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Altered Lipid Composition and Differential Changes in Activities of Membrane-Bound Enzymes of Erythrocytes in Hepatic Cirrhosis

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Lipid composition, fluidity, and Na^+,K^+–adenosine triphosphatase (ATPase), Mg^{2+}-ATPase, and acetylcholinesterase (AChE) activities of erythrocyte membranes were examined in comparison to plasma lipid composition and lecithin:cholesterol acyltransferase (LCAT) activities in 39 patients with hepatic cirrhosis due to viral hepatitis (Child-Pugh class A, n = 12; class B, n = 13; and class C, n = 14). Plasma LCAT activities decreased and the plasma free–cholesterol to phospholipid molar ratio (C/PL) increased with progressive severity of hepatic cirrhosis. C/PL and fluorescence polarization (inverse of fluidity) of erythrocyte membranes also increased with disease progression (C/PL: Child-Pugh A, 0.911 ± 0.010; B, 0.941 ± 0.011; C, 0.979 ± 0.028; and normal, 0.798 ± 0.010; fluorescence polarization: Child-Pugh A, 0.348 ± 0.002; B, 0.351 ± 0.002; C, 0.355 ± 0.002; and normal, 0.340 ± 0.002). There was a correlation between C/PL and fluorescence polarization of erythrocyte membranes ($r = .629$, $P < .001$). Na^+,K^+-ATPase activity of erythrocyte membranes did not differ between cirrhotic patients and normal subjects. On the other hand, Mg^{2+}-ATPase activity decreased in Child-Pugh C cirrhosis. AChE activity was decreased in Child-Pugh A cirrhosis, and decreased further in Child-Pugh B and C cirrhosis. AChE and Mg^{2+}-ATPase activities correlated inversely with fluorescence polarization ($r = -.652$, $P < .001$ and $r = -.381$, $P < .01$, respectively). These results suggest that the free-cholesterol content of erythrocyte membranes increased in parallel with a decrease in plasma LCAT activity with progression of severity of hepatic cirrhosis, and that a resultant decrease in membrane fluidity affected Mg^{2+}-ATPase and AChE activities but not Na^+,K^+-ATPase activity. These membrane-bound enzyme activities may show different dependencies on membrane fluidity.

LECITHIN-CHOLESTEROL acyltransferase (LCAT) synthesized in the liver is secreted into plasma, where it catalyzes the esterification of cholesterol by transferring the long-chain fatty acyl residue from lecithin to unesterified cholesterol. In chronic liver disease, plasma lipoproteins become enriched in cholesterol as a result of reduced LCAT activity[1,2] and eventually cause an increase of free cholesterol in the membranes of erythrocytes and platelets.[3-5] It has been suggested that the movement of cholesterol between plasma lipoproteins and membranes depends on the proportion of cholesterol in lipoproteins and in the membranes; this can be determined by measuring the amount of cholesterol relative to the amount of phospholipid, ie, the free–cholesterol to phospholipid molar ratio (C/PL).[6,7] Cooper et al[5] showed reduced plasma LCAT activity and a strong correlation between C/PL of low-density lipoproteins and C/PL of erythrocyte membranes in patients with alcoholic cirrhosis.

In cholestatic liver disease, plasma C/PL increases due to decreased plasma LCAT activities[8] and other factors such as LP-X, which is enriched in free cholesterol and lecithin.[9] As a result, these abnormal lipoproteins lead to accumulation of free cholesterol in erythrocytes.[8] Owen et al[3] reported that C/PL of erythrocyte membranes was increased and fluidity was decreased in human cholestatic liver disease. It was also demonstrated that the plasma C/PL could be correlated with C/PL of renal cortical brush-border membranes in bile duct–ligated rats, and that the increased C/PL of the membranes led to reduced membrane fluidity.[2,10]

Changes in membrane lipids and fluidity are known to influence such physiologically important membrane enzymes as Na^+,K^+–adenosine triphosphatase (ATPase) and hormone-responsive adenylate cyclase.[11] In cholestatic liver disease, Jackson and Morgan[12] reported that the increased C/PL of erythrocyte membranes was associated with reduction of ouabain-insensitive sodium efflux, but that ouabain-

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sensitive sodium efflux was not altered. Na^+-dependent D-glucose transport increased as a result of altered lipid composition of renal cortical brush-border membranes in bile duct–ligated rats.[10] Reports have been lacking with regard to the changes in lipid composition and fluidity of extrahepatic cell membranes and the associated alteration in membrane function in parenchymal liver disease, especially nonalcoholic hepatic cirrhosis.

