can come at the expense of quality, stability and cost. The faculty's experience (over nearly ten years) of an existing facility indicated more permanency than change.

On the question of flexibility, it seems that everyone has strong (but often divergent) views about what is necessary. In my visits to other comparable laboratories, I came across one whose staff were adamant about the need for flexibility in design. I found that virtually every laboratory was uniquely arranged and that change was common whenever faculty changed. Upon closer questioning, I became convinced that the different arrangements were more a result of individual preferences than of science. Because the flexibility was offered, each new faculty member felt they had to create a unique arrangement. Given choice, the ability to choose becomes important. On the other hand, many laboratories have very similar four-person modules with side and center benches that researcher after researcher has accepted unquestioningly because they were there and because they were functional. Similarity is also a testament to the fact that permanency is more likely than change.

For the Whitehead Institute, casework quality and stability were valued more than the ability to rearrange casework using only in-house staff. The plan did, however, ensure that the structural design and the layout of pipe runs were such that any single laboratory space could be gutted or even combined with adjacent laboratory space, without disrupting research in abutting spaces, particularly those above and below.

Conclusion

Although many details of the design were refined as the design progressed, there was never a wholesale revision in the layout. I conclude this was largely a result of the timely resolution of fundamental organizational and design issues. The architect's design can only match the user's needs if the user makes them known through the planners.

In the next issue of *TIBS*, the architect describes how the planning program was realized.

Emerging Techniques

Cryo-electron microscopy of vitrified biological specimens

J. Dubochet, M. Adrian, J. Lepault and A. W. McDowall

Rapid freezing has long promised the most faithful structural presentation of biological material for electron microscopy. With the recent emergence of methods for vitrifying specimens, this promise seems about to be fulfilled.

Electron microscopy has profoundly influenced our image of living matter in the 50 years since its invention, but unfortunately, the most abundant constituent of living things, water, has invariably been excluded from these studies. Electron microscopes must operate under high vacuum, and water evaporates at such low pressure. The construction of humid environmental chambers to preserve water in the specimen proved difficult[1]. The idea of preventing evaporation by cooling the specimen[2] was more successful[3], but this approach suffered from a major limitation: water crystallizes on freezing, inducing severe structural damage.

The recent discovery that liquid water can be cooled into a vitreous ('glass-like') solid, without crystallization, came

