CELL FUSION INDUCED BY LYSOLECITHIN

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1. Introduction

It has been proposed that under appropriate circumstances some of the lipids of biological membranes may be organised in globular micelles that are in dynamic equilibrium with the bimolecular leaflet structure [1,2]; it has also been suggested that a primary requirement for the fusion of two membranes is that both of the membranes involved have a relatively high proportion of their phospholipid molecules in the micellar configuration [3]. On this hypothesis any situation, no matter how it is produced, which favours the micellar arrangement should tend to facilitate membrane fusion. As a consequence of its wedge shape, lysolecithin causes bilayers of lecithin to break down into globular micelles [4,5]. The presence of lysolecithin in membranes would therefore be expected to lead to a high proportion of membrane lipids being organised in globular micelles, and to facilitate membrane fusion [3]. The present experiments have been undertaken to see if treatment of erythrocytes with exogenous lysolecithin would result in the fusion of plasma membranes and the formation of polykaryocytes or giant cells.

2. Materials and methods

Blood from the brachial veins of adult hens was collected in a citrate anticoagulant [6]. The cells were washed twice with anticoagulant, twice with saline (0.15 M NaCl) and resuspended in saline. The lysolecithin used was a gift from Dr. G.R.Webster; it was chromatographically pure (fatty acid composition: 67.2% palmitic, 32.8% stearic). For lysolecithin treatment, cells were suspended (approximately 4×10^8 cells/ml) in a solution made from equal volumes of sodium chloride solution (0.15 M) and sodium acetate buffer (0.15 M, pH 5.6). The cell suspension (1 ml) was brought to 37°C, added to a lysolecithin solution (0.7 ml; from 0.2 to 0.8 mg/ml in saline), and incubated at 37°C for 30 sec. Cells were fixed in 2.5% glutaraldehyde [7], followed by osmium tetroxide containing ruthenium red [8]; the blocks were stained with uranyl acetate [9] and with lead citrate when necessary [10]. Fixed cells were embedded in Araldite [11] and studied with an A.E.I. EM6b electron microscope.

3. Results

Hen erythrocytes treated with lysolecithin are rapidly lysed and their haemoglobin is released. The ghosts produced behave as if their surfaces are sticky and, under the phase-contrast microscope, ghosts can clearly be seen to adhere to one another.

When washed hen erythrocytes are treated at 37°C with lysolecithin (pH 5.6), some ghosts that are formed fuse together to form large syncytia; sections of these syncytia have been seen to contain up to about 50 nuclei. This phenomenon has not been seen with erythrocytes incubated at 37°C in buffer free from lysolecithin. Fig. 1 a illustrates the appearance of a part of a polykaryocyte formed in the presence of lysolecithin. The cytoplasm between the nuclei may contain some residual haemoglobin. Convoluted membraneous material is also present (arrows figs. 1a,b), which is thought to be derived from plasma membrane that became superfluous when the individual erythrocytes