The purpose of this study was to examine lipid composition and fluidity of erythrocyte membranes in comparison to plasma lipid composition and LCAT activities in postviral hepatic cirrhosis classified according to Child-Pugh grading, and to investigate whether the activities of three membrane-bound enzymes, Na^+,K^+-ATPase, Mg^{2+}-ATPase, and acetylcholinesterase (AChE), are altered in these patients.

SUBJECTS AND METHODS

Patients

Patients were classified according to Child-Pugh grading. Fourteen age-matched (60.3 ± 2.1 years) normal subjects served as controls. Thirty-nine patients (age: Child-Pugh A, 63.0 ± 3.0 years; B, 60.8 ± 1.9; and C, 60.3 ± 3.5) with hepatic cirrhosis due to viral hepatitis were studied. Clinical data for the patients are listed in Table 1. Diagnosis of cirrhosis was made on the basis of histologic examination using liver biopsy for 14 patients with Child-Pugh A and B cirrhosis or clinical and laboratory findings for the other patients. All patients were hepatitis C virus antibody–positive (Child-Pugh A, n = 11; B, n = 12; and C, n = 12) or hepatitis B surface antigen–positive (Child-Pugh A, n = 1; B, n = 1; and C, n = 2). Daily alcohol intake was less than 80 g for all patients and healthy controls. None of the patients and healthy controls had other diseases that modulate lipid metabolism in erythrocytes, such as diabetes mellitus, hyperlipidemia, and renal disease. Informed consent was obtained from each patient before inclusion in the study.

Reagents

Tetrahydrofuran and 1,6-diphenylhexa-1,3,5-triene were obtained from Wako Pure Chemical Industries (Tokyo, Japan). All other chemicals were of analytic grade.

Preparation of Erythrocyte Membranes

Venous blood was obtained from patients and healthy controls after overnight fasting and then mixed with an anticoagulant. After the plasma and buffy coats had been separated by centrifugation and removed, erythrocytes were washed three times with isotonic Tris-hydrochloride, pH 7.6, and the membranes were prepared using the method reported by Hanahan and Ekholm.[13] The membranes were then suspended in an equal volume of 20-mOsm Tris hydrochloride, pH 7.6, and stored under liquid nitrogen until analyzed. Measurements of enzyme activities, lipid composition, and fluidity of membranes were performed within 1 week after preparation of the membranes. Protein content was measured using the method reported by Lowry et al.[14]

Lipid Analysis of Erythrocyte Membranes and Plasma

Lipids were extracted from erythrocyte membranes using the method reported by Bligh and Dyer[15] and from plasma using the procedure reported by Folch et al.[16] Aliquots of lipid extracts from membranes and plasma were used for cholesterol, phospholipid, and fatty acid determinations. Cholesterol level was measured with a commercial enzymatic kit (Wako Pure Chemical Industries), and total phospholipid content was estimated as inorganic phosphorus after digestion with sulfuric acid.[17] Erythrocyte membrane phospholipids were separated on silica gel A150 (Whatman, Clifton, NJ) with one-dimensional thin-layer chromatography using chloroform: methanol:isopropanol:0.25% KCl:triethylamine 30:9:25:6:18 (vol/vol). Spots were visualized with Rhodamine 6G vapor and scraped from the plate, and then phospholipid levels were measured. After fatty acid transmethylation,[3] fatty acid composition in erythrocyte membranes was analyzed by a gas-liquid chromatography (GC-14A; Shimadzu, Tokyo, Japan) equipped with a 50-m HR-SS-10 column with a 0.25-mm inner diameter (Shinwa, Kyoto, Japan).

LCAT Activity

LCAT activity was measured using proteoliposomes (apolipoprotein A-1:lecithin:cholesterol molar ratio, 0.8:250:12.5) as substrate according to the method reported by Chen and Albers.[18]

Fluorescence Polarization

The fluorescent probe diphenylhexatriene was used to label erythrocyte membranes.[19] Diphenylhexatriene was stored in tetrahydrofuran at a concentration of 2 mmol/L and was diluted immediately before use to 2,000-fold with 20-mOsm Tris hydrochloride, pH 7.6. The colloidal solution obtained was sonicated for 20 minutes and then mixed with an equal volume of erythrocyte membranes (final concentration, 20 μg protein/mL). The mixture was incubated at 37°C for 1 hour to incorporate diphenylhexatriene into the lipid matrix.

Steady-state measurements of the degree of fluorescence polarization were made at 25°C in triplicate with the fluorescence polarization analyzer FS-501-A (Union Giken, Tokyo, Japan), which indicates the intensities of polarized light emitted in parallel (Ix) and perpendicularly (Iy). The polarization ratio (P) was obtained from the equation $P = (Ix - Iy)/(Ix + Iy)$.

