THE GROWTH AND VIABILITY OF SIXTY-THREE SPECIES OF MARINE BACTERIA AS INFLUENCED BY HYDROSTATIC PRESSURE[1]

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ABSTRACT

The hydrostatic pressure of sea water, which increases approximately 0.1 atmosphere per meter of depth, was found to affect the viability, reproduction, and morphology of 63 stock cultures of marine bacteria representing several genera. Many of the cultures were killed at 27° C by pressures ranging from 200 to 600 atm, although some few reproduced at 600 atm. Initial inoculum concentrations of the various bacteria appeared to influence their ability to reproduce or to tolerate high pressures. Pressures exceeding 400 atm inhibited the fission of certain bacteria without stopping their growth, thereby resulting in bizarre cells, some of which formed long filaments.

INTRODUCTION

The precursory observations of Certes (1884), Regnard (1891), ZoBell and Johnson (1949), and ZoBell and Oppenheimer (1950) indicate that hydrostatic pressures of the order of 200 to 600 atm have a pronounced effect on the vertical distribution and activities of bacteria in the sea. The significance of these observations is suggested by the fact that pressures exceeding 200 atm prevail in more than 90% of the area of the oceans. Approximately half of the oceans of the world are deeper than 4,000 m, at which depth the hydrostatic pressure is about 400 atm.

From the illustrative data in Table I it will be observed that the pressure of sea water increases with depth by approximately 0.1 atm/m, the pressure-depth gradient being influenced somewhat by latitude, temperature, and salinity. One atmosphere is equivalent to 14.696 lb/in^2, 760 mm Hg, 1.0332 kg/cm^2, or to 1.0133 bars.

Living bacteria have been found in virtually all samples of marine sediments examined for their presence regardless of water depth (ZoBell, 1946). Until recently the greatest depth at which bacteria

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TABLE I. Selected Pressure-depth Gradients in Atmospheres Per Meter in the Sea for Different Depths, Salinities, Temperatures, and Latitudes (Adapted From Data Given By Bjerknes and Sandström, 1910)

Dynamic depth (m)	*Salinity* ‰	*Temperature* °C	*Latitude 30°* atm/m	*Latitude 60°* atm/m
0	32	0	0.099,414	0.099,403
0	32	20	0.098,831	0.099,092
0	35	0	0.099,375	0.099,638
0	35	20	0.099,052	0.099,314
5,000	35	0	0.101,757	0.102,026
5,000	35	5	0.101,660	0.101,929
10,000	35	0	0.103,952	0.104,225

had been found was 5,800 m, but during the last year the Galathea Deep Sea Expedition extended the lower limits of the biosphere to 10,460 m. In several samples of sediments collected from depths exceeding 10,000 m in the Philippine Trench, ZoBell (1952) demonstrated the presence of bacteria that were physiologically active at pressures of the order of 1,000 atm. In fact, the well being of many bacteria collected from abyssal oceanic depths appears to depend upon high pressure. The purpose of this paper is to record the effect of pressure upon the activities of 63 different species of marine bacteria, all of which were taken from depths of less than 6,000 m.

EXPERIMENTAL METHODS

The bacteria were subjected to pressures up to 600 atm by means of apparatus described by ZoBell and Oppenheimer (1950). Essentially the apparatus consisted of thick-walled steel cylinders which have an inside diameter of 1 ⅜ inches and which are long enough (11 inches) to accommodate two dozen small culture tubes. The latter were fitted with no. 000 neoprene stoppers which functioned as pistons to subject the aqueous contents of the tubes to virtually the same pressure as the hydraulic fluid filling the steel cylinder. Preliminary experiments proved that pressure up to 1,000 atm could be applied and released ten times successively by means of the connecting hydraulic pump without the contents of the closed tubes becoming contaminated.

The culture medium consisted of sea water enriched with 0.5% peptone, 0.1% yeast extract, and 0.01% ferric phosphate. Following autoclave sterilization the pH of the medium was 7.6. Sufficient volume of such sea water medium was inoculated with bacteria in their logarithmic phase of growth to fill the number of culture tubes required

for any one experiment. This ensured having comparable numbers and kinds of bacteria at the beginning of each experiment. The inoculated medium was pipetted aseptically into sterile culture tubes, leaving approximately 0.5 ml of air at the top. The tubes were closed with sterile stoppers preparatory to placing them in pressure cylinders. After displacing the air from the cylinders by filling the space between the culture tubes with water, the cylinders were closed and immersed in a water bath at 27° C. Sufficient time (about 10 minutes) was allowed for the contents of the cylinders to attain this temperature. Then each cylinder in turn was connected to the hydraulic pump and the desired pressure applied. The valve was closed, the pump disconnected from the pressurized cylinders, and the latter were returned to the water bath to provide for the incubation of the bacterial cultures at 27° C.

