Decreased erythrocyte membrane fluidity and altered lipid composition in human liver disease

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Abstract Abnormal plasma lipoproteins in patients with liver disease are associated with characteristic changes in erythrocyte membrane lipid composition. The membranes are enriched in cholesterol and phosphatidylcholine and both the cholesterol/phospholipid and phosphatidylcholine/sphingomyelin molar ratios are increased. Phospholipid fatty acid composition is also abnormal; the proportions of arachidonic acid and stearic acid are decreased and that of palmitic acid raised. In this study we have examined the effects of these membrane lipid abnormalities on membrane fluidity. Erythrocyte membrane fluidity was assessed in 30 patients with a variety of liver diseases and in 25 normal subjects using the hydrophobic, fluorescent probe 1,6-diphenylhexa-1,3,5-triene and the values were related to their lipid composition. Membrane fluidity was significantly decreased in the patient erythrocytes (lipid order parameter, $S_v[37°C] = 0.713 \pm 0.018$, mean ± S.D. compared to 0.686 ± 0.008 in the normal subjects, $P < 0.001$) and correlated significantly with the cholesterol/phospholipid ratio ($r = 0.88$, $P < 0.001$). The fluidity of lipid extracts from the membranes of patient erythrocytes was also decreased, suggesting that decreased membrane fluidity was mainly a consequence of altered lipid composition rather than protein abnormalities. Incubation of patient erythrocytes for 20 hr with normal, heated plasma removed the excess cholesterol without affecting the phosphatidylcholine/sphingomyelin ratio or phospholipid fatty acid composition; following incubation the fluidity of these membranes was similar to that of normal membranes. We conclude that in liver disease changes in the composition of the phospholipid bilayer matrix in the erythrocyte membrane have little influence on its fluidity; the reduced fluidity is predominantly a result of increases in cholesterol relative to phospholipid.—**Owen, J. S., K. R. Bruckdorfer, R. C. Day, and N. McIntyre.** Decreased erythrocyte membrane fluidity and altered lipid composition in human liver disease. *J. Lipid Res.* 1982. **23:** 124–132.

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Cell plasma membranes are thought to consist of a fluid lipid bilayer with which various proteins are associated (1). The proteins may be attached to the membrane surface (the peripheral proteins) or be embedded in or span the bilayer (the integral proteins). These proteins act as receptors or are responsible for transport or enzymatic processes in the cell membrane. There is evidence that such membrane protein functions may be influenced by the properties of the lipid bilayer matrix, including its fluidity. For example, changes in fluidity may affect the lateral mobility and clustering of proteins, their vertical orientation or their conformation (2–4).

The fluidity of biological membranes is mainly determined by their lipid composition. Cholesterol plays a key role since it appears to maintain the bilayer matrix in an "intermediate fluid state" (5) by regulating mobility of phospholipid fatty acyl chains. An increase in the amount of cholesterol relative to phospholipid has been shown by a variety of physico-chemical techniques to decrease fluidity in both biological and artificial membranes (6). The cholesterol/phospholipid molar ratio is not the only determinant of membrane fluidity; the phospholipid composition (7–9) and the length and degree of unsaturation of the phospholipid fatty acyl chains affect fluidity (6, 7). Lipid-protein interactions within a biological membrane may also modify fluidity and so both the lipid/protein ratio and types of protein present may be important (10, 11).

In patients with liver disease, abnormalities in the composition of the plasma lipoproteins (12) are associated with corresponding changes in the erythrocyte membrane lipid composition. The membranes are enriched in cholesterol and phosphatidylcholine and both the cholesterol/phospholipid and phosphatidylcholine/sphingomyelin molar ratios are raised (13, 14). These changes in membrane lipid composition can affect the structure and properties of erythrocytes; their shape and deformability is abnormal (13) and they have a decreased permeability to sodium (14).