Na^+,K^+-ATPase and Mg^{2+}-ATPase Activities

To measure ATPase activity, 0.4 mL membrane suspension was added to 0.6 mL incubation medium containing ATP at 37°C for 30 minutes. The final composition of incubation medium was as follows: 3 mmol/L $MgCl_2$, 100 mmol/L NaCl, 20 mmol/L KCl, 30 mmol/L Tris hydrochloride (pH 7.4), 0.5 mmol/L EDTA, and 1.0 mmol/L ATP with or without 0.4 mmol/L ouabain. The reaction was terminated by addition of 0.1 mL 50% trichloroacetic acid and centrifuged at 4°C. Inorganic phosphate content in the supernatant

Table 1. Clinical Data for Patients With Hepatic Cirrhosis

Cirrhosis Level	Age, years	Sex (M:F)	ALT (IU/L)	Total Bilirubin (mg/dL)	Albumin (g/dL)	Prothrombin Time (%)	Child-Pugh Score
A	63.0 ± 3.0	7:5	45.8 ± 7.7	1.18 ± 0.11	3.42 ± 0.11	63.7 ± 2.1	5.9 ± 0.1
B	60.8 ± 1.9	8:5	54.9 ± 7.8	1.68 ± 0.21	3.03 ± 0.07	62.2 ± 3.8	8.0 ± 0.3
C	60.3 ± 3.5	10:4	63.1 ± 13.2	3.71 ± 1.10	2.61 ± 0.09	52.8 ± 5.6	10.7 ± 0.3

NOTE. Values are mean ± SEM.

Abbreviation: ALT, alanine aminotransferase.

was measured using the method reported by Jenkins and Marshall.[20]

AChE Activity

AChE activity of erythrocyte membranes was measured using the method reported by Ellman et al.[21] Acetylcholine iodide (final concentration, 0.075 mol/L) was used as the substrate.

Statistics

All results are expressed as the mean ± SEM. The difference between mean values of groups was tested by the Wilcoxon test and Kruskal-Wallis test. Correlation coefficients were obtained by linear regression.

RESULTS

Individual values for C/PL of erythrocyte membranes in cirrhotic patients are shown in Fig 1. In hepatic cirrhosis, C/PL of erythrocyte membranes increased with progressive severity of cirrhosis. This increase in C/PL of erythrocyte membranes resulted from an increase in cholesterol content (Table 2). In phospholipids of erythrocyte membranes, phosphatidylcholine content increased and phosphatidylethanolamine content decreased with progressive severity of hepatic cirrhosis (Table 3). Fatty acid compositions of erythrocyte membranes are listed in Table 4. Polyunsaturated fatty acids and the unsaturation index[22,23] did not change in hepatic cirrhosis. Saturated fatty acid levels in Child-Pugh A cirrhosis and monounsaturated fatty acid levels in Child-Pugh C cirrhosis were slightly increased. Plasma lipid composition and LCAT activity of erythrocyte membranes from cirrhotic patients are listed in Table 5. Plasma C/PL increased in parallel with a decrease in LCAT activity. There was a significant correlation between C/PL

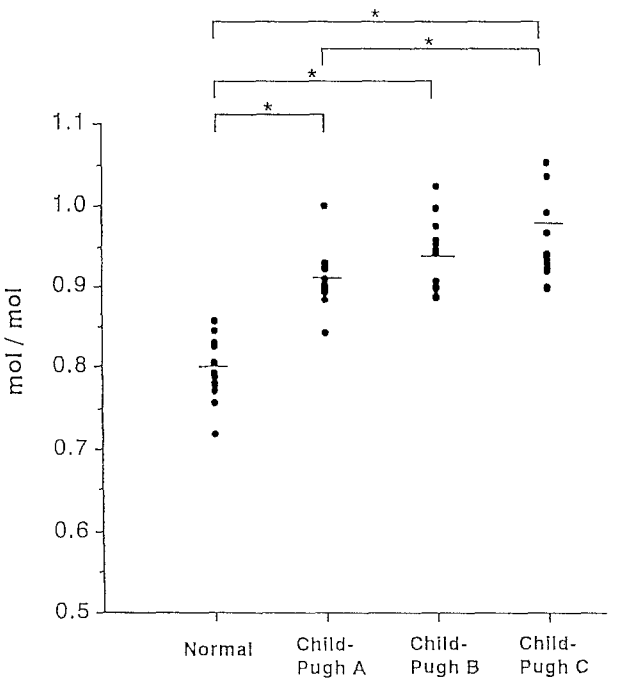

Fig 1. C/PL of erythrocyte membranes from patients with hepatic cirrhosis. Bars indicate the mean of values in each group. *$P < .01$.

