J. Membrane Biol. 64, 187–193 (1982)

The Journal of
Membrane Biology

State of Water in Extremely Halophilic Bacteria: Heat of Dilution of *Halobacterium halobium*

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Summary. Heat of dilution of *Halobacterium halobium* was measured when thick pastes of the bacteria, harvested throughout a complete growth cycle, were lysed by mixing with 40 times their volume of water in a microcalorimeter. A series of comparative measurements was made with pastes of bacteria previously disrupted by freezing and thawing but otherwise identical to the pastes of whole bacteria. The frozen-thawed pastes gave endothermic values some 18% greater than those obtained with intact bacteria; the difference was highly significant. Evidence was obtained that the mechanical component of bursting did not contribute to the difference between whole and lysed bacteria. On the other hand, when a correction was applied for heat of mixing of intracellular salts with extracellular NaCl, such as occurs when the bacteria lyse, the difference between whole and disrupted organisms was largely eliminated from exponential phase halobacteria but not from those harvested in stationary phase. It is concluded that there is no evidence, as reflected in heat of dilution, of "abnormal" solution properties of the cytosol of young halobacteria, which are rich in potassium. On the other hand, and paradoxically, some doubt remains about stationary phase organisms whose cytosol has a much higher Na^+ content (and Na/K^+ ratio) than the cytosol of exponential phase bacteria.

Key words *Halobacterium halobium* · *halobacteria* · halophilic bacteria · heat of dilution · microcalorimetry · cell water · cell K^+

Introduction

The possibility that the Halobacteriaceae might maintain their very high internal potassium concentrations by a mechanism that is partly dependent on cytoplasmic factors has been the subject of various investigations (summarized by Brown & Sturtevant, 1980) of the physics and physical chemistry of these bacteria. The investigations have been associated with two schools of thought based on whether or not K^+ is "bound" within the cell. Although most investigators favor conventional (i.e. membrane controlled) solute retention, an objective assessment of the evidence leaves the basic question essentially unresolved. Part of the difficulty, as we have already noted (Brown & Sturtevant, 1980), stems from restricting the possibilities to the concept of binding. The evidence against binding in any stoichiometric sense seems to be compelling but that does not necessarily exclude other physico-chemical peculiarities of the cytosol such as a modified solvent structure or a quasi-crystalline arrangement of solutes within it, or some other method of lowering the activity coefficient of K^+ and/or KCl within the cell by a mechanism not so far recognized in nonbiological solutions. Ordering of solvent or solute might be expected to restrict diffusion of the solute whereas a lowering of the activity coefficient would effectively diminish the concentration gradient across the plasma membrane. Either phenomenon should therefore assist in retaining potassium against the enormous concentration gradients that prevail in these organisms.

"Abnormal" solution properties of this type should be detectable by appropriate thermodynamic techniques. We have already reported (Brown & Sturtevant, 1980) one such series of observations involving the measurement by differential scanning calorimetry of the apparent latent heat of fusion of intact and disrupted *Halobacterium halobium*. The results did not suggest any abnormality of the bacterial cytosol. It is, however, quite difficult to predict how a hypothetical ordering of a solution might affect enthalpy changes observed during a freezing transition; some physico-chemical abnormalities of the cytosol could remain undetected by this technique.

It is much less likely that solute or solvent ordering (which would be reflected in a diminished entropy), or a lowered activity ciefficient of the solute would escape detection in measurements of the enthalpy of dilution. *Halobacterium* spp lyse readily on dilution of the suspending medium, the process involving, among other things, disaggregation of the cell envelope. These bacteria therefore provide an excellent opportunity to measure the heat of dilution of their cytosol provided suitable controls are used to account for the enthalpy changes of all the other ill-defined

