, it appeared that *S. faecalis* has

little capacity to concentrate this low-molecular-weight sugar, which presumably enters cells by nonconcentrative, pressure-insensitive processes.

Pressure did not significantly alter lactic acid yields from ribose; average molar ratios of lactate produced to ribose consumed were 0.98 at 1 atm and 1.08 at 408 atm. Control exponential growth rate constants for TRM-medium cultures were only about one-half those for TGM-medium cultures. The main basis for this difference in growth rate is probably a difference in the rate at which ATP is supplied for growth because, when *S. faecalis* is growing in a rich medium, catabolites do not significantly contribute intermediates for macromolecular synthesis (2). In all, it appeared that the pressure sensitivity of *S. faecalis* growth in TRM medium with ribose as main catabolite was determined primarily by catabolic reactions.

Pressure effects in other media. The pressure sensitivity of *S. faecalis* growth in a series of tryptone-Marmite media with different catabolites was found to vary markedly. To assess this variability, we grew paired cultures, one in TGM medium and one in the same medium with glucose replaced by another catabolite. We then compared the pressure responses of the cultures. Relative activation volumes for growth and control growth rates are presented in Table 1. It is apparent that cells degrading maltose at 306 atm were somewhat less sensitive to pressure than were those degrading glucose. Cells degrading gluconate, lactose, ribose, or pyruvate were more sensitive than those degrading either glucose or maltose. In fact, cells degrading pyruvate were so pressure sensitive that they would not grow at 306 atm; we had to use a pressure of only 170 atm and TRM-medium cultures for comparison. Pressure sensitivity appeared to be correlated inversely with growth rate; this in turn was probably correlated with the rate at which ATP was supplied for growth, because the cells were in a rich medium and because catabolites do not supply significant amounts of synthetic precursors.

TABLE 1. *Pressure sensitivity of S. faecalis growth in relation to catabolite supply*

Catabolite	Control growth rate constant (hr^{-1})[a]	Pressure (atm)	Relative ΔV^*
Maltose	0.198	306	0.90[b]
Glucose	0.157	306	1.00[b]
Gluconic acid	0.089	306	1.44[b]
Ribose	0.088	306	1.73[b]
Lactose	0.075	306	1.74[b]
Ribose	0.088	170	1.00[c]
Pyruvic acid	0.068	170	2.28[c]

[a] The growth rate constants given are those obtained for unpressurized control cultures in the experiments described in the table. All values are averages of at least duplicate experiments. The experimental temperature was 24 C.

[b] Values calculated by dividing ΔV^* for growth with the indicated catabolites by that for growth with glucose.

[c] Values calculated by dividing ΔV^* for growth with the indicated catabolites by that for growth with ribose.

The catabolite supply for growth also had an effect on the maximal pressures at which cultures would grow. Pyruvate-degrading cultures would not grow at pressures above ca. 300 atm; cultures degrading ribose or gluconate would not grow at pressures above ca. 450 atm; and those degrading glucose, maltose, or lactose would not grow in a 72-hr period at pressures above ca. 550 atm. Cells in the cultures at 550 atm had generation times more than 10 times those of control cells (ΔV^* greater than 100 ml/mole).

Buffer effects. The addition of 0.1 M phosphate buffer to TGM medium had little effect on the pressure sensitivity of *S. faecalis* growth from the relatively large inocula we used, nor did the buffer significantly change control rates of growth or glycolysis even though these rates were sustained for longer periods in buffered media. In contrast, 0.1 M imidazole buffer markedly increased the sensitivity of growth to pressure so that ΔV^* for growth at 306 atm was nearly twice that of unbuffered cultures. However, imidazole also reduced control growth rates and uncoupled glycolysis from growth even at 1 atm.

In some of our early experiments with small inocula, phosphate buffer reduced the pressure sensitivity of growth in TGM medium, but this effect did not occur when large inocula were used. In all, it seemed that the effects of pressure on growth could not be explained in terms of changes in the ionization states of buffers in cultures.

