

Platelet function and platelet lipid composition in the dyslipoproteinemias¹

Kamini M. Shastri, Angelina C. A. Carvalho,² and Robert S. Lees

Arteriosclerosis Center, Massachusetts Institute of Technology, Cambridge, MA;
Coagulation Research Laboratory and Cardiac Unit, Massachusetts General Hospital, and
Department of Medicine, Harvard Medical School, Boston, MA

Abstract Blood platelets from eight patients with hyperbetalipoproteinemia (type II) were more reactive to aggregating agents in vitro than those of 13 type IV patients or 12 normal subjects. Platelets of two patients with abetalipoproteinemia were also slightly hyperreactive in comparison with normal platelets. However, in one patient with Tangier disease the platelets were distinctly hyporeactive to aggregating agents. Total platelet phospholipid concentration (PL) was elevated in the four groups of patients studied but was highest in the platelets of type IV patients. Platelet-free cholesterol (FC) was significantly higher than normal in all of the dyslipoproteinemias studied. The FC was highest in the two patients with abetalipoproteinemia. The FC/PL molar ratio was normal in all but the abetalipoproteinemic patients, in whom it was markedly elevated. Therefore, there is no apparent correlation between FC/PL molar ratio and platelet behavior in vitro. Analysis of individual platelet phospholipids in the four patient groups showed that platelets in Tangier disease had very low concentrations of lysolecithin and phosphatidylinositol (PI) in comparison with normal platelets and the other disease states. Our findings suggest that lysolecithin and phosphatidylinositol may be involved in the structure or function of the sites which modulate platelet response to aggregating agents. — **Shastri, K. M., A. C. A. Carvalho, and R. S. Lees.** Platelet function and platelet lipid composition in the dyslipoproteinemias. *J. Lipid Res.* 1980. **21**: 467–472.

Supplementary key words abetalipoproteinemia · hyperlipoproteinemia · Tangier disease · phospholipids

The incidence of atherosclerosis and its thrombotic complications is much increased in certain types of hyperlipoproteinemia (1, 2), while in some lipoprotein deficiency states atherosclerosis is uncommon if it occurs at all (2). Since the blood platelets appear to play an important role in initiating the thrombotic complications of atherosclerosis and may be involved in atherogenesis (3, 4), we and others have investigated platelet function in the disorders of lipoprotein metabolism. Platelets from patients with hyperbetalipoproteinemia have increased sensitivity in vitro to aggregating agents, as well as increased nucleotide release (5), while platelets from patients with hypertriglycerid-

emia exhibit normal in vitro reactivity to ADP and collagen and hypersensitivity only to epinephrine.

Investigations into the mechanism of these changes in platelet behavior suggest that platelet composition may be closely related to platelet function. Enrichment of platelets with cholesterol in vitro, for instance, increases their sensitivity to aggregation (6), while cholesterol feeding to primates shortens platelet half-life in vivo (7).

Since tissue lipid abnormalities are usually more striking in the hypolipidemic states than in the hyperlipidemias, we have investigated platelet function in two lipoprotein deficiency diseases, abetalipoproteinemia and Tangier disease, compared it with the results of similar studies in the common Type II and Type IV hyperlipoproteinemias, and have related platelet function in all four groups of patients to platelet lipid composition.

MATERIALS AND METHODS

Patient selection

Two patients with well-documented abetalipoproteinemia (2) had complete absence of beta and pre-beta lipoproteins and chylomicrons from their plasma. Eight patients (Type II according to the Frederickson-Lees classification (8)) (five males and three females) were studied; all had at least one first-degree relative

Abbreviations: PL, phospholipid; FC, free cholesterol; PI, phosphatidylinositol; Type II, familial hypercholesterolemia; Type IV, hyperprebetalipoproteinemia; PS, phosphatidylserine; SF, solvent front; SM, sphingomyelin; PE, phosphatidylethanolamine; LL, lysolecithin; PRP, platelet-rich plasma; TLC, thin-layer chromatography.