Table 2. Lipid Composition of Erythrocyte Membranes From Patients With Hepatic Cirrhosis

Subjects	Free Cholesterol (μmol/mg protein)	Phospholipid (μmol/mg protein)	C/PL (mol/mol)
Cirrhosis			
A	0.475 ± 0.009*	0.522 ± 0.011	0.911 ± 0.010*§
B	0.487 ± 0.015*	0.519 ± 0.018	0.941 ± 0.011*†
C	0.505 ± 0.017*	0.517 ± 0.012	0.979 ± 0.028*‡
Normal	0.427 ± 0.012‡‖	0.535 ± 0.013	0.798 ± 0.010‡‖

NOTE. Values are mean ± SEM.
*$P < .01$ *v* normal subjects.
†$P < .05$.
‡$P < .01$: *v* Child-Pugh A.
§$P < .05$.
‖$P < .01$: *v* Child-Pugh B.

of erythrocytes and C/PL of plasma ($r = .603$, $P < .001$). Erythrocyte membrane C/PL also showed a correlation with plasma LCAT activity ($r = .833$, $P < .001$). Fluorescence polarization values for erythrocyte membranes in each group are shown in Fig 2. Fluorescence polarization increased (ie, fluidity decreased) with disease progression. Since C/PL, the phosphatidylcholine to sphingomyelin ratio, and fatty acid composition are determinants of membrane fluidity,[11,24,25] we performed multiregression analysis on the contribution of these parameters to erythrocyte membrane fluidity in this study. We found that C/PL could be correlated with the fluorescence polarization of erythrocyte membranes ($r = .629$, $P < .001$). No correlation was found between fluorescence polarization and either the phosphatidylcholine to sphingomyelin ratio or unsaturation index of fatty acids.

AChE activity was decreased in Child-Pugh A cirrhosis, and decreased further with progression of hepatic cirrhosis (normal, 3.86 ± 0.15 μmol/mg protein/min; Child-Pugh A, 3.31 ± 0.16; B, 3.03 ± 0.21; and C, 2.57 ± 0.19; Fig 3). Mg^{2+}-ATPase activity decreased only in Child-Pugh C cirrhosis (normal, 0.284 ± 0.018 μmol/mg protein/h; Child-Pugh A, 0.256 ± 0.020; B, 0.271 ± 0.008; and C, 0.195 ± 0.014; Fig 4). Na^+,K^+-ATPase activity did not differ between cirrhotic patients and normal subjects (normal, 0.296 ± 0.015 μmol/mg protein/h; Child-Pugh A, 0.255 ± 0.022; B, 0.315 ± 0.014; and C, 0.292 ± 0.022; Fig 5). AChE and Mg^{2+}-ATPase activities showed a correlation with fluorescence polarization ($r = .652$, $P < .001$ and $r = .381$, $P < .01$, respectively; Figs 6 and 7).

DISCUSSION

In liver disease, plasma LCAT activity has been shown to decrease, probably due to its decreased synthesis in the liver.[26,27] Plasma LCAT activity also decreased in a stepwise manner from Child-Pugh A to C cirrhosis in our patients. The decrease in LCAT activity resulted in an increase in the plasma C/PL. As shown by Cooper et al[5] for alcoholic cirrhosis, we confirmed that an elevated plasma C/PL showed a strong correlation with erythrocyte membrane C/PL in postviral cirrhosis.

Our findings clearly demonstrate that fluorescence polar-

Table 3. Phospholipid Composition of Erythrocyte Membranes From Patients With Hepatic Cirrhosis

Subjects	Phospholipid Composition				
	SM (%)	PC (%)	PS + PI (%)	PE (%)	PC to SM Ratio
Cirrhosis					
A	25.5 ± 0.6	31.5 ± 0.7†	16.5 ± 0.3*	26.5 ± 0.3†‖	1.25 ± 0.05†
B	25.7 ± 0.5	33.2 ± 0.5†	16.6 ± 0.2*	24.5 ± 0.7†‡	1.30 ± 0.04†
C	27.6 ± 0.9	36.2 ± 1.0†§‖	17.4 ± 0.4†	18.7 ± 1.7†§‖	1.33 ± 0.05†
Normal	26.0 ± 0.2	28.9 ± 0.3§¶	15.8 ± 0.2‡‖	29.2 ± 0.2§¶	1.11 ± 0.02§¶

NOTE. Values are mean ± SEM.
Abbreviations: SM, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine.
*$P < .05$.
†$P < .01$, *v* normal subjects.
‡$P < .05$.
§$P < .01$, *v* Child-Pugh A.
‖$P < .05$.
¶$P < .01$ *v* Child-Pugh B.

