

Platelet lipid composition and platelet aggregation in human liver disease

James S. Owen,¹ Ronald A. Hutton, Richard C. Day, K. Richard Bruckdorfer, and Neil McIntyre

Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, University of London, London, WC1N 1BP and Departments of Haemophilia and Medicine, Royal Free Hospital, Pond Street, London, NW3 2QG, United Kingdom

Abstract Abnormal plasma lipoproteins in patients with liver disease are associated with an increase in erythrocyte cholesterol concentration and a raised erythrocyte cholesterol/phospholipid molar ratio. We hypothesized that their platelets would also have an increased cholesterol/phospholipid ratio and that this might affect aggregation in vitro. Platelet aggregation by adrenaline and ADP was measured in 34 patients with a variety of liver diseases and in 20 normal subjects and the values were related to platelet lipid composition. The platelet cholesterol/phospholipid ratio was 13% higher in the patients and correlated closely with erythrocyte cholesterol/phospholipid ratio. Platelet aggregation was reduced in most of the patients and inversely correlated with the cholesterol/phospholipid ratio. Cross-incubation and hemostasis studies indicated that there were no inhibitory factors present in the plasma; the defect was in the platelets. In contrast, other workers have shown that cholesterol-rich platelets, either from patients with Type IIa hyperlipoproteinemia or prepared in vitro, aggregate more readily than normal platelets. However, the phospholipid and fatty acid compositions of our patient platelets were also abnormal: the lecithin/sphingomyelin ratio was increased and was inversely correlated with aggregation; the proportion of arachidonic acid was decreased and positively correlated with the aggregation. In our patients with liver diseases the effects of the altered phospholipid and fatty acid composition presumably overrode those of the increased cholesterol content so that instead of enhanced aggregation, only reduced or normal aggregation was seen. We conclude that the reduced platelet aggregation seen in liver disease may reflect a decrease in arachidonic acid availability for prostaglandin and/or thromboxane production.—Owen, J. S., R. A. Hutton, R. C. Day, K. R. Bruckdorfer, and N. McIntyre. Platelet lipid composition and platelet aggregation in human liver disease. *J. Lipid Res.* 1981. **22**: 423–430.

Supplementary key words arachidonic acid · membrane fluidity · platelet cholesterol · platelet phospholipids

In many patients with liver disease erythrocyte cholesterol and phosphatidylcholine concentrations are increased and the cholesterol/phospholipid ratio is raised (1). The excess lipid is acquired from the abnormal plasma lipoproteins (2, 3) and the resulting

decrease in membrane fluidity (4, 5) is associated with an abnormal permeability to sodium (6) and with an enhanced ability of the cells to fuse in vitro in the presence of a chemical fusogen (7). We have suggested that similar membrane lipid changes may occur in other cells and affect their function (3).

Incubation of platelets with cholesterol-rich liposomes increases their cholesterol content and their cholesterol/phospholipid molar ratio (8). These changes are accompanied by a decrease in membrane fluidity (9). The platelets of patients with Type IIa hyperlipoproteinemia also have a raised cholesterol/phospholipid ratio (10). In both cases the platelets exhibit increased sensitivity to the aggregating agents ADP and adrenaline (8, 10). In this study we have tested the hypothesis that platelets from patients with liver disease might have an increased cholesterol/phospholipid ratio and that this might affect their ability to aggregate in vitro.

MATERIALS AND METHODS

Patients

Thirty-four patients with liver disease of varying severity were studied. All were in-patients of the Department of Medicine at the Royal Free Hospital, London. The diagnosis was established by a variety of appropriate investigations including liver biopsy, cholangiography, or operation. Twelve patients had obstructive jaundice (seven, intrahepatic; five, extrahepatic), twenty-one had non-fulminant parenchymal liver disease (seven, alcoholic cirrhosis; two, alcoholic hepatitis; four, cryptogenic cirrhosis; six, chronic active hepatitis; two, drug-induced hepatitis) and in

¹ Address correspondence to Dr. J. S. Owen, Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, University of London, 8 Hunter Street, London, WC1N 1BP, U.K.

one there was both biliary obstruction and parenchymal dysfunction (cholestatic viral hepatitis). The comparison subjects were healthy medical and laboratory staff. Neither the patients nor the comparison group had received drugs known to affect platelet aggregation in the 10 days prior to being studied. After overnight starvation, 60 ml of venous blood was withdrawn and platelets (11) and erythrocytes (6) were isolated as described previously.

Platelet and erythrocyte lipid composition

Lipids were extracted from washed platelets by the method of Folch, Lees, and Sloane Stanley (12) and from washed erythrocytes with isopropanol and chloroform (13). Aliquots of the lipid extracts were taken for cholesterol (14) and phospholipid (15) estimations which were carried out in duplicate. Platelet and erythrocyte phospholipids were separated on silica gel H (Merck) by 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 (16). In preliminary experiments, individual fractions were identified by comparison with authentic phospholipid standards (Sigma Chemical Co., St. Louis, MO) and by use of specific chemical spray techniques (16). The fractions were located with iodine, scraped from the plate, and the phospholipids were measured as inorganic phosphorus after digestion with H_2SO_4 (15).