¹ This work was presented in part at the annual meeting of The Federation of American Societies for Experimental Biology, April 1976, and in part at the American Heart Association Meeting, November, 1976.

² Present address: Veterans Administration Hospital, 111, Davis Park, Providence, RI 02908.

with either hypercholesterolemia or xanthomatosis. Each patient had more than one lipoprotein electrophoretic pattern characteristic of type II.

Eleven patients (all males) with familial hyperprebetalipoproteinemia (Type IV) were selected after documentation of an increase in plasma triglycerides and prebetalipoproteins with normal plasma cholesterol and beta lipoprotein concentrations.

Thirteen normal individuals (seven males and six females) were selected from healthy laboratory personnel. They were known to have normal platelet function and normal plasma lipid and lipoprotein concentrations.

One patient with well-documented Tangier disease (9) and complete absence of normal alpha lipoproteins was studied.

All patients and normal subjects had not taken any medications for at least 15 days prior to blood collection. Blood was withdrawn after a 12-hr fast. All were on free normal diet at time of study.

Preparation of platelets and plasma for aggregation

Blood was collected through siliconized needles into plastic syringes. Nine volumes of blood were added to one volume of 3.8% aqueous sodium citrate in plastic tubes. Samples were centrifuged at 23°C for 10 min at 100 *g*; the resultant platelet-rich plasma (PRP) had a platelet count of 200,000 to 250,000 per microliter. On microscopic examination, PRP had <1% red blood cells and only an occasional leukocyte. The remaining blood was centrifuged at 3000 *g* for 15 min at 23°C. The platelet count of this platelet-poor plasma (PPP) was below 20,000 per microliter. Platelets were analyzed within 30 min of blood collection.

Platelet aggregation

Platelet aggregation was studied as described previously, according to a modification of the method of Born (5, 10). Adenosine diphosphate and 1-epinephrine were obtained from Sigma Chemical Co., St. Louis, MO and soluble calfskin collagen from Worthington Biochemical Co., Freehold, NJ, and prepared as described previously (5). The aggregometer (Chronolog Corp., Broomall, PA) was set up so that the percent transmittance of platelet-rich plasma was recorded as 0, and that of platelet-poor plasma as 100. Each aggregating agent was tested to determine the lowest concentration capable of inducing pen deflection from 0 to maximum transmittance. The maximum transmittance to each aggregating agent, which included the first and second wave of aggregation, was defined as "full response" and ranged from 65 to 100% in the normal individuals.

Preparation of platelets for lipid analysis

Sixty to 80 ml of blood was drawn from each subject into disodium EDTA at a final concentration of 7 mM. Platelet-rich plasma was separated by centrifugation at 23°C for 10 min at 100 *g*. The aliquots of PRP were combined in a 50-ml round bottom polystyrene tube and centrifuged at 4°C for 30 min at 3000 *g*. The resulting platelet pellet was resuspended in 20–30 ml of modified Ringer's solution (KCl 4 mM, NaCl 107 mM, NaHCO₃ 20 mM, Na₂SO₄ 2 mM, EDTA 2 mM, 1% dextrose; pH 7.1–7.2). Platelet count of the pellet ranged from 3 to 5 × 10⁹. The platelets were washed three times and frozen at –30°C for no longer than 2 weeks under nitrogen until lipid extraction. Platelet concentrates obtained from two units of whole blood were prepared as described above and phospholipids were fractionated by thin-layer chromatography to confirm *R_f* of minor platelet phospholipids including phosphatidylinositol and lysolecithin. These platelet pellets were extracted immediately and after 3, 6, and 12 weeks of storage at –30°C to test the effects of storage.

Platelet lipid analysis

Lipid extraction of the washed platelet pellets was carried out by the method of Folch, Lees, and Sloane Stanley (11) using redistilled chloroform and methanol with BHT (50 mg/liter) as an antioxidant. Extracts were filtered through solvent-washed filter paper; an aqueous wash was not performed to avoid loss of polar phospholipids. Because only a single patient with Tangier disease and two with abetalipoproteinemia were studied, the lipid analyses for each of those three patients were performed on three separate occasions on platelets from blood drawn especially for each analysis.

