These early stages in development are at present under investigation and may provide a useful indication of the mechanism of virus replication in the plant.

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[1] Smith, K. M., in *Protoplasmatologia*, edit. by Heilbrum, L. V., and Weber, F., **4**, Sect. 4*a*, 16 (Springer-Verlag, Vienna, 1958).
[2] Caldwell, J., and Robb, S. M., *Nat. Dahlia Soc. Ann.*, 29 (1962).

CYTOLOGY

Negatively Stained Lipoprotein Membranes

In a recent communication Finean and Rumsby[1] have cast doubt on the reliability of the electron microscopic image obtained from negatively stained preparations of lipoprotein membrane structures (myelin, mitochondria, microsomes and red cell ghosts). Although we agree with their remarks about the need for caution in interpreting such electron micrographs, it is felt that further comment is necessary concerning some of the published literature[2,3] mentioned by them.

During the examination of sub-cellular components[2] a comparison was made between: (*a*) unfixed negatively stained preparations; (*b*) preparations fixed with either formaldehyde or osmium tetroxide, followed by negative staining; and (*c*) conventional thin sections[4,5]. Owing to the low osmolarity of the 1 and 2 per cent phosphotungstate used for negative staining, it was found to be necessary with structures sensitive to the osmotic pressure of the surrounding medium (for example, nerve ending particles, mitochondria) to fix the particles before negative staining in order to prevent obvious disruption. Liver mitochondria are particularly sensitive. With osmotically insensitive structures (for example, myelin fragments) similar results were obtained with fixed and unfixed material. However, exposure of unfixed material to the hypotonic phosphotungstate was kept as brief as possible. Under these conditions negatively stained preparations were found to possess structural features directly comparable with those of positively stained sectioned material as is clearly illustrated in the published micrographs[2].

We consider that inferences drawn from the X-ray diffraction study of air-dried material in the absence of a suitable negative staining agent do not necessarily apply to material dried in the presence of a negative stain. As with positive staining, the mechanism of negative staining is not fully understood, but it is probable that the electron dense material sets in a rigid 'glass' and dries more rapidly than does the biological material in the absence of the stain. This may well prevent some of the changes seen in material air-dried in the absence of negative staining from occurring.

Turning to the question of biological and artificial membranes studied by several authors[3,6], it has been suggested by Finean and Rumsby that the patterns observed after treatment with saponin are a result of changes occurring during the final stages of drying. As indirect evidence that this is not so is the observation that the birefringence of a lecithin–cholesterol dispersion in water is rapidly lost by the addition of a dilute (1 per cent) saponin solution, thus indicating a loss of the lamellar structure in the myelinics. Bangham and Horne reported at a recent meeting of the British Biophysical Society that the same basic structures could be seen in lecithin–cholesterol preparations treated with saponin after treatment with buffered osmium and then air-dried. It was suggested that although dehydration effects may still be present, the separation of lipid components to form the same basic patterns under these conditions was unlikely.

The observations of Lucy and Glauert[7], on the increase in length of the helical components with time in mixtures of lecithin, cholesterol and saponin, lend support to the view that these structures are produced in the liquid phase.

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[1] Finean, J. B., and Rumsby, M. G., *Nature*, **197**, 1326 (1963).
[2] Horne, R. W., and Whittaker, V. P., *Z. Zellf.*, **58**, 1 (1962).
[3] Bangham, A. D., and Horne, . W., *Nature*, **196**, 952 (1962).
[4] Gray, E. G., and Whittaker, V. P., *J. Physiol.*, **153**, 35P (1960).
[5] Gray, E. G., and Whittaker, V. P., *J. Anat.* (Lond.), **96**, 79 (1962).
[6] Glauert, A. M., Dingle, J. T., and Lucy, J. A., *Nature*, **196**, 953 (1962).
[7] Lucy, J. A., and Glauert, A. M. (to be published).

X-ray diffraction studies of the air drying of myelin have included experiments in which myelin fractions from brain were dried in the presence of phosphotungstate. It is clear that this material does contain layering which electron microscopy of thin sections shows to be similar to that of the normal preparation, but it also includes additional layered systems which are significantly different. The drying of such specimens was probably appreciably slower than that achieved in the preparation of negatively stained specimens for electron microscopy, but the possibility of the existence of more than one type of layering in the latter preparations is clearly indicated.

Our suggestion concerning the remarkably regular hexagonal system seen in negatively stained preparations of saponin-treated cell membranes, lecithin–cholesterol dispersions, and cholesterol monolayers was that this particular geometrical arrangement might have been formed during the final stages of dehydration. The observation that the birefringence of lipid droplets is decreased by the addition of saponin certainly indicates an interaction in the hydrated state but not necessarily a loss of lamellar structure nor the formation of hexagonal or helical systems. Comparisons of the swelling of brain lipid pellets in distilled water and in saponin solutions (up to 50 per cent saponin) have shown very similar sequences of changes in X-ray diffraction patterns, all patterns so far being readily interpretable in terms of a layered structure. Similar diffraction sequences have also been observed using sodium cholate, sodium deoxycholate, or sodium dodecyl sulphate, all of which will dramatically reduce the birefringence of lipid droplets in the way that saponin does, but which have not yet been shown to form hexagonal or helical systems. It would seem to us that the state of hydration at which such systems are formed remains unknown.

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GENETICS

Sex Ratios under Natural Selection

Several workers[1,2] have shown the numerical equality of two sexes (that is, a 1 : 1 sex ratio) to be selectively favoured in relation to the parental effort involved in the rearing of offsprings to maturity. More recently, Kalmus and Smith[3] have argued in support of the adaptive superiority of this ratio on the basis of such considerations as those of hybridity, recombination potential and the level of inbreeding as influenced by sex ratio variations. However, their arguments are not valid in general and at best seem to apply only to the monogamous species. In fact, widely deviant sex ratios are reported to occur in the natural populations of both polygamodiœcious and gynodiœcious species[4,5], and Lewis[5] has suggested that sex ratio adjustments frequently through a preponderance