Cholesterol-rich erythrocytes may be prepared by incubation with cholesterol-rich phospholipid dispersions (15). The resulting cells have several abnormal properties (16) including decreased membrane fluidity (17). In contrast, an increase in phosphatidylcholine/sphingomyelin ratio is associated with increased erythrocyte membrane fluidity (8, 9). In the present study, we have

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examined the effects of increased cholesterol content and abnormal phospholipid composition on the fluidity of erythrocyte membranes in patients with liver disease. Erythrocyte membrane fluidity in patients was assessed by means of a hydrophobic fluorescent probe and compared to that of normal subjects; the values were then related to the lipid content of the membrane. In addition, the fluidity measurements were repeated in patient erythrocytes that had been incubated with normal, heated plasma to remove their excess cholesterol whilst leaving their phospholipid composition unchanged.

MATERIALS AND METHODS

Patients

Thirty in-patients with liver disease of varying severity were studied. The diagnosis was established in various ways including liver biopsy, cholangiography, or surgery. Eight patients had obstructive jaundice (six, intrahepatic; two, extrahepatic), twenty had non-fulminant parenchymal liver disease (eight, alcoholic cirrhosis; one, alcoholic hepatitis; seven, chronic active hepatitis; three, cryptogenic cirrhosis; one, drug-induced hepatitis) and in two there was both biliary obstruction and parenchymal dysfunction (cholestatic viral hepatitis). Many of the patients had target and spur cells on stained peripheral blood smears (13). No patient had severe reticulocytosis and only three had reticulocyte counts greater than 2% (3.5, 5.0, and 5.5%). Two siblings with abetalipoproteinemia were also studied (M.J. and S.J., documented in (18)). The comparison subjects were healthy medical and laboratory staff. Informed, verbal consent was obtained from all patients prior to blood withdrawal.

Reagents

Tetrahydrofuran and 1,6-diphenylhexa-1,3,5-triene were obtained from the Aldrich Chemical Company. Hanks' balanced salt solution and penicillin were from Flow Laboratories, and adenine, inosine, and bovine serum albumin were from the Sigma Chemical Company. Taurocholate and glycocholate (sodium salts, A grade) were bought from Calbiochem Ltd., Bishops Stortford, Herts, U.K. Silica gel was purchased from E. Merck AG and all solvents were redistilled before use. Plasma was isolated from out-dated AB-positive blood, heated at 56°C for 30 min, and clarified by centrifugation.

Incubation procedures

Venous blood was obtained from the subjects and mixed with an anticoagulant (1 mg/ml disodium EDTA). Erythrocytes were separated by centrifugation at 4°C, the plasma and buffy coat were removed, and the cells were washed three times with Hanks' solution. To remove excess cholesterol from patient erythrocytes, one portion of the cells was suspended at a hematocrit of about 10% in a mixture of Hanks' solution and heated plasma from AB-positive blood (1:3, v/v) containing pencillin (500 units/ml). The suspension was rotated continuously ("Rolamix", Luckham Ltd., Sussex, U.K.) at a constant temperature of 37°C for 6 hr, after which the medium was changed and the incubation continued for a further 14 hr. Erythrocytes from normal subjects and a further portion from patients were similarly incubated for 20 hr but with the heated plasma from AB-positive blood replaced by heated, autologous plasma. Adenine (final concentration, 2 mM) and inosine (10 mM) were added during the final hour of incubation.

Preparation of erythrocyte membranes

After the incubation period, the erythrocytes were washed three times with isotonic Tris–HCl, pH 7.6, and membranes were prepared by osmotic lysis as described by Hanahan and Ekholm (19). The membranes were suspended in an equal volume of 20 mOsM Tris–HCl, pH 7.6, and kept overnight on ice. Their protein content was estimated by the method of Lowry et al. (20) using bovine serum albumin as standard.