Free cholesterol was determined by the method of Rudel and Morris (12). Some samples were analyzed by Auto Analyzer (13) as well; the results were essentially identical. Lipid phosphorus was estimated by Bartlett's method (14).

Thin layer chromatography

Phospholipid separation was carried out by one-dimensional thin-layer chromatography (TLC), in CHCl₃–CH₃OH–CH₃COOH–H₂O 100:60:16:8 (15). Precoated silica gel H TLC plates (20 × 30 cm × 0.26 mm, Applied Science Laboratories, State College, PA) and individually prepared plates gave compatible results. Thin-layer chromatography plates were prewashed with the developing solvent before use, activated at 110°C for 60 min, then cooled in a desiccating cabinet. The tank was saturated with the develop-

ing solvent for 60 min before use. Samples were applied with prewashed disposable plastic micro-applicators (Applied Science Laboratories). Phospholipid standards (obtained from Serdary Res. Lab., Ltd., London, Ontario, Canada) were run in parallel with the samples. After running at least 15 cm the plates were air-dried and the phospholipid bands were visualized in an iodine chamber. Each spot was then scraped from the plate by the method of Parker and Peterson (16) for determination of lipid phosphorus. Solvent blanks and blank areas from each TLC plate were used as controls.

Statistical analysis

The compositional data was analyzed by Student's *t* test with a Bessel correction for small sample size. All lipid results are expressed as mean \pm SD. Since the concentrations of aggregating reagents were distributed lognormally (5), the *t* test for platelet aggregation data was performed on the logarithm of reagent concentration.

RESULTS

Plasma lipid composition

The patients with abetalipoproteinemia had low plasma cholesterol concentrations and essentially no plasma triglycerides, as expected (Table 1). The patient with Tangier disease had a low normal plasma cholesterol and elevated triglycerides, as is characteristic of that disease. The concentration of total and LDL cholesterol in the plasma of the type II patients had significantly elevated plasma triglycerides.

Platelet aggregation

The results of platelet aggregation studies are shown in Fig. 1. In contrast to the response of the normal platelets, the platelets of the patient with Tangier disease aggregated only to the extent of 25% (no second wave), even with concentrations of ADP (20, 40 μ M) up to 10-fold greater than required to aggregate normal platelets. Similar results were obtained with epinephrine. Even at concentrations of 25 and 50 μ M, aggregation was not complete. However, the platelets of the patient with Tangier disease aggregated normally in response to collagen. The differences from normal were even more marked when $\geq 20\%$ aggregation was used as an end point (17).

The platelets from the abetalipoproteinemic patient aggregated normally to ADP were borderline hyperreactive to epinephrine and collagen. Platelets from subjects with Types II and IV hyperlipoproteinemia behaved as described previously (5); the Type II

TABLE 1. Characteristics of the patients studied

Group	Number of Subjects	Total Plasma Cholesterol	Plasma Triglycerides
		<i>mg/100 ml</i>	
Normal	13	184 \pm 23 ^a	65 \pm 29
Tangier disease	1	42 \pm 8	195 \pm 11
Abetalipoproteinemia	2	34 \pm 4	0
Type II	8	334 \pm 71	105 \pm 57
Type IV	11	171 \pm 30	297 \pm 94

^a Mean \pm 1 SD.

patients' platelets were hyperreactive to all three aggregating agents, while those of Type IV patients were hyperreactive only to epinephrine.

Platelet lipid composition

The lipid analysis of platelets from normal and dyslipoproteinemic subjects is shown in Table 2. Platelet-free cholesterol in the normal group was 65 \pm 4.2 μ g/10⁹ platelets. Unlike the plasma, where most of the cholesterol is esterified, essentially all the platelet cholesterol is in the free form (18). Platelet cholesterol was significantly higher than normal in all the dyslipoproteinemias and was highest in the two patients with abetalipoproteinemia (*P* < 0.001). Total platelet phospholipids were also elevated in all four patient groups. The free cholesterol to phospholipid molar ratio was normal, except in the abetalipoproteinemic patients, in whom it was markedly high.