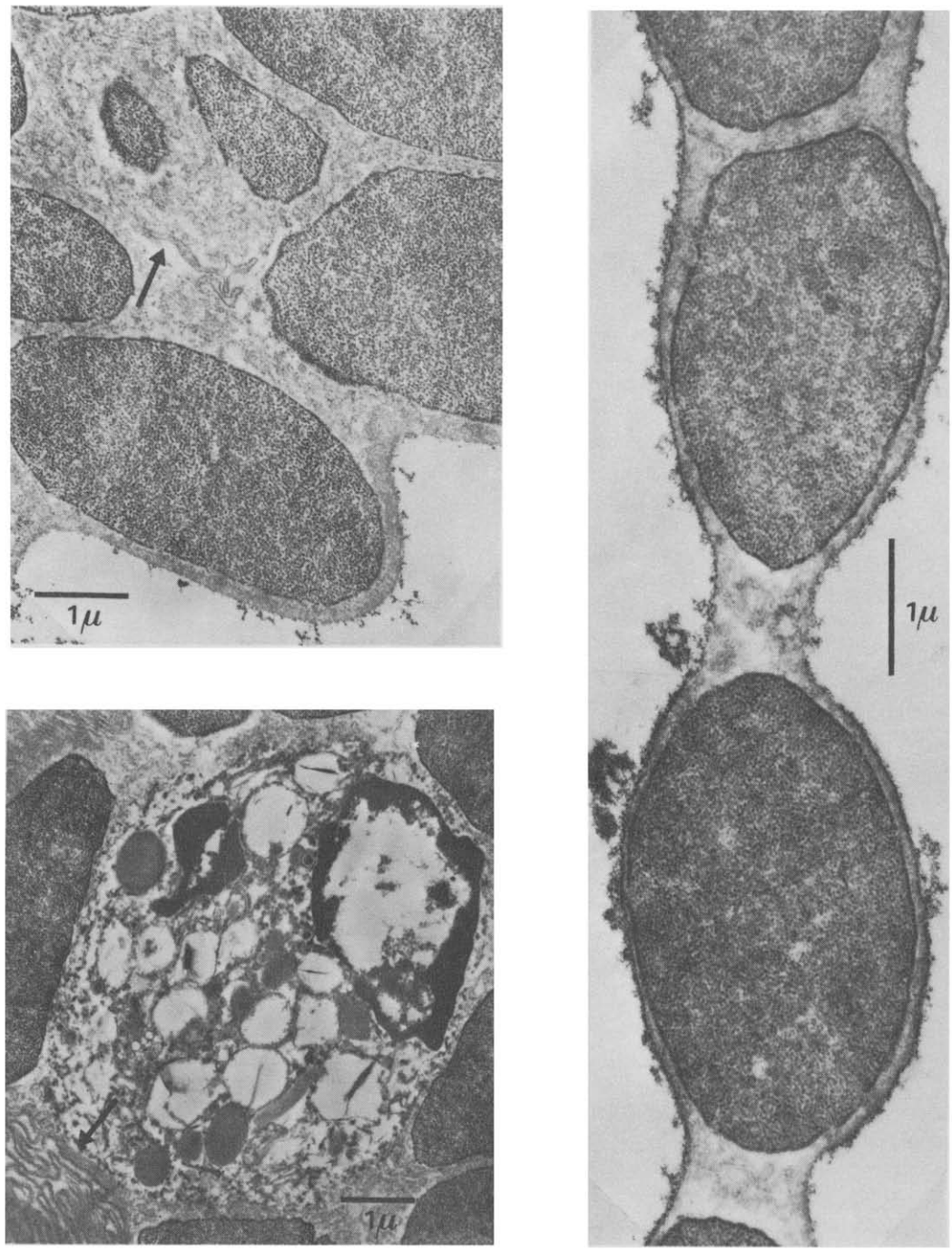

Fig. 1. Electron micrographs of thin sections of fused avian blood cells.

fused together. Where a giant cell is only one nucleus wide at its extremity, the chain of cells observed provides a clear indication of the occurrence of cell fusion (fig. 1c).

The formation of syncytia can be very rapid, and those shown here were produced within 30 sec of bringing the erythrocytes in contact with lysolecithin (0.47 mg/ml). However, with this final concentration of lysolecithin, the polykaryocytes appear to disintegrate quite quickly as a result of membrane damage. Experiments are being undertaken to investigate the sequence of morphological changes that is involved when fusion occurs more slowly; it seems from preliminary findings that the process is initiated by the formation of small cytoplasmic bridges as with cell fusion that is induced by inactivated Sendai virus [12]. Since white cells have been observed within the syncytia (fig. 1b), it is apparent that the action of lysolecithin, like that of Sendai virus, is not restricted to erythrocyte membranes. A limited number of giant cells have been observed by phase contrast and electron microscopy in which nuclear fusion has evidently also taken place subsequent to the fusion of two or three individual erythrocyte ghosts.

4. Discussion

Although fusion of lipoprotein membranes is of great importance in cell biology, relatively little is known about the molecular mechanisms that underlie this process. The present report appears to be the first in which the fusion of biological membranes has been induced by a simple chemical substance that is known to have a direct effect on the integrity of lipoprotein membranes.

Most of the information that is available on membrane fusion has been derived from the fusion of cells that is induced by viruses: this process has stimulated interest in the mechanisms of fusion since the hybrid cells obtained are invaluable for investigations on human genetics and on differentiation [13,14]. Fusion induced by lysolecithin, if it can occur without drastic cytotoxic effects, may possibly be developed to provide a controllable technique for producing cell hybrids without using viruses.

There are interesting similarities between our observations and those made on virus-induced fusion of erythrocytes [12]. For example, in the virus-induced fusion of hen erythrocytes with HeLa cells, haemolysis was complete or nearly so before fusion took place. It does not necessarily follow, however, that lysolecithin is actually involved in the mechanisms by which biological membranes fuse in response to viruses or to any other agent. It has indeed recently been concluded from analytical studies that lysolecithin is not an important factor in the fusion of hamster kidney cells induced by a para-influenza virus; no cell fusion was seen when these cells were treated with exogenous lysolecithin at a physiological pH [15].

The possibility that viral enzymes are involved in the virus-induced fusion of cells has frequently been considered. The ability of lysolecithin to cause cell fusion would, however, appear to be consistent with the thesis [16] that lysosomal enzymes are concerned in the formation of syncytia by viruses or by other means, in view of the fact that lysosomes contain phospholipase A [17,18]. Our findings also favour the proposals that the formation or liberation of lysolecithin may precede the secretion of catecholamines from chromaffin cells [19], and that lysolecithin may be involved in the fusion of primary lysosomes with digestive vacuoles and with the plasma membrane [3].

In conclusion, the observations reported here are thought to support the possibility that the formation of micelles of lipid or lipoprotein within membranes, under the influence of a variety of appropriate agents, may be a general mechanism for the fusion of biological membranes.

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