Erythrocyte membrane lipid analysis

Lipids were extracted from a portion of the erythrocyte membrane suspension with isopropanol and chloroform (21) and aliquots were taken for cholesterol (22) and phospholipid estimations (23) which were carried out in duplicate. Erythrocyte membrane phospholipids were separated on silica gel H (Merck) using two-dimensional thin-layer chromatography with chloroform–methanol–aqueous ammonia 65:35:5 (by vol) as the first solvent and chloroform–acetone–methanol–acetic acid–water 50:20:10:10:5 (by vol) as the second (24, 25). The fractions were located with iodine vapor and scraped from the plate, and the phospholipids were measured as inorganic phosphorus after digestion with H_2SO_4 (23). Phospholipid fatty acid composition was measured by gas–liquid chromatography. A portion of the lipid extract was transmethylated by heating at 70°C for 3 hr under N_2 in 5% (v/v) H_2SO_4 in dry methanol. The fatty acid methyl esters were separated at 175°C on a 150 cm column of 10% EGSS-X on Gas-Chrom P, 100/120 mesh; detection was by flame ionization. In preliminary experiments it was established that incubation of patient erythrocytes with heated plasma from AB-positive blood to remove excess cholesterol did not significantly change either membrane phospholipid pattern or membrane phospholipid fatty acid composition. This finding was consistent with the observation of other workers (15) and

therefore these phospholipid analyses were routinely carried out only in membranes prepared from those erythrocytes incubated with autologous plasma.

Preparation of liposomes from erythrocyte membrane lipids

In four patients and in four normal subjects, a portion of the total lipid extract from their erythrocyte membranes, each containing 0.7 mg of phospholipid, was evaporated to dryness under a stream of N_2. Ten ml of 20 mOsM Tris-HCl, pH 7.6, was added and a dispersion of the lipid extract was prepared under N_2 by sonication with an external probe for two periods of 1 min at 20°C (Rapidis Ultrasonic Bath, Model 180 at maximum setting). No lipid residue was apparent and the entire liposome preparation was immediately labeled with diphenylhexatriene for fluorescence polarization measurements.

Fluorescence polarization measurements

Labeling of erythrocyte membranes and liposomes. The hydrophobic, fluorescent probe, diphenylhexatriene, was used to label erythrocyte membranes and liposomes (7). The diphenylhexatriene was stored in tetrahydrofuran at a concentration of 2 mM and immediately before use it was diluted 2000-fold by injection into vigorously stirred 20 mOsM Tris–HCl, pH 7.6. The colloidal solution obtained was sonicated for 20 min and then mixed with an equal volume of erythrocyte membranes (final concentration 50 μg protein/ml) or liposomes (final concentration 35 μg phospholipid/ml). The mixtures were incubated at 37°C for 1 h to incorporate the diphenylhexatriene into the lipid matrix.

Fluorescence polarization. Steady-state measurements of the degree of fluorescence polarization (P) were made at 25°C and 37°C in triplicate or quadruplicate using the Elscint MV-1a microviscosimeter which directly records the polarization ratio $P = \frac{Ix - Iy}{Ix + Iy}$, where Ix and Iy are the intensities of the polarized light emitted in parallel and perpendicular, respectively, to the incident beam. The steady-state fluorescence anisotropy, r_s is given by the relation

$$r_s = \frac{Ix - Iy}{Ix + 2Iy} = \frac{2P}{3 - P}$$

Following the extensive studies of Shinitzky and Barenholz (26), r_s has been related to the microviscosity of the membrane by applying the Perrin equation for rotational depolarization (reviewed in (26)). The basis of these calculations is that the isotropy and freedom of the diphenylhexatriene depolarizing rotations in the membrane lipid bilayer are identical to those in an isotropic reference oil. Recent time-resolved fluorescence anisotropy measurements of diphenylhexatriene in pure phospholipid liposomes (27–29), in cholesterol-containing liposomes (30, 31), and in cell membranes (32, 33) suggest that this central assumption, upon which membrane microviscosity measurements are calculated, may be incorrect; the depolarizing rotations of diphenylhexatriene are anisotropic and so r_s depends on the structural order within the membranes as well as kinetic properties such as microviscosity. Recognizing this, Jähnig (10) has sought to improve the usefulness of r_s measurement by relating it to the lipid order parameter S_v, where v indicates the mean position of diphenylhexatriene along the fatty acid acyl chains, through the equation $r_\infty = 2/5\ S_v^2$ where r_∞, a non-zero limiting fluorescence anisotropy when the probe is immobilized, is given by $r_\infty = 9/8\ r_s - 1/20$.

Although these relationships have been successfully applied to phospholipid and cholesterol-containing liposomes, it has not yet been established whether they will hold for all membranes. In this study we have therefore presented both P and S_v values as indices of membrane fluidity, an increase in P or S_v signifying a reduction in fluidity.

