therefore that divalent cations bring about the complete rupture of phosphatidylserine vesicles, with production of structures which have lost the form of closed vesicles. This conclusion suggests the need for caution in interpreting vesicle 'permeability increases' under the influence of agents other than Ca^{2+} and Mg^{2+}. Regarding membrane fusion, it seems that the Ca^{2+}–phosphatidylserine system is an inappropriate model for biological membrane interaction, as the final product of the reaction between these components is not vesicular, although some initial fusion may occur on addition of Ca^{2+} to the suspension. Identification of the factors essential to fusion must await the development of a reliable assay for the occurrence of this process in artificial membrane systems.

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1. Poste, G. & Nicolson, G. L. (eds) *Cell Surface Reviews* Vol. 5 (North-Holland, Amsterdam, 1978).
2. Papahadjopoulos, D. & Poste, G. *Biophys. J.* **15,** 945–948 (1975).
3. Papahadjopoulos, D. *et al. Biochim. biophys. Acta* **465,** 579–598 (1977).
4. Maeda, T. & Ohnishi, S.-I. *Biochem. biophys. Res. Commun.* **60,** 1509–1516 (1974).
5. Prestegard, J. H. & Fellmeth, B. *Biochemistry* **13,** 1122–1126 (1974).
6. Papahadjopoulos, D., Vail, W. J., Pangborn, W. A. & Poste, G. *Biochim. biophys. Acta* **448,** 265–283 (1976).
7. Gingell, D. & Ginsberg, L. in *Cell Surface Reviews, Vol. 5* (eds Poste, G. & Nicolson, G. L.) 791–833 (North-Holland, Amsterdam, 1978).
8. Papahadjopoulos, D. & Watkins, J. C. *Biochim. biophys. Acta* **135,** 639–652 (1967).
9. Papahadjopoulos, D. *Biochim. biophys. Acta* **211,** 467–477 (1970).
10. Papahadjopoulos, D., Nir, S. & Ohki, S. *Biochim. biophys. Acta* **266,** 561–583 (1972).
11. Papahadjopoulos, D., Hui, S., Vail, W. J. & Poste, G. *Biochim. biophys. Acta* **448,** 245–264 (1976).
12. Allen, R. J. L. *Biochem. J.* **34,** 858–865 (1940).
13. Papahadjopoulos, D. & Bangham, A. D. *Biochim. biophys. Acta* **126,** 185–188 (1966).

Uptake of charged lipid vesicles by isolated tomato protoplasts

LIPID vesicles act as carriers of biologically active materials into animal cells[1–3]. A similar role might be envisaged for vesicles, for example, in the introduction of foreign DNA into plant protoplasts in genetic modification experiments[4], or in the initiation of virus infection in plant protoplasts[5]. The amount of DNA which enters fed protoplasts is difficult to determine as is the multiplicity of virus which initiates infection in the poly-L-ornithine (PLO) (ref. 5) and polyethylene glycol (PEG) (ref. 6) procedures. To overcome these limitations of existing methods for the introduction of materials into protoplasts, I report here a vesicle system with the potential to fuse with the negatively charged plasmalemma[7]. Single membraned ('hollow') vesicles were considered more suitable to combine membrane instability with large 'load' volume, required to facilitate carriage of large molecules/virus particles into the protoplast and to release their content synchronously within.

Tomato protoplasts were isolated as previously described[8]. Positively charged vesicles were prepared as follows: equal volumes of 10 μM lecithin and 1 μM stearyl amine were dissolved in chloroform–methanol (4:1, v/v) and evaporated to dryness[9]. The residue was resuspended at 2.5 mg ml^{-1} in phosphate buffered mannitol (PBM, 12% w/v mannitol in 50 mM sodium phosphate buffer, *p*H 7.0) containing 0.01% w/v fluorescein diacetate and shaken vigorously by hand to give vesicles of about 0.002–0.006 mm diameter. These were loaded with fluorescein diacetate to facilitate study. The vesicles were collected by centrifugation at 50 *g* for 10 min. Vesicles were washed by resuspension, recentrifugation in PBM. Small vesicles remained in the supernatant and were discarded.