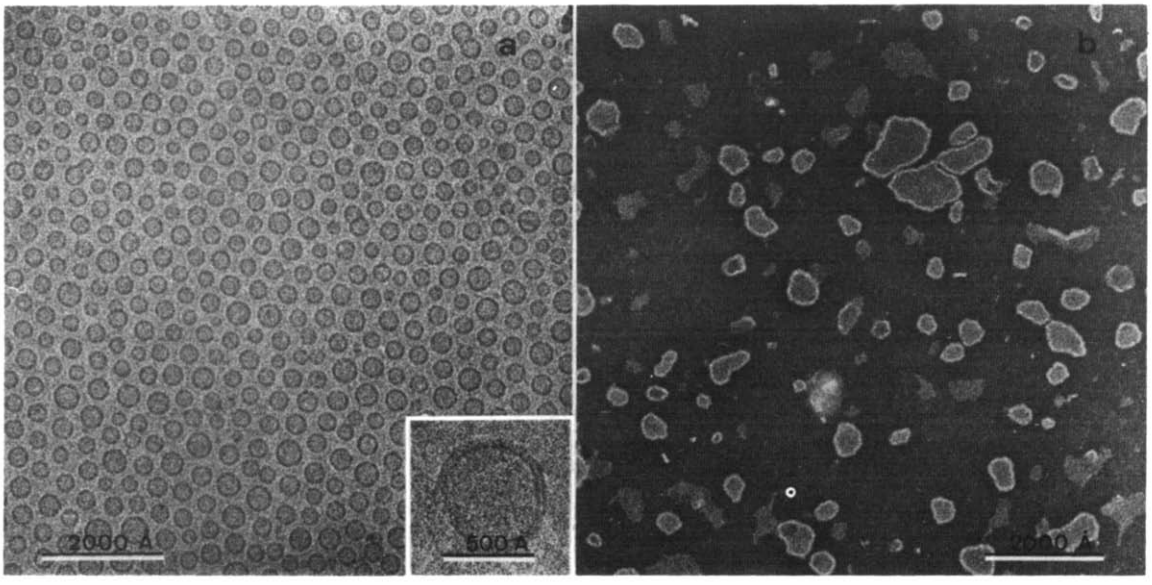

Fig. 1. Soya bean phospholipid vesicles obtained by dialysis of the lipids (1 mg ml^{-1}) solubilized in 1% neutral detergent. (a) Thin film vitrification: thin layer of unstained, unsupported vitrified solution. Insert: enlarged view of a vesicle from another preparation. (b) Conventional preparation, negatively stained with 2% sodium phosphotungstate, pH 7.2.