In the last eighteen patients studied, platelet fatty acid composition was determined 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 some additional subjects, the fatty acid composition of individual platelet phospholipids was measured. In these cases, the diacyl and plasmalogen ethanolamine glycerophospholipids were resolved by spraying the lipid track with 5 mM $HgCl_2$ to convert the plasmalogen fraction to lysophosphatidylethanolamine, prior to development in the second solvent (17). The individual phospholipids were located by spraying with aqueous Rhodamine-6G (0.01%, w/v) and transmethylated as before.

Investigation of hemostatic mechanism

Platelet aggregation was quantitated by nephelometry (18), the fall in absorbance of platelet-rich plasma being determined 3 min after addition of a standard amount of aggregating agent. Adenosine diphosphate, adrenaline, collagen (Sigma Chemical Co.), and

Ristocetin (Abbott Laboratories, Queensborough, U.K.) were used. In seven patients the platelet-rich plasma contained less than 150,000 platelets per μ liter, making measurement of aggregation unreliable. These patients were therefore excluded from statistical analyses of the aggregation results. For cross-over studies, platelet-rich plasma from normals or patients was diluted with an equal volume of normal platelet-poor plasma or with that of patients and, after incubation for 30 min at room temperature, aggregation was tested as before. Adenine nucleotides were measured by the firefly luminescence method (19). Coagulation studies were carried out by standard procedures (20).

Statistics

All results are expressed as means \pm S.D.; statistical differences were determined by Students' *t* test and the correlation coefficients by either linear regression (*r*) or Spearman's rank test (*r_s*) (21).

RESULTS

Platelet and erythrocyte lipids

A comparison of erythrocyte and platelet lipid composition between patients and normal subjects is shown in **Table 1**. The cholesterol concentration and the mean cholesterol/phospholipid molar ratio were significantly increased ($P < 0.001$) in erythrocytes from patients. The composition of the phospholipids was also changed in patient erythrocytes with an increase in the proportion of phosphatidylcholine and a decrease in sphingomyelin, phosphatidylethanolamine, and phosphatidylserine (Table 1). These results were in agreement with our previous observations (7) and those of other workers (1, 2). The mean cholesterol concentration was significantly higher in patient platelets but the phospholipid content was unchanged. There was a significant increase in the mean cholesterol/phospholipid molar ratio in platelets from patients (0.65 ± 0.05 compared to 0.57 ± 0.02 in the normal group, $P < 0.001$) and only nine patients had values within the normal range (**Fig. 2**). A close correlation existed between platelet cholesterol/phospholipid ratio and erythrocyte cholesterol/phospholipid ratio for the patients ($r = 0.83$, $P < 0.001$) (**Fig. 1**). The phospholipid composition of patient platelets was altered with an increase in the phosphatidylcholine fraction ($43.5 \pm 1.9\%$ compared to $40.2 \pm 1.5\%$ in the normals, $P < 0.001$) and decreases in the phosphatidylethanolamine and sphingomyelin fractions (Table 1). The phosphatidylcholine/sphingomyelin molar

TABLE 1. Lipid composition of erythrocytes and platelets from patients with liver disease and from normal subjects^a

Subjects	Cholesterol (nmol/10 ⁸ erythrocytes and nmol/10 ⁸ platelets)	Phospholipid	Cholesterol/ phospholipid (mol/mol)	% Total Lipid Phosphorus (mol %)									
				LPC ^b	SM	PI	PS	PC	PE	PA			
Erythrocytes													
Normal (20)	3.55 ± 0.24	4.10 ± 0.32	0.87 ± 0.03	1.2 ± 0.4	26.5 ± 1.0	0.5 ± 0.3	13.1 ± 1.1	29.4 ± 1.3	27.5 ± 1.3	1.8 ± 0.5			
Patients (34)	4.78 ± 0.57 ^c	4.62 ± 0.58 ^c	1.04 ± 0.12 ^c	0.8 ± 0.4 ^d	22.8 ± 3.2 ^e	0.7 ± 0.4	12.0 ± 1.6 ^d	40.7 ± 6.9 ^e	20.9 ± 2.9 ^e	2.1 ± 0.7			
Platelets													
Normal (20)	24.9 ± 4.0	43.6 ± 7.3	0.57 ± 0.02	1.1 ± 0.5	19.1 ± 1.1	2.7 ± 1.1	9.2 ± 1.2	40.2 ± 1.5	27.6 ± 2.3	N.D.			
Patients (34)	27.8 ± 4.1 ^c	43.0 ± 6.5	0.65 ± 0.05 ^c	1.2 ± 0.3	16.8 ± 1.1 ^e	2.9 ± 0.9	9.3 ± 1.1	43.5 ± 1.9 ^e	26.2 ± 1.9 ^c	N.D.			

