AN ABSTRACT OF THE THESIS OF

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(Name) (Degree) (Major)

Date thesis is presented May 19, 1965

Title SOME PHYSIOLOGICAL STUDIES ON CELLS OF VIBRIO MARINUS GROWN AT 4 C AND 15 C

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Abstract approved
(Major professor)

Cells of an obligately psychrophilic marine bacterium, Vibrio marinus, strain MP-1, were grown at 4 C and 15 C and studied for possible physiological differences. No differences were noted in sugar fermentation or temperature-shift growth studies; in both cases adequate nutrients were available. Some differences were noted in viability retention and manometric studies; adequate nutrients were not available. Fifteen C cells were more heat stable than 4 C cells. Fifteen C cells also utilized glucose at a higher rate than 4 C cells. These differences were attributed to differences in intra-cellular organization. The results indicated that in order to correlate laboratory results with in situ conditions, marine microorganisms should be studied when grown at the temperature of their natural habitat and without an abundance of nutrients.

SOME PHYSIOLOGICAL STUDIES ON CELLS OF *VIBRIO MARINUS* GROWN AT 4 C AND 15 C

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1966

APPROVED:

Redacted for Privacy

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Date thesis is presented July 19, 1965

Typed by Lucinda M. Nyberg

ACKNOWLEDGMENT

Without the instruction and confidence of Dr. R. Y. Morita, the financial aid of U. S. P. H. S. Training Grant 5 T1 GM 704 MIC, and the use of the facilities of Oregon State University this thesis could never have begun. Without the love, encouragement, and example of my husband this thesis would never have been completed. Thank you.

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INTRODUCTION

Temperature is one of the most important environmental factors which affects the growth of microorganisms. It helps to govern the growth rates, nutritional requirements, and general metabolic activities of cells. The chemical composition of lipids and enzymic composition of cells are also affected by temperature (15).

It is commonly observed that various diatoms, dinoflagellates, and bacteria grow more rapidly in the laboratory at temperatures 10 to 20 C higher than in their natural environment (3, 29). Since biogeographical surveys of marine phytoplankton have considered temperature an important factor, many terms such as arctic, boreal, temperate, and tropical have been used to imply a decisive effect of temperature upon growth and survival (3). The discrepancy between the sea temperature during periods of abundant growth and the optimum laboratory temperature of growth has been observed with various organisms (2, 7, 9, 28).

Because of the above observations, the question of possible physiological differences between cells grown at their original isolation temperature and at their optimum laboratory temperature becomes important. If there are no differences in cell physiology at various

temperatures, laboratory studies should aid materially in understanding the natural environment. However, if there are differences physiologically, such laboratory data must be interpreted with caution when it is used in relation to ecological situations.

Microbiologists have divided bacteria into three major divisions on the basis of their temperature characteristics of growth; these are the thermophilic, mesophilic, and psychrophilic bacteria. A great deal of work has been done to determine reasons for these temperature-based growth characteristics. Most researchers (1, 6, 13, 16, 20) have compared two different organisms, although some (27) have dealt with only one.

The recent isolation (24) and description (5) of an obligately psychrophilic marine bacterium, Vibrio marinus, strain MP-1, which functions in its natural habitat at 3 - 4 C and optimally in the laboratory at 15 C afforded an opportunity to investigate the possibility of physiological differences within one organism when grown at two temperatures. This organism grows well at both its original environmental temperature and its optimum laboratory temperature (23), so a comparison could be made.

REVIEW OF LITERATURE

In the chapter on temperature in Treatise on Marine Ecology and Paleoecology (12), Gordon Gunter stated, "Temperature is the most important single factor governing the occurrence and behavior of life". Braarud (3) has emphasized the importance of correlating information gained from field and laboratory studies for a fuller understanding of the marine environment, especially with reference to the effects of temperature, salinity, light, and inorganic nutrients. He has observed that cold-water diatoms and dinoflagellates grow best under laboratory conditions at temperatures which are 10 - 20 C higher than in their natural habitats. However, no data was presented on possible physiological differences in organisms grown at different temperatures. The lack of data emphasized the neglect of research in this important area.

Microbiologists have become interested in the differences between psychrophilic, mesophilic, and thermophilic bacteria. Koffler (20) noted that when exposed to heat, the cytoplasmic proteins from mesophilic bacteria were more subject to coagulation than those from thermophiles. Ingraham and Bailey (16) demonstrated that the temperature coefficients for the oxidation of glucose, acetate, and formate for facultative phychrophiles were lower than those for mesophiles. In cell-free extracts, however, the temperature coefficients

of the mesophiles were lowered to those of the psychrophiles. Their results suggested that the difference in the temperature response of psychrophilic and non-psychrophilic microorganisms was associated with the integrity of cellular organization. When classifying bacteria in accordance with their characteristics, Frank (6) noted that variations in incubation temperatures could cause some differences in the expression of those characteristics. Upadhyay and Stokes (27) demonstrated some interesting fermentation differences with a facultative psychrophile when they varied the incubation temperature. Baxter and Gibbons (1), in comparing respiratory activities of a psychrophilic and mesophilic yeast, obtained results which suggested that the temperature characteristics of the mechanisms for transporting solutes into these organisms were the main factors which determined the minimum growth temperature. Rose and Evison (25) have recently shown that the sugar transport mechanisms differ in sensitivity to near-zero temperatures in the mesophiles and psychrophiles studied.

No one has investigated psychrophilic marine microorganisms to ascertain if laboratory and in situ results can be meaningfully interpreted and correlated.

METHODS AND MATERIALS

Organism

Vibrio marinus MP-1 was isolated from a water sample taken at a depth of 1,200 m in the North Pacific Ocean (44 39.2' N lat, 125 34.9' W long). The water temperature was 3.24 C. Colwell and Morita (5) have taxonomically described this organism. It has a temperature maximum of 20 C and a temperature optimum of 15 - 16 C, as described by Morita and Haight (24). Morita and Albright (23) have shown that 15 C grown cells (15 C cells) are in the late logarithmic growth phase at 12 hours; 4 C grown cells (4 C cells) are in this phase at 24 hours. A stock culture was kept at 15 C and transferred once a month. Before use as inoculum in any experiment, cells were transferred twice at 12 or 24-hour intervals for 15 C and 4 C cells, respectively.