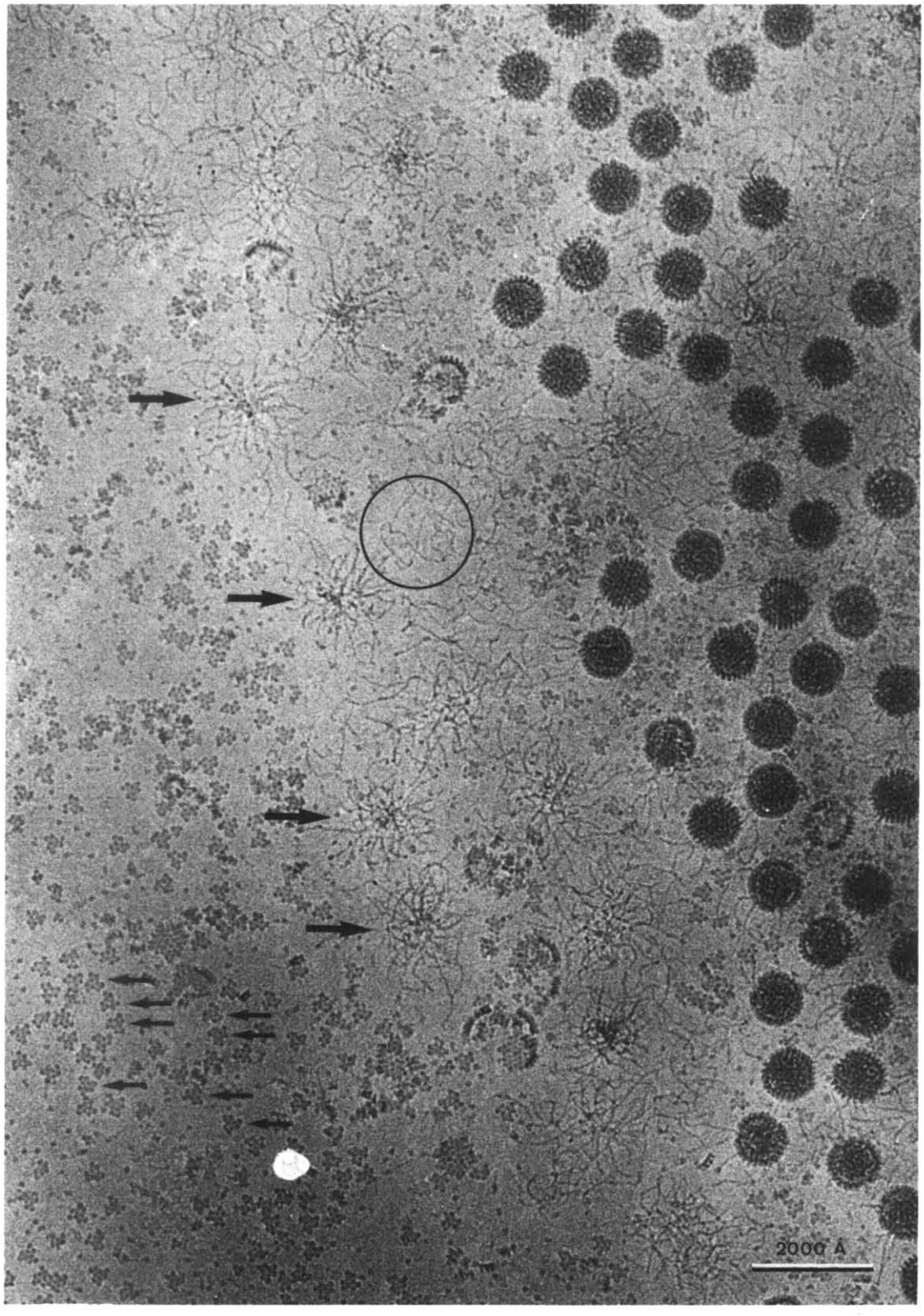
2000 Å

Fig. 2. Strongly underfocused image of an unstained, unsupported vitrified layer of a partially disrupted Adenovirus suspension. Some groups of nine (G9) are marked by small arrows. Partially open nuclear cores are marked by large arrows and a DNA loop is circled. (See opposite.)

as a surprise[4,5]. After many decades of unsuccessful attempts, hope of achieving vitrification had faded, and workers in the field were either convinced that it was impossible in principle[6] or that the required cooling-rate could not be achieved in practice. When it was achieved, the critical factor for successful vitrification proved to be the size of the specimen. It is fortunate that, on reducing sample-size to increase cooling-rate, vitrification becomes easy at the dimensions suitable for electron microscopy.

This ability to vitrify water began the good fortune of cryo-electron microscopists. Soon after, the difficult problem of forming a sufficiently thin, stable layer of suspension was solved elegantly by spanning the unsupported liquid over the holes in a grid[7]. Furthermore, the ability to cut vitrified material into thin sections opened new possibilities for cryo-ultramicrotomy. To help matters further, the advantages of cryo-electron microscopy seem already to be obtained around −160°C, so that convenient and cheap liquid nitrogen can be used for refrigeration. Finally, it is fortunate for electron microscopists (and those financing them) that of all the various designs of cryosystems explored, the simplest give the best results. From these beginnings, cryo-electron microscopy of vitrified biological material has been developed into a practical technique[7–11] which we will illustrate here with examples.

A simple technique

An uncoated electron microscope grid is clamped vertically in forceps, several centimeters above a pot of liquid ethane cooled in liquid nitrogen. A drop of suspension (containing the specimen) is applied to the grid and most of the liquid is then removed by blotting with filter paper for a few seconds. The thin liquid layer is remarkably self-stabilizing, so the method of blotting is not critical. The specimen is plunged into the cryogen (liquid ethane) where it vitrifies. It is then transferred to liquid nitrogen and mounted in the cryospecimen holder of the electron microscope. If the subsequent transfer from liquid nitrogen into the high vacuum of the microscope is sufficiently rapid, there is no need for sophisticated thermal insulation around the specimen. Observations are made at temperatures around −160°C using the minimum dose of electrons.

This description suggests that the method is very easy, which is true for liquid suspensions; however, the procedure for bulk material that requires cryo-sectioning is more involved. Nor do the problems end when the sample has been prepared, because imaging frozen hydrated specimens is difficult. Micrographs are recorded with large defocusing values to make the best possible use of phase contrast[12], and this can complicate image interpretation. Finally, electron-beam damage, frequently forgotten when metal-shadowed or stained specimens are observed, is always a source of concern with unstained, hydrated material.

Artefact-free electron microscopy?

The high quality of structural preservation in vitrified preparations has been demonstrated in several publications[7,13–16]. We claim it compares favourably with conventional electron microscopical methods for all specimens

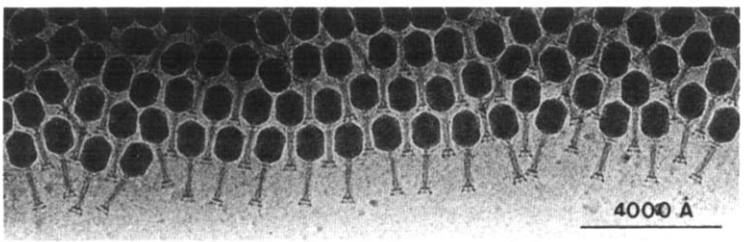

Fig. 3. Small portion of a thin vitrified layer of unsupported T4 bacteriophages.