^a Results are expressed as means ± S.D. for the number of subjects given in parentheses, except for platelet phospholipid composition when only 24 patients were studied.

^b LPC, lysophosphatidylcholine; SM, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; N.D., not detected.

^c $P < 0.05$.

^d $P < 0.01$.

^e $P < 0.001$.

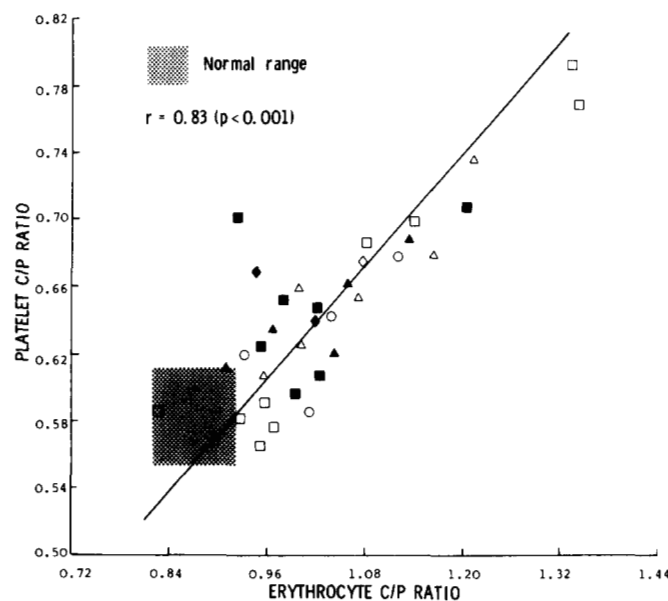


Fig. 1. Correlation between the cholesterol/phospholipid molar ratio (C/P) in platelets and in erythrocytes from patients with liver disease (■, intrahepatic obstructive jaundice; ▲, extrahepatic obstructive jaundice; □, alcoholic liver disease; △, chronic active hepatitis; ○, cryptogenic cirrhosis; ◆, drug-induced hepatitis; ◇, cholestatic viral hepatitis).

ratio was significantly higher in patient platelets (2.60 ± 0.23 compared to 2.11 ± 0.13 for the normal subjects, $P < 0.001$) (Fig. 1). Platelet fatty acid composition was also abnormal in the patients and the proportion of arachidonic acid was significantly decreased ($23.9 \pm 3.4\%$ compared to $31.5 \pm 4.0\%$) (Table 2). The arachidonic acid content of the major individual phospholipid classes was determined in seven patients, each of whom had a total platelet arachidonic acid content less than that of the normal range. The mean proportion of arachidonic acid in each of the individual patient phospholipid classes was decreased (Table 3), and although there was considerable variation within the separate classes there was no clear indication of an asymmetric depletion to account for the total arachidonic acid reduction.

Platelet studies

Aggregation of patient platelets was usually reduced with one or more of the aggregating agents and the mean values were lower for both adrenaline-induced aggregation ($51 \pm 32\%$ compared to $69 \pm 14\%$ for the normal subjects, $P < 0.05$) and ADP-induced aggregation ($60 \pm 25\%$ compared to $72 \pm 18\%$, $P < 0.1$). No consistent pattern was seen in the decreased response; the primary wave was reduced in some cases while in others secondary aggregation due to impairment of the release reaction was affected more.

There were significant inverse correlations (Table 4)

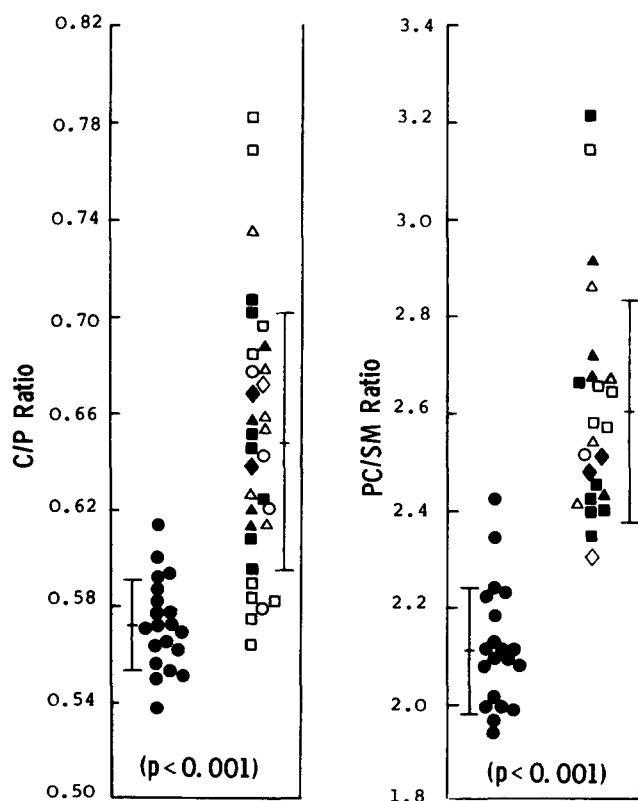


Fig. 2. Platelet cholesterol/phospholipid (C/P) and phosphatidylcholine/sphingomyelin (PC/SM) molar ratios in normal subjects (●) and in patients with liver disease (■, intrahepatic obstructive jaundice; ▲, extrahepatic obstructive jaundice; □, alcoholic liver disease; △, chronic active hepatitis; ○, cryptogenic cirrhosis; ◆, drug-induced hepatitis; ◇, cholestatic viral hepatitis). The mean \pm S.D. of the results is indicated by the bars.