Growth Media

The medium used in most experiments was 2216 E or a variation in which glucose was added (2216 EG). One liter of 2216 E was composed of 26.25 g Rila marine salts (Rila Products, Teaneck, New Jersey), 5.0 g neopeptone (Difco), 1.0 g yeast extract (Difco), 0.1 g ferric phosphate, and about 900 ml of distilled water. The pH of this mixture was adjusted to 7.2 with sodium hydroxide. It was boiled,

cooled, filtered, and again adjusted to pH 7.2. Distilled water was added to bring the final volume to 1,000 ml. The medium was dispensed as needed, and autoclaved for 20 minutes at 121 C and 15 psi. For one liter of 2216 EG, 0.5 g glucose was added after filtering. Stock cultures were grown in 2216 E. The two transfers before use as inoculum were into 2216 EG.

A basic salt solution (BSS) plus phenol red broth base (PRBB) and the appropriate sugar were used in the sugar fermentation experiments. One liter of BSS was composed of 24.0 g sodium chloride, 0.7 g potassium chloride, 5.3 g magnesium chloride ($MgCl_2 \cdot 6H_2O$), 7.0 g magnesium sulfate ($MgSO_4 \cdot 7H_2O$), and distilled water to 1,000 ml. To this was added 16.0 g of PRBB (Difco). The BSS-PRBB medium was divided into 100 ml aliquots. To each of these aliquots 0.5 g of a sugar was added. These sugar media were dispensed in 10.0 ml portions into 18 x 150 mm metal capped test tubes containing Durham fermentation tubes. Sterilization was effected by autoclaving for 15 minutes at 121 C and 15 psi.

All media and solutions were cooled to the appropriate temperature before use.

Sugar Fermentation Studies

One drop of 15 C cell inoculum was added to duplicate tubes of sugar medium. The tubes were incubated without shaking in a

controlled-environment incubator-shaker (New Brunswick Psychro-Therm) at 15 C. They were checked visually for the production of acid and gas at 12-hour intervals for 48 hours.

A similar procedure was employed using 4 C cell inoculum and a 4 C incubation temperature. Visual checks were made at 12-hour intervals for 96 hours.

Temperature-shift Growth Studies

Twelve 500-ml Erlenmeyer flasks, each fitted with a sidearm nephelometer tube and containing 100 ml of 2216 EG, were used. Six were inoculated with 5.0 ml of 15 C cells and six with 5.0 ml of 4 C cells. These flasks were incubated at their respective temperatures in PsychroTherms, each with a reciprocating mechanism at 200 strokes per minute with a 2.54-cm stroke. At appropriate time intervals the rate of growth was estimated by reading optical density (OD) at 525 mμ in a Bausch and Lomb Spectronic 20 spectrophotometer (spectrophotometer). A flask containing 100 ml of 2216 EG was used as the 100 percent transmittance standard. When the cells were into the logarithmic growth phase, the flasks were incubated in duplicate as follows: 15 C cells at 15 C (control), 15 C cells at 10 C, 15 C cells at 4 C, 4 C cells at 4 C (control), 4 C cells at 10 C, and 4 C cells at 15 C. A gyrotory shaker (New Brunswick) water bath was used to maintain the 10 C temperature. Growth rate was again

followed in the spectrophotometer until a new trend was established.

Viability Retention Studies

Ten ml of 15 C cell inoculum were added to each of three Fernbach flasks containing 300 ml of 2216 EG. After 12 hours incubation with shaking at 15 C in the PsychroTherm the cells were aseptically harvested by centrifugation in a Sorvall RC-2 refrigerated centrifuge (RC-2) at 0 C and 10,400 x g for ten minutes. All further steps were carried out at 0 C unless otherwise indicated. The cells were suspended in a buffer of the following composition: 24.22 g tris (hydroxymethyl) aminomethane (Sigma 7-9), 26.25 g Rila marine salts, concentrated hydrochloric acid to adjust the pH to 7.2, and distilled water to 1,000 ml total volume. This Tris-HCl 75 percent sea water buffer will hereafter be referred to as buffer. Since all procedures in this experiment were performed aseptically, the buffer was autoclaved before use. The cells were washed once with buffer and suspended so that a 1:100 dilution gave an OD of 0.150 at 600 mμ. A duplicate series of 18 x 150 mm metal capped tubes, each containing 9.0 ml of buffer, had been placed in a polythermostat (24) with temperatures ranging from 5.0 to 32.3 C. One ml of the concentrated cell preparation was added to each tube. At twenty minute intervals 0.1 ml aliquots were removed and added to duplicate tubes containing 9.0 ml of 2216 EG. These tubes were incubated at 15 C. Growth was

determined visually at 28, 72, and 96 hours.

A similar procedure was employed using 4 C cell inoculum. The 1:100 dilution gave an OD of 0.180 at 600 mμ. The polythermostat temperatures ranged from 5.6 to 34.2 C. Final incubation was at 15 C.

At the end of three hours heating the duplicate tubes at each temperature from the polythermostat were combined. The cells were removed by centrifugation in the RC-2 at 0 C and 7,710 x g for ten minutes. The supernatants were decanted and kept on ice until used. A Beckman Model DB recording spectrophotometer was used to scan these heat-shock supernatants from 340 to 250 mμ. Where necessary the supernatants were diluted with buffer to obtain OD values within the range of reliability. This procedure was carried out for both the 15 C and 4 C cells.

Manometric Studies

The cells were prepared as in the Viability Retention Studies, except that aseptic techniques were not used and a 1:100 dilution gave an OD of $0.220 \pm .010$ at 600 mμ. The Warburg flasks contained 1.8 ml buffer, 0.2 ml 20 percent potassium hydroxide, 1.0 ml concentrated cell preparation, and 0.2 ml substrate. A 20 micromoles per ml concentration of glucose was used as substrate; distilled deionized water was substituted in endogenous studies. To standardize these

experiments, all steps from start to finish were performed in a timed sequence. Oxygen uptake was determined using standard Warburg manometric technique. Fifteen C cells and 4 C cells were studied at both 4 C and 15 C.