0022-2631/82/0064-0187 $01.40

events that accompany lysis and subsequent dilution. The present paper describes a series of measurements of heat of dilution of intact and disrupted *H. halobium*. The function of the disrupted bacteria was to provide a control system of overall chemical composition identical to that of the paste of intact bacteria but differing physically from it in that the intracellular fluid was already "disorganized" and mixed with the extracellular fluid. Disruption was achieved by freezing and thawing which does not cause dissolution of the cell envelope but has been shown to allow mixing of intra- and extracellular solutions (Lanyi & Silverman, 1972; Brown & Sturtevant, 1980). As previously (Brown & Sturtevant, 1980), the rationale was that only an empirical approach was possible with such a complex system and any hypothetical abnormalities should be reflected in a difference between whole and lysed bacteria rather than in absolute values. Because of this complexity, we again had difficulty in predicting the direction of any possible difference between whole and disrupted bacteria.

Materials and Methods

Organism

Halobacterium halobium was maintained, cultured and harvested as described by Brown and Sturtevant (1980) except for the following differences. The growth medium used Oxoid peptone (1% wt/vol) instead of Bacto tryptone and yeast extract, the experimental culture was inoculated (5% vol/vol) with an exponentially growing culture and incubation temperature was 30 °C. At intervals throughout a growth cycle, portions of the culture were withdrawn, centrifuged and drained as described by Brown and Sturtevant (1980). A weighed sample of the paste of whole bacteria was transferred directly to the calorimeter (*see below*). A tube containing the "control" paste (to be lysed by freezing and thawing) was sealed and stored in liquid N_2.

Calorimetry

(i) The Instrument and its Use

A batch calorimeter was constructed to a design of P. Monk and W.W. Forrest. It is similar in principle to an instrument described by Wadsö (1968). It consisted, in essence, of a stainless steel box (length 245 mm, height 120 mm, width 145 mm) precisely filled with aluminum blocks to function collectively as a heat sink. Two fused silica reaction vessels (external dimensions $40 \times 40 \times 10$ mm) were fitted into spaces within the assembly of aluminum blocks. The reaction vessels were divided by a 30-mm-high bulkhead into two compartments, a small one (compartment 1, internal length 10 mm) and a large one (compartment 2, internal length 22 mm). The free space above the bulkhead allowed mixing of the contents of the two compartments when the vessel was inverted. Access to each compartment was gained by stoppered holes in the top of the vessel. The vessels were mounted between two Cambion "thermoelectric modules" wired in parallel for each vessel and in opposition series for the pair of vessels. The circuit was connected to a Keithley Model 148 nanovoltmeter and thence to a recorder. The calorimeter block was immersed in a water bath at 25 °C (24.70 ± 0.01 °C), access to the reaction vessels being obtained through two stoppered ports projecting above the water. The block was mounted on a spindle which connected through a waterproof bearing to a crank handle on the outside of the bath, the crank being used to rotate the block.

The instrument was calibrated against the heat of neutralization of HCl with NaOH (Berger, 1969). For this purpose HCl (0.01 M, 0.4, 0.8 or 1.2 ml) was added to compartment 1 and NaOH (0.01 M, 3.6, 3.2 or 2.8 ml, respectively) to compartment 2 of the test vessel. The reference vessel contained water in compartment 1 and NaOH (0.01 M) in compartment 2 in volumes equal to those in the test vessel. All fluids had been brought to 25 °C before transfer to the calorimeter. The access holes in the reaction vessels and the loading ports of the block were closed and the instrument was allowed to stabilize thermally. This took 40–60 min.

The contents of the two compartments in each vessel were mixed by rotating the calorimeter block. The following tipping procedure was adopted since it was found to minimize the shift in the baseline that commonly occurred after the solutions were mixed; it also allowed complete mixing of the contents of the two compartments. The block was rotated steadily through 360° counterclockwise, 360° clockwise and 90° counterclockwise. This left the reaction vessels on their sides with compartment 2 uppermost. Returning the block to its original upright position did not always allow complete mixing of the two fluids; it never did when bacteria were used since they became viscous on lysis and tended to stick to the sides and bottom of compartment 1.

