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TRANSIENT BREAKDOWN OF THE PERMEABILITY BARRIER OF THE MEMBRANE OF *ESCHERICHIA COLI* UPON HYPOOSMOTIC SHOCK

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Summary

The leak of intracellular solutes which occurs when *Escherichia coli* is washed with hypoosmotic solutions was studied. This leak is not dependent on the actual osmolarity of the washing fluid but only upon the difference between its osmolarity and that of the incubation medium. The solute which makes up for the difference in osmotic pressure can be any of a number of salts and sugars tested with the exception of ethylene glycol, glycerol and propane diol.

The intracellular solutes which leak out, include neutral and anionic sugars accumulated by specific transport systems, endogenous nucleotides and K^+. In contrast acid precipitable macromolecules were not detected in the washing fluid. The osmotic shock caused no permanent damage to the retention capacity and transport ability of the cells. The efficiency of the osmotic downshock was found to depend on its suddenness and the breakdown of the barrier, the leak and the restoration of the barrier seemed to be over in less than two seconds.

A combination of osmotic shock with cold shock increased the leak but the cold sensivity appeared more variable with strains and culture conditions than osmotic sensitivity.

The mechanism of the breakdown of the permeability barrier is discussed and the role of the compression of the bilayer by the turgor pressure is suggested in analogy with the electrostriction effect.

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Abbreviations used: TMG, 1-*S*-methyl-β-thiogalactopyranoside.

Introduction

The most universal function of cell membranes is to oppose the free diffusion of solutes from the medium to the cytoplasm and vice versa.

In bacteria it has been recognized that the permeability barrier, in the absence of specific transport systems, precludes the influx of most hydrophilic nutrient molecules at a rate compatible with normal growth. Pools of hydrophilic solutes built up by active transport systems leak out only at very high concentration gradients [2].

Increased membrane permeability as monitored by the leak of an accumulated metabolic pool has been described several times at the two extremes of the physiological temperature range, close to 0° C [5] or close to 45° C [6,7].

Permeability changes in various experimental conditions have had important methodological consequences in the measurement of intracellular pools. Nearly all methods used for measuring intracellular concentrations include the separation of cells from medium, and washing is generally included. Since separation of the cells and washing are not instantaneous, to minimize leakage from the cell during this time the use of low temperature was advocated [4]. Several authors however have described leakage upon cold shock [3,6,7]. Leder, in a detailed study [8] concluded that a hyperosmotic wash can protect against cold shock. Conversely alkali halides have been described as inhibitors of sugar uptake [9,10]. But hypoosmotic media were known not to cause lysis of bacteria, and it is well known that bacteria are tolerant to a wide range of osmotic pressure in their growth medium. In the present article we describe the effect of a hypoosmotic shock on the retention of intracellular solutes.

Materials and Methods

Strains of *E. coli* used in this study are listed in Table I. They were grown in medium 63 containing 100 mM potassium phosphate buffer (pH 7.2), 12 mM $(NH_4)_2SO_4$, 2.6 mM $MgSO_4$, 0.004 mM $FeSO_4$ at temperatures ranging from 22 to 40° C in aerated conical flasks with 4 g/l carbon source, inducers and required supplements specified in each experiment. Measurement of accumulated radioactive substrates was made by rapid filtration on nitrocellulose filter membranes of 0.45-μm pore size (millipore HA) followed by washing. As a standard procedure washing was done at room temperature using 2 5-ml portions of the incubation medium without the transport substrate.

TABLE I

STRAINS OF *ESCHERICHIA COLI* USED

E. coli K12 3300, HFc, $Lac^+ i^-$, thi^-
E. coli K12 3000, HFc, $Lac^+ i^+$, thi^-
E. coli K12 2000, F^-, $Lac^+ i^+$, thi^-
E. coli K108 galactokinase deficient (collection of G. Buttin)
E. coli W3092 CY^- galactokinase deficient $lacY^-$ isolated by Wu [11]
E. coli P4 X AJ19 met^- isolated by J. Jimeno [12] from P4 X as glucuronic acid isomerase deficient.
E. coli AR13 isolated by A. Robin [13] from DF 1070 as a gluconic acid kinase deficient.
E. coli K1059 i^+ F^- by transduction of $\phi 80$ *dlac* (M. Koyiyama) into K1059 $Lac^+ i^-$ isolated by P. Overath [14]

Serial measurements, where the only variable was the washing solution, were made 10—40 min after the beginning of uptake, during which the intracellular pool remained constant. Leak of substrate was estimated by comparison with the samples which retained the highest radioactivity, usually the sample submitted to the standard washing.