Following four days' incubation the cylinders were reconnected to the hydraulic pump and gauge for checking the terminal pressure. After releasing the pressure, the culture tubes were removed from the cylinders for examination for evidence of growth as indicated by increased cloudiness or turbidity of the medium. Those tubes showing no evidence of bacterial growth under pressure were incubated an additional four days at room pressure in order to ascertain if the bacteria were still viable. The abundance and viability of bacteria in some tubes was estimated by standard plate count procedures. The contents of other tubes were examined by means of a phase microscope at a magnification of 970x.

RESULTS

In order to assess the effect of the abundance of bacteria in the inoculum upon the pressure tolerance of bacteria, four representative species were introduced into sea water medium to give initial populations ranging from 10 to 10^7 cells/ml. After four days' incubation at different pressures the bacterial population was determined by plate counts (Table II). When incubated at pressures of 1, 200, or 400 atm, the final bacterial population was not perceptibly affected by the initial number of cells present within the range of 10^2 to 10^6 cells/ml. Comparable results were not obtained when the initial bacterial population was less than 10^2 cells/ml. This suggests that an initial low bacterial population results in a more prolonged lag phase probably due to the presence of fewer pressure-tolerant cells in the smaller inocula. Therefore in subsequent experiments the medium was inoculated with enough cells to give initial bacterial populations of approximately 10^3 cells/ml.

As shown by the data in Table II, there was generally a decrease in

TABLE II. Numbers of Viable Cells per ml of (a) Culture No. 643, (b) *Serratia marinorubra*, (c) *Sarcina pelagia*, and (d) Culture No. 38:172 as Indicated by Plate Counts After Sea Water Broth Cultures Containing the Indicated Inocula Had Been Incubated at Different Pressures for Four Days at 27° C

Cultural organism	*Inoculum cells/ml*	*Hydrostatic pressures in atmospheres*			
		one	*200*	*400*	*600*
(a)	3×10^{0}	5×10^{7}	1×10^{7}	2×10^{8}	0
	3×10^{2}	2×10^{7}	5×10^{7}	9×10^{7}	8×10^{2}
	3×10^{4}	1×10^{7}	2×10^{7}	4×10^{7}	6×10^{4}
	3×10^{7}	3×10^{7}	3×10^{7}	7×10^{6}	4×10^{6}
(b)	2×10^{1}	5×10^{7}	9×10^{6}	6×10^{5}	7×10^{1}
	2×10^{3}	6×10^{7}	3×10^{7}	5×10^{6}	8×10^{4}
	2×10^{5}	9×10^{7}	5×10^{7}	7×10^{6}	8×10^{4}
	2×10^{7}	5×10^{7}	3×10^{7}	7×10^{7}	7×10^{6}
(c)	2×10^{-1}	1×10^{7}	3×10^{3}	1×10^{5}	0
	2×10^{1}	3×10^{7}	7×10^{7}	4×10^{7}	1×10^{3}
	2×10^{3}	1×10^{8}	5×10^{7}	3×10^{7}	3×10^{4}
	2×10^{5}	3×10^{7}	4×10^{7}	4×10^{7}	7×10^{6}
(d)	6×10^{0}	4×10^{7}	4×10^{7}	6×10^{3}	2×10^{1}
	6×10^{2}	9×10^{7}	4×10^{7}	5×10^{5}	2×10^{2}
	6×10^{4}	5×10^{7}	4×10^{7}	7×10^{7}	2×10^{3}
	6×10^{6}	1×10^{8}	5×10^{7}	5×10^{7}	6×10^{6}

the bacterial population of medium incubated at 600 atm. Employing turbidity as a criterion of growth or reproduction, it was found that only seven out of a total of 63 species tested reproduced at 600 atm. The pressure tolerance of the 63 species, representing 10 different genera, recorded in Table III, is summarized as follows:

Number of bacterial cultures which:	*Hydrostatic pressure at 27° C*			
	1 atm	*200 atm*	*400 atm*	*600 atm*
Reproduced	63	48	35	7
Showed no change	0	10	17	33
Were killed	0	5	11	23

In interpreting these results, it should be noted that the organisms had been maintained in the laboratory for several years since their isolation from the sea. Whether their pressure tolerance changed during this period is problematical, although significantly those tolerating the highest pressures in general are species commonly found at greater depths in the sea. Most of the organisms, described by ZoBell and Upham (1944), were originally isolated from shallow water

TABLE III. Multiplication of Marine Bacteria as Indicated by Relative Turbidity in Sea Water Broth After Eight Days' Incubation at Different Hydrostatic Pressures at 27° C

	Hydrostatic pressures in atmospheres			
*Name or number of culture**	*one*	*200*	*400*	*600*
Achromobacter stenohalis	++++	−	−	K
Achromobacter aquamarinus	++++	++++	+++	−
Achromobacter stationis	++++	++++	++++	−
Achromobacter thalassius	++++	K	K	K
Actinomyces halotrichis	++	−	−	−
Actinomyces marinolimosus	++++	++++	−	−
Bacillus imomarinus	++++	−	−	−
Bacillus cirroflagellosus	++++	++	−	−
Bacillus epiphytus	+++	++	++	K
Bacillus submarinus	++++	+++	+++	−
Bacillus thalassokoites	++++	+++	+	−
Bacillus filicolonicus	++++	−	K	K
Bacillus abysseus	++++	++++	+++	−
Bacillus borborokoites	++++	++++	++++	+++
Flavobacterium marinotypicum	++++	++++	++++	−
Flavobacterium marinovirosum	++++	++++	++	−
Flavobacterium neptunium	++	+	−	−
Flavobacterium okeanokoites	++++	++++	+	−
Micrococcus aquivivus	++++	++++	++++	++++
Micrococcus sedimenteus	++++	++++	++++	K
Micrococcus maripuniceus	++++	++++	−	−
Micrococcus infimus	+++	++++	−	−
Micrococcus sedentarius	++++	++++	++++	−
Micrococcus euryhalis	++++	K	K	K
Pseudomonas enalia	++++	+++	−	K
Pseudomonas neritica	++++	++++	+++	K
Pseudomonas azotogena	++++	K	K	K
Pseudomonas vadosa	++++	++++	++++	−
Pseudomonas oceanica	++++	+++	++++	−
Pseudomonas felthami	++++	++++	++	−
Pseudomonas aestumarina	++++	−	K	K
Pseudomonas membranula	+	−	K	K
Pseudomonas stereotropis	++++	+++	+	K
Pseudomonas coenobios	++++	++++	++++	−
Pseudomonas obscura	++++	++++	++	−
Pseudomonas pleomorpha	++++	++++	++	−
Pseudomonas marinopersica	++	K	K	K
Pseudomonas periphyta	++	−	K	K
Pseudomonas hypothermis	++++	K	K	K
Pseudomonas perfectomarinus	++++	++++	+++	+++
Pseudomonas xanthochrus	++++	−	−	−

TABLE III. (*Continued*)

*Name or number of culture**	*Hydrostatic pressures in atmospheres*			
	one	*200*	*400*	*600*
Sarcina pelagia	++++	+++	+++	−
Serratia marinorubra	++++	++++	+++	−
Vibrio marinopraesens	++++	++++	+++	−
Vibrio algosus	++++	++++	++	+
Vibrio adaptatus	++++	+++	+++	K
Vibrio marinoflavus	+++	+++	+	K
Vibrio ponticus	++++	+++	++	K
Vibrio phytoplanktis	++++	++++	++++	+++
Vibrio haloplanktis	++++	++++	++++	+
Vibrio marinovulgaris	++++	+++	−	−
Vibrio marinofulvus	++++	++++	++	−
Vibrio marinagilis	++++	+	−	−
Vibrio hyphalus	++++	++++	K	K
Number 516	++++	++++	++	K
Number 549	++++	++++	+++	K
Number 595	++	−	−	−
Number 623	++	++	−	−
Number 632	++++	−	−	−
Number 633	++++	++++	−	−
Number 639	++++	++	K	K
Number 643	++++	++++	++++	++++
Number 689	++++	+++	−	K

* All except the numbered cultures have been described by ZoBell and Upham (1944).