Phospholipid fractionation

When the platelet concentrate prepared from one unit of blood was applied to a TLC plate, complete separation of all major phospholipids was obtained. In particular, there was good resolution between phosphatidylinositol (PI) and phosphatidylserine (PS). Table 3 shows the platelet phospholipid composition for each of the four disease states. In absolute terms the amount of each phospholipid per platelet was increased almost across the board. The deviations from normal in platelet phospholipid distribution are much greater in the two lipoprotein deficiency states than they are in the two syndromes of lipoprotein excess. In Tangier disease, lysolecithin and phosphatidylinositol are uniquely low, not only as a percent of platelet phospholipid but also in absolute amount of those components per platelet.

Platelets of both type II and IV subjects (Table 3) had a significant increase in phosphatidylinositol (PI) and the minor lipids that are at the solvent front (SF), compared with the normal. Relatively small decreases in sphingomyelin (SM) in type II and phosphatidylserine (PS) in type IV patients were found. In the

PLATELET AGGREGATION IN FOUR DYSLIPOPROTEINEMIAS

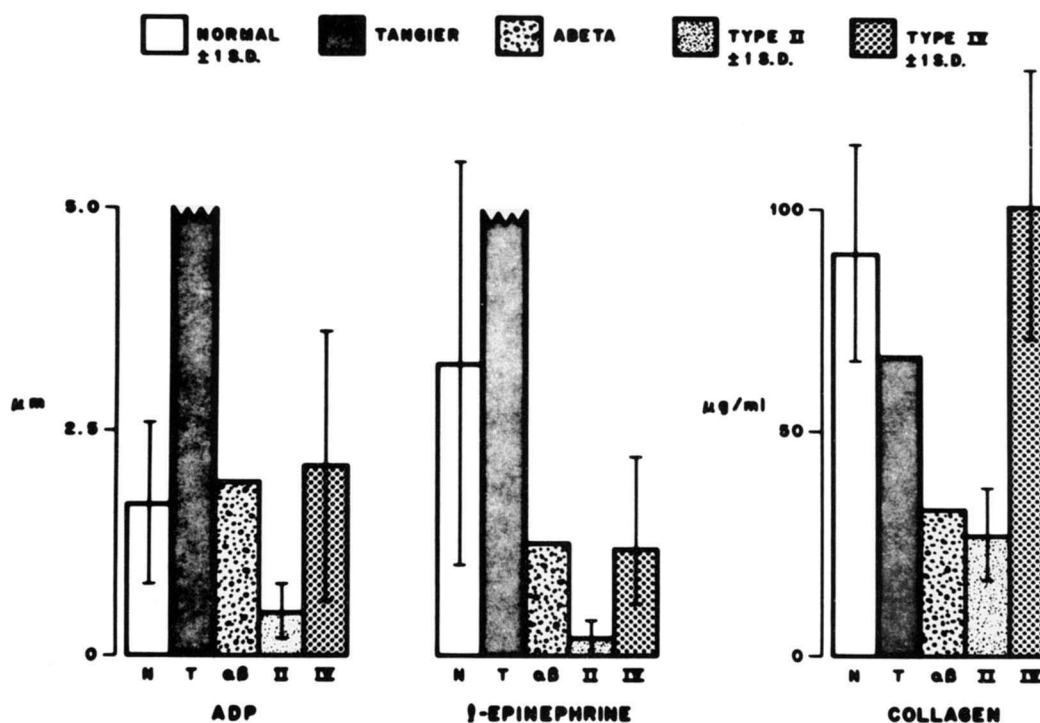


Fig. 1. Platelet aggregation in four dyslipoproteinemias. The bars labeled N represent values obtained in normal individuals, those labeled T, the patient with Tangier disease, $\alpha\beta$, the patients with abetalipoproteinemia, II, those with familial hyperbetalipoproteinemia, and IV, those with familial hypertriglyceridemia. The left-hand set of bars represents the threshold concentrations for complete ADP-induced aggregation, except in the Tangier patient (see text), the middle set that to epinephrine, and the right-hand set the response to collagen. The standard deviations shown by the error bars were calculated on the logarithm of reagent concentrations, as explained in the Methods section.

patient with Tangier disease the platelets had a significant increase in PS, PE and SF lipids and a significant decrease in PI, SM and LL compared with normal.