The bacterial pastes were manipulated as follows. A paste of whole or lysed bacteria was transferred to the test vessel by means of a plunger-operated micropipette fitted with a tared glass capillary. Approximately 0.1 g paste (accurately weighed) was deposited in compartment 1; water (3.9 ml) was added to compartment 2. The reference vessel contained water (0.1 ml in compartment 1 and 3.9 ml in compartment 2). The instrument was allowed to stabilize as above after which the contents of the two compartments were mixed by the tipping procedure already described. When the baseline had again stabilized, the calorimeter block was rotated once more to ensure that there was no further heat evolution or uptake and that mixing had been complete the first time. After a run, the contents of each reaction vessel were removed by suction, the vessels were rinsed several times with water followed by acetone and then dried in a stream of air.

(ii) Treatment of Results

The area under the peak was measured with a planimeter. When the baseline shifted the area was assessed by each of the following methods (see Fig. 1):

Method 1. The area *OPC*, *O* being the origin of the peak, *P* its summit and *C* the point from which the final baseline is extended backwards to intersect either the leading edge of the peak or its downward extension.

Method 2. (*a*) When the final baseline was lowered, the area *OPA* was derived by a linear extention of the original baseline to intersect the trailing edge of the peak at *A*. (*b*) When the final baseline was raised the area *OPCA* was measured. This area was obtained by dropping the perpendicular, *CA*, *C* being the point from which the final basline was extended backwards and *A* the point of intersection of that perpendicular with the forward extension of the original baseline.

Method 3. The area *BPC* was derived by a linear backwards exten-

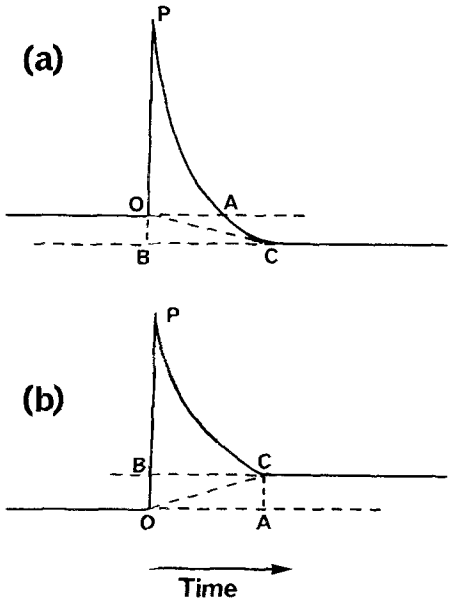

Fig. 1. A diagrammatic representation of two endothermic peaks illustrating the methods of measuring area when the baseline shifted (*see text*). In (*a*) the baseline is lowered after the thermal event, in (*b*) it is raised. For endothermic peaks, lapsed time increase from left to right

sion of the final baseline to intersect the leading edge of the peak (*b*) or its downward extension (*a*).

Method 4. The mean of methods 2 and 3, that is (*a*), $(OPA+BPC)/2$ or (*b*), $(OPCA+BPC)/2$.

When the baseline was stable, all four methods were, of course, identical.

(iii) Determination of Water Content

The water content of bacterial pastes was determined by drying a sample of paste on a tared watch glass at 100 °C for 15–18 hr.

Results and Discussion

Figure 2 shows a representative exothermic peak obtained by mixing HCl (0.01 M, 1.2 ml) with NaOH (0.01 M, 2.8 ml). Figure 3 is a calibration curve for the instrument derived in this way. Figure 4 is a representative heat of dilution curve for whole bacteria. It is endothermic and gives an example of a raised baseline. Figure 5 is an analogous curve for lysed bacteria. In this case the baseline is stable but that is coincidental.

Table 1 shows the application of the four methods of deriving peak area to the determination of heat of dilution of KCl (4 molal).