between adrenaline-induced aggregation and both the cholesterol/phospholipid ratio ($r_s = -0.41$, $P < 0.05$) and the phosphatidylcholine/sphingomyelin ratio ($r_s = -0.58$, $P < 0.01$). By contrast, there was a positive correlation between the proportion of platelet arachidonic acid and adrenaline-induced aggregation ($r_s = 0.70$, $P < 0.01$). Similar results were obtained when ADP was the aggregating agent, but the correlation coefficients were usually lower. Cross-over studies were carried out on the first sixteen patients investi-

gated; these indicated that the defect was probably in the platelets themselves since the patients' platelet-poor plasma did not inhibit (and in four cases enhanced) the aggregation of normal platelets, nor did normal platelet-poor plasma correct the defect in the patients' platelets.

Total adenine nucleotide level was generally lower in platelets from patients compared to normals and in a few cases the ATP/ADP ratio was above the normal range. However, the aggregation defect did not correlate with this or the ADP concentration, suggesting it was not due to a deficiency of endogenous ADP.

Coagulation studies

The prothrombin time, partial thromboplastin time, and thrombin time were prolonged in many of the patients although fibrinogen and von Willebrand factor were normal or elevated. Fibrin(ogen) degradation products were usually less than $8 \mu\text{g/ml}$ and never greater than $12 \mu\text{g/ml}$. No fibrin monomers were detected. Patient antithrombin III level was within the normal range of 75–125% for all but one patient (69%).

DISCUSSION

In the present study, *in vitro* platelet aggregation was reduced in patients with liver disease; plasma factors did not appear to be involved suggesting that the defect was in the platelet itself. The platelet lipid composition in the patients differed from that in normal subjects; they had an increased cholesterol/phospholipid ratio, an altered phospholipid composition, and a decreased arachidonic acid content. These lipid changes may explain the reduced ability of the platelets to aggregate in response to ADP and adrenaline.

In erythrocytes from patients with liver disease, there is evidence that their excess cholesterol and phosphatidylcholine is acquired from the abnormal lipoproteins present in the plasma (10, 22). Erythrocyte fatty acid composition is also abnormal in liver

TABLE 2. Fatty acid composition of platelets from patients with liver disease and from normal subjects^a

Subjects	% Total Fatty-acids						
	16:0	16:1	18:0	18:1	18:2	20:3	20:4
	(mol %)						
Normal	19.6 \pm 3.1	0.7 \pm 0.7	24.0 \pm 2.9	17.1 \pm 1.7	6.1 \pm 1.4	1.0 \pm 0.3	31.5 \pm 4.0
Patients	22.3 \pm 3.2 ^b	1.6 \pm 0.8 ^c	22.1 \pm 2.2 ^b	24.5 \pm 3.4 ^d	4.5 \pm 0.9 ^d	1.1 \pm 0.5	23.9 \pm 3.4 ^d

^a Results are expressed as means \pm S.D. There were 18 subjects in each group.

^b $P < 0.05$.

^c $P < 0.01$.

^d $P < 0.001$.

TABLE 3. Arachidonic acid content in the major individual phospholipids of platelets from patients with liver disease and from normal subjects^a

Subjects (No.)	% Arachidonic Acid in Fatty Acids of Individual Phospholipids			
	Diacyl-PE ^b	Plasmalogen-PE	PS	PC
	(mol%)			
Normal (4)	41.0 ± 3.7	95.1 ± 5.1	18.5 ± 5.7	16.6 ± 2.4
Patients (7)	33.1 ± 10.3	77.9 ± 15.5	12.3 ± 8.8	12.5 ± 1.9

^a Results are expressed as means ± S.D.

^b Diacyl-PE, diacyl ethanolamine phosphoglyceride; plasmalogen-PE, plasmalogen ethanolamine phosphoglyceride; PS, phosphatidylserine; PC, phosphatidylcholine. No arachidonic acid was detected in sphingomyelin while insufficient patient platelets were available to measure accurately the fatty acid composition in lysophosphatidylcholine and phosphatidylinositol.

disease with a decreased content of polyunsaturated fatty acids (23). In the present study, similar changes were observed in the lipid composition of patient platelets and the close correlation between the erythrocyte and platelet cholesterol/phospholipid ratio suggests the same underlying mechanism is responsible. The increase in platelet cholesterol/phospholipid ratio was only 13% compared to that of 20% in the erythrocyte and the increase in the proportion of phosphatidylcholine was also smaller in the platelets. However, in the present study, only whole platelet lipids were estimated and it seems likely (10) that the changes in the plasma membrane lipids were greater. The alterations in platelet phospholipid and fatty acid profiles were of particular interest because platelets, unlike erythrocytes, are able to synthesize both phospholipid and fatty acids (24) and might have maintained a normal membrane composition of these lipids in the presence of abnormal plasma lipoproteins.