Results

Transport and retention of solutes in incubation media of various osmotic strengths

In the experiment represented in Fig. 1, an exponential culture of *E. coli* strain K12 3300 grown on M63 glycerol at 37°C was centrifuged and resuspended in media containing tenfold diluted M63 glycerol plus various concentrations of NaCl to make up osmotic pressures ranging from 30 to 530 mosM, at identical bacterial densities, equilibrated at 25°C in a shaking bath for 10 min, then supplemented with 1 mM [^{14}C]methyl-β-D-thiogalactoside (TMG). 1-ml samples were filtered at close intervals and rinsed with the same solution as used for incubation. Initial velocity, time course and steady state level of TMG uptake were closely similar whatever the osmotic strength of the incubation medium.

Intracellular concentration was found to be independent of osmotic strength of the medium for many solutes with the notable exception of K^+ ion. Intracellular K^+ is known to increase with the osmotic strength of the medium.

The misleading experiment

When exploring the effect of a supposed inhibitor upon an active uptake process, the inhibitor can be added before the transport substrate or at the steady state of accumulation. The experiment shown in Fig. 2 with bacteria incubated in M63 with or without 0.2 M NaCl (or 0.2 M KCl) is typical for an

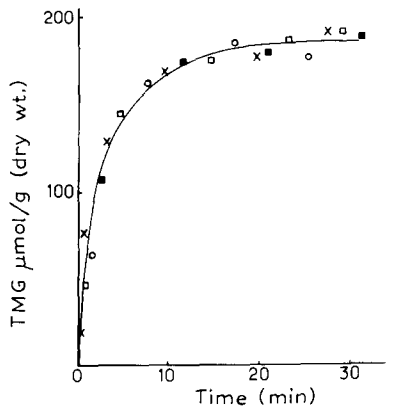

Fig. 1. TMG uptake in media of different osmotic pressure. A culture of *E. coli* strain K12 3300 grown on M63 glycerol at 37°C was centrifuged and resuspended in media containing 10-fold diluted 63 glycerol plus various concentrations of NaCl to make up osmolarities of 30 mosM (X———X), 230 mosM (□———□), 430 mosM (○———○) and 530 mosM (■———■) at 25°C. 1 mM [^{14}C]TMG was added and 1-ml samples were filtered and rinsed with the same solution as used for incubation.

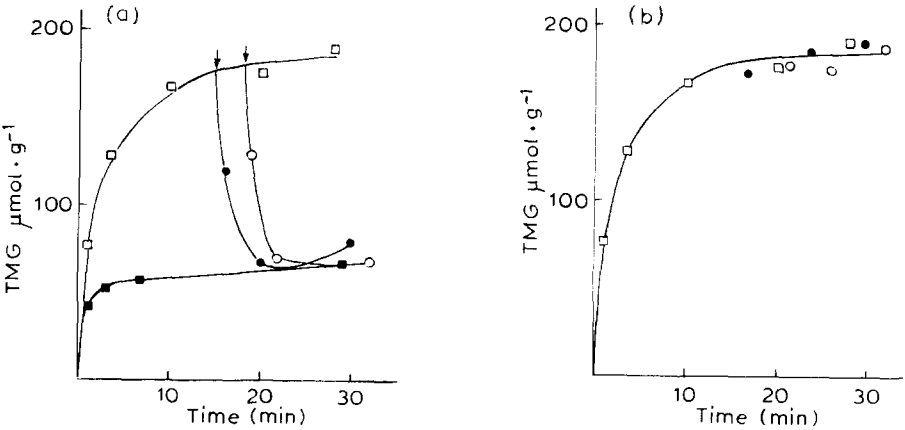

Fig. 2. The "inhibitory effect" of salts on TMG uptake and its cancellation by isoosmotic wash. (a) *E. coli* strain K12 3300 grown on M63 glycerol at 37°C was incubated in the same medium at 25°C. 1 mM [^{14}C]TMG was added. 1-ml samples were filtered at close intervals and rinsed with the same medium (□———□). Another portion was incubated with 0.2 M NaCl in addition (■———■). 0.2 M NaCl (●———●) and 0.2 M KCl (○———○) were added (arrows) and 1-ml samples were filtered and rinsed with M63. (b) Same experiment, but alternate samples were rinsed with M63 complemented with 0.2 M NaCl. Same symbols.

inhibitor (2a), but when all samples were washed after filtration with M63 + 0.2 M NaCl (2b) the inhibitory effect disappeared. In fact, from the results of Fig. 1, showing that NaCl in the incubation medium had no major effect on TMG uptake, it seems plausible that Fig. 2b reflects the level of TMG in the bacteria during incubation more closely than Fig. 2a and that the samples in Fig. 2a have lost a part of their TMG pool during the wash with M63 after an incubation in M63 + 0.2 M NaCl. M63 is in these circumstances a hypoosmotic medium. In contrast, hyperosmotic shock during washing or during incubation has no adverse effect on the TMG pool.