DISCUSSION

Platelets of patients with hyperbetalipoproteinemia (Type II) are hyperreactive to all aggregating agents, while those of type IV patients were hyperreactive only to epinephrine, when compared with normal platelets. In marked contrast, the platelets from the patient with Tangier disease were unreactive to ADP or epinephrine but exhibited normal aggregation and complete release to collagen. In abetalipoproteinemia, aggregation to ADP was within the normal range, while that to epinephrine was similar to type IV and that to collagen similar to the platelets of patients with type II. This is a surprising result since abetalipoproteinemia is characterized by complete absence

of both beta and prebeta lipoproteins, while type II and IV hyperlipoproteinemia imply an excess of one or the other of these lipoprotein species.

We studied platelet lipid composition in an effort to explain the platelet functional findings, expecting to find some reflection in platelet lipids of the striking lipoprotein differences among the four groups of patients. Unlike the plasma, where most of the cholesterol is esterified, cholesterol in platelets is almost all in the free form (18). Platelet cholesterol was significantly higher than normal in type II patients, as reported by Shattil et al. (6), but it was also high in the other diseases studied, and highest in the two patients with abetalipoproteinemia. Total phospholipids were elevated in all dyslipoproteinemias, but were highest in the type IV patients.

The platelet free cholesterol to phospholipid molar ratio was normal in all groups except the patients with abetalipoproteinemia, in whom it was markedly high. Therefore, no clear explanation for the aggregation abnormalities was forthcoming from these results.

TABLE 2. Platelet lipid composition

	Platelet Count	Phospholipid ^a	Free Cholesterol	Free Cholesterol Phospholipid
	$\times 10^9$	$\mu\text{g}/10^9$ platelets		molar ratio
Normal subjects (13) ^b	4.8 ± 2.0^c	9.2 ± 1.6	64.7 ± 14.6	0.54 ± 0.07
Tangier disease (1)	3.6^d	12.3 ± 0.62	98.6 ± 9.1	0.64 ± 0.05
Abetalipoproteinemia (2)	3.8^d	13.0 ± 0.16	239 ± 10.6	1.47 ± 0.05
Type II (8)	4.1 ± 1.3	14.7 ± 2.2^c	106.6 ± 28.3^c	0.58 ± 0.12
Type IV (13)	3.5 ± 1.9	20.9 ± 6.7^e	130.7 ± 47.9^e	0.52 ± 0.11

^a Lipid phosphorus.^b Number of subjects.^c Standard deviation.^d 3.6–3.9.^e $P < 0.001$.

Quantitation of the individual platelet phospholipids showed that the deviations from normal were much greater in the lipoprotein deficiency states than in the diseases of lipoprotein excess. The platelets of the patient with Tangier disease, which were hyporeactive to ADP and epinephrine were uniquely low in two different phospholipid classes—lysolecithin and phosphatidylinositol. Lysolecithin and phosphatidylinositol were low not only as a percent of total platelet phospholipid in Tangier disease, but also in absolute amount of these components per platelet. Despite wide variations in the relative composition of the platelet phospholipids in the other three groups of dyslipoproteinemias, platelet aggregation was normal or increased, as were the concentrations of LL and PI.

A direct relationship between platelet phospholipid distribution and platelet aggregation and release has been suggested (19) and demonstrated in vitro (20) in normal platelets. Bills, Smith, and Silver (21) have demonstrated that normal human platelets have a mechanism for incorporating the polyunsaturated fatty acid, arachidonic acid, from plasma into their phospholipids and, in response to the aggregating agent thrombin, mechanisms for hydrolyzing it from

platelet phosphatidylcholine and phosphatidylinositol. Similar data have been obtained by Rittenhouse-Simmons (22). Furthermore, Bills et al. (21) demonstrated that thrombin stimulates two types of platelet phospholipase A_2 activities, one which specifically releases arachidonic acid from PC and another which releases it from PI. Therefore it is possible that the increase in PI in our patients with heightened platelet response is an important determining factor of platelet hypersensitivity to aggregating stimuli. Alternatively, these platelets may have an increase in platelet phospholipase A_2 or abnormalities in fatty acid distribution in each phospholipid class. The latter hypothesis is now under evaluation.