Micro-Kjeldahl analyses of the cells used in the experiments were made. One liter of the digestion mixture was composed of 0.150 g cupric selenate ($CuSeO_4$), 144 ml concentrated sulfuric acid, and distilled deionized water to bring the final volume to 1,000 ml. After four hours of digestion, direct Nesslerizations were performed. Color was read at 450 mμ in the spectrophotometer using ammonium sulfate as the standard. A conversion factor of 6.25 was used to calculate the mg of protein per ml of cells.

RESULTS

The results of the sugar fermentations are shown in Table I. No gas was produced with any sugar at either incubation temperature. Sugars which gave a strongly acidic reaction did so at both 15 C and 4 C. Sugars giving an acidic reaction were, except for rhamnose, the same at the two temperatures. The weakly acidic reactions found with some sugars were similar at both temperatures. There were, therefore, with one exception, no differences in the sugar fermentation patterns of 15 C and 4 C cells.

The growth curves of 15 C cells in the Step-down experiments are shown in Figure 1. Each slope was the average of two experiments. The control (15 C cells at 15 C) had a slope of 2.15. Incubation at 10 C gave a slope of 1.70. At 4 C the slope became 0.80. This indicated that 15 C cells grew fastest at 15 C and slower at lower temperatures.

Figure 2 illustrates the growth curves of 4 C cells in the Step-up experiments. Again, each slope was the average of two experiments. The control (4 C cells at 4 C) had a slope of 0.74. Note that this was similar to the slope of 15 C cells at 4 C. Incubation of 10 C gave a slope of 1.55, which was slightly less than for the 15 C cells at 10 C. At 15 C the slope became 2.10, which was almost the same as the slope of the 15 C control. It appeared that 4 C cells grew slowest at

TABLE I. SUGAR FERMENTATIONS.

Sugar	Incubation Temperature 15 C*	4 C**
Fructose	+++	+++
Galactose	+++	+++
Glucose	+++	+++
Levulose	+++	+++
Maltose	+++	+++
Arabinose	++	++
Mannose	++	++
Rhamnose	++	+
Xylose	++	++
Dulcitol	+	+
Inositol	+	+
Lactose	+	+
Mannitol	+	+
Raffinose	+	+
Salicin	+	+
Sorbitol	+	+
Trehalose	+	+

*24 hours incubation
**96 hours incubation

Note: +++ strongly acidic; tube yellow throughout
++ acidic; tube yellow with narrow red surface band
+ weakly acidic; tube orange with narrow red surface band

4 C and faster at higher temperatures. Initial growth temperature did not seem to effect cell growth at other temperatures.

Table II illustrates the viability of 15 C cells after exposure to temperatures ranging from 5.0 to 32.3 C for zero to 180 minutes. No cells remained viable after only 20 minutes exposure to 32.3 C. After 60 minutes cells exposed to 26.6 C were no longer viable.

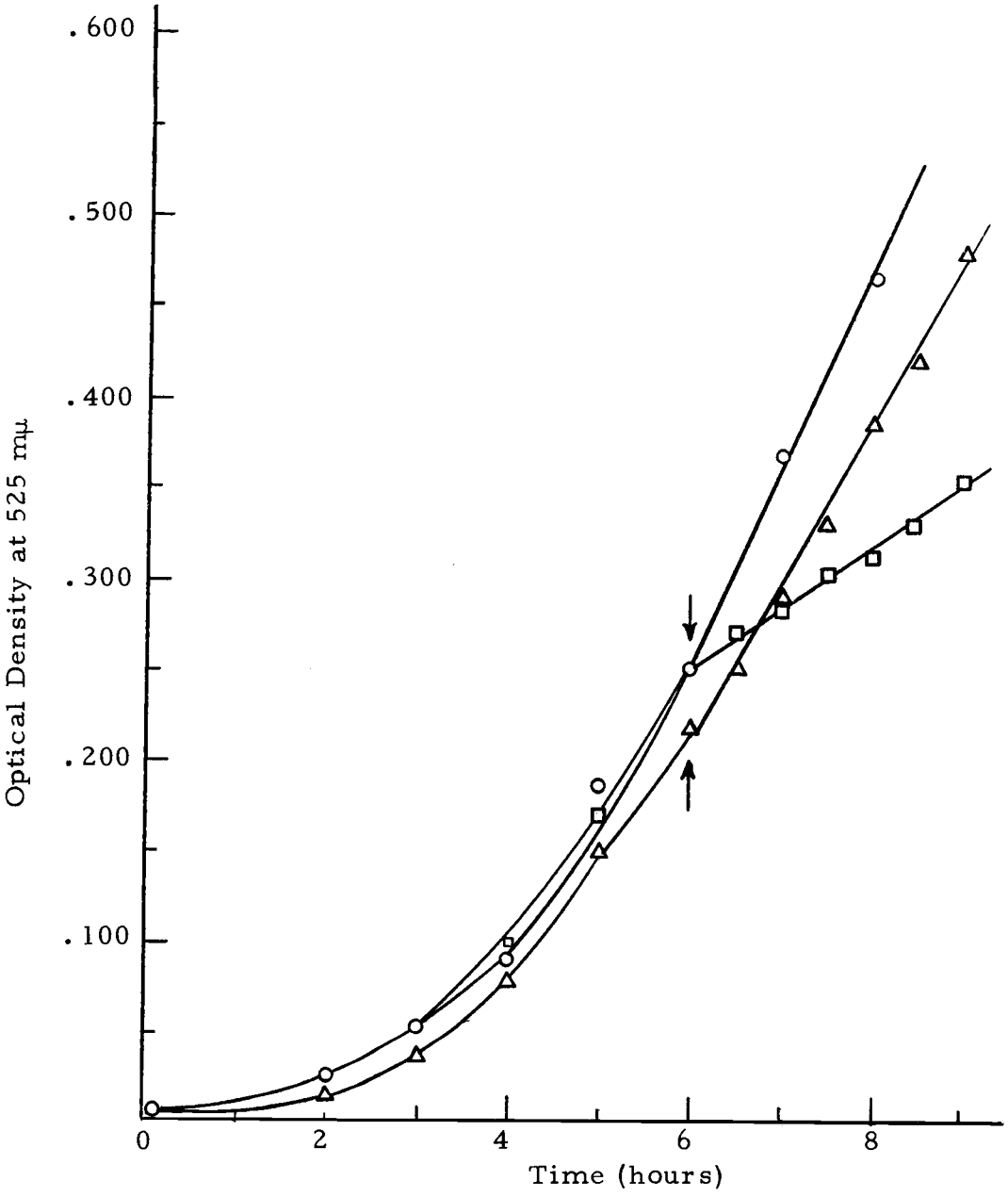

Figure 1. Step-down temperature-shift growth curves using 15 C grown cells of Vibrio marinus. Arrows indicate the time of temperature shift.