Statistics

All results are expressed as means ± S.D.; statistical differences were determined by Student's *t* test and the correlation coefficients by linear regression.

RESULTS

Erythrocyte membrane lipids

A comparison of erythrocyte membrane cholesterol and phospholipid contents between patients with liver disease and normal subjects is shown in **Table 1.** Cholesterol concentration and mean cholesterol/phospholipid molar ratio were significantly increased ($P < 0.001$) in erythrocyte membranes from patients. The phospholipid concentration of the patient erythrocyte membranes was normal but its composition was changed, as shown previously for whole erythrocytes (13, 14, 24). The phosphatidylcholine fraction and the phosphatidylcholine/sphingomyelin molar ratio were increased in the patient membranes, while the proportions of phosphatidylethanolamine and sphingomyelin were reduced (Table 1). Erythrocyte membrane phospholipid fatty acid composition was also abnormal in the patients; the proportion of palmitic acid was significantly increased and that of stearic acid and arachidonic acid significantly decreased (**Table 2**). Incubation of patient erythrocytes with heated normal plasma removed excess cholesterol leaving a membrane with a normal cholesterol/phospholipid ratio

TABLE 1. Lipid composition of erythrocyte membranes from patients with liver disease and from normal subjects

Erythrocyte Membrane Source	Cholesterol	Phospholipid	Cholesterol/ Phospholipid	PC/SM[c]	% Total Lipid Phosphorus					
					LPC	SM	PI	PS	PC	PE	PA
	nmol/mg protein		*mol/mol*	*mol/mol*	*mol %*						
Normals (25)[a]	587 ± 82	691 ± 94	0.85 ± 0.04	1.11 ± 0.10	1.0 ± 0.4	26.1 ± 1.3	0.7 ± 0.4	13.8 ± 1.1	29.2 ± 1.4	27.2 ± 1.1	2.1 ± 0.7
Patients (30)[a]	725 ± 119[e]	698 ± 107	1.05 ± 0.14[e]	1.84 ± 0.59[e]	1.0 ± 0.6	22.6 ± 3.2[e]	0.9 ± 0.7	12.6 ± 2.0[d]	39.6 ± 6.0[e]	21.1 ± 2.9[e]	2.6 ± 1.0
Patients (30)[b]	629 ± 106	738 ± 108	0.84 ± 0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Erythrocytes were incubated for 20 hr in heated, autologous plasma as described in Materials and Methods. Results are expressed as means ± S.D. for the number of subjects given in parentheses; n.d., not determined.
[b] Erythrocytes were incubated in heated normal plasma.
[c] LPC, lysophosphatidylcholine; SM, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid.
[d] $P < 0.05$.
[e] $P < 0.001$.

TABLE 2. Phospholipid fatty acid composition of erythrocyte membranes from patients with liver disease and from normal subjects[a]

Fatty Acid	Normals (25)	Patients (30)
16:0	27.5 ± 3.6	34.1 ± 4.6[b]
16:1	0.8 ± 0.6	2.2 ± 1.1[b]
18:0	25.8 ± 3.1	20.2 ± 3.9[b]
18:1	16.1 ± 2.7	17.0 ± 3.1
18:2	10.5 ± 1.8	10.4 ± 2.8
20:3	1.7 ± 1.0	1.8 ± 1.3
20:4	17.6 ± 3.0	14.4 ± 3.1[b]

[a] Results are expressed in mol percent of the major fatty acids with retention times up to and including arachidonic acid. The values given are means ± S.D. for the number of subjects shown in parentheses.
[b] $P < 0.001$.

(Table 1) but, as discussed in the Materials and Methods section, an abnormal phospholipid composition.

Erythryocyte membrane fluidity

The fluidity in erythrocyte membranes from patients with liver disease was significantly lower (i.e., P and S_v were increased), $P < 0.001$, than in those from normal subjects whether measured at 37°C or 25°C (**Table 3**). When lipids were extracted from erythrocyte membranes of four patients in which there were marked decreases in membrane fluidity (S_v [37°C] = 0.731 ± 0.010 compared to 0.688 ± 0.008 in four normal membranes, $P < 0.001$), their fluidity was also significantly reduced (S_v [37°C] = 0.730 ± 0.013 compared to 0.683 ± 0.007 in lipid extracts from the four normal subjects, $P < 0.001$). Removal of excess cholesterol from the patient erythrocytes increased membrane fluidity, but at 37°C it was still significantly lower than in normal membranes; at 25°C it was essentially equal to that of the normal membranes (Table 3).