Platelet aggregation in the patients was either reduced or normal; in no case could enhanced aggregation be demonstrated. In interpreting these results, we have considered whether the defect in platelet function was due to abnormalities in the plasma or in the platelet itself. Reduced aggregation has been reported for platelets from patients with Laennec's cirrhosis and was thought to be due to increased fibrinogen degradation products (FDP) in the plasma (25). High levels of FDP were not found in our patients, suggesting that there had been no substantial activation of the coagulation system *in vivo*. A comparison of the aggregation results from cirrhotic patients with those from patients with obstructive jaundice supported this conclusion; no major differences were noted between these two groups even though the cirrhotic patients were likely to have had some activation of their coagulation system as a consequence of inflammatory liver disease (26). It is unlikely that other plasma factors caused decreased aggregation since addition of

patient platelet-poor plasma to normal platelets did not decrease aggregation. These observations suggest that the main platelet defect in liver disease lies within the platelet itself, a conclusion consistent with the observations of other workers (27). One explanation for such a defect is splenomegaly; the spleen preferentially sequesters the larger and hemostatically more active platelets and so its enlargement could explain both reduced aggregation and thrombocytopenia in liver disease (27). However, grouping our patients into those with and without splenomegaly or into those with and without decreased platelet counts did not reveal any obvious differences in their platelet aggregability.

A second possible explanation for platelet dysfunction in liver disease is an abnormality in their lipid composition. Physiological platelet function is controlled by stimulatory and inhibitory pathways and *in vitro* measurement of platelet aggregation is an artificial and relatively crude method of monitoring *in vivo*

TABLE 4. Correlation coefficients between platelet lipids and platelet aggregation in patients with liver disease^a

Variables (No.)	Spearman's Rank Correlation Coefficient (<i>r_s</i>)	Significance
Cholesterol, ADP (27)	-0.05	N.S. ^b
Cholesterol, adrenaline (27)	-0.29	N.S.
C/P, ADP (27)	-0.41	<i>P</i> < 0.05
C/P, adrenaline (27)	-0.41	<i>P</i> < 0.05
PC/SM, ADP (23)	-0.25	N.S.
PC/SM, adrenaline (23)	-0.58	<i>P</i> < 0.01
%20:4, ADP (13)	0.52	<i>P</i> < 0.05
%20:4, adrenaline (13)	0.70	<i>P</i> < 0.01

^a Aggregation was measured as the maximum percentage decrease in absorbance within 3 min of the addition of ADP or adrenaline (final concentrations 2 μmol/liter) for the number of subjects given in parentheses.

^b N.S., not significant; C/P, cholesterol/phospholipid molar ratio; PC/SM, phosphatidylcholine/sphingomyelin molar ratio; 20:4, arachidonic acid.

function. Aggregating agents such as ADP and adrenaline bind to receptors on the plasma membrane of the platelet and the ensuing aggregation is associated with activation of a specific phospholipase A₂ which releases arachidonic acid from certain membrane phospholipids (28–30). The free arachidonic acid is rapidly converted via the cyclic endoperoxides, prostaglandin G₂ and prostaglandin H₂, to thromboxane A₂. The latter, probably by inhibiting membrane-bound adenylate cyclase and so decreasing cAMP concentration, induces the release of intrinsic ADP, and other substances, which sustains platelet aggregation leading to formation of a primary hemostatic plug (31). Opposing these effects are a number of substances which degrade or neutralize the aggregating agents or which elevate the levels of cAMP, a potent inhibitor of platelet aggregation *in vitro*. In our patients the ADP- and adrenaline-induced platelet aggregation were inversely correlated with the platelet cholesterol/phospholipid and phosphatidylcholine/sphingomyelin ratios and positively correlated with the proportion of arachidonic acid. Any of these changes in platelet lipid composition might affect the stimulatory or inhibitory pathways in platelet aggregation.

Shattil et al. (10) reported an increase of 8% in the cholesterol/phospholipid ratio of platelets from patients with Type II hyperlipoproteinemia; the same authors raised the cholesterol content of normal platelets by *in vitro* incubation with cholesterol-rich liposomes (8). In contrast to our results, platelets from both these studies exhibited increased sensitivity to the aggregating agents ADP and adrenaline although the response to collagen and thrombin was unchanged. We have confirmed that normal platelets aggregate more readily with ADP and adrenaline after enrichment with cholesterol, and we have also shown that aggregation of cholesterol-poor platelets is inhibited.² The reason for the increased sensitivity of cholesterol-rich platelets to aggregating agents is not clear. Initially it was suggested (9) that a decrease in membrane fluidity, associated with the increase in cholesterol/phospholipid ratio, might affect receptor function by a change in either the lateral mobility and clustering of receptors or in their vertical orientation, all of which may affect binding of the ligand (32–35). However, Insel et al. (36) established that neither the number nor affinity of the α -adrenergic receptors depend on the platelet's cholesterol content. The same group of investigators has reported that cholesterol-rich platelets had an increased level of basal adenylate cyclase while hormonally responsive adenylate cyclase was decreased (37). However, they have been unable to con-