Effects of pressure on cell size. Many bacteria become enlarged when grown under high pressure because the process of cell division is inhibited more than cell growth. This type of selective inhibition was not apparent in *S. faecalis* cultures grown under pressures of up to 408 atm. Cells from pressurized cultures were microscopically indistinguishable from those grown at 1 atm. Moreover, average turbidity coefficients for 700-nm light and a 1-cm light path were 1.28 cm^2/mg for control cultures, 1.42 cm^2/mg for cultures grown at 306 atm, and 1.41 cm^2/mg for cultures grown at 408 atm. Direct counts of over 1,000 cells per sample indicated no significant difference in cell counts between pressurized and control cultures of the same optical density. For

example, cultures with an optical density of 0.3 contained an average of 7.5×10^8 cells/ml. Calculations indicated that average cell dry weights were 3.1×10^{-10} mg/cell, 2.8×10^{-10} mg/cell, and 2.9×10^{-10} mg/cell for cultures grown at 1, 306, and 408 atm, respectively. Differences in cell size and extinction coefficient appeared to be minor and in keeping with the slower growth rates of pressurized cultures.

Nongrowing cells showed a tendency to undergo autolysis under pressure, as indicated by a decline in optical density over periods of 8 hr or more after growth had ceased. Therefore, cultures used in determining dry weights, especially those used for yield coefficients, were harvested either before or soon after exponential growth was complete.

Volume changes under pressure. We had previously found that *S. faecalis* cultures growing at 1 atm in TGM medium increase in volume mainly because of volume changes of glycolytic reactions (7, 12). The total volume change was related to the difference in molecular volumes of lactic acid and glucose and to the volume change associated with lactic acid neutralization. The latter volume change depends on the buffer present and is particularly large for phosphate-buffered media. Our enzymatic analyses indicated that the products of *S. faecalis* glycolysis are the same under pressure as at 1 atm. Still, it seemed possible that the bacterium might have some way under pressure of circumventing the relatively large volume increase associated with glycolysis, especially since part of the volume change is due to buffer reactions. The organism might also have been able to couple reactions accompanied by volume decreases to glycolysis and so convert the process to one with less dilatation.

Volume changes of cultures under pressure were measured with both volumeters and pycnometers (Table 2). Average volume increases measured by the two methods were in reasonable agreement, although the volumeter tended to give lower values. (In subsequent experiments with another bacterium, the agreement between the two methods was much closer.) Following the growth period, the contents of the pycnometers were examined microscopically for contamination and assayed for lactic acid. The average volume increase of 15.7 ml per mole of lactic acid produced is nearly the same as the values reported previously by us for cultures grown at 1 atm.

Apparently, then, pressure did not induce any major change in the type of metabolism used to supply ATP for growth. This result seemed at first surprising, but we calculated the work required for a dilatation of 15.7 ml/mole of lactate at 410 atm as

TABLE 2. *Volume changes of S. faecalis cultures grown under pressure in relation to lactate production*

Pressure (atm)	Incubation time (hr)	ΔV (as % culture volume)		ΔV (ml/mole of lactate)
		Volumeter	Pycnometer	
270	16	0.017	0.029	24.1[a]
270	17	0.052	0.078	12.2
270	18	0.071	0.116	14.7
410	17	0.110[b]	0.074[b]	16.6
410	24	0.045	0.083	17.7
	+21[c]	0.088[b]		
410	21	0.022	0.033	9.0
	Avg	0.058	0.069	15.7

[a] Lactate assays were performed on pycnometer samples only.

[b] If the values indicated are neglected, average ΔV values of 0.041 for the volumeter method and 0.065 for the pycnometer method are obtained.

[c] In this experiment, the volumeter was kept under pressure for a second night, and a higher ΔV was obtained.

$$15.7 \frac{\text{cm}^3}{\text{mole}} \times 410 \text{ atm} \times 1033.3 \frac{\text{g/cm}^2}{\text{atm}} = 6.65 \times 10^6 \text{ g cm/mole}$$

The energy derived from glycolysis is approximately 29,000 cal/mole of lactate (15) or 12.37×10^8 g cm/mole. Therefore, the work of dilatation is

$$\frac{100 \times 6.65 \times 10^6}{12.37 \times 10^8} = 0.54\%$$

of the energy obtained from forming 1 mole of lactic acid. This percentage does not seem to be an excessive price to pay for using glycolysis as a source of ATP under pressure. Even at the deepest spot in the Pacific Ocean (11 km down), the work of dilatation would require only 1.5% of the available glycolytic energy.