No individual membrane fluidity value found in modified patient erythrocytes was above (i.e., an S_v value lower than) the normal range. The results suggest that the increased cholesterol content of patient erythrocytes is the main determinant of the decrease in membrane fluidity. Support for this is evident in **Table 4,** which shows that the highest correlation coefficient between membrane fluidity and membrane lipid composition is obtained for the cholesterol/phospholipid ratio ($r = 0.88$, $P < 0.001$), and in **Fig. 1,** which shows a close relationship between the two. Weak correlations existed between the phospholipid fatty acid composition and membrane fluidity (Table 4), but there was no significant correlation between the phosphatidylcholine/sphingomyelin ratio and membrane fluidity ($r = 0.04$), even when excess cholesterol was removed ($r = -0.20$).

This was surprising; in erythrocytes from patients with abetalipoproteinemia the phosphatidylcholine/sphingomyelin ratio is decreased and Cooper, Durocher,

TABLE 3. Fluidity of erythrocyte membranes from patients with liver disease and from normal subjects

Erythrocyte Membrane Source	P		S_v	
	37°C	25°C	37°C	25°C
Normals (25)[a]	0.287 ± 0.005	0.327 ± 0.005	0.686 ± 0.008	0.750 ± 0.008
Patients (30)[a]	0.304 ± 0.011[c]	0.335 ± 0.008[c]	0.713 ± 0.018[c]	0.762 ± 0.012[c]
Patients (30)[b]	0.292 ± 0.006[d]	0.326 ± 0.005	0.693 ± 0.010[d]	0.749 ± 0.009

[a] Erythrocytes were incubated for 20 h, in heated, autologous plasma as described in Materials and Methods. Membrane fluidity is expressed as the degree of fluorescence polarization (P) or the lipid order parameter (S_v) and results are given as means ± S.D. for the number of subjects shown in parentheses.
[b] Erythrocytes were incubated in heated normal plasma.
[c] $P < 0.001$.
[d] $P < 0.01$.

and Leslie (9) considered it the major factor responsible for the reduced fluidity of their erythrocyte membranes. Patients with abetalipoproteinemia usually have a small (about 10%) increase in erythrocyte cholesterol/phospholipid ratio. To confirm the conclusions of Cooper et al. (9), we removed the excess cholesterol from erythrocytes of two patients with abetalipoproteinemia and subsequently measured the fluidity of their membranes. In agreement with the results from our patients with liver disease, removal of excess cholesterol increased the fluidity compared to the original membranes. However, the fluidities were still lower than the normal range (i.e., an increased S_v, **Table 5**).

Effect of bile salts on erythrocyte membrane fluidity

The influence of the taurine and glycine conjugates of cholic acid on the fluidity of erythrocyte membranes from a normal subject and from a patient with liver disease is shown in **Table 6.** Addition of either bile salt to normal membranes had no effect on their fluidity even at concentrations as high as 1 mM. In patient membranes the effect was minor at 0.1 mM and 0.2 mM. but at higher concentrations there was clear evidence of an increase in fluidity.

TABLE 4. Correlation coefficients between erythrocyte membrane fluidity and lipid composition in human liver disease[a]

Lipid	P	S_v
Cholesterol	0.25	0.25
Cholesterol/phospholipid	0.89[c]	0.88[c]
Phosphatidylcholine/sphingomyelin	0.03	0.04
% 16:0	0.42[b]	0.41[b]
% 18:0	0.14	0.14
% 20:4	−0.33	−0.33

[a] Membrane fluidity at 37°C is expressed as the degree of fluorescence polarization (P) or lipid order parameter (S_v).
[b] $P < 0.05$.
[c] $P < 0.001$.