Table 4. Fatty Acid Composition of Erythrocyte Membranes From Patients With Hepatic Cirrhosis and Normal Subjects

Composition	Cirrhosis			Normal Subjects
	A	B	C	
Saturated fatty acids (%)	42.7 ± 0.3†	42.3 ± 0.4	41.8 ± 0.2§	41.5 ± 0.3
16:0	25.9 ± 0.4†	25.7 ± 0.3†	25.9 ± 0.4†	22.9 ± 0.3
18:0	16.7 ± 0.2†	16.7 ± 0.2†	15.9± 0.4†	18.7 ± 0.2
Monounsaturated fatty acids (%)	16.6 ± 0.3	17.2 ± 0.2	18.2 ± 0.4*§‖	17.2 ± 0.3
16:1	0.8 ± 0.1†	0.8 ± 0.1†	0.9 ± 0.2†	0.3 ± 0.1
18:1	15.8 ± 0.3†	16.4 ± 0.2*	17.3 ± 0.3§‖	16.9 ± 0.2
Polyunsaturated fatty acids (%)	40.7 ± 0.4	40.5 ± 0.5	40.0 ± 0.5	41.3 ± 0.4
18:2	10.7 ± 0.3	11.1 ± 0.4	11.6 ± 0.7	10.8 ± 0.5
20:3	1.6 ± 0.3	1.9 ± 0.2	2.0 ± 0.2*	1.6 ± 0.2
20:4	13.8 ± 0.3	12.5 ± 0.4*‡	12.5 ± 0.3*§	13.8 ± 0.5
20:5	1.7 ± 0.1†	2.0 ± 0.2	1.7 ± 0.2*	2.4 ± 0.2
22:5	2.5 ± 0.1†	2.7 ± 0.1	2.8 ± 0.2	2.9 ± 0.7
22:6	10.4 ± 0.3	10.3 ± 0.2	9.5 ± 0.4	9.8 ± 0.2
Unsaturation index	181.4 ± 1.9	180.4 ± 2.1	178.7 ± 2.6	183.9 ± 1.6

NOTE. Values are mean ± SEM. Unsaturation index calculated as Σ percentage of fatty acid × number of double bonds.
*$P < .05$.
†$P < .01$, *v* normal subjects.
‡$P < .05$.
§$P < .01$, *v* Child-Pugh A.
‖$P < .05$ *v* Child-Pugh B.

Table 5. Plasma Lipid Composition and LCAT Activity of Patients With Hepatic Cirrhosis

Subjects	Total Cholesterol (mmol/L)	Free Cholesterol (mmol/L)	Phospholipid (mmol/L)	C/PL (mol/mol)	LCAT Activity (nmol cholesterol esterified/h/mL)
Cirrhosis					
A	3.29 ± 0.15†	1.16 ± 0.03	2.11 ± 0.08†	0.553 ± 0.010†‖	49.1 ± 2.9†
B	3.20 ± 0.32*	1.22 ± 0.12	2.08 ± 0.21	0.592 ± 0.011†‡	40.8 ± 5.7†
C	2.59 ± 0.23†‡	0.96 ± 0.10*‡‖	1.55 ± 0.15†§‖	0.617 ± 0.018†‡	27.1 ± 3.2†§‖
Normal	4.32 ± 0.32§‖	1.28 ± 0.07	2.56 ± 0.08§	0.498 ± 0.017§¶	155.2 ± 8.1§¶

NOTE. Values are mean ± SEM.
*$P < .05$.
†$P < .01$, *v* normal subjects.
‡$P < .05$.
§$P < .01$, *v* Child-Pugh A.
‖$P < .05$.
¶$P < .01$: *v* Child-Pugh B.

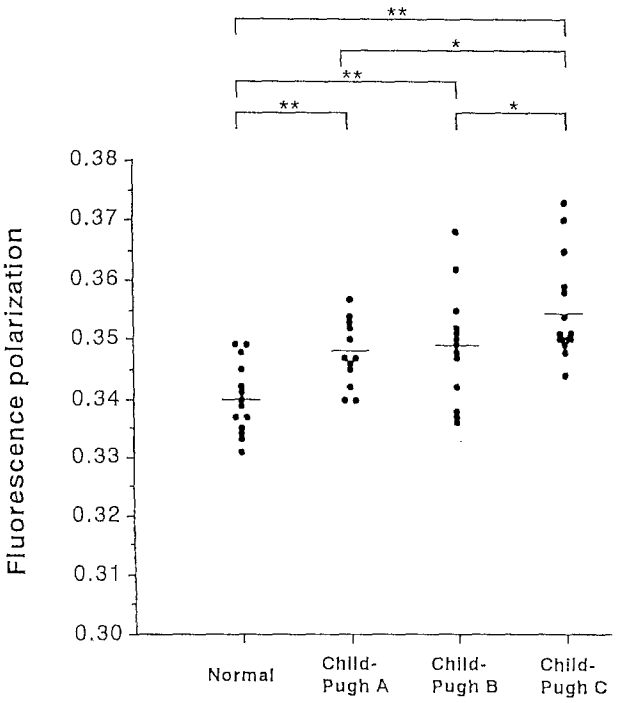

Fig 2. Fluorescence polarization of erythrocyte membranes from patients with hepatic cirrhosis. Bars indicate the mean of values in each group. *P < .05, **P < .01.