It is possible that in hyperlipidemias the platelets contain more arachidonic acid in PI than in normal platelets. Since a number of oxygenated derivatives of arachidonic acid, including prostaglandin G_2 , H_2 and thromboxane A_2 , have potent effects on platelet function they are now considered to play an important role in hemostasis and thrombosis (23). Since only trace amounts of free arachidonic acid are found in platelets (18), that which is oxygenated during platelet aggregation must be released from platelet phospholipids.

TABLE 3. Platelet phospholipid composition

Origin	LL	SM	PC	PI	PS	PE	SF	
(% lipid phosphorus in phospholipids)								
Normal subjects (13) ^a	4.3 ± 1.2^b	5.5 ± 1.4	16.2 ± 1.3	25.8 ± 1.4	5.4 ± 1.2	14.2 ± 1.3	22.7 ± 2.5	5.5 ± 1.8
Tangier disease (1)	5.0 ± 0.6	2.8 ± 0.4	11.9 ± 0.5	26.1 ± 0.9	2.8 ± 0.4	17.1 ± 0.8	28.0 ± 0.8	6.2 ± 0.9
Abetalipoproteinemia (2)	6.0 ± 0.5	9.5 ± 0.5	13.9 ± 0.7	19.5 ± 0.4	9.6 ± 0.5	10.1 ± 0.6	23.7 ± 0.7	7.7 ± 0.5
Type II (8)	5.0 ± 1.6	5.9 ± 2.0	13.5 ± 1.0^c	25.3 ± 3.3	6.8 ± 1.8^d	13.8 ± 1.7	23.0 ± 1.7	6.7 ± 1.5
Type IV (13)	5.4 ± 1.9	5.6 ± 1.5	16.3 ± 1.3	25.4 ± 1.8	6.8 ± 1.4^e	12.4 ± 1.8^e	21.5 ± 1.8	6.6 ± 1.9

^a Number of subjects.^b Mean \pm SD.^c $P < 0.001$.^d $P < 0.05$.^e $P < 0.01$.

Stimulation of platelet phospholipid synthesis has been shown to occur after addition of the aggregating agents ADP (24), thrombin (20), and epinephrine (25). Hydrolysis of platelet membrane phospholipids is thought to be an integral step in the mechanisms of platelet release. Schick, Kurica, and Chacko (26) have proposed that "phospholipids" are situated near the active site or "receptor" on the platelet surface and function as modulators of the platelet release reaction. Binding of the aggregating agent with its receptor site may induce conformational changes in the adjacent phospholipases, rendering them catalytically active.

In summary, our data show that a marked decrease in the concentration of LL and PI in platelets of a patient with Tangier disease correlates with poor platelet response to ADP and epinephrine, while the increase in platelet reactivity in types II and IV hyperlipoproteinemia correlates best with increased platelet PI. A greater content of PI and possibly its fatty acid content in type II and type IV platelets might be related to the greater reactivity of these platelets in vitro. Perhaps more likely, platelet arachidonic acid content may be altered by the changes which we have observed in platelet phospholipids and these changes relate to the observed pathophysiological abnormalities. This hypothesis is now under investigation. ■

The authors gratefully acknowledge the excellent typing of the manuscript by Jean Charpentier. This work was supported in part by grants HL-23591-01 and HL-21460-02 from the National Heart and Lung Institute for a Specialized Center of Research in Arteriosclerosis.