DISCUSSION

In the present study, the mean fluidity of erythrocyte membranes from 30 patients with various liver diseases was significantly decreased compared to that of 25 normal subjects. The reduction in fluidity was a consequence of altered lipid composition; it appeared to be due to an increased membrane cholesterol/phospholipid ratio. This conclusion is consistent with the results of Vanderkooi et al. (34), who studied two patients with alcoholic cirrhosis, and of Kutchai, Cooper, and Forster (35), who studied one patient with spur cell anemia associated with liver disease. Surprisingly, the increased phosphatidylcholine/sphingomyelin ratio in our patients had little influence on the change in fluidity and we have tentatively concluded that its effect was balanced by a decreased content of unsaturated fatty acyl chains in the membrane.

While the fluid nature of biological membranes is well recognized, the term "membrane fluidity" is ambiguous; it refers to several aspects of the dynamic structure of membranes including a variety of molecular motions of both the lipid and protein constituents (25, 33, 36). The structural and dynamic properties of the membrane lipid matrix have been extensively studied using such techniques as nuclear magnetic resonance, electron spin resonance, and fluorescence polarization (25, 33). Of these, fluorescence polarization is the most convenient as a routine method and is widely used in conjunction with the extrinsic, hydrophobic probe diphenylhexatriene. This probe is located deep within the hydrophobic core of the lipid bilayer with its rod-like structure aligned parallel to the phospholipid acyl chains (37) and offers several advantages in its spectral properties and sensitivity (26, 38). As discussed in the Materials and Methods section, the extrapolation of steady-state fluorescence anisotropy measurements to the quantitation of membrane microviscosity is inappropriate, although it may be useful for

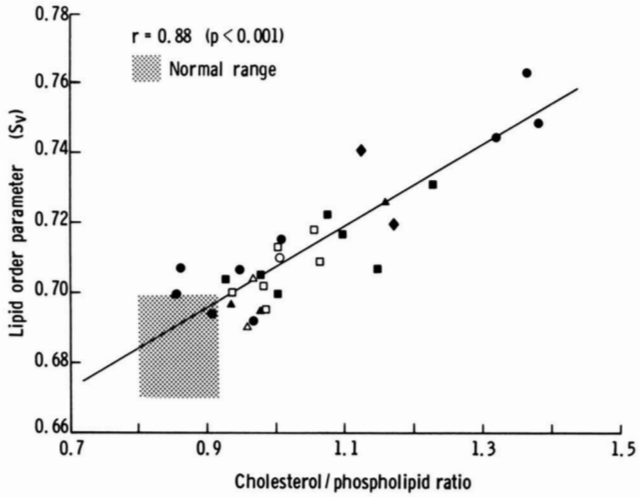

Fig. 1. Correlation between membrane cholesterol/phospholipid molar ratio and membrane fluidity, expressed as the lipid order parameter, S_v (37°C), in erythrocytes from patients with liver disease (□, intrahepatic obstructive jaundice; △, extrahepatic obstructive jaundice; ●, alcoholic liver disease; ■, chronic active hepatitis; ▲, cryptogenic cirrhosis; ♦, cholestatic viral hepatitis; ○, drug-induced hepatitis).

qualitative comparisons. Accordingly, we have interpreted our steady-state measurements in terms of the lipid order parameter, S_v, proposed by Jähnig (10), which incorporates the contribution of both the order and the dynamic state of the membrane components to the fluorescence anisotropy. Nevertheless, it should be emphasized that the technique represents an overall average of lipid order and clearly cannot describe adequately the highly heterogeneous nature of the fluidity of biological membranes.

Proteins in a biological membrane perturb the lipid environment and, depending on their nature and concentration, influence membrane fluidity (10, 11, 39). However, erythrocyte membrane protein content appeared normal in our patients, as indicated by an unchanged phospholipid/protein ratio, and although protein composition abnormalities have been reported in liver disease (40) they were relatively minor and so unlikely to have been a major factor in lowering membrane fluidity. The marked reduction in fluidity of lipid extracts from our patient erythrocytes suggests that the decreased membrane fluidity was predominantly a consequence of altered lipid composition rather than protein abnormalities. Nevertheless, a contribution to decreased membrane fluidity mediated via changes in protein constituents cannot be completely excluded. Relevant to this is our observation that the abnormal apoprotein content of high-density lipoprotein from patients with liver disease induces marked echinocyte formation when added to a suspension of normal erythrocytes (41). These transformed cells had small decreases in membrane fluidity, presumably caused by alteration in the structure of the protein cytoskeleton (42) as a consequence of apoprotein binding to the erythrocyte surface (43). We suggested that this may also occur in vivo in patients with liver disease.