Adaptation to hyperosmotic medium is rapid; 1 min after raising the osmotic pressure of the medium cells behave as those incubated more than ten minutes in hyperosmotic medium with regard to loss of TMG upon hypoosmotic shock.

Hypoosmotic shock by dilution

The phenomenon described above does not depend on a special state of the bacteria collected on the filter. The same effect can be observed when the incubation medium is suddenly diluted in a hypoosmotic medium, as shown in Fig. 3. In this experiment, 1 or 4 vols. of prewarmed distilled water have been poured into the incubation flask, while TMG concentration was kept constant. Sample volume was adjusted so as to contain the same amount of bacteria, and washing on the filter was made with the original M63.

TMG leaked out into the incubation medium of lowered osmotic strength, but soon the loss was followed by a compensatory uptake so that a few minutes later the pool again reached the normal steady state value. This experiment shows that the effect of the hypoosmotic shock is very brief and that no permanent damage is caused to the uptake and retention mechanisms. Viable

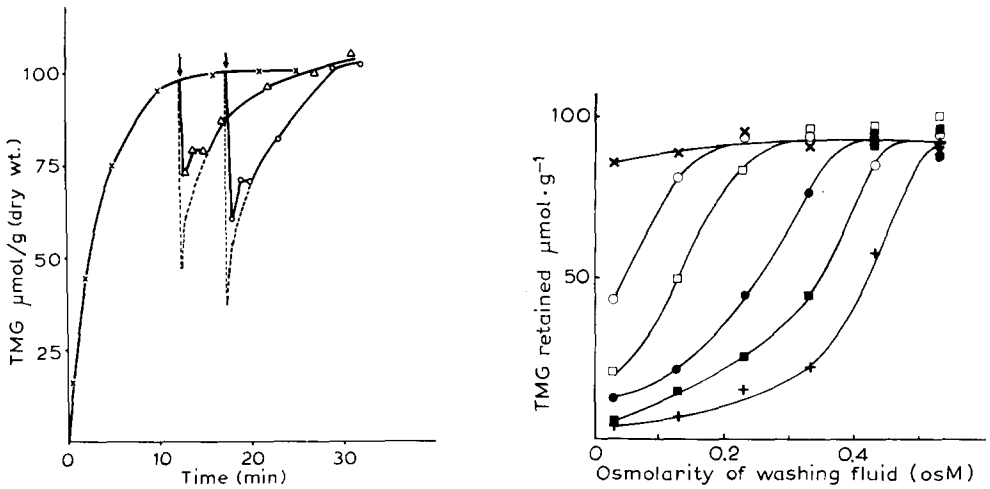

Fig. 3. Transient effect of osmotic downshock on the TMG pool. *E. coli* strain K12 3300 grown on M63 glycerol at 37°C was incubated at a cell density of 125 μg/ml at 25°C and supplemented with 1 mM [^{14}C]TMG. At the stationary phase of accumulation 1 (△———△) or 4 (○———○) vols. of distilled water containing TMG were added. Sample volume was adjusted so as to contain the same amount of bacteria. All samples were rinsed on the filter with M63.

Fig. 4. The effect of difference in osmotic pressure between incubation medium and washing solution upon retention of the TMG pool. *E. coli* K12 strain 3300 grown on M63 glycerol at 37°C were centrifuged and suspended in 10-fold diluted M63 (30 mosM) (X———X), supplemented with NaCl in order to obtain a medium having an osmotic pressure of 130 (○———○), 230 (□———□), 330 (●———●), 430 (■———■) and 530 (+———+) mosM. Each incubation mixture was supplemented with 1 mM [^{14}C]TMG. The temperature of incubation and washing was 25°C. At the stationary phase of accumulation of TMG, samples were filtered and washed with each of the different media. The per cent retention of [^{14}C]TMG is represented as a function of osmolarity of the washing medium. Osmolarities were calculated as the sum of molar concentrations of all solutes, anions and cations taken separately. No attempt was made to account for activity coefficients different from 1.

counts were unchanged when bacteria were diluted in M63 or in distilled water and then plated using the soft agar technique.