It should be noted that volume changes measured by means of volumeters or pycnometers are those for complete reactions. They are not necessarily equal to volumes of activation (ΔV^*), which can be estimated only from pressure sensitivities of reactions (12).

DISCUSSION

A major aim of biological pressure studies is to be able to understand cellular responses to pressure in biochemical and physiological terms. The experimental results presented in this paper illustrate some of the difficulties that may be encoun-

tered in attempting to identify the biochemical basis for barosensitivity of any particular organism, even one such as *S. faecalis* with relatively complex nutrient requirements and a restricted array of catabolic enzymes. Much of the recent work on biological pressure effects has focused on synthetic reactions. Certainly, the cell size and compositional changes induced by pressure in organisms such as *E. coli* suggest an important role for the process of initiation of chromosome replication in determining barotolerance. However, our findings indicate that catabolism may also play a major role. Thus, barotolerance of *S. faecalis* growing in TRM medium appeared to be determined primarily by the pressure sensitivity of the cell's ribose-degrading system. Presumably the pressure-sensitive structures here are enzymes involved in ribose breakdown to three-carbon and two-carbon fragments.

The finding that *S. faecalis* cells did not become grossly enlarged or morphologically deformed when growing at high pressures indicates that the response of the coccus to pressure differs fundamentally from that of *E. coli*, even when ATP is being produced in excess and noncatabolic reactions are the major determinants of barotolerance. Thus, there is a parallel between pressure responses and responses to the amino acid analogue *p*-fluorophenylalanine. Both agents induce giant-cell formation in *E. coli* strain B, whereas neither has this effect on *S. faecalis*.

Attempts to define barotolerance of any particular microorganism encounter a number of problems. In this paper, we have mainly used growth rate as an index of tolerance. Others have used cell survival under pressure as an index (22). Survival may or may not be related to ability to grow, depending on experimental conditions. Another commonly used index of tolerance (22), which is probably usually related to growth rate under pressure, is the maximal pressure permitting growth in some specified time period. These indices can probably all be related to microbial ecology in compressed environments such as the oceans' depths, but it is clear that barotolerance can be defined only in relation to some fixed set of nutritional and physiological conditions. In fact, many organisms that are considered to be barotolerant may be so only in certain natural or concocted media and not in others. Thus, a bacterium at the bottom of the Challenger Deep of the Pacific Ocean may be barotolerant in its natural environment but may not be tolerant when placed in common bacteriological culture media. Temperature, of course, is also of major importance in determining barotolerance.

At present, there is not sufficient experimental data for making very extensive comparisons of pressure sensitivities of specific biochemical processes such as glycolysis and protein synthesis to ascertain whether certain organisms produce enzymes that are less sensitive to pressure than are the analogous enzymes in other organisms. In his review of pressure effects on fungi, Morita (14) presented a table giving rates of ethyl alcohol production from glucose by nongrowing yeast cells suspended in *p*H 5.6 phosphate buffer at 27 C for 3 hr. We used these rates to calculate ΔV^* values for yeast glycolysis, and the results indicated a pressure sensitivity similar to that of streptococcal glycolysis, even though our experimental conditions were different. For example, at ca. 400 atm, ΔV^* for yeast glycolysis was 52 ml/mole, and that for streptococcal glycolysis was 41 ml/mole. Clearly, more comparisons of this sort are needed to give us a feeling for just how barotolerance should be defined, but a great deal of experimental work will be required before valid comparisons can be made.