Membrane fluidity may be influenced by lipid composition in several ways; it depends on the cholesterol content, whether other neutral lipids are present (7, 44) and on phospholipid composition and phospholipid fatty acid pattern. Such membrane lipid analyses are usefully expressed as ratios or proportions rather than absolute amounts per mg of membrane protein; these are independent of variation in membrane protein content and are convenient indicators of lipid-lipid interactions within biomembranes. An increased cholesterol content appeared to be the major factor responsible for the decreased membrane fluidity in our patients; there was a close correlation between fluidity and cholesterol/phospholipid ratio and the fluidity increased towards normal values when excess cholesterol was removed. This conclusion is consistent with the linear decrease in membrane fluidity observed during the enrichment of normal

TABLE 5. Fluidity of erythrocyte membranes from two patients with abetalipoproteinemia[a]

Erythrocyte Membrane Source	Incubated with Autologous Plasma			Incubated with Normal Plasma	
	Cholesterol/ Phospholipid	Phosphatidylcholine/ Sphingomyelin	S_v	Cholesterol/ Phospholipid	S_v
	mol/mol	*mol/mol*		*mol/mol*	
M.J.	0.98	0.58	0.726	0.87	0.712
S.J.	0.99	0.61	0.717	0.85	0.702
Normals (25)	0.80–0.93	0.91–1.32	0.670–0.699	n.d.[b]	n.d.[b]
Liver disease patients (30)	0.85–1.37	1.07–3.96	0.690–0.749	0.77–0.92	0.675–0.709

[a] Erythrocytes were incubated for 20 hr in either heated autologous plasma or heated normal plasma as described in Materials and Methods. Membrane fluidity at 37°C is expressed as lipid order parameter (S_v). Results for normal subjects and patients with liver disease are given as ranges for the number of subjects shown in parentheses.
[b] Not determined.

TABLE 6. Effect of bile salts on erythrocyte membrane fluidity[a]

Bile Salt Concentration	Fluidity, S_v [37°C]			
	Normal Erythrocytes		Patient Erythrocytes	
	Taurocholate	Glycocholate	Taurocholate	Glycocholate
mM				
0	0.674		0.702	
0.1	0.672	0.674	0.700	0.700
0.2	0.674	0.674	0.699	0.699
0.4	0.674	0.672	0.690	0.694
1.0	0.672	0.672	0.682	0.685

[a] Erythrocyte membranes from a normal subject and from a patient with chronic active hepatitis were labeled with diphenylhexatriene and then mixed with bile salt solutions to give final concentrations as indicated. Membrane fluidity was measured at 37°C in quadruplicate and expressed as the lipid order parameter, S_v.

erythrocytes in cholesterol by incubation with cholesterol-rich phospholipid dispersions (16, 17). No neutral lipids, other than cholesterol, could be detected by thin-layer chromatography in the erythrocyte membranes of our patients.

Several factors suggested that alterations in phospholipid composition of our patient erythrocytes had little influence on their membrane fluidity. There was no correlation between phosphatidylcholine/sphingomyelin ratio and fluidity; when excess cholesterol was removed from the erythrocytes, the mean membrane fluidity was similar to that of normal subjects and did not correlate significantly with the phosphatidylcholine/sphingomyelin ratio. Moreover, fluidity measurements in individual patient membranes with a high phosphatidylcholine/sphingomyelin ratio did not consistently fall on one side of the regression line shown in Fig. 1. By contrast, Cooper (16) has stated, without presenting experimental evidence, that certain patients with a raised erythrocyte phosphatidylcholine content may have increased membrane fluidity; we found no individual patient with an erythrocyte membrane fluidity above the normal range, even when excess cholesterol had been removed.