Leakage depends on the extent of hypoosmotic change

TMG was accumulated to the steady state by 6 bacterial suspensions made in media of osmotic strength ranging from 30 to 530 mosM. Samples from each incubation were filtered and washed with each of the 6 media so that all combinations of initial and final osmotic pressures were utilized.

The size of the remaining TMG pool is plotted vs. the osmotic strength of the washing fluid in Fig. 4, and this gives 6 sigmoid curves (or parts of sigmoids) for the 6 incubation mixtures, each shifted compared to its neighbours in the order of increasing initial osmotic strength. When the same data are represented vs. the difference in osmotic strength between the incubation medium and the washing fluid (not shown), the 6 curves can be approximated by a single sigmoid curve showing that the main parameter determining the breakdown of the permeability barrier is the extent of the hypoosmotic shock.

In the experiment represented in Fig. 5, incubation was made in media of various osmotic strengths made up with 10-fold diluted M63 by the addition of suitable amounts of NaCl or sucrose, and washed after filtration with M63/10.

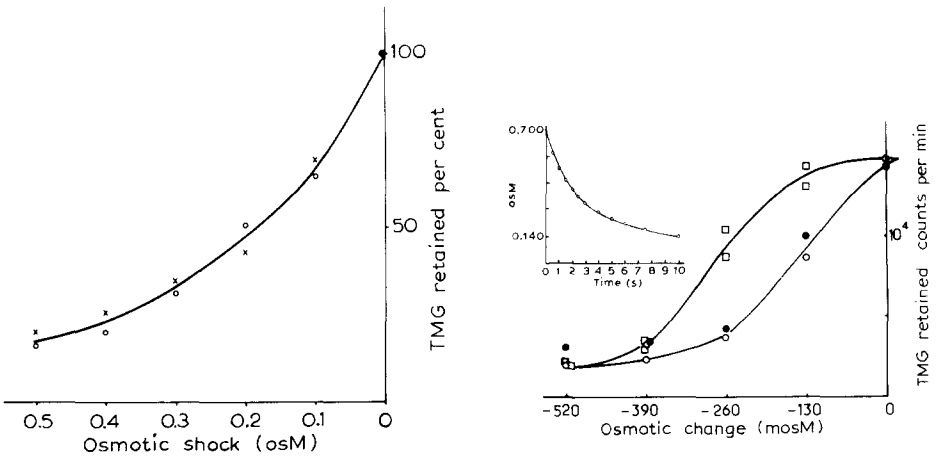

Fig. 5. Comparison between osmotic effects of NaCl and sucrose. *E. coli* strain K12 3300 grown on M63 glycerol at 37°C were centrifuged and suspended in a 10-fold diluted M63 at 25°C. By addition of NaCl (X———X) or sucrose (○———○) the osmolarity was adjusted to 130, 230, 330, 430 and 530 mosM. 1 mM [^{14}C]TMG was added. At the stationary phase of accumulation, samples were filtered and washed with tenfold diluted M63 at 25°C. The per cent retention of TMG is represented as a function of difference in osmolarity.

Fig. 6. Comparison between the effects of sudden and progressive osmotic shocks. *E. coli* was diluted 5-fold in mixtures of M63 and H_2O in different proportions to give the final osmotic changes given by the abscissa. TMG retained was measured by filtration (without washing) 30 s after start of dilution. Three different techniques of dilution were used: pipetting the suspension into the dilution fluid (●———●), pipetting the dilution fluid upon the suspension (○———○) or introducing the dilution fluid by a peristaltic pump into the tube containing the suspension under constant stirring, over a period of 10 s (□———□). The theoretical curve of the time course of change in osmolarity for this procedure is represented in the insert.

The resulting curves of the remaining TMG pool are superimposed when plotted vs. the osmolarity of the incubation medium, but not when plotted vs. its molarity.

Influence of the rate of osmotic change and duration of the leak

In the experiments described, the osmotic change was sudden and could suggest, that only a sudden osmotic change can cause the breakdown of the membrane barrier. An attempt was made to compare the effect of sudden and progressive osmotic change.

