it consisted of a double spot. Under these latter conditions, the ceramides from bovine brain sphingomyelin migrated as a single spot.

The virus and the milk-fat-globule membrane, however, significantly differed in their molecular species of phosphatidylcholines. Indeed, whereas the phosphatidylcholines from the milk-fat-globule membrane did not contain more than 50% of saturated fatty acids, up to 70% of the fatty acids from the virus phosphatidylcholines were saturated, implying that the virus contained some disaturated species of phosphatidylcholine. The phosphatidylcholines from the virus were submitted to hydrolysis by phospholipase A2 (EC 3.1.1.4) from Crotalus adamanteus venom by Lands & Merkl (1963) to study the distribution of the various fatty acids between the 1- and 2-positions. The monoenoic and polyenoic fatty acids were entirely recovered at the 2-position, thereby suggesting that the positional specificity was not abolished during the infection of the mammary cell. However, the presence of fully saturated phosphatidylcholines in the viral envelope may reflect some metabolic disturbances occurring during the neoplastic transformation of the infected cells.

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Chemically Induced Cell Fusion in vitro of Erythrocytes from Patients with Liver Diseases

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The increased cell diameter and osmotic resistance of erythrocytes from patients with hepatobiliary diseases is associated with an increased content of cholesterol, and to a lesser extent an increase in phosphatidylcholine, of the cells (Cooper & Jandl, 1968; Werre et al., 1970). It has been demonstrated by freeze-etch electron microscopy that the lipid-rich abnormal lipoprotein LP-X found in cholestasis can fuse with erythrocyte membranes (Verkleij et al., 1976). Intercellular fusion of hen erythrocytes can be induced by chemical fusogens such as glycerol mono-oleate (Ahkong et al., 1973). We have shown that enrichment of hen erythrocytes with cholesterol from liposomes strikingly increases their susceptibility to fusion by chemical fusogens and by Sendai virus (M. J. Hope, K. R. Bruckdorfer & J. A. Lucy, unpublished work). In the present study, erythrocytes of abnormal lipid composition obtained from patients with a variety of hepatobiliary disorders were compared with cells from healthy control subjects with respect to fusion induced by glycerol mono-oleate.

Venous-blood samples, all taken on the same day, were obtained from six patients with either alcoholic cirrhosis, acute drug-induced hepatitis, extrahepatic cholestasis, primary biliary or cryptogenic cirrhosis, and from four controls. Each sample of packed erythrocytes, which was washed three times in a citrate anti-coagulant solution (De Gowin et al., 1949), was divided for extraction and analysis of lipids, and counting of cell numbers with a Coulter counter. An evaluation of fusion in triplicate after incubation with ultrasonically dispersed glycerol mono-oleate (200 mM) in a modified Eagle's medium at 37°C was made as described previously (Ahkong et al., 1973). The percentage polykaryocytosis (number of cells involved in fusion expressed as a percentage of the total cell number) was determined by observation with a Zeiss standard WL phase-contrast
Table 1. Lipid content and percentage polykaryocytosis induced by glycerol mono-oleate in erythrocytes from patients with liver diseases and from healthy subjects

Samples of blood from patients and control subjects were taken, and the erythrocytes washed three times in a citrate anti-coagulant solution (DeGowin et al., 1949). Packed erythrocytes were counted with a Coulter counter and analysed for cholesterol and individual phospholipids. Fusion was evaluated in triplicate after incubation with ultrasonically dispersed glycerol mono-oleate (200mm) at 37°C as previously described (Ahkong et al., 1973). Percentage polykaryocytosis was calculated from the number of cells involved in fusion expressed as a percentage of the total cell number after 20min incubation with glycerol mono-oleate, followed by fixation with 5% (v/v) glutaraldehyde. All results are expressed as means ± s.d.; statistical differences were determined by Student's t test. The correlation coefficients (r) shown were derived from data obtained from all subjects (patients and control subjects).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Cholesterol Content (fmol/cell)</th>
<th>Total phospholipid</th>
<th>Cholesterol/phospholipid ratio</th>
<th>Phosphatidylcholine</th>
<th>Phosphatidylserine</th>
<th>Percentage polykaryocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 4)</td>
<td>3.18 ± 0.22*</td>
<td>3.81 ± 0.06</td>
<td>0.84 ± 0.05</td>
<td>1.13 ± 0.02</td>
<td>0.48 ± 0.08</td>
<td>7.7 ± 1.9</td>
</tr>
<tr>
<td>Patients (n = 6)</td>
<td>4.15 ± 0.70*</td>
<td>3.84 ± 0.51</td>
<td>1.12 ± 0.08*</td>
<td>1.30 ± 0.30</td>
<td>0.58 ± 0.13</td>
<td>12.4 ± 4.6*</td>
</tr>
<tr>
<td>Correlation coefficient r (lipid against percentage polykaryocytosis)</td>
<td>0.83</td>
<td>0.50</td>
<td>0.66</td>
<td>0.81</td>
<td>0.71</td>
<td>—</td>
</tr>
</tbody>
</table>

*Statistically different from control values (P < 0.05).
microscope of cells fixed in 5% (v/v) glutaraldehyde after 20 min incubation with glycerol mono-oleate. Fused cells were discernible by their size; the number of cells involved in fusion was calculated as a percentage of the total cell number in 10–15 fields for each incubation.

Although considerable individual variation occurred, statistically significant increases in cell cholesterol concentration and cholesterol/phospholipid molar ratios were found in the cells from patients with liver diseases (Table 1). The erythrocytes of some patients also exhibited increased concentrations of phosphatidylcholine and phosphatidylserine, but not of other phospholipid fractions, and there was no difference in the mean values for total phospholipid in each group. Glycerol mono-oleate-induced cell fusion was more extensive in the erythrocytes of the patients with liver diseases. Taking data from all subjects studied (patients and control subjects), there was a positive correlation between percentage polykaryocytosis and cholesterol content or cholesterol/phospholipid ratio, but not with the total concentration of phospholipid. A correlation was observed between cell fusion and the cellular concentration of phosphatidylcholine or phosphatidylserine, but not with other phospholipids.

From these observations it appears that increases in membrane lipids facilitate cell fusion. A mechanism for fusion has been proposed in which the intercalated membrane particles are excluded from the regions of membrane involved in fusion (Ahkong et al., 1975). Enrichment of bovine erythrocytes with cholesterol decreases the density of intramembranous particles (Deuticke & Ruska, 1976) and this has also been demonstrated in cells from patients with severe cholestasis (Verkleij et al., 1976). Furthermore, in erythrocytes from these patients, fusion of lipoprotein LP-X coincides with an increase in smooth areas in the fracture faces of the membrane. We therefore suggest that increases in membrane lipid concentrations, especially cholesterol, may facilitate phase separations of protein-free areas in the lipid bilayer, permitting fusion to occur more readily.

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Some Properties of Liposomes of Different Sizes

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Liposomes are unilamellar or concentric multilamellar bilayered phospholipid vesicles. They have been proposed as carriers of therapeutic agents in the treatment of enzyme-deficiency states and also in cancer chemotherapy, and their applications have been extensively reviewed (Tyrrell et al., 1976). One problem inherent in such an approach is the direction of liposomes to specific target tissues. The fate of intravenously injected liposomes of various lipid compositions has been documented (Jonah et al., 1975), as well as the blood clearance of unilamellar and multilamellar liposomes (Juliano & Stamp, 1975). In the present study we have investigated the fate in vivo of intravenously injected small unilamellar (25 nm diameter) and large multilamellar liposomes (up to 1 μm