Membrane Fluidity and Membrane Fusion

By J. I. HOWELL, Q. F. AHKONG, F. C. CRAMP, D. FISHER, W. TAMPION and J. A. LUCY (*Department of Biochemistry, Royal Free Hospital School of Medicine, London WC1N 1BP, U.K.*)

Hen erythrocytes fuse to form large unstable syncytia on treatment with lysophosphatidylcholine in solution (Howell & Lucy, 1969; Poole *et al.*, 1970) and more-stable giant cells with lysophosphatidylcholine in micro-droplets of lipid (Ahkong *et al.*, 1972). Long-chain *cis*-unsaturated fatty acids and short-chain saturated acids (and certain derived esters) also induce erythrocytes to fuse into giant cells, but long-chain saturated compounds are inactive (Lucy *et al.*, 1971).

It is noteworthy that the active fatty acids have relatively low melting points and endothermic transition temperatures (Chapman, 1968). Interestingly, chemically induced cell fusion is itself markedly temperature-dependent. Thus treatment of hen erythrocytes (2.3×10^8 cells/ml) with dispersed glycerol mono-oleate (294 nmol/ml) at pH 5.6 or 7.4 in an iso-osmotic salt-cacodylate buffer containing Dextran 60 (80 mg/ml) and glucose (1 mg/ml) gave no fusion within 30 min at 0° and 17°C, but at 27°, 37°, 50° and 60°C extensive fusion began at 23–27, 8–9, 2–3 and less than 1 min respectively.

Lysophosphatidylcholine (Bangham & Horne, 1964) and linoleic acid (Galliard, 1971) cause the concentric lamellar organization of negatively stained phosphatidylcholine liposomes to disintegrate into globular units and to form undulating, apparently highly mobile, lamellae respectively. Similar structures to those observed by Galliard (1971) are reproducibly seen with the electron microscope when egg phosphatidylcholine (lecithin) (1.25 mg/ml) interacts with oleic acid, decanoic acid, glycerol mono-oleate, selachyl alcohol and retinol (all at 1.25 mg/ml), each of which readily causes fusion of hen erythrocytes. These structures are not observed in preparations of the fusing agents alone. Glycerol dioleate, glycerol trioleate, palmitic acid and stearic acid, which do not fuse erythrocytes, have no effect on the organization of intact phosphatidylcholine liposomes.

Spin-label spectra indicate that the addition of oleic acid, but not of stearic acid, to lipid-deficient sarcoplasmic membranes produces a fluid membrane structure that is apparently required for activity of the membrane-bound Ca^{2+} -dependent adenosine triphosphatase (Seelig & Hasselbach, 1971). It is suggested, by analogy, that those fatty acids and other substances that appear to mobilize the structure of liposome membranes may induce erythrocytes to fuse by increasing membrane fluidity.

We thank the Medical Research Council for financial support.

- Ahkong, Q. F., Cramp, F. C., Fisher, D., Howell, J. I. & Lucy, J. A. (1972) *J. Cell Sci.* **10**, in the press
 Bangham, A. D. & Horne, R. W. (1964) *J. Mol. Biol.* **8**, 660
 Chapman, D. (1968) in *Biological Membranes* (Chapman, D., ed.), p. 125, Academic Press, London and New York
 Galliard, T. (1971) *Eur. J. Biochem.* **21**, 90
 Howell, J. I. & Lucy, J. A. (1969) *FEBS Lett.* **4**, 147
 Lucy, J. A., Ahkong, Q. F., Cramp, F. C., Fisher, D. & Howell, J. I. (1971) *Biochem. J.* **124**, 46P
 Poole, A. R., Howell, J. I. & Lucy, J. A. (1970) *Nature (London)* **227**, 810
 Seelig, J. & Hasselbach, W. (1971) *Eur. J. Biochem.* **21**, 17

The Fusion of Hen Erythrocytes by Non-Ionic Surface-Active Adjuvants

By Q. F. AHKONG, F. C. CRAMP, D. FISHER, J. I. HOWELL, W. TAMPION and J. A. LUCY (*Department of Biochemistry, Royal Free Hospital School of Medicine, London WC1N 1BP, U.K.*)

Retinol (Dresser, 1968), lysophosphatidylcholine (Westphall *et al.*, 1970) and DL-sphingosine (Youngner & Axelrod, 1963) are known to have adjuvant properties. Oleic acid (Dresser, 1961) and the esters Arlacel A (Spitznagel & Allison, 1970), Span 20 (Dresser, 1961) and Span 80 (Gall, 1966) also have adjuvant activity. Dresser (1968) and Spitznagel & Allison (1970) have both noted that many adjuvants labilize lysosomal membranes, but it is noteworthy also that retinol, oleic acid and glycerol mono-oleate (Lucy *et al.*, 1971), lysophosphatidylcholine (Poole *et al.*, 1970) and DL-sphingosine sulphate induce cell fusion.

Several commercial preparations of non-ionic surface-active agents have therefore been tested for their ability to fuse chicken erythrocytes at pH 7.4, by using the experimental system reported by Lucy *et al.* (1971). Positive results were obtained with emulsions of the following unsaturated or short-chain saturated fatty acid esters (2 mg/ml): Arlacel A (mannide mono-oleate), Span 20 and 80 (sorbitan monolaurate and mono-oleate), sucrose mono-laurate, ethylene glycol mono-oleate and propylene glycol mono-oleate. Significantly, fusion was not observed with the saturated esters tested, namely Span 40 and 60 (monopalmitate and monostearate esters of sorbitol), nor with the corresponding esters of ethylene glycol (cf. Howell *et al.*, 1972).

The impure samples of Arlacel A, Span 80 and ethylene glycol mono-oleate used have been separated by a combination of ion-exchange and adsorption chromatography into mono-oleate and dioleate fractions. The mono-oleate fractions (0.1 mg/ml), but not the dioleate fractions, induced fusion of