Membrane Fluidity and Membrane Fusion

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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.

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

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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

hen erythrocytes. The mono-oleate fraction from ethylene glycol mono-oleate also produced changes in the structural organization of egg lecithin phosphatidylcholine liposomes resembling those produced by glycerol mono-oleate (cf. Howell *et al.*, 1972).

It seems from these experiments that a relationship may exist between the ability of some substances to alter the organization of the lipids of membranes in such a way as to lead to membrane fusion and their adjuvant activity. The effects of certain adjuvants may be at least partly on the membrane systems of macrophages (Asherson & Allwood, 1969). Conceivably cell fusion *per se* induced by some adjuvants may be important if cytoplasmic connexions between macrophages and lymphocytes (Schoenberg *et al.*, 1964; Clarke *et al.*, 1971) are of functional immunological significance.

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DEMONSTRATION

Analysis of Enzyme Mechanisms and Metabolic Pathways

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Trial-and-error model building is frequently the best method of interpreting experimental results obtained from 'freeze-clamp' metabolite assays or from measurements of enzyme kinetics. Previously this type of work has been the province of specialist computer programmers; however, the present demonstration illustrates a computer model-building system that requires no programming knowledge and that is sufficiently versatile to handle any biochemical process that can be broken down into a sequence of partial reactions.

The model to be tested can be typed into the computer in ordinary chemical notation, for instance:

(where LDH stands for lactate dehydrogenase) and so forth, until the whole model has been entered with the steps in any order and without format restrictions. This section may be very complex with many different

enzymes, substrates and allosteric effectors. There are facilities for entering estimates of rate constants and for providing optional initial values for the concentrations of the intermediates.

The system responds either with a steady-state solution describing the flow of material between the intermediates in the model or with a time-course for the intermediate concentrations if this has been specifically requested or if no steady-state solution exists. This solution can now be compared with the experimental results and the necessary alterations made to the model before another test is made.

When used 'on line' the program is obeyed interactively, the results being typed out as these become available and a chance being given to modify the model while the system is running. The necessary differential equations are set up by the computer and solved automatically. The system chooses between matrix inversion for simple cases and numerical integration for non-linear or time-varying problems.

During the demonstration there will be opportunities for visitors to run problems of their own choosing. Please contact the author if you wish to save time by typing in large models before the demonstration. The maximum size for enzyme mechanisms is well over 100 intermediates; however, there is little point in testing such complex models without comprehensive experimental results. Large metabolic models can also be accommodated; however, for demonstration purposes such problems should not contain more than 20 different intermediate names.