Osmotic changes were performed by dilution at different rates. The rapid method consisted of pipetting 4 vols. of hypoosmotic medium into a sample of bacterial suspension which had accumulated TMG to the steady state, filtering 30 s later (a time lapse short enough to prevent substantial recovery), or else the sample was pipetted into the hypoosmotic fluid. In the slow method the hypoosmotic fluid was introduced by a peristaltic pump over an interval of 10 s, while the same suspension was agitated in a vortex. It can be seen in Fig. 6 that the 10-s program was much less efficient in causing a leak of TMG than dilution by pipetting. We also tried to measure the time it would take to complete the breakdown of the barrier, the leak of the solute and the restoration of

TABLE II

DURATION OF THE LEAK OF SOLUTE

A bacterial suspension (*E. coli* W3092 CY^-) having accumulated to the steady state [^{14}C]galactose from a 10^{-6} M solution was diluted 10-fold with distilled water. Samples were filtered * 30 s after dilution. At short intervals between dilution and filtration the osmotic downshock (—300 mosM) was reversed by addition of concentrated NaCl.

Time of addition of NaCl solution restoring the initial osmotic pressure (s)	Intracellular galactose (cpm)
0	13666
2	8389
4	8277
6	8572
10	7792
15	7914
30	8757

* The filter was not rinsed. Contamination by the galactose in solution remaining on the filter in a control without bacteria was 60—80 counts.

** NaCl was added to the distilled water before dilution of the bacterial suspension, hence no osmotic shock.

efficient retention. To do this an instantaneous hypoosmotic shock was followed by an instantaneous reestablishment of the initial osmotic pressure by addition of NaCl. The interval between hypo- and hyperosmotic change was varied between 2 and 30 s. When the hypo- and hyperosmotic solutions were added at the same time, there was no leak. When the two additions were separated by 2 s the same leak was observed as with an interval of 30 s (Table II). Assuming that the return to the initial osmotic pressures stops the increase in permeability and starts the "healing" of the membrane barrier, and so stops the leak of solutes, the experiment suggests that all these events are terminated after 2 s.

The osmoactive solute

A number of different solutes have been tried in experiments similar to that represented for NaCl in Fig. 2a. The following solutes added to a final concentration of 0.2 M to the incubation mixture gave rise to a breakdown of the capacity for TMG retention when omitted from the washing fluid: NaCl, KCl, NH_4Cl, $(NH_4)_2SO_4$, $HCOONH_4$, $(NH_4)_2$ HPO_4, mannitol, sucrose, xylitol, arabitol, ribitol, sorbitol. No osmotic shock occurred upon omission of glycerol, ethylene glycol and propane di-1,2-ol.

The nature of molecules which leak out upon hypoosmotic shock

Table III gives the list of various transport substrates together with the strains which accumulate high pools. All of these leaked out during hypoosmotic shocks as did TMG. The list includes a cation, neutral sugars, anionic sugars and a phosphate ester.

A special mention has to be made of K^+, the most abundant solute in the cytoplasm of *E. coli*. Fig. 7 shows the leak of K^+ as a function of the hypoosmotic shock (see also ref. 6).

Since K^+ content of *E. coli* in M63 is approximately 800—850 μequiv per g

TABLE III

SUBSTRATES LEAKING UPON HYPOOSMOTIC SHOCK

Substrate	Strain
TMG (1-*S*-methyl-β-D-thiogalactoside)	K12 3300, K12 3000, K12 2000 K1059 i^+, AR13 *
TDG (β-galactosyl 1-*S*-β-D-thiogalactoside)	K12 3300
1-*O*-methyl-α-D-glucoside and its phosphate ester	K12 3300, P4 × AJ19
K^+	K12 3300
D-gluconate	AR 13 **
D-glucuronate	P4 × AJ19
D-galactose (accumulated by the β-methyl-galactose permease)	W 3092 CY^-, K 108

* All strains except 3300 had to be induced by growth in the presence of 0.5 mM isopropyl-β-D-thiogalactoside

** Induced by growth in the presence of 0.5 mM gluconate

dry weight, of which some 85—95% (700—800 μequiv) leak out if these are accompanied by a similar amount of anions, the loss of endogenous solute pools must be detectable by a loss of weight. The experiment actually showed that a distilled water wash as compared to a wash by M63 removed approx. 20.5% of the dry weight of the bacterial pellet (after correction for the dry weight of the interstitial fluid which was 1.8% of the original dry weight). Bacteria grown on [^{14}C]glucose as carbon source released a large amount of ^{14}C radioactivity upon a distilled water wash but no significant amount of the ^{14}C released was precipitable with 10% trichloroacetic acid.

The nucleotide pool of bacteria appeared also sensitive to the osmotic shock. The release of ultraviolet absorbing material as a function of osmotic shock is shown in Fig. 8. The washing fluid was lyophilized and paper chromatography showed that a variety of ultraviolet-absorbing molecules were removed from the cell by the procedure.