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LITERATURE CITED

1. Albright, L. J. 1969. Alternate pressurization-depressurization effects on growth and net protein, RNA and DNA synthesis by *Escherichia coli* and *Vibrio marinus*. Can. J. Microbiol. **15**:1237-1240.
2. Bauchop, T., and S. R. Elsden. 1960. The growth of micro-organisms in relation to their energy supply. J. Gen. Microbiol. **23**:457-469.
3. Bergmeyer, H. U. 1963. Methods of enzymatic analysis. Academic Press Inc., New York.
4. Ceriotti, G. 1955. Determination of nucleic acids in animal tissues. J. Biol. Chem. **214**:59-70.
5. Chumak, M. D., and T. P. Blokhina. 1964. Influence of high pressure on the accumulation of organic acids during glucose fermentation of barotolerant bacteria. Mikrobiologiya (Transl.) **33**:200-204.
6. Corner, T. R., and R. E. Marquis. 1969. Why do bacterial protoplasts burst in hypotonic solutions? Biochim. Biophys. Acta **183**:544-558.
7. Fenn, W. O., and R. E. Marquis. 1968. Growth of *Streptococcus faecalis* under high hydrostatic pressure and high partial pressures of inert gases. J. Gen. Physiol. **52**:810-824.
8. Forrest, W. W. 1967. Energies of activation and uncoupled growth in *Streptococcus faecalis* and *Zymomonas mobilis*. J. Bacteriol. **94**: 1459-1463.
9. Gale, E. F. 1947. The assimilation of amino acids by bacteria. J. Gen. Microbiol. **1**:53-76.
10. Landau, J. V. 1970. Hydrostatic pressure on the biosynthesis of macromolecules, p. 45-70. *In* A. M. Zimmerman (ed.), High pressure effects on cellular processes. Academic Press Inc., New York.
11. Marquis, R. E. 1970. Fluoroamino acids and microorganisms, p. 166-192. *In* F. A. Smith (ed.), Handbook of experimental pharmacology vol. XX/2. Springer-Verlag, Berlin.
12. Marquis, R. E., and W. O. Fenn. 1969. Dilatometric study of streptococcal growth and metabolism. Can. J. Microbiol. **15**:933-940.
13. Marsland, D. 1956. Protoplasmic contractility in relation to gel structure: Temperature-pressure experiments on cytokinesis and amoeboid movement. Int. Rev. Cytol. **5**:199-227.
14. Morita, R. Y. 1965. The physical environment for fungal growth. 2.

Hydrostatic pressure, p. 551-557. *In* G. C. Ainsworth and A. S. Sussman (ed.), The fungi. Academic Press Inc., New York.

15. Neilands, J. B., and P. K. Stumpf. 1955. Outlines of enzyme chemistry. John Wiley & Sons, Inc., New York.
16. Pollard, E. C., and P. K. Weller. 1966. The effect of hydrostatic pressure on the synthetic processes in bacteria. Biochim. Biophys. Acta **112**:573-580.
17. Tilney, L. G., Y. Hiramoto, and D. Marsland. 1966. Studies on the microtubules of heliozoa. III. A pressure analysis of the role of these structures in the formation and maintenance of axopodia of *Actinosphaerium nucleofilum* (Barrett). J. Cell Biol. **29**:77-95.
18. Yayanos, A. A., and E. C. Pollard. 1969. A study of the effects of hydrostatic pressure on macromolecular synthesis in *Escherichia coli*. Biophys. J. **9**:1464-1482.
19. Zimmerman, A. M. 1969. Effects of high pressure on macromolecular synthesis in synchronized *Tetrahymena*, p. 203-225. *In* G. M. Padilla, G. L. Whitson, and I. L. Cameron (ed.), The cell cycle. Academic Press Inc., New York.
20. Zimmerman, A. M. 1970. High pressure effects on cellular processes. Academic Press Inc., New York.
21. Zimmerman, A. M., and D. Marsland. 1964. Cell division: Effects of pressure on the mitotic mechanisms of marine eggs (*Arbacia punctulata*). Exp. Cell Res. **35**:293-302.
22. ZoBell, C. E. 1970. Pressure effects on morphology and life processes of bacteria, p. 85-103. *In* A. M. Zimmerman (ed.), High pressure effects on cellular processes. Academic Press Inc., New York.
23. ZoBell, C. E., and A. B. Cobet. 1964. Filament formation by *Escherichia coli* at increased hydrostatic pressures. J. Bacteriol. **87**:710-719.
24. ZoBell, C. E., and C. H. Oppenheimer. 1950. Some effects of hydrostatic pressure on the multiplication and morphology of marine bacteria. J. Bacteriol. **60**:771-781.