○ 15 C control
△ 15 C to 10 C
□ 15 C to 4 C

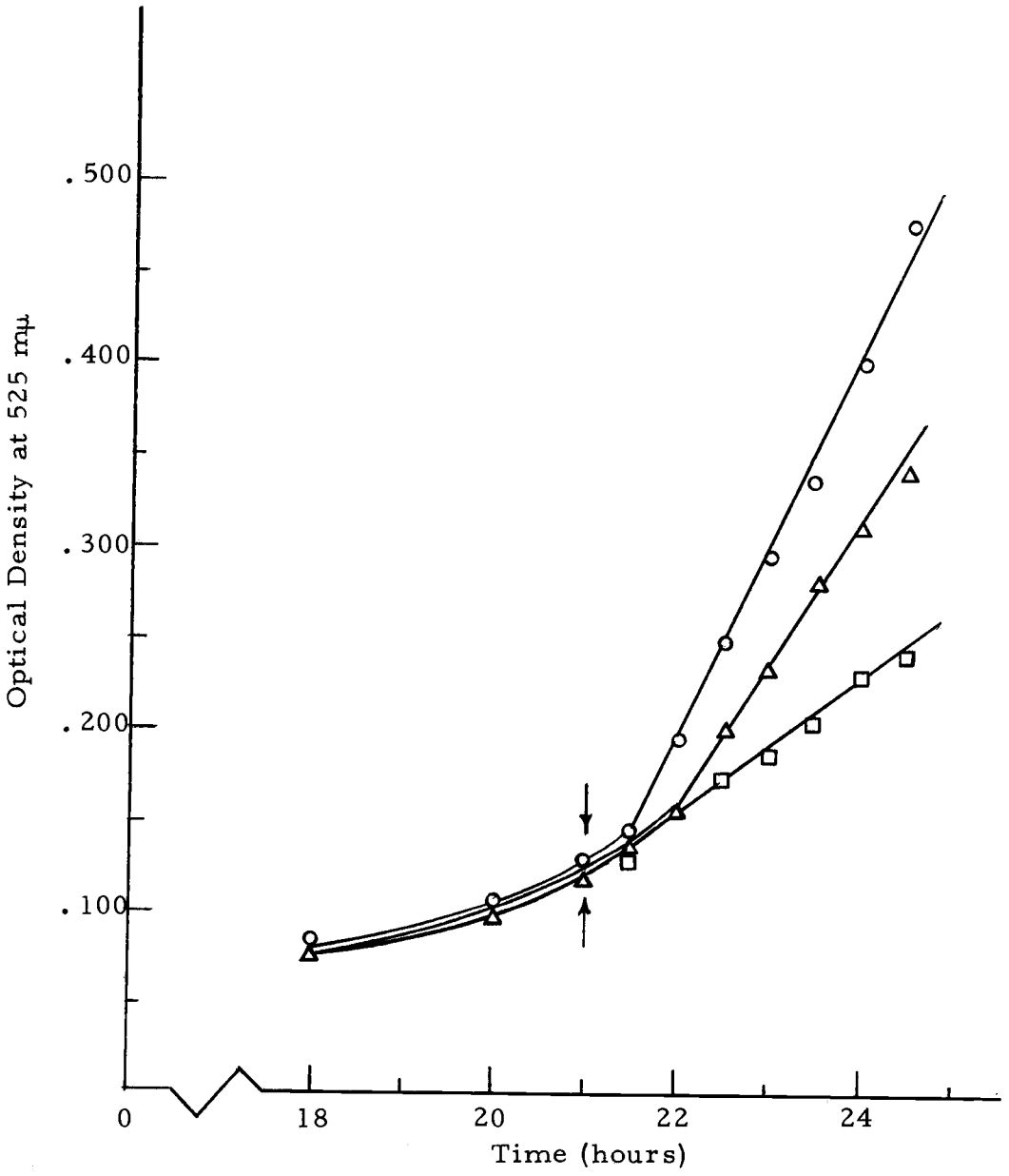

Figure 2. Step-up temperature-shift growth curves using 4 C grown cells of Vibrio marinus. Arrows indicate the time of temperature shift.

○ 4 C to 15 C △ 4 C to 10 C □ 4 C control

TABLE II. VIABILITY OF VIBRIO MARINUS MP-1 GROWN AT 15 C FOLLOWING EXPOSURE TO HEAT.

Time (min)	Exposure Temperature (C)								
	5.0	15.0	20.5	22.8	24.6	26.6	28.4	30.6	32.3
0	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	0
60	+	+	+	+	+	0	0	0	0
80	+	+	+	+	+	0	0	0	0
100	+	+	+	+	+	0	0	0	0
120	+	+	+	+	+	0	0	0	0
140	+	+	+	+	+	0	0	0	0
160	+	+	+	+	+	0	0	0	0
180	+	+	+	+	+	0	0	0	0

Table III shows the viability of 4 C cells after exposure to temperatures ranging from 5.6 C to 34.2 C for zero to 180 minutes. No cells remained viable after 20 minutes exposure to 30.6 C. After 60 minutes no cells were viable at 26.6 C. At 24.7 C viability was lost by 80 minutes. After 140 minutes even a temperature of 22.8 C was inimical. It appeared that 15 C cells were less heat labile than 4 C cells.

The absorption spectra of the supernatants of 15 C cells are plotted in Figure 3. More ultra-violet absorbing material was released as the temperature increased. Figure 4 is a similar plot for 4 C cells. Comparing the increase with Figure 3, more material left 4 C cells than 15 C cells.

TABLE III. VIABILITY OF VIBRIO MARINUS MP-1 GROWN AT 4 C FOLLOWING EXPOSURE TO HEAT.

