# SHORT COMMUNICATIONS

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## The intracellular pH of *Escherichia coli*

The intracellular pH has been determined for a wide variety of cells by different methods[1]. A technique involving the distribution of the weak acid 5,5-dimethyl-2,4-oxazolidinedione (DMO) depends upon the equilibration across the cell membrane of the non-ionized molecules, associated with the lack of penetration by the ionized molecular species. The degree of ionization on the two sides of the cell membrane will depend on the pH. This method was introduced by WADDELL AND BUTLER[2]. The extensive use of this and other methods has recently been reviewed by WADDELL AND BATES[3].

The internal pH of *Escherichia coli* cells has been estimated indirectly from the photodynamic inactivation of bacteriophage DNA by methylene blue and light[4]. The pH of *Thiobacillus thiooxidans* cells ruptured with a French pressure cell after exposure to two media of different pH's has been measured[5]. In addition, direct measurements of the internal $H^+$ content of *Streptococcus faecalis* have been reported (ref. 6; F. M. HAROLD, personal communication).

We chose the facultative anaerobe *E. coli* to explore the possible effects of alterations in metabolism and environment on the cell's internal pH. This communication presents intracellular pH values obtained by the DMO method for *E. coli* suspended in media with pH values ranging from 5 to 9. Measurement of the pH of sonicated cells gave values that were in agreement with those obtained by the DMO method.

*E. coli* ML308 cells were grown in mineral Medium 63 (ref. 7), supplemented with 50 mM NaCl and 1% casein hydrolysate (Tryptone, Difco) at 37° with vigorous agitation in four 500-ml batches in 2-l erlenmeyer flasks. The cells were harvested at midexponential phase and resuspended in 25 ml 50 mM NaCl or diluted Medium 63–NaCl (1:10, v/v, with water), *plus* 1.0 ml chloramphenicol at 1 mg/ml water. The absorbance of a cell suspension suitably diluted with Medium 63–NaCl was determined in a Klett–Summerson colorimeter with a No. 42 filter.

An aliquot (3.0 ml) of the cell suspension was added to each of 8 centrifuge tubes. The cells were centrifuged in a Servall centrifuge with the SS34 rotor at 15–20° at 39000 × *g* for 20 min. The supernatant fluid was carefully decanted and the sides of the centrifuge tube dried with cotton swabs. Each pellet (A) was resuspended in 5.0 ml 50 mM NaCl or diluted Medium 63–NaCl, and 0.1 ml of 0.145 M [$^{14}$C]DMO (New England Nuclear Corp.) at 0.007 $\mu$C/$\mu$mole was added. The pH was adjusted to the desired value with HCl or NaOH. The suspension was centrifuged at 15–20° for 20 min at 39000 × *g*, the supernatant fluid B carefully decanted, and the sides of the centrifuge tubes swabbed.

Abbreviation: DMO, 5,5-dimethyl-2,4-oxazolidinedione.

The pH of the supernatant fluid B was determined with the Radiometer 22 pH meter. Aliquots of supernatant fluid B were counted in a liquid scintillation counter with BRAY's[8] solution.

Each pellet (B) (0.6–0.8 g wet weight) was acidified with 1.0 ml 5 M $NaH_2PO_4$ and extracted with 5 ml of toluene–ethyl acetate (1:1, v/v) by the method of POOLE *et al.*[9]. Duplicate aliquots (2.0 ml) of the toluene–ethyl acetate extracts were counted. The recovery of DMO by this extraction exceeded 98%.

The ratio of extracellular to intracellular fluid in the cell pellets was determined from the total pellet water and the sucrose space in the pellet. The dry weight of the pellet B was determined after drying the cells to constant weight in a desiccator at reduced pressure over anhydrous $CaCl_2$ for 24 h at 37° and by exposure to 80° for 2 days in an oven. The volume of pellet water was calculated from the difference between the wet and dry weights of the pellet. The sucrose space was determined by adding 0.1 ml of 0.5 mM [$^{14}$C]sucrose at 3 $\mu$C/$\mu$mole instead of [$^{14}$C]DMO to the pellet A, under the same conditions. After the second centrifugation, the pH and radioactivity of supernatants B were measured. The resultant pellet B was then suspended again in 5.0 ml diluted Medium 63–NaCl and recentrifuged. The radioactivity in supernatant C was counted, and represents more than 98% of the sucrose contained in the original pellet. All volume measurements were performed gravimetrically; the specific gravity of the pellet was taken as 1.05 (ref. 10). It should be pointed out that determination of the extracellular fluid volume based on the inulin space may lead to errors of as much as 50% in the calculation of the intracellular volume. The error is derived from the effects of plasmolysis. Sucrose, unlike inulin, can penetrate the cell wall, being excluded by the cell membrane from the intracellular fluid[10,11].

The values obtained from DMO measurements were inserted in the equation[2]:

$$pH_i = pK'_a + \log\{[C_r(1 + V_r)][1 + 10^{(pH_e - pK'_a)}] - 1\}$$

where $pH_i$ is the intracellular pH, $pH_e$ is the (extracellular) pH of supernatant fluid B, $C_r$ is the ratio of the counts/min per $\mu$l of total pellet water to the counts/min per $\mu$l

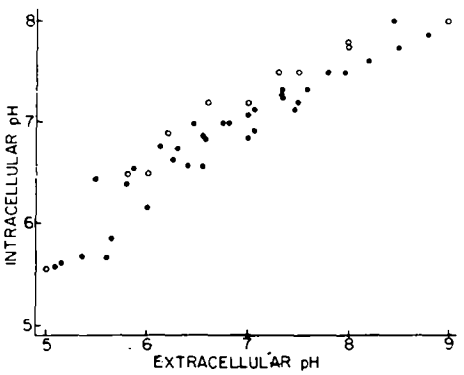


Fig. 1. The intracellular pH of *E. coli* exposed to media of various pH's. ●, derived from the distribution of [$^{14}$C]DMO between cells and medium at various pH's, as described above; ○, values for the pH of sonicated cells. For sonication each washed pellet A (0.65 g wet wt.) was resuspended in 5 ml water and the pH adjusted to the desired value with HCl or NaOH. The cells were centrifuged at 39000 × *g* for 20 min and resuspended in 5 ml water. After sonication for three 30-sec bursts at 1.7 A at 0–5° in an MSE sonicator, the pH was measured.

of supernatant B, $V_r$ is the ratio of extracellular water volume to intracellular water volume, and $pK'_a$ is the apparent ionization constant of DMO at 25° (6.32).

The value obtained for the ratio of extracellular to intracellular fluid volume of the cell pellets ($V_r$) was 0.60 and was not significantly affected by the pH of the medium. The limitations and assumptions of the DMO method, and the reliability of results have been discussed[12,13].

When *E. coli* cells were exposed to media of pH ranging from 5 to 9, the intracellular pH was found to vary with the medium pH (Fig. 1). These data indicate that the internal pH varies over a range of at least 2 units. The $pH_i$ values at $pH_e$ less than 7.3 were consistently higher than the $pH_e$, while at $pH_e$ values above 7.3, the $pH_i$ was lower, indicating that the cell's cytoplasm has a buffering capacity, which tends to adjust the internal pH toward neutrality.

The results obtained with the [$^{14}$C]DMO method were confirmed by direct pH measurement of sonicated cells exposed to media with the same pH range (Fig. 1). The values found for the $pH_i$ are average ones because of the heterogeneity of the cellular contents. Under the experimental conditions used, that is, with centrifugation lasting 20 min, the cells are most probably in an essentially anaerobic state.

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