firm this observation (36) and now consider that cholesterol incorporation has little effect on adenylate cyclase and that the associated increase in aggregation is not a result of decreased cAMP production. More recently it has been suggested that the increased aggregation of cholesterol-rich platelets is a consequence of enhanced release of arachidonic acid from phospholipids and its increased conversion to thromboxane A₂ (38). This proposal is attractive but other interpretations of the data are possible (39).

Phospholipid composition may also influence membrane fluidity (40, 41), while specific phospholipids have been implicated in the coupling of receptors to adenylate cyclase (42, 43). Platelets from our patients, unlike those studied by Shattil et al. (8, 10), had a significant increase in their phosphatidylcholine/sphingomyelin ratio; this would be expected (40, 41) to oppose the decrease in platelet membrane fluidity associated with a raised cholesterol/phospholipid ratio (9). We did not measure platelet membrane fluidity in the present study, but in erythrocytes from patients with liver disease we have shown that it is the increased cholesterol/phospholipid ratio, rather than changes in phospholipid composition, that determines the extent to which membrane fluidity is decreased (5). This suggests that the membrane fluidity in platelets from our patients would be decreased in a similar manner to those studied by Shattil and co-workers even though there were changes in their phospholipid composition; it would also suggest that the observed differences in platelet aggregability between the various types of cholesterol-rich platelets cannot be explained by differences in membrane fluidity.

Platelet fatty acid composition was abnormal in our patients. This was not measured in the studies of Shattil et al. (8, 10) but it is likely that it was normal since they studied either normal subjects or patients with Type IIa hyperlipoproteinemia in whom the fatty acid composition of the plasma lipoproteins is not markedly abnormal (44). The cellular content and release of arachidonic acid from phospholipids are considered to be rate-limiting factors in prostaglandin and thromboxane A₂ synthesis (29, 45, 46). The 24% decrease in arachidonic acid seen in the platelets of our patients may therefore have reduced its availability for thromboxane A₂ synthesis and so led to decreased platelet aggregation. However, neither the specific phospholipids utilized nor the mechanisms involved are yet established, although some of the discrepancies reported may be explained either by the use of different subcellular fractions and aggregating agents or by the fact that the distribution of radioactive arachidonic acid in prelabeled platelets may differ from its endogenous distribution (29, 30, 39). Current evidence sug-

² Hutton, R. A., and J. S. Owen, Unpublished experiments.

gests that there are two pathways through which arachidonic acid can be released from platelet phospholipids. One is a phospholipase A₂ activity, acting predominantly on diacyl ethanolamine phosphoglyceride (30, 47) and to a lesser extent on phosphatidylcholine, to yield arachidonic acid and the corresponding lysophospholipids. The second pathway initially involves the action of a phosphatidylinositol-specific phospholipase C (48–50); the liberated diglyceride may then be acted on either by a diglyceride lipase to yield arachidonic acid (49) or by a diglyceride kinase to form phosphatidic acid (30, 51). The exact balance between these two pathways is not yet clear. However, it is likely that both pathways of arachidonic acid release were affected in our patients: the proportion of arachidonic acid in the diacyl ethanolamine phosphoglyceride and phosphatidylcholine fractions of our patients was reduced; the general decrease of arachidonic acid found throughout the major phospholipid classes suggests it would be reduced in phosphatidylinositol, although insufficient patient platelets were available to actually measure its content in this fraction.

The exact role of platelet lipid composition in affecting platelet aggregation remains to be established. However, in our patients with liver diseases, the effects of the altered phospholipid and fatty acid composition must have overridden those of the increased cholesterol content so that, instead of increased aggregation, only reduced or normal aggregation was seen. Whether these effects can indeed be attributed to decreased arachidonic acid availability must await further investigation. ■

We thank Professor J. A. Lucy for his interest in this study and the provision of laboratory facilities, Professor Dame Sheila Sherlock for allowing us to study patients under her care, and Mr. J. Morrison for the platelet fatty acid measurements. J.S.O. thanks the Wellcome Trust for an Inter-Disciplinary Research Fellowship and R.C.D., the Prophit Fund and the British Heart Foundation for research scholarships.

Manuscript received 22 August 1979 and in revised form 14 October 1980.