ization increased (ie, fluidity decreased) as the severity of hepatic cirrhosis progressed (Fig 2). Lipid composition of the membranes affects membrane fluidity.[25] As a result of decreased LCAT activity, erythrocyte membranes of our patients had higher levels of both phosphatidylcholine and free cholesterol, both of which are substrates of LCAT. Cholesterol makes membranes rigid, whereas phosphatidylcholine has the opposite effect. Multiple regression analysis showed that an increased C/PL was responsible for the decreased membrane fluidity, and that there was no correlation between the phosphatidylcholine to sphingomyelin ratio and fluorescence polarization in this study. These results agreed well with the report by Owen et al[4] that the reduced fluidity of erythrocyte membranes was predominantly a result of an increased C/PL in liver disease, and also with another report that the fluorescence polarization of renal cortical brush-border membranes correlated well with C/PL but not with the phosphatidylcholine to sphingomyelin ratio in bile duct–ligated rats.[10] The other factor that modulates membrane fluidity is fatty acid composition: an increase of unsaturated fatty acids causes an increase in membrane fluidity.[11,25] The changes in fatty acid composition of erythrocyte membranes (Table 4) were in agreement with those reported by Owen et al.[3] In our study, there was no correlation between the fluorescence polarization and unsaturation index of fatty acids. These results suggest that the fatty acid composition of erythrocyte membranes did not affect the fluidity of these membranes in patients with postviral hepatic cirrhosis.

Protein-lipid interactions and fluidity play a pivotal role in many physiologic processes that take place in cell membranes, such as the activity of membrane-bound enzymes. In this study, we measured activities of three membrane-bound enzymes of erythrocytes, Na^+,K^+-ATPase, Mg^{2+}-ATPase, and AChE, in postviral hepatic cirrhosis. We found that Na^+,K^+-ATPase activity of the membranes was not altered in all clinical stages of cirrhotic patients, whereas Mg^{2+}-ATPase activity decreased in Child-Pugh C cirrhosis and AChE activity was already decreased in Child-Pugh A cirrhosis and decreased even further in Child-Pugh B and C cirrhosis.

Our patients with postviral cirrhosis showed no change in Na^+,K^+-ATPase activity of erythrocyte membranes, which had an increased C/PL and decreased membrane fluidity. The effect of changes in lipid composition and fluidity on Na^+,K^+-ATPase activity of erythrocyte membranes is controversial. There have been two reports on the relationship between C/PL and Na^+,K^+-ATPase activity of erythrocyte membranes using phospholipid liposomes.[28,29] Giraud et al[28] depleted human red blood cells of cholesterol by incubation with phospholipid vesicles and measured the Na^+ efflux. Under their experimental conditions, cholesterol depletion increased the maximal rate of Na^+ efflux but decreased the apparent affinity for internal Na^+. Incerpi et al[29] reported that an increase of cholesterol content by incubation with phospholipid liposomes resulted in decreased membrane fluidity and stimulation of Na^+,K^+-

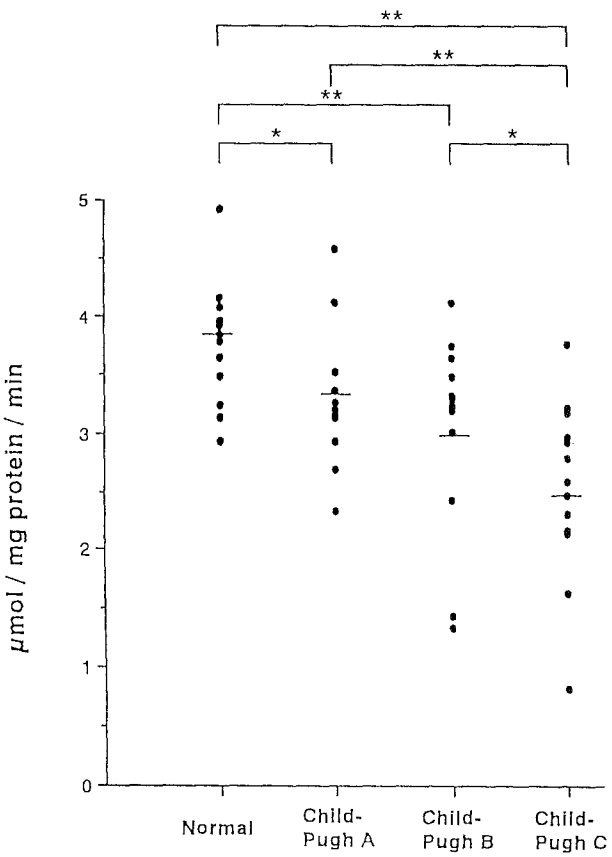

Fig 3. AChE activities of erythrocyte membranes from patients with hepatic cirrhosis. Bars indicate the mean of activities in each group. *P < .05, **P < .01.