Time (min)	Exposure Temperature (C)								
	5.6	15.1	20.7	22.8	24.7	26.6	28.4	30.6	34.2
0	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	0	0
60	+	+	+	+	+	0	0	0	0
80	+	+	+	+	0	0	0	0	0
100	+	+	+	+	0	0	0	0	0
120	+	+	+	+	0	0	0	0	0
140	+	+	+	0	0	0	0	0	0
160	+	+	+	0	0	0	0	0	0
180	+	+	+	0	0	0	0	0	0

The results of the manometric studies are shown in Figures 5 and 6. Each slope was the average of the two most illustrative of several experiments. Figure 5 shows oxygen uptake with glucose as substrate. Correction for endogenous uptake has been made. The slope of the line of oxygen uptake was 2.17 for cells grown and tested at 15 C, 1.25 for cells grown at 4 C and tested at 15 C, 1.17 for cells grown at 15 C and tested at 4 C, and 0.69 for cells grown and tested at 4 C. Figure 6 gives the results of the endogenous studies. The slope of the line of oxygen uptake was 2.33 for cells grown and tested at 15 C, 1.40 for cells grown at 4 C and tested at 15 C, 0.64 for cells grown at 15 C and tested at 4 C, and 0.42 for cells grown and tested at 4 C.

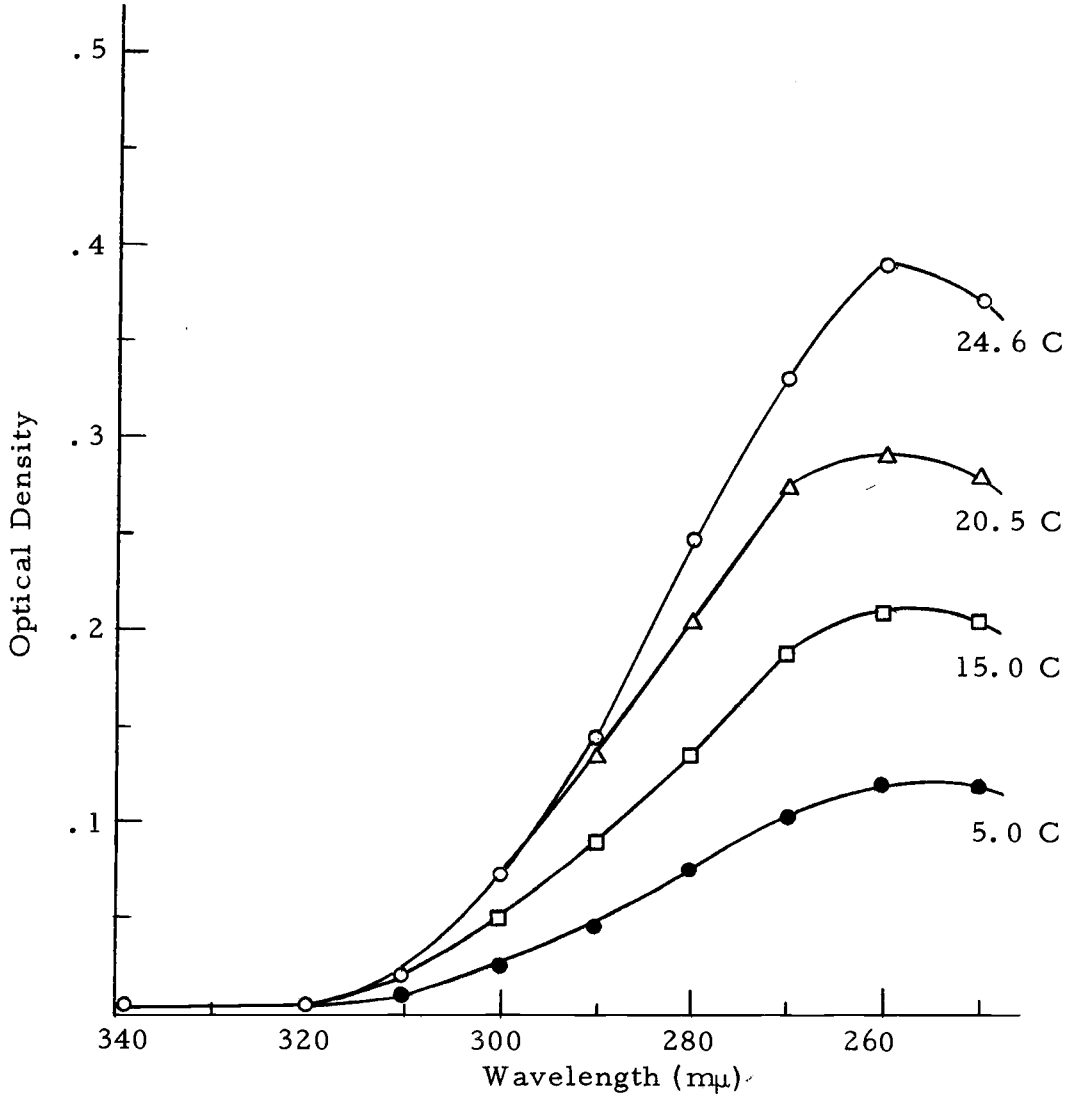

Figure 3. Absorption spectra of supernatants from heat-shocked cells of Vibrio marinus grown at 15 C. The cells were heated at the designated temperatures and removed by centrifugation at 0 C.