Samples were withdrawn at successive stages of a growth cycle. There were *a priori* reasons for expecting culture age to influence the results (Brown & Sturtevant, 1980) but the variability was such that

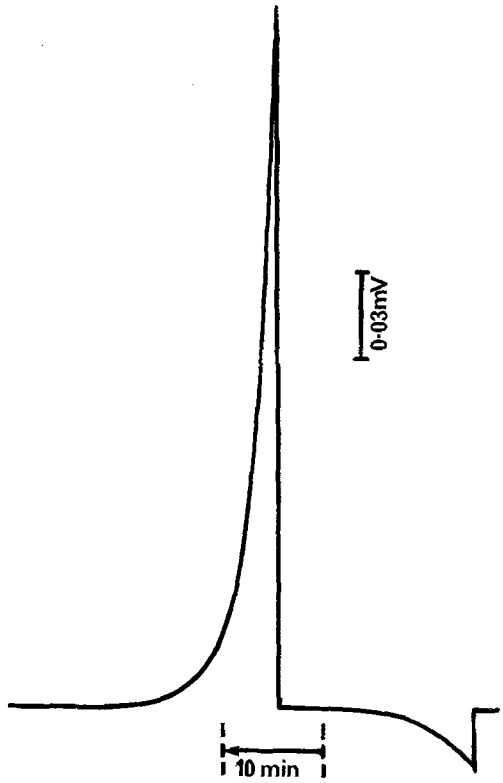

Fig. 2. An exothermic peak produced by neutralizing HCl with NaOH for the calibration of the instrument. In this case 1.2 ml HCl (0.01 M) was used (*see text*). Lapsed time increased from right to left

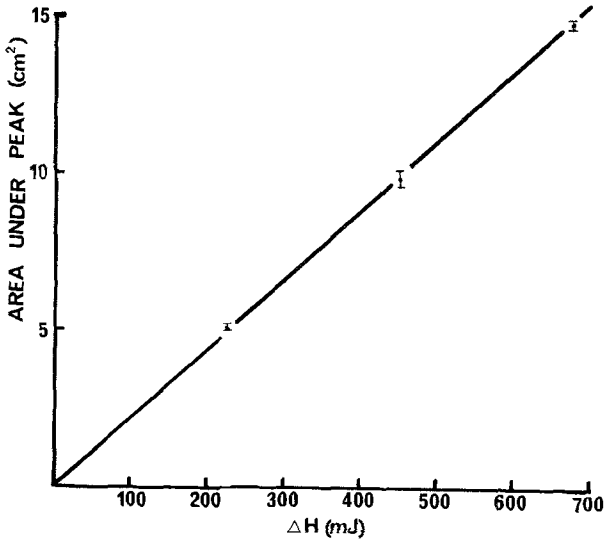

Fig. 3. A calibration curve derived by method 1 (*see text*) from heat of neutralization of HCl with NaOH. The error bars denote standard deviation for four determinations

an unreasonably large number of samples would have been necessary to demonstrate any progressive effect of age. Nevertheless, it was possible to divide the samples into two major groups representing bacteria harvested during exponential growth and from the

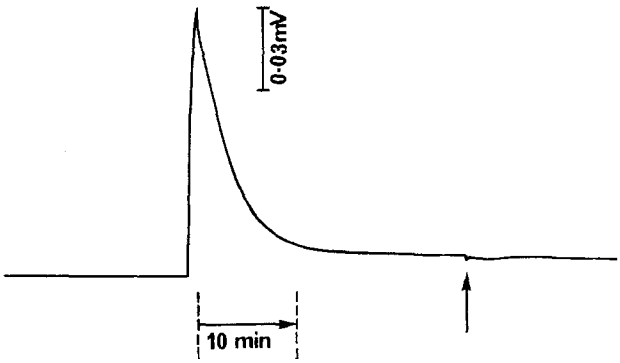

Fig. 4. A curve depicting heat of dilution of a paste of whole *Halobacterium halobium*. It is endothermic and illustrates an upwards shift of the baseline. The vertical arrow denotes a second tipping of the calorimeter block to ensure that mixing had been complete the first time

