Microbiology Comment provides a platform for readers of *Microbiology* to communicate their personal observations and opinions in a more informal way than through the submission of papers.

Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others. In other instances we may have a useful bit of methodology that we would like to share.

The Editors hope that readers will take full advantage of this new section and use it to raise matters that hitherto have been confined to a limited audience.

John Freer
Editor-in-Chief

What's it like inside prokaryotic cells?

We no longer regard prokaryotic cells as 'bags of enzymes', but how are we to picture and know their internal composition and architecture (2, 3)?

Much of recent work on internal concentrations of solutes and ions in eubacterial halophiles (5, 8, 9) has been marred by doubts about the cell volumes used in the calculations, resulting in widely varying – including hyper – internal osmolalities. Escape from this difficulty by offering amounts of intracellular solutes per mg dry weight of cells (6) is no answer. Accurate determinations of intracellular volumes are mandatory for evaluation and discussion of the cell's osmotic conditions and true internal environment (2, 3).

Most modern methods for determining cell volumes (4, 7) measure the total space of cell pellets occupied by a penetrant molecule, and the extracellular space, using a non-penetrant molecule. The intracellular volume is then the difference between the two. Unfortunately, any, even transient, changes in cell permeability will alter the proportion of extracellular space, and hence the intracellular volume. Furthermore, any stickiness or clumping of cells will decrease proportions of intercellular space, thus increasing the apparent intracellular volume.

The worst of it is that there are no 'controls' for such adventitious changes in cell pellets – only replication and consistency of results.

I would appeal to all interested in the 'true intracellular environment' of micro-organisms to come to grips with this problem and obtain valid, repeated and consistent values for intracellular volumes (1, 2). It is worth the effort if we want to understand intracellular conditions and events.

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Plasmid expansion?

I recently encountered a problem which stemmed from the conventions concerning the estimation of size in DNA molecules. This in turn caused me to reflect upon the implied accuracy of oft-quoted sizes for plasmids. In the early days of plasmid DNA isolation, it was customary to determine the sizes of plasmids by sedimentation rates in preformed alkaline sucrose gradients (1) or by contour-length of plasmid open circles visualized by electron-

microscopy (4). Sizes were usually given in megadaltons (MDa). Since that time the sizes of some plasmids have been revised in the light of mapping studies involving restriction fragment analysis of whole plasmids and the sizes are frequently given in kilobase pairs (kb). For example, the size of RP4 was shown to be larger (approx. 60 kb) than previously determined (3).

In studying plasmids present in plant pathogenic bacteria, I recently obtained a strain of *Escherichia coli* K12 (39R861), carrying four plasmids which are useful as size standards. These plasmids, RA1, X, S-a and NTP168 are 98, 42, 23.9 and 4.6 MDa, respectively, and this strain is available as NCTC 50192 from the National Collection of Type Cultures, UK. Strain 39R861 was constructed by E. J. Threlfall and others (7) and although this prototrophic, NA⁺ host is assumed to be recombination-proficient, nevertheless the four plasmids appear to remain as stably inherited replicons, which show no size variation after repeated subculture (E. J. Threlfall, pers. comm.).

The problem that can arise is related to the use that is made of this strain. It was developed to provide epidemiologists, working in clinical practice, with an additional means of comparing isolates. The primary aim was not to determine precise sizes, but rather to identify strains having similar-sized (and hence potentially related) plasmids and to size

GUIDELINES

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

Approval for publication rests with the Editor-in-Chief, who reserves the right to edit letters and/or to make a brief reply. Other interested persons may also be invited to reply. The Editors of **Microbiology** do not necessarily agree with the views expressed in **Microbiology Comment**.

Contributions should be addressed to the Editor-in-Chief via the Editorial Office.