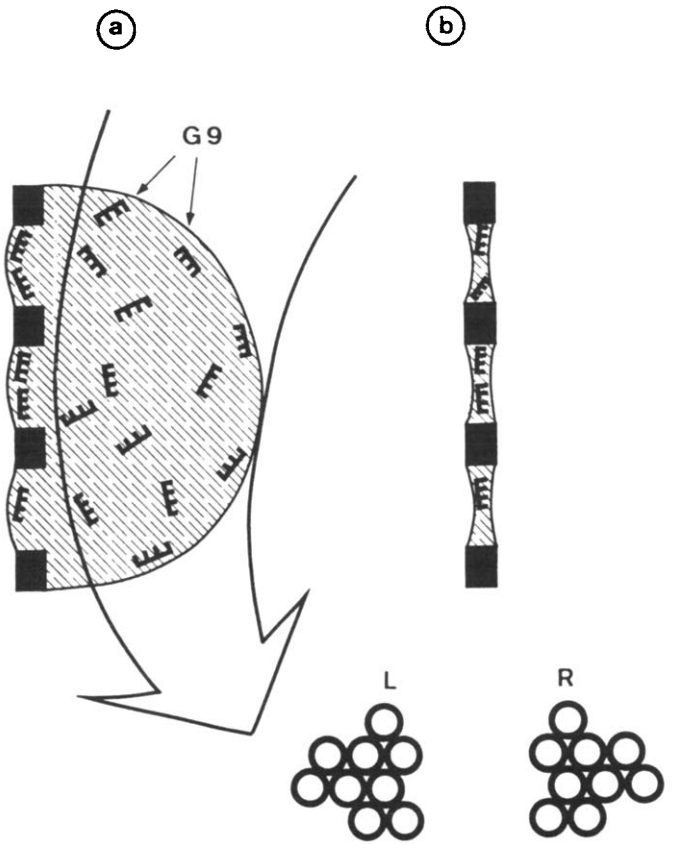

Fig. 4. Schematic explanation for the asymmetry of the G9 observed in Fig. 2. Depending on which side they are seen from, the G9 are seen as L or R. When the drop of suspension is put on the grid, those G9 which are close to the surface take a fixed orientation (a). Removing most of the drop leaves a sample which represents only the left-side surface of the bulk sample (b).

so far studied in detail. For example, Fig. 1 shows a concentrated suspension of lipid vesicles prepared by the thin film vitrification method (a) and by conventional negative staining (b). In the vitrified sample, the regular arrangement of vesicles in the thin liquid layer is also preserved; the insert shows that the lipid bilayer is clearly resolved. A second example (Fig. 2) shows the high contrast that can be obtained with adequate defocusing. In this vitrified thin layer of partially disrupted Adenovirus, many groups of nine capsomeres (G9; small arrows), and partially open nuclear cores (large arrows), are visible, together with more or less complete virions. Loops of DNA radiating from nuclear cores are also displayed (circle).

It may seem surprising that unstained vitrified specimens can provide so much high-resolution information. The explanation lies with the signal to noise ratio. The signal from unstained, unsupported, hydrated objects is low, but far from negligible. It arises from the difference in mass density between the biological material (e.g. 1.35 g cm^{-3} for compact protein) and the vitrified medium (e.g. 0.93 g cm^{-3} for vitrified water), which is substantial. Judicious use of phase contrast allows this difference to be exploited to the full. In addition, noise in the specimen is low: there are no contributions from stain or supporting film, and the specimen is not damaged during preparation. We must stress here that the excellent preservation of biological structures during vitrification is not only an expectation based on theoretical considerations, but has been experimentally confirmed whenever objective comparison between the native and vitrified state was possible. This was true, for example, for the diameter of spherical viruses[7], and the structure of insect flight muscle[15].

From these observations, we thought at first that vitrification offered the long cherished goal of artefact-free electron microscopy. A closer look shows that the reality is more complex.