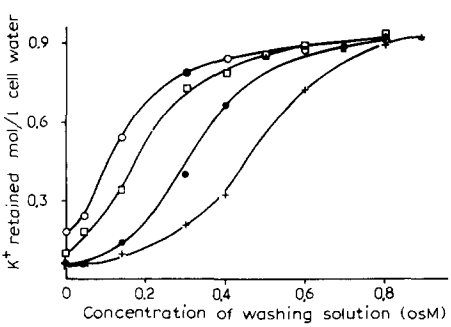

Fig. 7. Retention of potassium ion upon osmotic shock. Culture of *E. coli* K12 strain 3300 grown on mineral medium containing among others 10 mM K^+ and adjusted with NaCl to different final osmotic strengths were equilibrated at 25° C, samples were filtered and washed on the filter twice with mineral medium of different osmolarities. K^+ retained was measured by flame photometry. It is expressed in μmol per mg dry weight. The point on each curve which corresponds to isoosmotic wash is designated by a small triangle in the symbol of the experimental point.

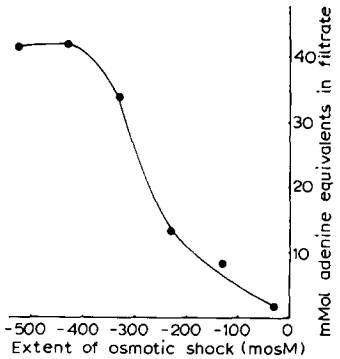

Fig. 8. Leak of endogenous ultraviolet-absorbing solutes upon osmotic downshock. Samples of an exponentially growing culture of *E. coli* K12 strain 3300 were centrifuged and resuspended in solutions of various osmotic strengths made up by 10-fold diluted M63 and the appropriate amounts of NaCl. Samples containing approximately 0.9 mg bacteria (dry weight) were filtered and rinsed with 2 ml H_2O on the filter. The washing fluid was collected and its absorbance at 260 nm expressed as equivalent nmol adenine is plotted vs. the osmotic downshock.

The effect of temperature and combination of cold shock with hypoosmotic shock

In experiments similar to that represented in Fig. 4, the temperature of the washing fluid was varied from 0 to 40°C in combination with the variation of its osmotic strength. Fig. 9a shows that the curves of TMG retention versus osmotic change have roughly the same shape but somewhat shifted at different temperatures of the washing fluid, the osmotic sensitivity increased when the

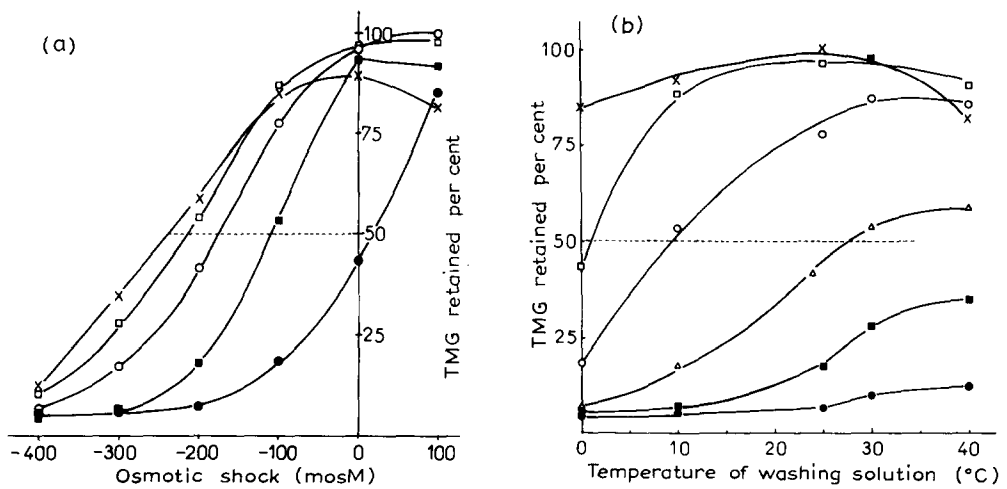

Fig. 9. Effect of combined osmotic and thermal shocks on the retention of a TMG pool. *E. coli* K12 strain 3300 was loaded with TMG to steady state at 25°C in a medium of 430 mosM. Samples were filtered and washed with solutions having osmolarities ranging from 0.030 to 0.530 and temperatures of 0, 10, 25, 30 and 40°C. a: TMG retained is plotted vs. osmotic downshock ●——● 0°C, ■——■ 10°C, ○——○ 25°C, □——□ 30°C and X——X 40°C. b: the same data are represented vs. temperature of the washing fluid having the following osmolarities in mosM: ●——●, 30; ■——■, 130; ▲——▲, 230; ●——●, 330; □——□, 430; (isotonic) and X——X, 530 (hypertonic). The intersections of the experimental curves with the dotted line of 50% retention from both graphs are reported on Fig. 10.