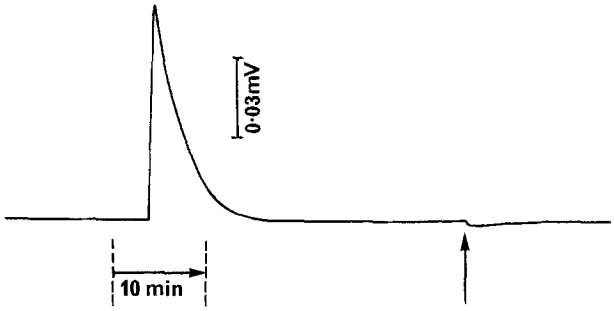

Fig. 5. A curve depicting heat of dilution of a paste of *Halobacterium halobium* previously disrupted by freezing and thawing. The vertical arrow denotes a second tip as in Fig. 4. The constancy of the baseline is unrelated to the history of the bacterial paste

Table 1. Heat of dilution of KCl (4 molal) with water

Method[a]	Heat of dilution[b] (ΔH, J/g-atom K)
1	1665± 28
2	1630± 4
3	1750±129
4	1690± 64

[a] "Method" in this and other Tables refers to the measurement of the area under the peak (*see text*).
[b] For these determinations, approximately 0.1 g (accurately weighed) of a KCl solution (4.00 molal) was diluted with 3.9 ml water. The reference cell contained 0.1 ml and 3.9 ml water. Values for ΔH are endothermic unless prefixed by a negative sign, in which case they are exothermic. Results are expressed ± standard deviation for three measurements.

Table 2. Heats of dilution of pastes of whole and disrupted *H. halobium*[a]

Method	ΔH (J/g H_2O)				Level of significance (P)
	Whole bacteria	(N)[b]	Lysed bacteria	(N)[b]	
1	7.02±2.35	(85)	8.20±2.50	(86)	<0.01
2	6.95±2.95	(87)	7.66±2.35	(84)	<0.1
3	8.31±2.83	(82)	10.44±3.94	(86)	<0.001
4	7.71±2.26	(81)	9.04±2.63	(84)	<0.001

[a] Values are quoted in this and subsequent Tables as the mean ± standard deviation.
[b] Number of samples.

stationary phase. A preliminary comparison was made, however, with pooled samples of all physiological ages ranging from early exponential to stationary phase.

The results are shown in Table 2. Heat of dilution was endothermic as expected and was greater for the pastes of frozen-thawed "controls" than for pastes of whole bacteria. The difference was highly significant by each of the methods used for measuring peak area.

The lysis of whole halobacteria, however, includes at least two events that do not occur to any significant extent in the dilution of the previously frozen and thawed pastes. These events are (*i*) the mixing of internal and external salts at the moment of bursting and (*ii*) possible thermal changes associated with the mechanical component of osmotic swelling and bursting. The first possibility was considered to be especially important in young bacteria with high potassium and low sodium contents with a major resultant difference in ion composition between the cytosol and extracellular fluid.

Brown and Sturtevant (1980) reported sodium and potassium contents of halobacterial pastes, prepared as in the present study, over a complete growth cycle. They also calculated, with some assumptions, that bacteria from the exponential growth phase contained 6.5 molal potassium and 0.7 molal sodium whereas stationary phase bacteria contained 2.7 molal potassium and 3.6 molal sodium. The assumption was made that the extracellular fluid consistently contained 4.9 molal NaCl (as in the growth medium) and negligible amounts of K^+. Solutions were therefore prepared to simulate, in NaCl and KCl concentrations, (*a*) the cytosol of young (exponential phase) and (*b*) old (stationary phase) halobacteria as well as (*c*) the extracellular fluid. The compositions of these solutions were, (*a*) 6.5 molal KCl+0.7 molal NaCl; (*b*) 2.7 molal KCl+3.6 molal NaCl; (*c*) 4.9 molal NaCl. The purpose was to determine separately the heat of mixing solutions (*a*) and (*b*) with (*c*). No attempt was made to obtain the corresponding information for the transition from exponential to stationary growth phase. Heat of mixing was determined by