Protoplast and vesicle counts were made and the populations mixed in the ratio of 1:1, protoplasts:vesicles, in a volume of 1 ml (1×10^6 protoplasts) of PMB. Samples were examined immediately after mixing and at 15 and 25 h after mixing. Incubation was in the dark at 25 °C. A random sample of 181 protoplasts examined immediately after mixing showed a total of 140 vesicles bound (Fig. 1, top). After 15 h, vesicles were detected in the central vacuole (Fig. 1, middle), no vesicles were detected in the medium or still adhering to the outer surface of the plasmalemma, nor was fluorescence detected in the medium. After 24 h, vesicles were no longer detected, but the vacuoles of many protoplasts were fluorescing (Fig. 1, bottom).

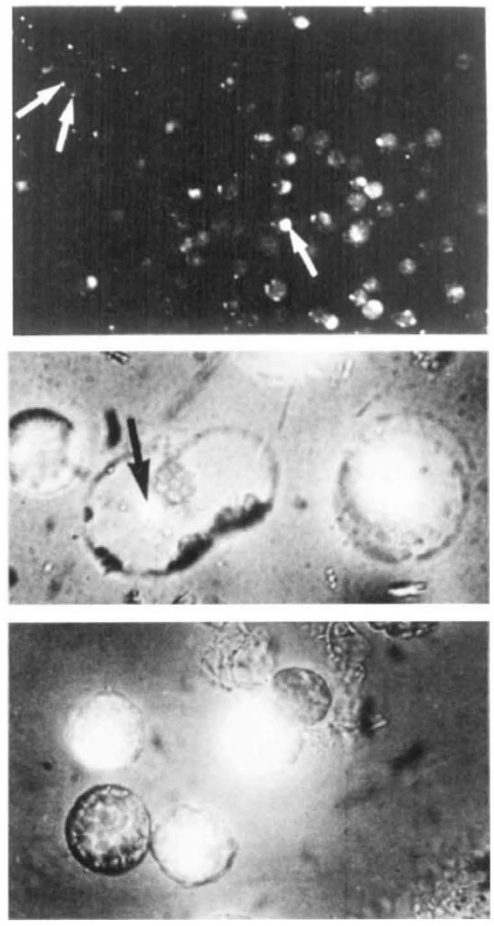

Fig. 1 Uptake of charged vesicles by isolated tomato protoplasts. Top, binding of vesicles to the plasmalemma, arrows indicate binding of one or more vesicles per protoplast. Middle, uptake of vesicles into the central vacuole, indicated by arrow. In the adjacent protoplast, the vesicle is releasing its contents. Bottom, some protoplasts showing intense fluorescence in the central vacuole following release of the vesicle contents.

Further work is in progress to prepare vesicles with less stable membranes than those described here, which on fusion with the plasmalemma may release their contents directly into the cytoplasm. The present study suggests that charged vesicles may provide a method of introducing quantifiable amounts of materials into plant protoplasts.

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1. Weissman, G. *Hosp. Pract.* **11,** 49–58 (1976).
2. Gregoriadis, G., *New Engl. J. Med.* **295,** 704–710; 765–770 (1976).
3. Tyrell, D. A., Heath, T. D., Colley, C. M. & Ryman, B. E. *Biochim. biophys. Acta* **457,** 259–302 (1976).
4. Hess, D. in *Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture* (eds Reinert, J. & Bajaj, Y. P. S.) 506–535 (Springer, Berlin, 1977).
5. Takebe, I. *A. Rev. Phytopathol.* **13,** 105–125 (1975).
6. Cassells, A. C. & Barlass, M. *Virology* **87,** 459–462 (1978).
7. Grout, B. W. W. & Coutts, R. H. A. *Plant Sci. Letters* **2,** 397–403 (1974).
8. Cassells, A. C. & Barlass, M. *Physiol. Plant* **42,** 236–242 (1978).
9. Bangham, A. D., Standish, M. M. & Watkins, J. C. *J. molec Biol.* **13,** 238–252 (1965).