The negligible influence of the phosphatidylcholine/sphingomyelin ratio on erythrocyte membrane fluidity in our patients does not fit with other experimental evidence: patients with abetalipoproteinemia have a decreased erythrocyte membrane phosphatidylcholine/sphingomyelin ratio and decreased fluidity (9); increasing the phosphatidylcholine/sphingomyelin ratio in sheep erythrocytes is accompanied by an increase in membrane fluidity (8). Our findings in two patients with abetalipoproteinemia support this; both patients had a reduced erythrocyte membrane phosphatidylcholine/sphingomyelin ratio that was associated with decreased fluidity even when excess cholesterol was removed from the membrane. Other studies are in agreement with these observations; phosphatidylcholine liposomes are more fluid than those of sphingomyelin (9, 39) whilst increasing the phosphatidylcholine/sphingomyelin ratio in mixed phospholipid-cholesterol dispersions increases the fluidity at each cholesterol/phospholipid ratio studied (7). The lower fluidity of natural sphingomyelin molecules compared to natural phosphatidylcholine has been explained (7, 9, 26) by their high content of saturated fatty acids, by the *trans* double bond in the sphingosine chains and by inter- and intramolecular hydrogen bonding of the free hydroxyl groups and amide linkages. However, the relative contribution of each to the decrease in fluidity is not known.

Why is the increased phosphatidylcholine/sphingomyelin ratio in the erythrocyte membranes of our patients with liver disease not associated with increased fluidity? One explanation is that the phospholipid fatty acid composition of their erythrocyte membranes is abnormal; the content of palmitic acid is increased and that of arachidonic and stearic acids is decreased. This finding is in agreement with that of Neerhout (45) and, since the sum of stearic and palmitic acid is constant, the overall effect expected would be a decrease in fluidity as a consequence of reduced polyunsaturated fatty acid content. We conclude that this might compensate for the increase in fluidity that would be expected with an increased phosphatidylcholine/sphingomyelin ratio. However, direct evidence for this conclusion has not been provided and recent reports suggest that changes in biological membrane fatty acid composition are not always accompanied by marked effects on membrane fluidity (46, 47). Whether other differences in the erythrocyte membrane of liver disease, such as its reduced phosphatidylethanolamine content or an alteration in protein composition, can also cause decreases in fluidity remains to be investigated. Nevertheless, our results appear to establish that the erythrocyte membrane of liver disease has a basic phospholipid bilayer matrix of essentially constant fluidity, irrespective of its composition, and that decreases in fluidity compared to normal membranes are predominantly determined by accumulation of excess cholesterol.

There is evidence that cholesterol and phospholipid (mainly phosphatidylcholine) molecules on the surface of the plasma lipoproteins exchange with their counterparts in the membranes of erythrocytes and other cells (48). In liver disease, net transfer of cholesterol and phosphatidylcholine occurs from the lipoproteins to the erythrocyte membrane (13, 49) and to platelets (24), presumably because these lipids, which accumulate in the plasma as a consequence of secondary lecithin-cholesterol acyltransferase deficiency, begin to saturate the lipoprotein surface and so shift the equilibrium (6, 49). Addition of plasma lipoproteins from patients with liver disease to the culture media of human skin fibroblasts increases cellular cholesterol/phospholipid ratio.[2] These results suggest that there may be widespread lipid abnormalities of cell membranes in liver disease. Whether these changes will be accompanied by decreased fluidity remains to be established. However, our results from the present study indicate that bile salts are unlikely to counteract a fluidity decrease; at bile salt concentrations up to 0.2 mM, an upper limit for the majority of patients with liver disease (50), there was little effect on erythrocyte membrane fluidity, whilst even at 1 mM the increase in fluidity was only moderate. If membrane fluidity is decreased, then there may be interference with a number of cellular processes, many of which are known to be disturbed in liver disease. These include the inward and outward transport of various compounds including water and electrolytes, the cellular response to drugs and hormones, the capacity of the cell for phagocytosis and endocytosis, and the processes of cell division and regeneration. Whether abnormalities in such cellular functions can indeed be related to changes in plasma membrane lipid composition and membrane fluidity deserves further attention, since it may lead to a clearer understanding of the metabolic abnormalities and cellular disturbances present in patients with liver disease.

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