REFERENCES

- Cooper, R. A. 1970. Lipids of human red cell membrane; normal composition and variability in disease. *Semin. Hematol.* **7**: 296–321.
- Cooper, R. A., M. Diloy-Puray, P. Lando, and M. S. Greenberg. 1970. An analysis of lipoproteins, bile acids, and red cell membranes associated with target cells and spur cells in patients with liver disease. *J. Clin. Invest.* **51**: 3182–3192.
- Owen, J. S., R. A. Hutton, M. J. Hope, D. S. Harry, K. R. Bruckdorfer, R. C. Day, N. McIntyre, and J. A. Lucy. 1978. Lecithin:cholesterol acyltransferase deficiency and cell membrane lipids and function in human liver disease. *Scand. J. Clin. Lab. Invest.* **38**: Suppl. 150, 228–232.
- Vanderkooi, J., S. Fischkoff, B. Chance, and R. A. Cooper. 1974. Fluorescent probe analysis of the lipid architecture of natural and experimental cholesterol-rich membranes. *Biochemistry.* **13**: 1589–1595.
- Owen, J. S., K. R. Bruckdorfer, and N. McIntyre. 1979. Decreased membrane fluidity in erythrocytes from patients with liver disease. *Biochem. Soc. Trans.* **7**: 1272–1274.
- Owen, J. S., and N. McIntyre. 1978. Erythrocyte lipid composition and sodium transport in human liver disease. *Biochim. Biophys. Acta.* **510**: 168–176.
- Hope, M. J., K. R. Bruckdorfer, J. S. Owen, and J. A. Lucy. 1977. Chemically induced cell fusion in vitro of erythrocytes from patients with liver diseases. *Biochem. Soc. Trans.* **5**: 1144–1146.
- Shattil, S. J., R. Anaya-Galindo, J. Bennett, R. W. Colman, and R. A. Cooper. 1975. Platelet hypersensitivity induced by cholesterol incorporation. *J. Clin. Invest.* **55**: 636–643.
- Shattil, S. J., and R. A. Cooper. 1976. Membrane microviscosity and human platelet function. *Biochemistry.* **15**: 4832–4837.
- Shattil, S. J., J. S. Bennett, R. W. Colman, and R. A. Cooper. 1977. Abnormalities of cholesterol-phospholipid composition in platelets and low-density lipoproteins of human hyperbetalipoproteinemia. *J. Lab. Clin. Med.* **89**: 341–353.
- Hardisty, R. M., R. A. Hutton, D. Montgomery, S. Richards, and H. Trebilcock. 1970. Secondary platelet aggregation: a quantitative study. *Brit. J. Haematol.* **19**: 307–319.
- Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497–503.
- Rose, H. G., and M. Oklander. 1965. Improved procedure for the extraction of lipids from human erythrocytes. *J. Lipid Res.* **6**: 428–431.
- Rudel, L. L., and M. D. Morris. 1973. Determination of cholesterol using *o*-phthalaldehyde. *J. Lipid Res.* **14**: 364–366.
- Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**: 466–468.
- Nelson, G. J. 1972. Quantitative analysis of blood lipids. In *Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism*. G. J. Nelson, editor. Wiley-Interscience, New York. 25–73.
- Cohen P., and A. Derksen. 1969. Comparison of phospholipid and fatty acid composition of human erythrocytes and platelets. *Brit. J. Haematol.* **17**: 359–371.
- Born, G. V. R. 1962. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature.* **194**: 927–929.
- Holmsen, H., I. Holmsen, and A. Bernardsen. 1966. Microdetermination of adenosine diphosphate and adenosine triphosphate in plasma with the firefly luciferase system. *Anal. Biochem.* **17**: 456–473.
- Denson, K. W. E., and R. Briggs. 1976. Laboratory diagnosis, tests of clotting function and their standardization. In *Human Blood Coagulation, Haemostasis and Thrombosis*. R. Briggs, editor. 2nd ed. Blackwells, Oxford. 310–364.

