# Osmotic Pools in Escherichia coli

Abstract. *Osmotically sensitive pools in* Escherichia coli *are released by treatment with warm water without loss in viability or of cytoplasmic components. The rates of protein release are linear and are inversely dependent on the osmolarity of the medium, and only discrete amounts are released. These pools, which are thought to be periplasmic and mesosomal, are stabilized with magnesium ions; material released in the presence of these ions may have been localized outside of these pools at the cell surface.*

Certain bacterial proteins appear to be localized outside of the cell membrane close to the surface. The cell membrane is the osmotic and permeability barrier lying beneath the cell wall, which serves as the limiting structural element of the cell; the membrane has the properties of a molecular sieve. Some of these proteins are thought to lie within the periplasm, a compartment enclosed between the wall and membrane. According to Heppel (*1*), these proteins are released selectively by an osmotic shock. The procedure used breaches the wall with ethylenediaminetetraacetate (EDTA) under hypertonic conditions, and the contents of the periplasm are expelled under hypotonic conditions. Penetrability of the wall by large molecules is increased by treatment with EDTA, perhaps because lipopolysaccharide panels are dislodged from the wall (*2*). Evidently, when turgor is increased by the lowering of the osmolarity of the medium in which the cells are suspended, the membrane expands against the breached, but still limiting, wall. The pressure thus generated forces periplasmic material into the medium. Heppel's analysis has suggested another means to test for localization between wall and membrane. By application of Poiseuille's law, the hypothesis can be substantiated by kinetic means because the rates of release of material through capillaries or pores should be related directly to the turgor of the cell and inversely to the osmolarity of the medium. Therefore, it may be possible to identify periplasmic materials according to osmotic criteria, and to distinguish them from surface-localized and cytoplasmic materials.

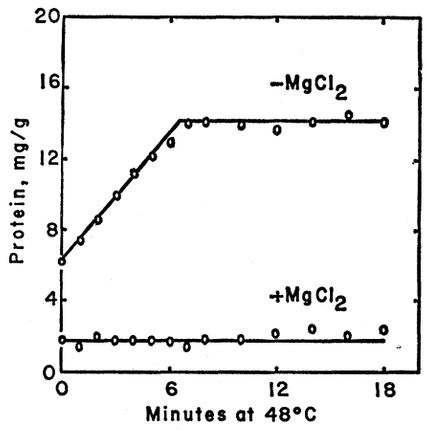


Fig. 1. Release of protein during warm-water treatment. The concentration of $MgCl_2$ is 0.01*M*.

Osmotic shock is unsuited for the proposed kinetic analysis because release is completed too rapidly and because a constant turgor is not maintained. Instead, a more suitable procedure may be the warm-water solubilization procedure used to obtain a sugar-binding protein from *Escherichia coli* A (*3*). Both procedures release similar amounts and kinds of protein, and both attack structures that are stabilized by divalent cations. Warm-water treatment can be used over convenient time intervals, under various conditions, and without prior treatment with EDTA, which might otherwise cause deleterious effects. Briefly, the latter procedure (*4*) consists of treatment of the cells in 0.1*M* buffer (*p*H 7.3) at room temperature followed by extraction at 48°C. By itself this treatment causes no irreversible effects, for viability and uptake of sugar are retained under appropriate conditions. Like osmotically shocked cells, the cells during warm-water treatment display a growth lag and are sensitive to actinomycin D; however, the presence of $Mg^{2+}$ during this treatment prevents the manifestation of both growth lag and sensitivity to actinomycin. Warm-water treatment does not release glucose-6-phosphate dehydrogenase, which is considered a cytoplasmic enzyme (see *1*).

The kinetics of protein release by warm-water treatment is a linear function of duration of treatment (Fig. 1). A constant rate of release is expected for a constant pressure differential between wall and membrane. The substantial amount of protein obtained after only the briefest contact at 48°C attests to the abruptness of the breaching process, while the discrete amount of protein released argues for the selectivity of the warm-water treatment. The inability to remove more than minimum amounts of protein by warm-water treatment in the presence of 0.01*M* $MgCl_2$ indicates that a structure that is stabilized with divalent cations is involved. The same minimum amounts of protein, which may have been bound at the surface, are obtained when the cells are briefly washed at 0°C.