++++ = good multiplication
+++ = fair multiplication
++ = less multiplication
\+ = poor multiplication
− = no multiplication, but organisms not killed
K = all bacteria in culture killed

TABLE IV. Morphological Variations of Marine Bacteria Induced by Hydrostatic Pressures Ranging from 200 to 600 atm After Four Days' Incubation at 27° C

Organism	*Morphological variations*
Bacillus abysseus	Increase in cell size and spore formation
Bacillus borborokoites	General increase in cell size
Micrococcus aquivivus	General increase in cell size
Sarcina pelagia	Increase in size and marked pleomorphism
Serratia marinorubra	Formation of long filaments and loss of motility
Vibrio phytoplanktis	Longer pleomorphic rods, granular, loss of motility
Culture no. 643	Long filament formation

environments and none of them came from depths exceeding 6,000 m where pressures are greater than 600 atm.

Examination of the bacteria with a phase microscope shortly after the cultures were removed from the pressure cylinders showed that many had undergone morphological alterations (Table IV). The commonest change in organisms that failed to reproduce at elevated pressures was an increase in cell size, especially in length. Pressure to the limit of tolerance nearly always induced marked pleomorphism and rendered the cells nonmotile. Several seemed to grow in size or length without any evidence of cell fission or reproduction.

DISCUSSION

Although our knowledge of the effects of pressure on organisms is still woefully scant, data summarized by Cattell (1936) show that pressures such as those that occur in the sea may have multiple physiological effects. Slightly increased pressures may be stimulatory, whereas higher pressures cause the attenuation or death of organisms. According to literature reviewed by Bridgman (1946), pressure affects the rate and direction of many chemical reactions. By virtue of its effect on solubilities and dissociation constants, the pH and Eh values of complex solutions may be affected by pressure. These and other properties of solutions affected by pressure may combine to influence the physiology and ecology of marine organisms in many ways.

Very little is known about the ability of organisms to adjust themselves to higher pressures. In the marine environment, where pressures range from one to somewhat more than 1,000 atm, organisms may become acclimatized to the pressures of their habitat range. The results presented in this paper substantiate the observations of ZoBell and Johnson (1949) that the pressure tolerance of marine bacteria is related to their depth habitat. The intolerance of certain bacteria for pressures exceeding 200 atm, for example, suggests that such organisms are not active in the sea at depths exceeding 2,000 m. On the other hand, finding bacteria at depths of several thousand meters that grow at atmospheric pressure but not at pressures isobaric with the environment where they are found raises the question whether such bacteria were active in situ or were in a state of dormancy.

Such questions must be answered before the marine microbiologist can hope to appraise the importance of bacteria as biochemical or geochemical agents in the deep sea. The effect of temperature must be taken into account in seeking the answers to such questions, because there is evidence that the pressure tolerance of organisms is

partly a function of the temperature. Although data to be detailed elsewhere show that the pressure tolerance of some bacteria is increased at elevated temperature, others seem to exhibit maximum pressure tolerance at the lowest temperature at which they are physiologically active.

While submarine pressures undoubtedly affect the distribution and physiological activities of bacteria, at no place in the sea will pressures prohibit the presence of bacteria, because mixed cultures have been shown to be alive and active at presssures higher than those characteristic of the most abyssal deeps.

SUMMARY

The optimal initial bacterial population in nutrient medium to give comparable results in pressure tolerance tests proved to be approximately 10^3 viable cells per ml.

Pressures ranging from 200 to 600 atm inhibited the normal growth of nearly all of the 63 different species of marine bacteria tested, although a few cultures of mixed microflora from deep sea sediments reproduced at pressures exceeding 1,000 atm. Out of 63 pure cultures tested, 15 failed to reproduce when subjected to a pressure of 200 atm at 27° C and 5 were killed in four days. Of the 28 that failed to reproduce at 400 atm 11 were killed, and of 56 that failed to reproduce at 600 atm 23 were killed by pressure.

The morphology of bacteria was affected by pressures ranging from 400 to 600 atm.

The distribution and physiological activities of bacteria in the sea are probably influenced by the hydrostatic pressures which increase per meter of depth by approximately 0.1 atm.

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