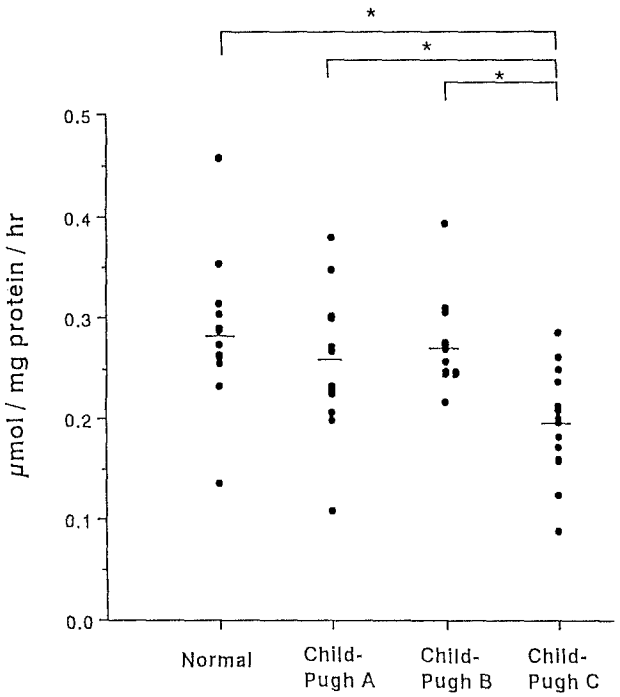

Fig 4. Mg^{2+}-ATPase activities of erythrocyte membranes from patients with hepatic cirrhosis. Bars indicate the mean of activities in each group. *$P < .01$.

ATPase activity in human erythrocyte membranes. Na^+,K^+-ATPase activity of erythrocyte membranes has been measured in some disease conditions. In insulin-dependent diabetic patients, Na^+,K^+-ATPase was reduced and showed

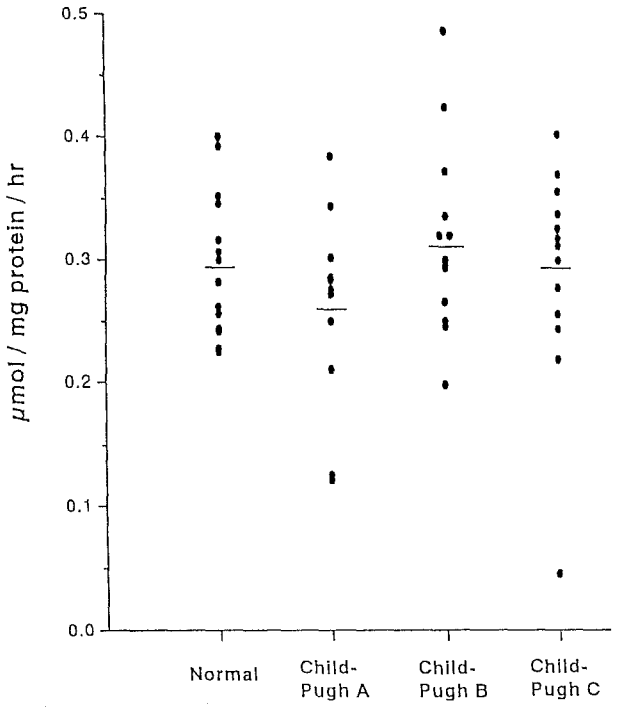

Fig 5. Na^+,K^+-ATPase activities of erythrocyte membranes from patients with hepatic cirrhosis. Bars indicate the mean of activities in each group.

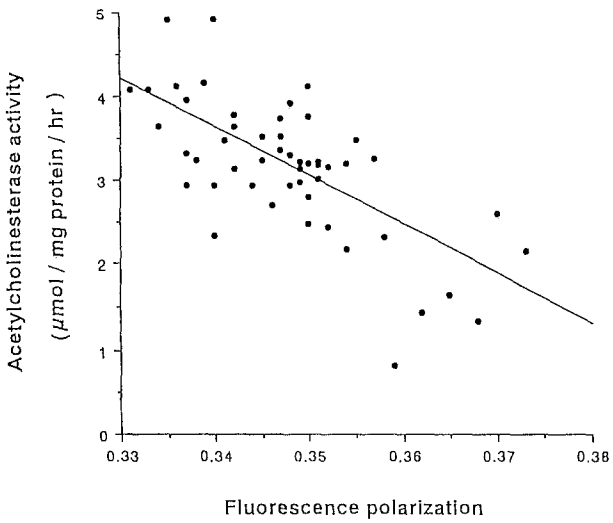

Fig 6. Correlation between AChE activity and fluorescence polarization of erythrocyte membranes from patients with hepatic cirrhosis. $r = -.652, P < .001$.