The rates of protein release by warm-water treatment are an inverse function of the osmolarity of the medium (Fig. 2) (*5*), which is expected because turgor is inversely related to osmolarity. The total amount of protein released at lower osmolarities (0.021*M*) is the same as that released at higher osmolarities (0.101*M*), provided the duration of treatment is limited. A second surge in protein release occurs after somewhat longer periods of treatment, and this suggests the existence of more than one periplasmic compartment or possibly a mesosomal compartment.

Surface binding may be distinguished from localization within the periplasm because surface components should be removed in the presence of $Mg^{2+}$ without resorting to warm-water treatment. For example, by proper adjustment of *p*H and ionic strength, but not by changes in osmotic pressure, the α-amylase of *Aspergillus oryzae* can be removed from and returned to mycelia in the presence of $Mg^{2+}$ at 0°C; in addition, the surface location of α-amylase is shown with fluorescent anti-

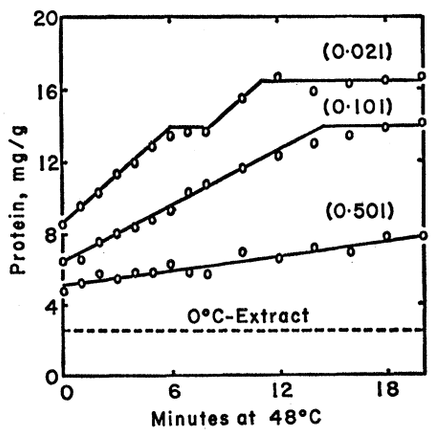


Fig. 2. Influence of osmolarity of the suspending medium on the release of protein during warm-water treatment. The osmolarities are indicated by figures in parentheses.

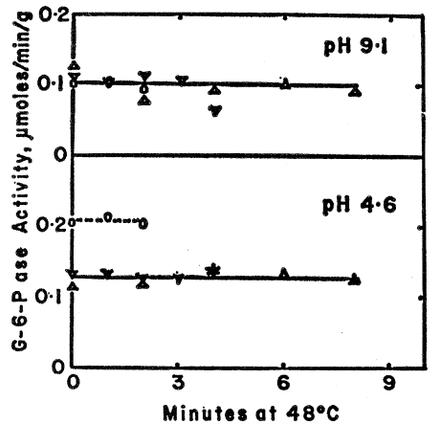


Fig. 3. Release of acid and alkaline phosphatases in the presence of $MgCl_2$ at various temperatures (○, 0°C; △, 24°C; ▽, 48°C). Those suspensions chilled to 0°C were subsequently treated at 48°C for 0, 1, and 2 minutes.

body technique (*6*). In the case of *E. coli* A, both acid and alkaline phosphatases are removed promptly and essentially independently of time and temperature, even in the presence of 0.01*M* $MgCl_2$ (Fig. 3) (*7*). That the amount of acid phosphatase obtained at 0°C is greater than that obtained at higher temperatures is reminiscent of the unexpected release of α-glucosidase from *Bacillus subtilis* at 0°C (*8*). The structural inferences from these results are consistent with cytochemical localization of alkaline phosphatase at the outer surface of the wall, and of acid phosphatase in a continuous compartment beneath the wall (*9*). Possibly, the location of acid phosphatase may be better described as being on a metabolically stabilized "plug" or "stopper" at an entry to the periplasm. Other factors must be involved because alkaline phosphatase is also described as being located in an intermediate layer of the wall or confined to the periplasm by the wall (*10*). Since warm-water treatment is not as effective with cells grown in enriched medium as with those grown in synthetic medium, some cellular fragility may have been encountered in my experiments. Less wall structure may have been deposited during growth in synthetic medium with the result that structural stability was decreased and the embedment of alkaline phosphatase within the wall was shallower.