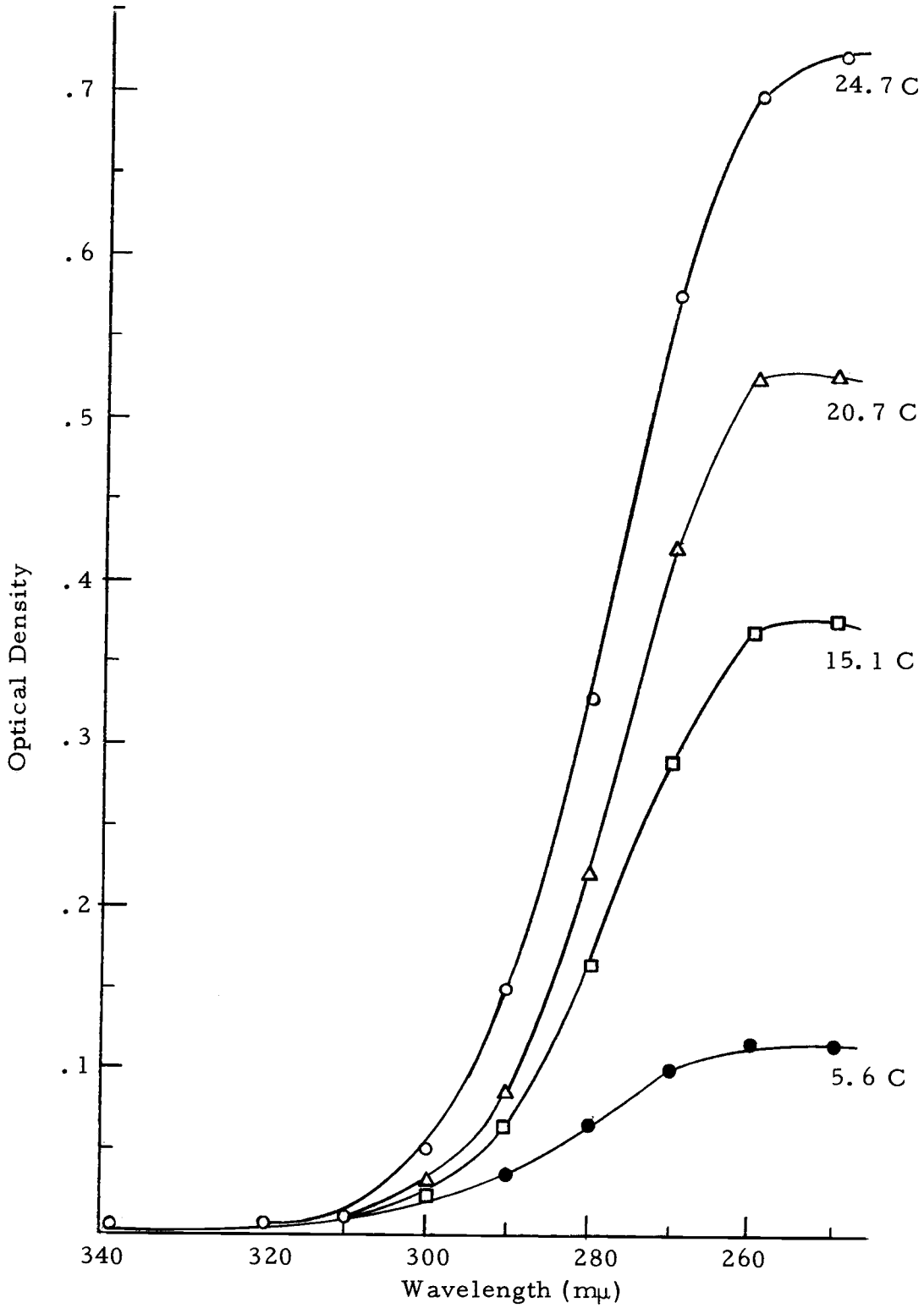

Figure 4. Absorption spectra of supernatants from heat-shocked cells of Vibrio marinus grown at 4 C. The cells were heated at the designated temperatures and removed by centrifugation at 0 C.

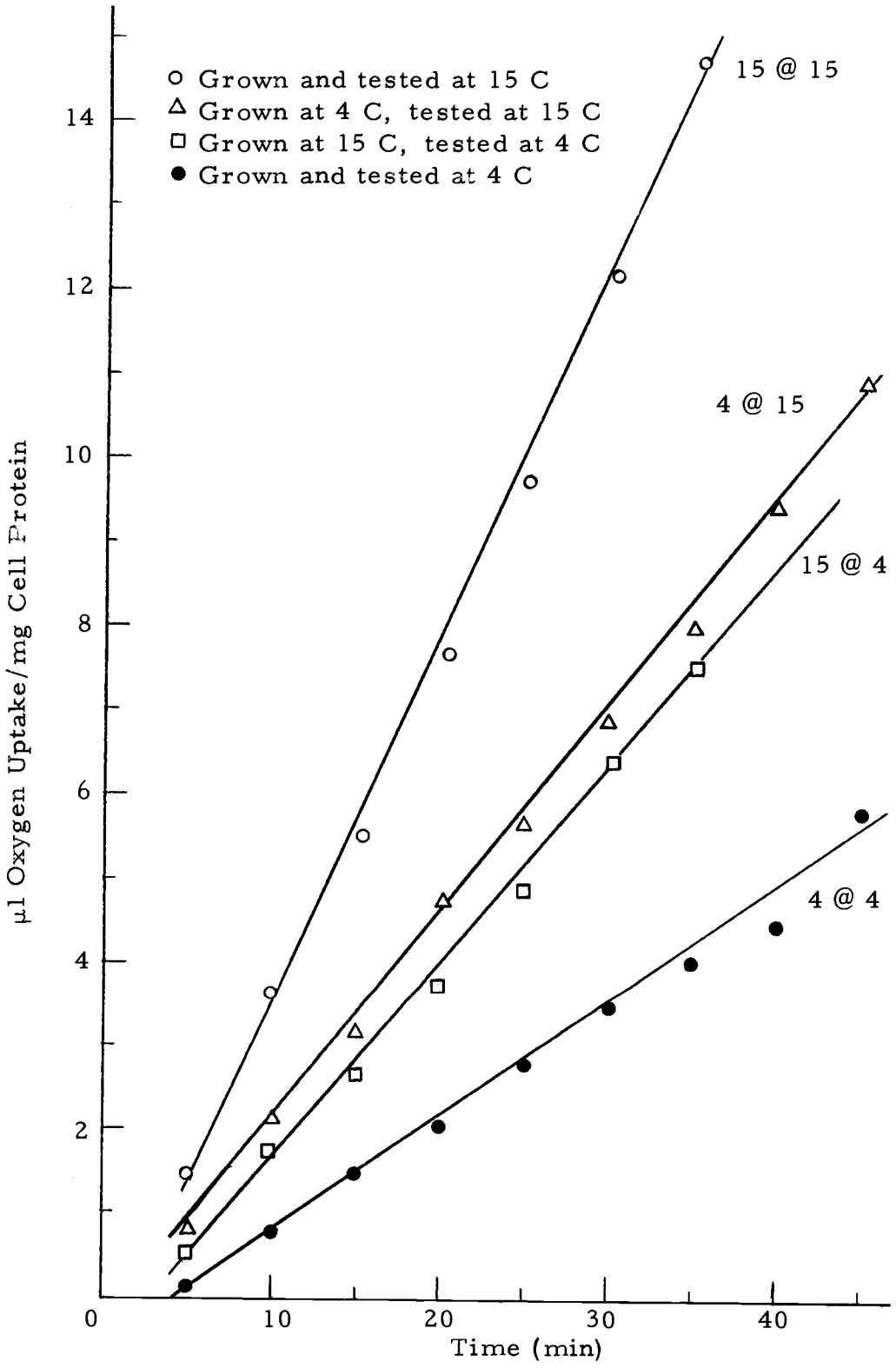

Figure 5. Oxygen uptake by cells of Vibrio marinus using glucose as substrate. Values corrected for endogenous control.