Table 3. Heats of dilution of *H. halobium* from the exponential growth phase. Results expressed in relation to water and K^+ contents

Method	ΔH (J/g H_2O)						Level of significance[b]
	Mixing[a] salts	Whole bacterial paste	(*N*)	Net	Lysed bacterial paste	(*N*)	(*P*)
1	−0.560	6.57±2.13	(31)	7.13	7.86±2.71	(30)	ca. 0.3
2	−0.552	6.19±2.04	(29)	6.74	6.95±2.26	(28)	>0.5
3	−0.610	8.22±3.18	(28)	8.83	10.06±3.20	(28)	ca. 0.15
4	−0.585	7.22±2.01	(28)	7.80	8.76±2.51	(28)	ca. 0.15
	(J/g-atom K)						
1	−218	2180± 706	(31)	2398	2608± 898	(30)	ca. 0.3
2	−215	2052± 675	(29)	2267	2307± 750	(28)	>0.5
3	−237	2727±1054	(28)	2964	3338±1061	(28)	<0.2
4	−228	2395± 667	(28)	2623	2906± 833	(28)	ca. 0.15

[a] The heat of mixing the salt solutions (*see text*) was calculated on the basis of the total water content of both solutions and of the potassium content of the simulated intracellular solution.
[b] The level of significance refers to the difference between the value for lysed bacteria and the corrected or net value for whole bacteria. For this calculation the standard deviation of the net value for whole bacteria was taken to be the same as that of the uncorrected value.

Table 4. Heats of dilution of *H. halobium* from the stationary growth phase. Results expressed in relation to water and K^+ contents[a]

Method	ΔH (J/g H_2O)						Level of significance
	Mixing salts	Whole bacterial paste	(*N*)	Net	Lysed bacterial paste	(*N*)	(*P*)
1	−0.026	6.94±2.14	(37)	6.97	8.18±2.30	(35)	<0.05
2	−0.019	6.76±2.29	(37)	6.78	7.95±1.87	(36)	<0.02
3	−0.064	8.08±2.09	(37)	8.14	9.85±3.32	(37)	<0.001
4	−0.043	7.41±2.00	(37)	7.45	8.63±2.61	(37)	<0.05
	(J/g-atom K)						
1	−19	5003±1545	(37)	5022	5896±1656	(35)	ca. 0.02
2	−14	4872±1650	(37)	4886	5732±1348	(36)	ca. 0.02
3	−48	5824±1508	(37)	5872	7098±2392	(37)	ca. 0.01
4	−32	5339±1441	(37)	5371	6220±1879	(37)	<0.05

[a] *See* footnotes to Tables 2 and 3.

adding solutions (*a*) or (*b*) (1.92 ml) to compartment 1 and solution (*c*) (2.08 ml) to compartment 2 of the calorimeter cell. These proportions were selected on the basis of Brown and Sturtevant's (1980) calculation of intra- and extracellular water content in the bacterial paste. The reference cell contained water in both compartments (1.92 and 2.08 ml as above). The specific gravities and water content of the salt solutions were known and results were expressed as J/g total water in the two solutions and as J/g-atom K.

The results are shown in Tables 3 and 4 for young and old bacteria, respectively. Heat of mixing was exothermic and thus would have diminished the total endothermic heat of dilution of the whole bacterial paste. When the heat of dilution of whole young bacteria (Table 3) was corrected for the heat mixing of the salts, the difference between the enthalpy of dilution of whole and lysed bacteria was greatly diminished but a small residual difference remained by all methods of calculation. This was so whether the enthalpy was expressed as a function of water or potassium or water content of the system. The difference was not statistically significant but it possibly would have been with a larger sample size. The uncorrected

difference between whole and lysed young bacteria was significant at about the 5% level.