21. Seigel, S. 1956. *In Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill Inc., New York. 202-213.
22. Cooper, R. A. 1977. Abnormalities of cell-membrane fluidity in the pathogenesis of disease. *N. Engl. J. Med.* **297**: 371-377.
23. Neerhout, R. C. 1968. Abnormalities of erythrocyte stromal lipids in hepatic diseases. *J. Lab. Clin. Med.* **71**: 438-447.
24. Deykin, D. 1976. Platelet lipids and platelet function. *In Platelets in Biology and Pathology*. (Research Monographs in Cell and Tissue Physiology, vol. 1. J. T. Dingle, gen. editor.) J. L. Gordon, editor. North-Holland, New York. 111-119.
25. Thomas, D. P., V. J. Ream, and R. K. Stuart. 1967. Platelet aggregation in patients with Laennec's cirrhosis of the liver. *N. Engl. J. Med.* **276**: 1344-1348.
26. Ballard, H. S., and A. J. Marcus. 1976. Platelet aggregation in portal cirrhosis. *Arch. Intern. Med.* **136**: 316-319.
27. Rubin, M. H., M. J. Weston, P. G. Langley, Y. White, and R. Williams. 1979. Platelet function in chronic liver disease: relationship to disease severity. *Dig. Dis. Sci.* **24**: 197-202.
28. Bills, T. K., J. B. Smith, and M. J. Silver. 1977. Selective release of arachidonic acid from the phospholipids of human platelets in response to thrombin. *J. Clin. Invest.* **60**: 1-6.
29. Marcus, A. J. 1978. The role of lipids in platelet function: with particular reference to the arachidonic acid pathway. *J. Lipid Res.* **19**: 793-826.
30. Brockman, M. J., J. W. Ward, and A. J. Marcus. 1980. Phospholipid metabolism in stimulated human platelets. Changes in phosphatidylinositol, phosphatidic acid, and lysophospholipids. *J. Clin. Invest.* **66**: 275-283.
31. Caen, J. P., S. Cronberg, and P. Kubisz. 1977. *In Platelets: Physiology and Pathology*. Stratton Intercontinental, New York. 34-45.
32. Brûlet, P., and H. M. McConnell. 1976. Lateral hapten mobility and immunochemistry of model membranes. *Proc. Natl. Acad. Sci. USA.* **73**: 2977-2981.
33. Borochoy, H., and M. Shinitzky. 1976. Vertical displacement of membrane proteins mediated by changes in microviscosity. *Proc. Natl. Acad. Sci. USA.* **73**: 4526-4530.
34. Armond, P. A., and L. A. Staehelin. 1979. Lateral and vertical displacement of integral membrane proteins during lipid phase transition in *Anacystis nidulans*. *Proc. Natl. Acad. Sci. USA.* **76**: 1901-1905.
35. Ganguly, P., and N. L. Gould. 1979. Receptor aggregation: a possible mechanism of platelet stimulation by thrombin. *Thromb. Res.* **15**: 879-884.
36. Insel, P. A., P. Nirenberg, J. Turnbull, and S. J. Shattil. 1978. Relationships between membrane cholesterol, α -adrenergic receptors, and platelet function. *Biochemistry.* **17**: 5269-5274.
37. Sinha, A. K., S. J. Shattil, and R. W. Colman. 1977. Cyclic AMP metabolism in cholesterol-rich platelets. *J. Biol. Chem.* **252**: 3310-3314.
38. Stuart, M. J., J. M. Gerrard, and J. G. White. 1980. Effect of cholesterol on production of thromboxane B₂ by platelets in vitro. *N. Engl. J. Med.* **302**: 6-10.
39. Owen, J. S., R. A. Hutton, and N. McIntyre. 1980. Platelet cholesterol content and platelet aggregation. *N. Engl. J. Med.* **302**: 1424.
40. Borochoy, H., P. Zahler, W. Wilbrandt, and M. Shinitzky. 1977. The effect of phosphatidylcholine to sphingomyelin mole ratio on the dynamic properties of sheep erythrocyte membrane. *Biochim. Biophys. Acta.* **470**: 382-388.
41. Cooper, R. A., J. R. Durocher, and M. H. Leslie. 1977. Decreased fluidity of red cell membrane lipids in abetalipoproteinemia. *J. Clin. Invest.* **60**: 115-121.
42. Houslay, M. D., T. R. Hesketh, G. A. Smith, G. B. Warren, and J. C. Metcalfe. 1976. The lipid environment of the glucagon receptor regulates adenylate cyclase activity. *Biochim. Biophys. Acta.* **436**: 495-504.
43. Birnbaumer, L. 1973. Hormone-sensitive adenyl cyclases. Useful models for studying hormone receptor functions in cell-free systems. *Biochim. Biophys. Acta.* **300**: 129-158.
44. Blaton, V. H., and H. Peeters. 1972. Integrated approach to plasma lipid and lipoprotein analysis. *In Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism*. G. J. Nelson, editor. Wiley-Interscience, New York. 275-314.
45. Hong, S. L., G. Patton, and D. Deykin. 1979. Arachidonic acid level in cellular lipids determines the amount of prostaglandins synthesized during cell growth in tissue culture. *Prostaglandins.* **17**: 53-59.
46. Trugnan, G., G. Bereziat, M-C. Manier, and J. Polonovski. 1979. Phospholipase activities in subcellular fractions of human platelets. *Biochim. Biophys. Acta.* **573**: 61-72.
47. Jesse, R. L., and P. Cohen. 1976. Arachidonic acid release from diacyl phosphatidylethanolamine by human platelet membranes. *Biochem. J.* **158**: 282-287.
48. Rittenhouse-Simmons, S. 1979. Production of diglyceride from phosphatidylinositol in activated human platelets. *J. Clin. Invest.* **63**: 580-587.
49. Bell, R. L., D. A. Kennerly, N. Stanford, and P. W. Majerus. 1979. Diglyceride lipase: a pathway for arachidonate release from human platelets. *Proc. Natl. Acad. Sci. USA* **76**: 3238-3241.
50. Bell, R. L., and P. W. Majerus. 1980. Thrombin-induced hydrolysis of phosphatidylinositol in human platelets. *J. Biol. Chem.* **255**: 1790-1792.
51. Call, F. L., II, and M. Rubert. 1973. Diglyceride kinase in human platelets. *J. Lipid Res.* **14**: 466-474.