The image of an interface

Figure 3 illustrates that the thin film vitrification method does not always give an exact representation of the bulk suspension. In this preparation of T4 bacteriophages, the thickness of the liquid film in the center of a grid square was smaller than that of the virions. They were therefore pushed to the periphery of the square, forming the close-packing arrangement shown on the figure. More subtle effects are apparent on closer observation of the groups of nine seen in Fig. 2. Firstly, it can be seen that most of the G9 are oriented parallel to the surface. We note secondly that G9 could be right-handed (R) or left-handed (L), depending on which side they are seen from (Fig. 4). Because of the symmetry of the unsupported thin layer of suspension, R and L groups would seem equally probable. This is not so, and in the extreme case depicted in Fig. 2, all G9 have the same L-handedness. The only explanation we can offer for this effect is illustrated in Fig. 4: when the drop was applied to the grid, those groups of nine close to the surface were oriented in a specific manner (Fig. 4a); most of the drop was then removed, leaving only the remnant of the left-side surface layer in the vitrified preparation (Fig. 4b).

Some might argue that the surface effects described above set a limit to the usefulness of the method. We feel that the contrary holds true. In the first place, surface effects are not just an irrelevant, disturbing artefact. Indeed, it must be hard to find anything in a cell which does not interact with a surface. The thin film vitrification method seems to provide a way to study these surface interactions. Secondly, we note that all the surface effects shown in this article are an expression of forces acting in the liquid medium, sometimes over a long distance. Conventional dry microscopy is obviously in a bad position to explore them. The 'liquid' structures observed in so many vitrified specimens, not only in relation to artificial interfaces but also in bulk biological samples, could well be the most far-reaching finding emerging from the new cryo-electron microscopy of vitrified specimens.

Acknowledgements

We thank Drs C. Devaux, E. Kellenberger and F. Pattus for samples. Dr J. M. Smith helped us with many valuable suggestions on the manuscript.

References

1 Parsons, D. F. (1974) *Science* 186, 407–414
2 Fernandez-Moran, H. (1960) *Ann. NY Acad. Sci.* 85, 689–713
3 Taylor, K. A. and Glaeser, R. M. (1974) *Science* 186, 1036–1037
4 Brüggeller, P. and Mayer, E. (1980) *Nature* 288, 569–571
5 Dubochet, J. and McDowall, A. W. (1981) *J. Microsc.* 124, RP3–4
6 Johari, G. P. (1977) *Philosophical Magazine* 35, 1077–1090
7 Adrian, M., Dubochet, J., Lepault, J. and McDowall, A. W. (1984) *Nature* 308, 32–36
8 Dubochet, J., Lepault, J., Freeman, R., Berriman, J. A. and Homo, J.-Cl. (1982) *J. Microsc.* 128, 219–237
9 Lepault, J., Booy, F. P. and Dubochet, J. (1983) *J. Microsc.* 129, 89–102
10 McDowall, A. W., Chang, J.-J., Freeman, R., Lepault, J., Walter, C. A. and Dubochet, J. (1983) *J. Microsc.* 131, 1–9
11 Chang, J.-J., McDowall, A. W., Lepault, J., Freeman, R., Walter, C. A. and Dubochet, J. (1983) *J. Microsc.* 132, 109–123
12 Lepault, J. and Pitt, T. (1984) *EMBO J.* 3, 101–105
13 Dubochet, J., Adrian, M., Teixeira, J., Kadiyali, R. K., Alba, C. M., MacFarlane, D. R. and Angell, C. A. *J. Phys. Chem.* (in press)
14 Dubochet, J., McDowall, A. W., Menge, B., Schmid, E. N. and Lickfeld, K. G. (1983) *J. Bacteriol.* 155, 381–390
15 McDowall, A. W., Hofmann, W., Lepault, J., Adrian, M. and Dubochet, J. (1984) *J. Mol. Biol.* 178, 105–111
16 Milligan, R. A., Brisson, A. and Unwin, P. N. T. (1984) *Ultramicroscopy* 13, 1–10

The authors are at the European Molecular Biology Laboratory, Postfach 10.2209, Meyerhofstr. 1, D-6900 Heidelberg, FRG.