substrate concentrations at 7.5, 7.0, 5 and 0.7–1.0mM for the sample 1 plasma, sample 2 plasma, normal plasma and erythrocyte cholinesterase respectively. With butyrylthiocholine as substrate, the values are 10, 7.5, and 7.5mM for the three plasma samples respectively. Values of $V_{\max}/2$ for the two 'silent' samples are similar to those for normal plasma, but differ from that for the erythrocyte cholinesterase by about five-fold when acetylthiocholine is the substrate.

With 5mM-acetylthiocholine as substrate the inhibitors 10nM-compound RO2-0683 [dimethylcarbamate of (2-hydroxy-5-phenylbenzyl)trimethylammonium bromide], 0.3mM-sodium fluoride and 10mM-nialamide hydrochloride inhibit both the normal serum cholinesterase and the erythrocyte cholinesterase by 95–98% and 15–19%, by 80–90% and 12–18%, by 45–58% and 30–35% and by 34–40% and 19–25% respectively. On the other hand sample 1 plasma and sample 2 plasma are unaffected by these inhibitors. Compound C51 (1,5 bis[4 allyl-dimethylammonium]-pentan-3-one dibromide) at 5 μ M inhibits the acetylthiocholine-hydrolytic activities of sample 1 plasma, sample 2 plasma, normal plasma and erythrocyte cholinesterase by 28%, by 58%, by 14–20% and by 95–98% respectively. Similarly incubation with 6% butanol inhibits sample 2 plasma cholinesterase by 15% and erythrocyte cholinesterase by 20–30%, whereas sample 1 plasma cholinesterase and normal plasma cholinesterase remain unaffected.

Gel electrophoresis of the two 'silent' plasma on the 7.5% polyacrylamide discs at pH8.6 by the method of Ornstein (1964) and Davies (1964) shows five enzymically active zones when stained with acetylthiocholine with similar mobilities to those present in normal plasma. Erythrocyte cholinesterase preparations contain three bands with different mobilities from those in the plasma samples. A considerable amount of erythrocyte cholinesterase remains at the origin. The enzyme bands of the 'silent' samples show less activity against butyrylthiocholine, and the reverse is true with the normal plasma cholinesterase.

Sample 1 plasma possesses the immunological and electrophoretic properties of normal plasma cholinesterase. On the other hand sample 2 plasma differs from sample 1 plasma (i) in not neutralizing a specific antiserum and (ii) in being inhibited by 6% butanol and by 5 μ M-compound C51.

Although the inhibition value by compound C51 of sample 2 plasma may indicate the presence of an erythrocyte cholinesterase, other inhibition values and electrophoretic properties suggest that the 'silent' cholinesterase variants are mutants of plasma cholinesterase and provide further evidence that they are heterogeneous. Because of the complex properties of the 'silent' samples, the cause

of the heterogeneity must be treated with caution until better characterization of the purified enzyme is possible.

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Cell Fusion without Viruses

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Aqueous dispersions of lysolecithin (lysophosphatidylcholine) can induce cell fusion and the formation of both homokaryons and heterokaryons (Poole, Howell & Lucy, 1970). Electron microscopy has now shown that fusion of avian erythrocytes by lysolecithin involves the establishment of small cytoplasmic bridges between cells. The polykaryocytes subsequently produced disintegrate rapidly owing to generalized membrane damage, but damage can be minimized by using lysolecithin in micro-droplets of glycerol trioleate. Only those regions of membrane in contact with the lipid droplets are then directly affected, and the droplets may be regarded as morphological equivalents of Sendai-virus particles. These experiments may be relevant to membrane fusion in the exocytosis of milk-fat droplets containing lysolecithin (Keenan, Morr, Olson, Yungmans & Patton, 1970).

The following procedure has been devised to determine the ability of defined chemicals to cause cell fusion. Hen erythrocytes in Eagle's medium or buffered saline at pH5.6 containing Dextran 60C (mol.wt. 77500) (80mg/ml) are treated with the test substances at 37°C for up to 2h, and bovine serum albumin is then added. Lysis accompanying cell fusion is thus minimized, except with aqueous lysolecithin, and the polykaryocytes remain intact for 24h. This allows the quantitative measurement of cell fusion by optical and electrical methods. Various degrees of cell fusion are seen with retinol, oleic, linoleic, linolenic, lauric acid glycerol monooleate, glycerol monolaurate and selachyl alcohol, and when Ca^{2+} ions are followed by F^- ions. Fusion apparently does not occur in this procedure with

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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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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