a correlation with an increase in membrane fluidity.[30] In cholestatic liver disease, Jackson and Morgan[12] studied Na^+ efflux of erythrocytes and found that ouabain-sensitive Na^+ efflux (ie, Na^+,K^+-ATPase) was not changed, but that cholesterol loading of normal erythrocytes could reduce Na^+ efflux. These conflicting results might be due to different experimental conditions or factors other than lipid composition and membrane fluidity. However, our results for hepatic cirrhosis did agree with those for cholestatic liver disease.[12,31]

In human erythrocyte membranes, three different proteins have been identified as Mg^{2+}-ATPase, although their physiologic roles remain equivocal.[32] Jackson and Morgan[12] also studied ouabain-insensitive Na^+ efflux (ie, Mg^{2+}-ATPase activity) and found that it decreased and showed an inverse correlation with the membrane C/PL in cholestatic liver disease. In postviral cirrhosis, we found that

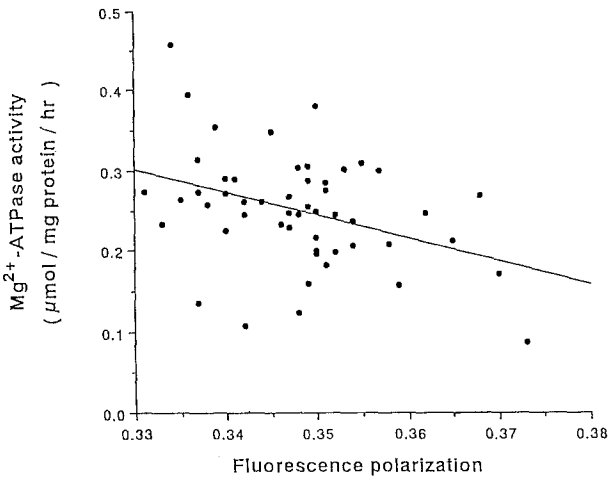

Fig 7. Correlation between Mg^{2+}-ATPase activity and fluorescence polarization of erythrocyte membranes from patients with hepatic cirrhosis. $r = -.381, P < .01$.

Mg^{2+}-ATPase activities decreased in Child-Pugh C cirrhosis and that there was an inverse correlation between Mg^{2+}-ATPase activity and fluorescence polarization of erythrocyte membranes.

This study demonstrated for the first time that AChE activity of erythrocyte membranes decreases in patients with hepatic cirrhosis. Cholesterol loading in rat erythrocyte membranes results in a decrease in AChE activity.[33] In erythrocytes from insulin-dependent diabetic patients, a significant correlation was found between AChE activity and membrane fluidity.[30] In our study, AChE activity of erythrocyte membranes decreased with progressive severity of hepatic cirrhosis (Fig 3), and an inverse correlation was found between enzyme activity and fluorescence polarization (Fig 6). These results indicate that in hepatic cirrhosis, as well as in other conditions, AChE activity of erythrocyte membranes is dependent on membrane fluidity, with the correlation being positive.

The mechanisms of differential changes in membrane-bound enzymes remain unclear. Na^+,K^+-ATPase is a protein that penetrates the membrane at active sites on each side of the membrane and acts as a sodium pump, whereas AChE is a protein that is anchored on the surface of the plasma membrane by an attached glycosyl-phosphatidylinositol.[34,35] One possibility is that Na^+,K^+-ATPase as a membrane-penetrating enzyme is resistant to a change in fluidity of erythrocyte membranes, whereas AChE is sensitive to the change. Alkaline phosphatase is another glycosyl-phosphatidylinositol–anchored protein.[34,35] Brasitus et al[36] reported that increasing the C/PL of rat proximal small intestinal microvillus membranes using a nonspecific lipid-transfer protein and cholesterol/phospholipid liposomes decreased alkaline phosphatase specific activity. Further studies on differential changes in activities of membrane-bound enzymes of erythrocytes are needed.

In conclusion, the cholesterol content of erythrocyte membranes increased with progressive severity of the disease, and a resultant decrease in membrane fluidity affected AChE and Mg^{2+}-ATPase activities but not Na^+,K^+-ATPase activity of erythrocyte membranes in hepatic cirrhosis. These membrane-bound enzymes may show different dependencies on membrane fluidity for their activities.

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