glycerol monopalmitate, glycerol monostearate, palmitic, stearic and elaidic acids.

Fusion of avian erythrocytes in Eagle's medium by glycerol mono-oleate is inhibited by serum albumin and by EDTA; Ca^{2+} ions facilitate fusion. Glycerol mono-oleate will also fuse hen erythrocytes with mouse LS fibroblasts. In preliminary experiments on cell hybrids formed from mouse 3T3TK-fibroblasts and wg3IMP⁻ hamster fibroblasts, clones of viable cells occur in HAT medium (Littlefield, 1963) 4–7 times more frequently with glycerol mono-oleate (50 $\mu\text{g}/\text{ml}$) than in untreated cultures. As glycerol mono-oleate damages cells less than lysolecithin, it may be the more useful compound for cell fusion without viruses in somatic genetics.

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Keenan, T. W., Morré, D. J., Olson, D. E., Yungmans, W. N. & Patton, S. (1970). *J. Cell Biol.* **44**, 80.

Littlefield, J. W. (1963). *Proc. natn. Acad. Sci. U.S.A.* **50**, 568.

Poole, A. R., Howell, J. I. & Lucy, J. A. (1970). *Nature, Lond.*, **227**, 810.

A Study of the Fusion of Highly Vacuolated Plant Protoplasts

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Highly vacuolated protoplasts from leaves (Power & Cocking, 1970) or from callus (Chupeau & Morel, 1970) are the preferred starting material for studies on the somatic hybridization of plants by the fusion of protoplasts.

By using a mixture of cell-wall-degrading enzymes (Power & Cocking, 1970) protoplasts were obtained from tobacco leaves and from tomato fruit. Protoplasts were aggregated together by centrifugation (140g for 5min) in 0.9M-sodium nitrate. Induced fusion in tobacco-leaf protoplasts, treated similarly, was observed electron-microscopically to be initiated by plasmalemma contact followed by breakdown of adpressed plasmalemmae over limited areas, resulting in the formation of regions of connecting cytoplasm somewhat reminiscent of virus-induced animal-cell fusion (Schneeberger & Harris, 1966). The presence of large vacuoles in these protoplasts prevents rapid coalescence, and fusion is not readily detectable optically, contrasting with the situation in relatively unvacuolated protoplasts (Power & Cocking, 1971). More extensive coalescence can be induced by further direct pressure, and from a mixture of highly vacuolated

tomato-fruit and tobacco-leaf protoplasts heterokaryon protoplasts were obtained that could be seen optically to contain plastids characteristic of both fruit and leaf.

Early stages in spontaneous fusion of protoplasts (Power, Cummins & Cocking, 1970) have also been studied electron-microscopically both in relatively unvacuolated seedling root tissues and in highly vacuolated leaf tissues. A prerequisite for fusion is the maintenance of contact of adjacent protoplasts via plasmodesmata during the enzyme treatment and the accompanied plasmolysis. As cell-wall degradation proceeds the plasmodesmata appear to expand, drawing the protoplasts together, facilitating cytoplasmic organelle interchange by the formation of regions of connecting cytoplasm: a phenomenon shedding new light on plasmodesmatal-endoplasmic reticulum relationships. Thus it would seem that, although of different origins, induced fusion and spontaneous fusion are both functionally similar.

Multinucleate spontaneously fused protoplasts can regenerate cell walls and divide (Power, Frearson & Cocking, 1971). It would seem possible that the same pattern of cell-wall regeneration, growth and division could hold for heterokaryon protoplasts formed by induced fusion, or by enzymic degradation of the cell-walls of chimaeral cell aggregates formed by the aggregation of protoplasts of different species (Pojnar & Cocking, 1968), since the presence of cytoplasmic connexions might behave in an analogous way to plasmodesmata, enabling spontaneous interspecies fusion to occur by repeating the cell-wall degradation.

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Chupeau, Y. & Morel, G. (1970). *C. r. hebd. Acad. Sci. Paris, Sér. D*, **270**, 2659.

Pojnar, E. & Cocking, E. C. (1968). *Nature, Lond.*, **218**, 289.

Power, J. B. & Cocking, E. C. (1970). *J. exp. Bot.* **21**, 64.

Power, J. B. & Cocking, E. C. (1971). *Sci. Prog.* (in the Press).

Power, J. B., Cummins, S. E. & Cocking, E. C. (1970). *Nature, Lond.*, **225**, 1016.

Power, J. B., Frearson, E. M. & Cocking, E. C. (1971). *Biochem. J.* (in the Press).

Schneeberger, E. E. & Harris, H. (1966). *J. Cell Sci.* **1**, 401.