Because of their lower potassium content, the correction applied to old bacteria was smaller than that used for young organisms and the residual difference between enthalpy of dilution of whole and lysed halobacteria from the stationary phase remained statistically significant (Table 4).

The second factor suggested as a possible contributor to the observed difference summarized in Table 2 was the enthalpy of the mechanical events associated with osmotic swelling and bursting of whole bacteria. Evaluation of this possibility is difficult but an indirect approach to doing so was made in the following way. When halobacterial suspensions are diluted in water, as in the current experiments, the overall lytic process includes disaggregation, effectively dissolution, of the cell envelope. In the presence of Mg^{2+} (0.02 M), however, the cell membrane remains intact although the outer glycoprotein layer is lost (Brown, Shorey & Turner, 1965).

A series of measurements was therefore made with $MgCl_2$ (0.02 M, 3.9 ml) instead of distilled water in compartment 2 of the calorimeter vessel. The reference vessel also contained $MgCl_2$ solution in compartment 2 with water as usual in compartment 1. The rationale was that, under these conditions, whole halobacteria would physically burst on dilution and the membrane would not disaggregate. Without $MgCl_2$, on the other hand, the temporal relation between physical bursting and membrane disaggregation was uncertain and, in any case, should be energetically different because of the weakening of the envelope as a prologue to ultimate disaggregation.

The results are shown in Table 5 for bacteria of all physiological ages. The enthalpy of dilution was greater in the presence of $MgCl_2$ than in its absence and the difference was marginally significant. The same effect was observed with the lysed bacterial pastes, however, and cannot therefore be attributed to a postulated mechanism involving an enhanced contribution by the physical process of bursting. The effect observed presumably reflected at least partly the elimination of membrane disaggregation from the total enthalpy measured. If this were the dominant effect, then membrane disaggregation is evidently exothermic. This last interpretation could be tested directly, of course, with isolated envelopes but, at this stage, the experiment was not considered warranted.

We can conclude with moderate confidence that the observed effects are not substantially attributable to the physical process of bursting. It remains, therefore to consider other possible reasons for the differences in heats of dilution of whole and disrupted bacteria.

Table 5. Effect of $MgCl_2$ (0.02 M) on heat of dilution of whole and disrupted bacterial pastes[a]

Method of measurement	ΔH (J/g H_2O)		Level of significance (P)
	$+MgCl_2$ (N)	$-MgCl_2$ (N)	
Whole bacteria:			
1	7.44±2.44 (27)	6.64±1.79 (42)	<0.15
2	7.65±2.85 (27)	6.39±1.72 (40)	<0.05
3	9.03±3.37 (27)	7.91±2.74 (41)	<0.2
4	8.34±2.31 (27)	7.01±1.73 (41)	<0.02
Lysed bacteria:			
1	8.54±2.21 (28)	7.67±2.38 (40)	ca. 0.15
2	7.91±2.10 (28)	7.33±2.22 (39)	ca. 0.3
3	10.74±2.48 (26)	9.55±3.40 (39)	ca. 0.15
4	9.77±2.75 (28)	8.41±2.74 (39)	<0.05

[a] The results were obtained over complete growth cycles and represent bacteria of all physiological ages.

Because of the variability of the material, a large number of samples was needed to demonstrate significance in this difference. There is no doubt from the raw results listed in Table 2, however, that whole bacteria do have a lower endothermic enthalpy of dilution than their counterparts that had been frozen and thawed. Equally, from Table 3 there is no doubt that the heat of mixing internal (predominantly potassium) salts with external salts (sodium chloride) is about sufficient to account for this difference in exponential phase bacteria.

The situation with stationary phase bacteria is not so simple. The correction for heat of mixing salts was smaller because of the higher content of sodium in old organisms and, after applying this correction, the difference in heat of dilution of whole and lysed bacterial pastes remained significant. This result was not expected since we believed that any solution "abnormalities" of the type postulated were more likely to occur in young bacteria that were energetically active and far from equilibrium with their environment than in old, metabolically sluggish bacteria that were substantially closer to equilibrium.