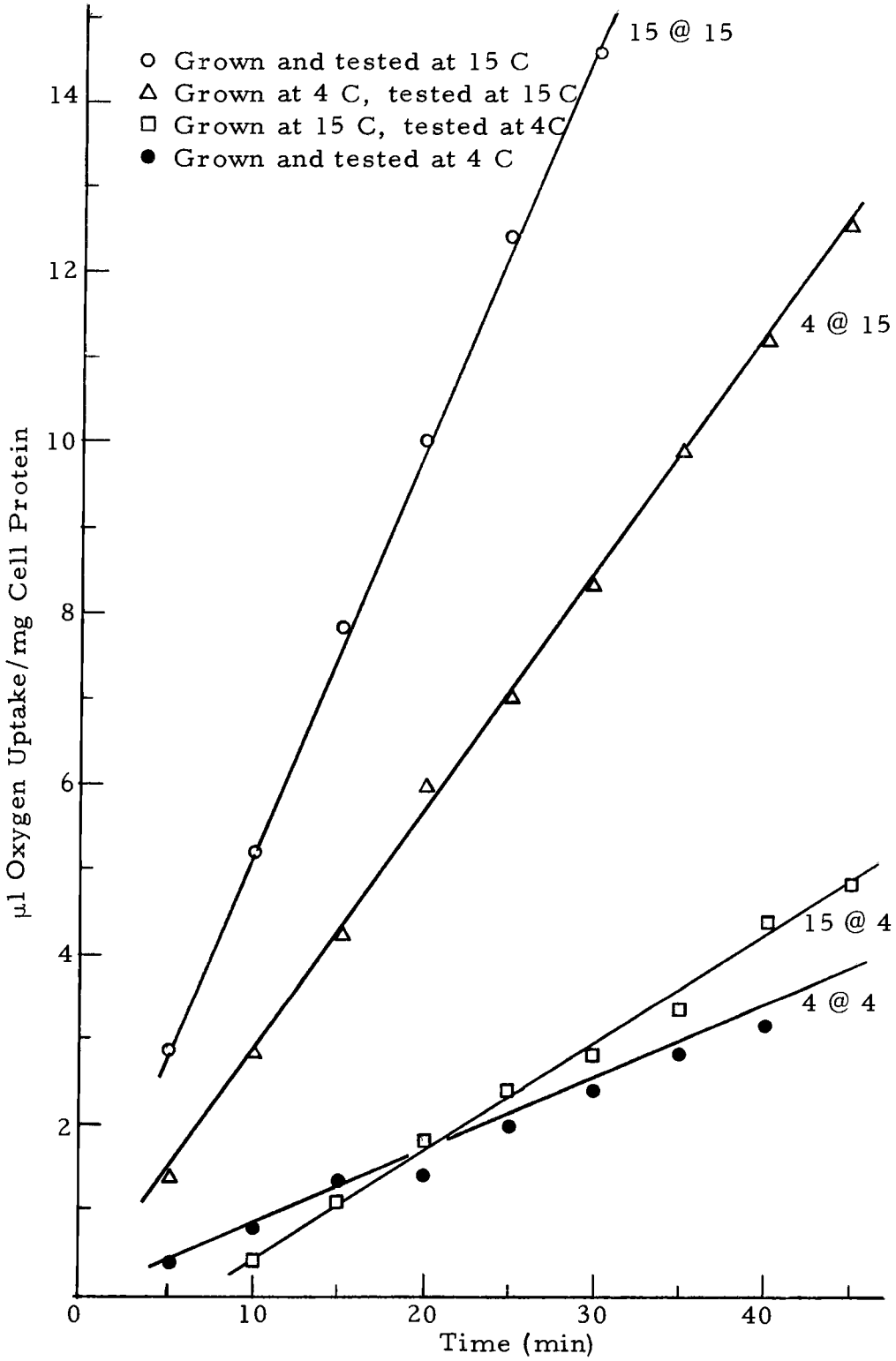

Figure 6. Endogenous oxygen uptake by cells of Vibrio marinus.

DISCUSSION

Although many marine organisms grow more rapidly at temperatures 10 to 20 C higher than the temperature of the environment from which they were isolated (29), there is little or no literature comparing the physiology of these organisms at the two temperatures. The optimum growth temperature of organisms as expressed in the laboratory may be the result of an adaptive process, i.e., a temperature controlled phenotypic expression of the genotype. Whether this expression is governed by temperature in marine organisms remains to be investigated. The purpose of these studies was to determine whether cells of Vibrio marinus MP-1 grown at environmental and optimum laboratory temperatures were physiologically different. In this discussion symbols such as 4 @ 15 and 15 @ 15 will be used. The former number will indicate the initial incubation temperature, the latter will indicate the temperature of secondary treatment.

This culture is an aerobic Vibrio; therefore if carbohydrates are attacked, no gas should be produced (4). If the incubation temperature affects enzymatic pathways, a lack of or difference in intensity of fermentation might be observed, as might gas production. Table I shows neither major fermentation differences nor induction of gas production. The one exception, rhamnose, is so slight as to seem insignificant. More refined techniques, such as titrating acidity or

using manometric methods, might reveal other or more definitive differences in levels of enzymatic activity. It can be concluded from these data that incubation at 4 C and 15 C does not affect sugar fermentation patterns.