With loss of compartmentalization, products of enzymic degradation are observed; among them, inorganic phosphate, acid-soluble nucleotides, and substances reacting with the Folin-Denis phenol reagent.

The attempts to localize cellular components according to osmotic criteria will undoubtedly yield useful information, especially by associating components with similar kinetics of release. Osmotically sensitive compartments outside of the membrane are inferred because certain components behave as though they are located between an osmotically driven surface and a porous limiting barrier. Although no direct evidence is offered, these components may have been located within the periplasm and mesosomes. Other components appear to be located outside of these structures, presumably on or within the wall. To some extent, the ease of release of surface components should correspond to the depth of localization within the surface structures external to the osmotic barrier. Structural inferences from this type of information will have to be correlated more closely with available cytological evidence (*11*).

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### References and Notes

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4. Cells of *E. coli* A in the logarithmic phase of growth were suspended at a concentration of 10 mg of dry weight per milliliter of 0.1*M* tris(hydroxymethyl)aminomethane-acetic acid, *p*H 7.3 buffer. After 1 hour at room temperature, the cells were harvested and resuspended in buffer warmed to 48°C. At intervals, samples were transferred to equal volumes of buffer chilled in ice. Extracts were analyzed for protein with a turbidity method (*3*). No nucleic acid precipitable in acid was observed.
5. Warm-water treatment was conducted with 0.01*M* buffer containing 0.01, 0.09, and 0.49*M* sucrose to provide for osmolarities of 0.021, 0.101, and 0.501, respectively.
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7. Phosphatase assays were performed at both *p*H 4.6 and *p*H 9.1 with 0.005*M* glucose-6-phosphate in 0.5*M* tris-acetate buffer for 2 hours at 37°C. Inorganic phosphate was determined by the method of R. L. Dryer, A. R. Tammes, J. I. Routh [*J. Biol. Chem.* **225**, 177 (1957)].
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12. Supported by a PHS fellowship 5 F3 AM-11,093. I thank R. R. Becker and Dorothy K. Fraser for useful discussions.

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## Lipoperoxidation of Lung Lipids in Rats Exposed to Nitrogen Dioxide

Abstract. *Absorption spectra characteristic of diene conjugation and typical for peroxidized polyenoic fatty acids can be induced in rat lung lipids after the rats have been exposed to a scant amount of nitrogen dioxide (1 part per million) for 4 hours. The peroxidative changes do not occur immediately but appear to reach a maximum between 24 and 48 hours after exposure. The prooxidant effect of this atmospheric pollutant in rat lung lipids may be partially prevented by prior treatment of the animal with large doses of alpha-tocopherol.*

Nitrogen dioxide ($NO_2$) is one of several toxicants present in our atmosphere. It is among the most toxic of the oxides of nitrogen and is an important and integral component in the complex of chemicals producing photochemical smog. Evidence has indicated that the effects of nitrogen dioxide on man and lower animals are confined primarily to the respiratory tract. Histological studies revealed that lung mast cells undergo considerable change when animals inhale $NO_2$ (*1*). Therefore, we have studied the effect of $NO_2$ on the lipids of rat lungs.

Young Sprague-Dawley rats (weighing 175 to 200 g and maintained on stock diet of Purina Chow) were used. In certain experiments, six rats were exposed to $NO_2$ [1 part per million (ppm) by volume] for a single 4-hour period (*2*), whereas in other experiments they were exposed for 4 hours daily during a 6-day period. After the initial 30 minutes, the $NO_2$ concentration in the chamber was maintained between 0.9 and 1.1 ppm. Comparably treated control animals were forced to inhale ambient air in the chamber for a similar 4-hour period immediately after exposure to $NO_2$. For the multiple exposures, ten rats were placed in a stainless steel and glass chamber (0.4 $m^3$) provided with 30 air changes per hour and laminar flow. During the exposure periods, the control animals were forced to inhale ambient air in an equivalent chamber. The $NO_2$ was metered into the incoming ambient air from a pressurized cylinder containing 1500 ppm in nitrogen. The $NO_2$ concentration inside the exposure chamber was continuously monitored with an instrument calibrated with respect to