To some extent, however, this expectation was also confounded by comparisons of the uncorrected values for ΔH. The mean ratio of ΔH for lysed/whole bacteria calculated by all four methods was 1.18 for both young and old bacteria. Furthermore, absolute values of ΔH were similar for young and old bacteria when expressed on the mass of water (mean values of 7.05 and 7.30 J/g H_2O, respectively) but not, of course, when expressed on the potassium content. The explanation of this lies largely in the very close similarity of the heat of dilution of NaCl and KCl.

Under our conditions, the heat of dilution of 4 molal NaCl was numerically greater than that of 4 molal KCl by 9.3% when expressed on the mass of solution, by 3.9% (3.860) on the number of moles of salt and 3.9% (3.878) on the mass of water.

Obviously NaCl was a major contributor to the observed ΔH under all conditions and, furthermore, the considerable change in potassium content that occurred during a growth cycle should not have affected absolute values of ΔH much, provided that potassium was present as KCl. This proviso was not wholly met, however, since potassium can exceed chloride by about 28% (mole/mole) (Christian & Waltho, 1962; Ginzburg, 1978). A substantial contribution by anions other than Cl^- to the neutralization of intracellular K^+ would presumably require a correction for heat of mixing on lysis different from those we have used, but for the present we cannot determine what that correction should be.

As they stand, the results indicate the following. There was a difference in the heat of dilution of pastes of whole halobacteria and control pastes previously disrupted by freezing and thawing. The disrupted pastes had, in all cases, the greater endothermic value. In young (exponential phase) bacteria, that difference was largely eliminated by applying a correction for the heat of mixing of internal salts (predominantly KCl) with external NaCl on the assumption that both K^+ and Na^+ were present within the cell entirely as the chlorides. The corresponding correction was not sufficient to overcome the difference between the two systems prepared from old (stationary phase) bacteria. Old bacteria have a much higher Na^+/K^+ ratio than young bacteria but the heat of dilution of NaCl is about the same as that of KCl. On the other hand the heat of mixing NaCl with a solution consisting predominantly of NaCl is obviously going to be quite small. This correction was not sufficient to overcome the differences between whole and lysed (old) bacteria.

Thus we are left with an anomalous situation in which young, exponential phase bacteria give results that, in the terms of this investigation, do not suggest any abnormality of their cytosol. On the other hand, old bacteria, which by many criteria including an analysis of their ion contents, are relatively close to equilibrium with their environment, give results that do suggest such an abnormality.

A possible source of error leading to this apparent anomaly is that the correction for heat of mixing applied to old bacteria was not large enough but, apart from the previous comment about the proportion of Cl^- in the cells, we have at present no evidence on which to examine such a possibility objectively. On the other hand, NaCl and KCl do affect solutions quite differently in several respects, some of which contributed to the effects of age on apparent latent heat of fusion of halobacteria described by Brown and Sturtevant (1980). Although the suggestion offends our intuition, it is thus possible that the relatively high proportion of NaCl that accumulates in old bacteria does indeed acquire some "abnormality" inside the cell which KCl (and other potassium salts) does not. If this is true, it might be related to the incompatible nature of NaCl in relation to enzyme function (Aitken & Brown, 1972) but this suggestion is highly speculative.

The technique has shown itself sufficiently sensitive to reflect at least some of the structural differences between whole and lysed bacteria. We can conclude with moderate confidence that there is no evidence at this level of "abnormal" physical chemistry (in the terms of the investigation) in the cytosol of young halobacteria. We are left with a dilemma with old bacteria, however, for which either a considerable refinement of the technique or a completely different approach is needed to resolve.

This work was supported by the Australian Research Grants Committee (D2-74/15525). We wish to thank Drs. P. Monk and W.W. Forrest for the design of the calorimeter and Dr. Forrest for help and advice with its installation.

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Received 1 April 1981