In the temperature-shift experiments a new growth rate for 15 @ 15 cells was noted with a 15 @ 4 shift. This rate was the same as 4 @ 4. Conversely, the 4 @ 15 rate corresponded to the 15 @ 15 rate. The 4 @ 10 and 15 @ 10 rates were nearly identical. In other words, no physiological differences were apparent. There was no extensive lag period. However, if the cells had been in synchronous growth, a lag might have been shown.

The fermentation and temperature-shift studies deal with growing cells in the presence of nutrients. Adaptive processes could readily take place, especially since all nutrient materials were supplied to the cells. This may be a reason why physiological differences of organisms grown at two temperatures have not been readily detected in the laboratory. Because of the above negative results, it was decided that nutrients should not be included in further attempts to demonstrate a difference between cells grown at the optimum laboratory temperature and at the original environmental temperature. This procedure could minimize adaptive processes by not supplying the necessary materials needed for adaptation.

It was thought possible that the viability retention capability of

4 C cells might be less than for 15 C cells at inimical temperatures. Table II shows that heating 15 C cells at 26.6 C for 60 minutes caused death of the inoculum; 180 minutes of heating at 24.6 C did not destroy viability. Table III indicates that 4 C cells can be destroyed with 80 minutes of heating at 24.7 C or 140 minutes of heating at 22.8 C. These data suggest that 4 C cells are more heat labile than are 15 C cells. The reasons for greater viability loss with 4 C cells are not known, but it has been suggested (10) to be a heat-induced rupture of the membrane. Since cell membranes are mainly lipoproteins (26), a qualitative difference of lipids in cells grown at the two temperatures might render one membrane more heat labile than the other. Some workers (17, 18, 22) have shown that the fatty acid constituents of lipids are more unsaturated at lower temperatures. This unsaturation could make the 4 C cell membrane more susceptible to heat. It has also been stated (19) that as temperature decreases, hydrophobic bonds of proteins are weakened. Any or all of these factors might account for the observed differences in viability retention. Figures 3 and 4 display more dramatically the differences in thermal sensitivity of 4 C and 15 C cells. Analysis of the supernatants from 15 @ 5.0 and 4 @ 5.6 cells indicates that a similar amount of 260 mμ absorbing material was released. This was probably due to endogenous metabolism. The appearance of ultra-violet absorbing material in supernatants has been observed in organisms respiring endogenously (8). The 4 @ 15.1

supernatant contained 1.8 times the amount of 260 mμ absorbing material as the 15 @ 15.0 supernatant. Similar values were found for 4 @ 20.7/15 @ 20.5 and 4 @ 24.7/15 @ 24.6 supernatants. The reasons for the greater amounts of material in the supernatants of 4 C cells are probably similar to those discussed for Tables II and III, i.e., there seems to be some membrane difference(s) between 4 C and 15 C cells.

Manometric techniques were used to study the response of intact cells with glucose as substrate (Figure 5). The 15 @ 15 slope is approximately double the 15 @ 4 slope; the 4 @ 15 slope is approximately double the 4 @ 4 slope. This is probably a simple Q_{10} effect (12, 15), i.e., as the temperature increases or decreases by ten degrees (in these studies by eleven degrees) the reaction rate will double or halve, respectively.

There may be a number of reasons for the differences in the 15 @ 15/4 @ 15 and 4 @ 4/15 @ 4 slopes. If it is assumed that the 4 C and 15 C cells are equally permeable to glucose at all temperatures within the growth range, the difference in slopes between 15 @ 4 and 4 @ 4 could be attributed to the presence of more glucose-utilizing enzymes in the 15 C cells. If however, permeability is not the same, the difference may be due to intra-cellular organization. The viability retention studies have indicated a possible membrane difference. Ingraham and Bailey's (16) work comparing the oxidation of glucose,

acetate, and formate by psychrophiles and mesophiles indicated that cellular integrity, not enzyme differences, was responsible for differences in rates of oxidation. Langridge (personal communication) has shown that partially purified preparations of malic dehydrogenase from 4 C and 15 C cells of Vibrio marinus MP-1 exhibited no difference in thermolability. These data indicate that intra-cellular organization is a more likely explanation for the slope differences than any enzymatic variations.

Endogenous respiration curves are shown in Figure 6. Endogenous respiration is lowest with 4 @ 4 cells. This may reflect the functioning of a cold-labile enzyme system. Langridge (21) has shown that malic dehydrogenase from this culture was partially inactivated at 4 C but could be reactivated upon elevation of the temperature to 15 C. The increase in slope shown by 4 @ 15 cells could be due to partial reactivation of the cold-labile enzyme system at the higher temperature. Since the intra-cellular organization of the 4 C cells may not be adaptable in the absence of nutrients to 15 C function, the observed slope is lower than for the 15 @ 15 cells. The 15 C cells are fully adapted for functioning at 15 C. Hence maximal endogenous activity is observed in the 15 @ 15 curve. However, with 15 @ 4 cells the cold-labile enzyme system may be partially inactivated, thereby accounting for the drastic decrease in slope.

Studies performed with nutrients present did not reveal any

physiological differences when Vibrio marinus MP-1 was grown at 4 C and 15 C. Studies performed without nutrients did show some differences which seem to indicate that intra-cellular organization and membrane integrity are involved. Since very little organic matter (11) is available in the oceans below the thermocline, and since lack of nutrients seems to physiologically affect this culture when incubation temperatures not synonymous with the original environmental temperature are used, it seems feasible to conclude that studies without nutrients would most closely approximate conditions in the natural habitat. It also becomes apparent that organisms should be kept at their original temperature from isolation through subsequent studies to insure proper understanding of the relationships between marine organisms and their environments.

SUMMARY

Cells of Vibrio marinus, strain MP-1, grown at 4 C and 15 C, were studied for possible physiological differences. There were no such differences evidenced by sugar fermentation or temperature-shift growth experiments. When viability retention after exposure to heat was studied, 15 C cells were more stable than 4 C cells. Manometric studies using glucose as substrate revealed that 15 C cells utilized substrate faster than 4 C cells. These differences, observed under conditions of nitrogen starvation, were attributable to differences in intra-cellular organization. Endogenous metabolism, as evidenced by manometric studies, also revealed differences between 4 C and 15 C cells.

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