temperature of the washing fluid decreased. Fig. 9b represents the same data with the thermal effects explicited. By interpolation of Figs. 9a and 9b combinations of osmotic and thermal changes which result in 50% loss of the pool can be determined and plotted as "iso-loss" curves exemplified in Fig. 10. The representation of 50% "iso-loss" curves has a comparative value. When the curve approaches a horizontal line, the thermal shock produces a relatively smaller effect, the osmotic shock exerts a predominant influence and conversely curves of higher slope describe situations where the cold shock has an increased effect on leakage.

Fig. 11 represents the 50% loss curves of *E. coli* strain K 1059 grown in the same medium with different supplements of unsaturated fatty acids, one was fed with oleic acid at 40° C, the other with the trans isomer elaidic acid at 40° C. The cold sensitivity greatly increased with the supplement of unsaturated fatty acid of the unnatural trans configuration.

Fig. 12 shows three sets of 50% "iso-loss" curves made each with a different bacterial culture because specific mutants were needed to accumulate different solutes. For each set the behaviour of two or three different solutes in the same conditions can be compared. In Fig. 12A, *E. coli* K12 strain 3300 on 63 glycerol was allowed to accumulate a pool of TMG via the lactose permease, a pool of methyl-α-D-glucopyranoside and its phosphate ester via the phospho-*enol*pyruvate-glucose phosphotransferase system and the leak of these solutes together with the leak of the endogenous pool of K^+ was observed under combined thermal and osmotic shocks. The osmotic sensitivity was highest for K^+ and lowest for the sugar phosphate while cold shock sensitivity was moderate. In Fig. 12B, strain AR13 doubly induced accumulated TMG via the lactose permease and gluconate via the gluconate permease. The 50% "iso-loss" curves of these two solutes in this strain are very close. Fig. 12C represents the "iso-loss" curves of glucuronate and of methyl-α-D-glucopyranoside and its

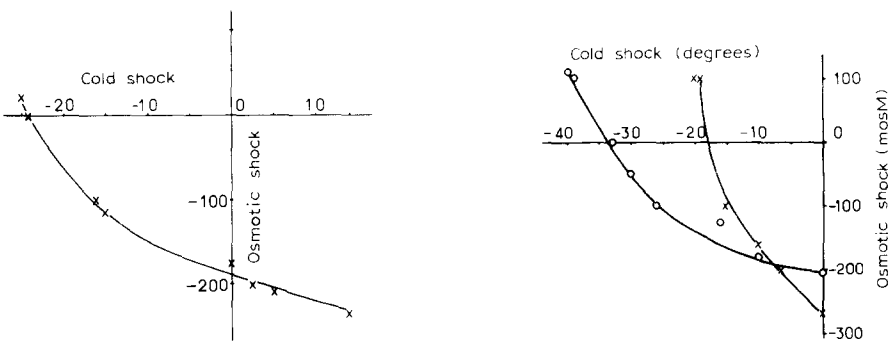

Fig. 10. Effect of combined osmotic and thermal shocks on the retention of a TMG pool, represented as 50% "iso-loss" curve. The intercept with the ordinate gives the size of the isothermal osmotic downshock causing 50% loss, the intercept with the abscissa gives the size of the isoosmotic cold shock causing 50% loss. The negative slope of the curve describes the cold sensitivity of the system compared to its osmotic sensitivity.

Fig. 11. 50% "iso-loss" curves of accumulated TMG pool from *E. coli* grown on different fatty acid complements. *E. coli* strain K 1059 (ϕ80 dlac$^+$) auxotrophic for unsaturated fatty acids, was grown with oleic acid (○———○) or with elaidic acid (X———X) supplements. TMG was accumulated at 40° C in a medium of 430 mosM. Filtration was followed by washing with solutions of temperatures ranging from 0 to 40° C and osmolarities from 30 to 530 mosM.