REFERENCES

1. Lees, R. S., and D. E. Wilson. 1971. The treatment of hyperlipidemia. *N. Engl. J. Med.* **284**: 186-195.
2. Lees, R. S., D. E. Wilson, G. Schonfeld, and S. Fleet. 1973. The familial dyslipoproteinemias. *Prog. Med. Genet.* **10**: 237-290.
3. Haerem, J. W. 1971. Sudden coronary death; the occurrence of platelet aggregates in the epicardial arteries of man. *Atherosclerosis.* **14**: 417-432.
4. Mustard, J. F., and M. A. Packham. 1972. Platelets and thrombosis in acute myocardial infarction. *Hosp. Pract.* **713**: 115-128.
5. Carvalho, A. C. A., R. W. Colman, and R. S. Lees. 1974. Platelet function in hyperlipoproteinemia. *N. Engl. J. Med.* **290**: 434-438.
6. Shattil, S. J., R. Anaya-Galindo, J. Bennett et al. 1975. Platelet hypersensitivity induced by cholesterol incorporation. *J. Clin. Invest.* **55**: 636-643.
7. Ross, R., and L. Harker. 1976. Hyperlipidemia and atherosclerosis. *Science.* **193**: 1094-1110.
8. Frederickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. *N. Engl. J. Med.* **276**: 34-44.
9. Brook, J. G., R. S. Lees, J. H. Yules, and B. Cusack. 1977. Tangier Disease (α -lipoprotein deficiency). *J. Am. Med. Assoc.* **238**: 332-334.
10. Born, G. V. R. 1962. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature (London).* **194**: 927-929.
11. 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**: 497-509.
12. Rudel, L. L., and M. D. Morris. 1973. Determination of cholesterol using *O*-phthalaldehyde. *J. Lipid Res.* **14**: 364-366.
13. Block, W. D., K. J. Jarrett, Jr., and J. B. Levine. 1966. An improved automated determination of serum total cholesterol with a single color reagent. *Clin. Chem.* **12**: 681-689.
14. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**: 466-468.
15. Skipski, V. P., R. F. Peterson, and M. Barclay. 1964. Quantitative analysis of phospholipids by thin-layer chromatography. *Biochem. J.* **90**: 374-378.
16. Parker, F., and N. F. Peterson. 1965. Quantitative analysis of phospholipids and phospholipid fatty acids from silica gel thin-layer chromatograms. *J. Lipid Res.* **6**: 455-460.
17. Walsh, P. N., F. I. Pareti, and J. J. Corbett. 1976. Platelet coagulant activities and serum lipids in transient cerebral ischemia. *N. Engl. J. Med.* **296**: 854-858.
18. Marcus, A. J., H. L. Ullman, and L. B. Safier. 1969. Lipid composition of subcellular particles of human blood platelets. *J. Lipid Res.* **10**: 108-114.
19. Schick, P. K., and B. P. Yu. 1974. The role of platelet membrane phospholipids in the platelet release reaction. *J. Clin. Invest.* **54**: 1032-1039.
20. Lewis, N., and P. W. Majerus. 1969. Lipid metabolism in human platelets II. De novo phospholipid synthesis and the effect of thrombin on the pattern of synthesis. *J. Clin. Invest.* **48**: 2114-2123.
21. Bills, T. K., J. S. 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.
22. Rittenhouse-Simmons, S. 1979. Production of diglyceride from phosphatidylinositol in activated human platelets. *J. Clin. Invest.* **63**: 580-587.
23. Hamberg, M., J. Svensson, and B. Samuelsson. 1975. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. Nat. Acad. Sci. (USA).* **72**: 2994-2998.
24. Lloyd, J. V., E. E. Nishizawa, J. Halder, et al. 1972. Changes in 32 P-labeling of platelet phospholipids in response to ADP. *Br. J. Haematol.* **23**: 571-585.
25. Deykin, D., and D. Snyder. 1973. Effect of epinephrine on platelet lipid metabolism. *J. Lab. Clin. Med.* **82**: 554-559.
26. Schick, P. K., K. B. Kurica, and G. K. Chacko. 1976. Location of phosphatidylethanolamine and phosphatidylserine in the human platelet plasma membrane. *J. Clin. Invest.* **57**: 1221-1226.