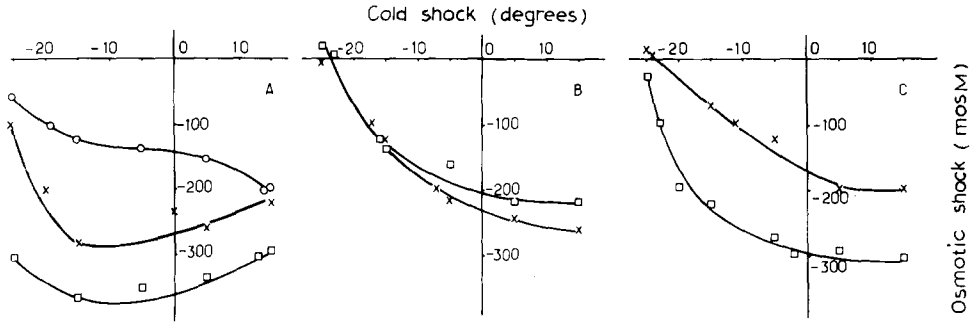

Fig. 12. 50% "iso-loss" curves for different solutes in several strains of *E. coli*. A (left) strain 3300 grown at 37°C incubated at 25°C. Pools: (○———○) K^+, (X———X) TMG, (□———□) α-methyl-glucose and its phosphate ester. B (middle) strain AR 13 grown on M63 glycerol, with IPTG 0.2 mM and K gluconate 0.5 mM at 37°C, pools: (□———□) gluconic acid, (X———X) TMG. C (right) strain P4 X AJ19, pools: (X———X) glucuronic acid, (□———□) α-methyl-glucose and its phosphate ester.

phosphate ester from strain AJ19. The osmotic shock sensitivity of the latter was again lower.

Discussion

The dramatic but transient breakdown of the permeability barrier of the bacterial membrane described here is the result of a rather modest hypoosmotic shock. The events which are likely to follow such shock can be inferred from what is known about the effects of a hyperosmotic shock. This is accompanied by shrinkage of the cell and so can be monitored by changes in light scattering.

Two phases could be distinguished: the first, obviously linked to the osmotic efflux of water, occurred in less than 100 ms; the second phase presumably due to compensatory fluxes of solutes can last a few minutes [15].

Hypoosmotic shock is followed by osmotic influx of water, which develops a hydrostatic pressure instead of swelling which is prevented by the rigid cell wall. The breakdown of the permeability barrier falls within this early period, which is followed by a compensatory flux of solutes which cancels the osmotic unbalance and permits the rejection of excess water.

Several hypotheses can be proposed for the mechanism of the membrane change. First, the water flux per se causes a change in membrane structure. This hypothesis is unlikely, since hyperosmotic shock, where a similar flux of water is thought to occur outward, does not cause a similar effect. The solvent drag cannot account for the leak of solutes, it is in the wrong direction.

The stretching of the membrane by the increasing volume of water would be particularly likely to create pores or channels. Nevertheless, we have strong reasons to suppose that stretch is negligible. It is well established that the osmotic pressure of the cytoplasm is higher than that prevailing in the medium, and consequently a hydrostatic pressure develops. When the rigid cell wall is damaged by lysozyme or penicillin the cytoplasm is seen to bulge across the opening until rupture. Therefore at steady state the cell membrane is already pressed in close contact with the rigid cell wall. The pressure, which increases during the early phase of hypoosmotic shock could be the cause of the

membrane change. As a comparison with the 2—4 atm extra pressure one can think of the effects of centrifugation. In routine conditions, a centrifugal field of 6000 × g submits the bacteria at 1 cm from the surface to a pressure of 6 atmospheres. Centrifugal cell separation was utilized to assess intracellular solute concentrations with results comparable to the filtration technique. No harm to the permeability barrier has ever been noticed in these conditions.

This is not a final statement but there is an attractive possibility, that the anisotropic pressure across the membrane is capable of causing changes distinct from the effects of an isotropic pressure. It is clear that due to the fluidity of the hydrocarbon chains the pressure would soon become isotropic, so the permeability could last milliseconds only. The mechanism suggested is reminiscent of the breakdown of electrical resistance experienced by black lipid membranes upon the establishment of a potential difference of a few hundred millivolts since this results in an electrostriction due to coulombian forces [16].

The available data do not justify any valid speculation about the actual configurational change responsible for the leakiness. The only rational question that can be asked is whether the membrane change upon hypoosmotic shock is similar to the change caused by low temperature. The effects of cold shock upon the retention of intracellular solutes were attributed to the thermal transition of the paraffinic chains to the liquid crystalline state. Certain membranes of high transition temperature, such as obtained by incorporating elaidic acid, become permanently leaky in the cold even if the cooling is slow. Although a hyperosmotic shock can counteract the effects of low temperature as stated by Leder and confirmed by our results, an equation of the membrane change upon cold shock and hypoosmotic shock should be viewed with caution.

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