Human Platelet Lipids and Their Relationship to Blood Coagulation

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Abstract

There is now reasonable agreement on the sequence of physiological and biochemical events leading to fibrin formation, and phospholipids are an important part of this process. The phospholipids are ordinarily provided by platelets, and phospholipids are an important part of this process. The phospholipids are an important part of this process. They are: a) blood coagulation; b) hemostasis; c) thrombosis. The tendency in the past to think of these as a single entity has led to confusion. For example, an increase in plasma lipids or postprandial lipemia was equated with a "hypercoagulable" state. This was presumed to be directly related to thrombosis. Arterial thrombi histologically resemble hemostatic platelet plugs in that there is a white "head" consisting of relatively intact platelets. Venous thrombi, on the other hand, more closely resemble clots as formed in the test tube. That is, they consist of a mixture of fibrin, entrapped red cells and leukocytes, as well as platelets. These are the so-called "red" thrombi. Thus, it is important to realize that these thrombi may not play a role in thrombosis and hemostasis, but may only be important for coagulation. These concepts have been discussed in detail in recent reviews of the subject (2-4).

Current Theory of Coagulation

Figure 1 shows the sequence of events leading to the formation of fibrin. Contact with a foreign surface, such as damaged endothelium or the cut edge of a blood vessel appears to activate Hageman factor. This in turn activates PTA and a pattern of biochemical transformations appears to ensue, which involves the activation of a previously inactive coagulation pathway.
protein (1,31). Note that the lipid contribution appears relatively late in the reactions of the intrinsic prothrombin activator system. Current thinking is that the lipid is derived from platelets which have already arrived at the site of blood vessel damage because of their role in hemostasis. Classical theories of platelet involvement in clotting propose that granules are discharged into the surrounding medium, which makes their lipid available to the coagulation process. Recent data in our own laboratory indicate that the platelet membrane has somewhat better clot-promoting capabilities than do the granules. We have proposed that the platelet granules are "storehouses" for metabolic substances concerned with other platelet functions, and the membrane lipid acts as a surface catalyst for coagulation. It is emphasized that these are merely theoretical proposals, and further investigation will be necessary before they can be considered unequivocal (5).

Conclusions drawn from studies on isolated platelet lipids must be considered in the light of their in vitro activity only. For example, phospholipids, especially those known whether this represents an artefact of the disruption, all the platelet lipids are probably available as a lipoprotein complex. In addition, when platelet homogenates are made, a soluble lipoprotein appears which possesses clot-promoting properties. It is not yet known whether this represents an artefact of the disruption procedure or whether the platelet actually contains soluble lipoprotein in an unbound form.

With these preliminary comments in mind, we would like to describe some of the methods utilized in our laboratory for the past ten years in the investigation of human platelet lipids.

**Methods and Materials**

**Methods of Collection and Lipid Extraction of Platelets**

Platelets were isolated from freshly collected blood (6). The appearance of platelets as seen by electron microscopy is illustrated in Fig. 2. They were washed and frozen within a period of 5 hr. Ordinarily, they were maintained at −20°C for periods up to 3 months prior to extraction. The subcellular platelet particles had a tendency to deteriorate at −20°C, as evidenced by discoloration and putrefaction. Therefore, the subcellular materials are now being stored at −85°C. Following venisection, all operations involved in platelet processing were carried out in a cold room at 4°C. Two main extraction procedures have been used. The first was based on the procedure of Bell and Alton (7). This involves suspension in four times the original volume of acetone for 30 min, following which the acetone was removed by centrifugation. This was repeated 4–6 times. The acetone-insoluble material was then extracted with ten times its volume of chloroform, and this was repeated twice, the third extraction being allowed to take place overnight. The yields appeared to be the same whether the procedure was carried out at 4°C or at room temp. This extraction procedure yielded less of the neutral lipid fraction and is still our procedure of choice for preparation of platelet or brain "cephalin" for use in blood coagulation tests as platelet substitutes.

In later studies, chloroform:methanol 2:1 was used. The procedures were always carried out under highly purified nitrogen, and the solvents were deoxygenated immediately before use (8). In this method, extraction of 1 g (wet weight) of platelet material yielded about 31 mg of crude lipid. The starting material

for each column separation was approximately 400 mg of lipid. Approx 13 g (wet weight) of platelets was homogenized in 150 ml chloroform-methanol in a Waring Blender under an atmosphere of nitrogen. The insoluble material was removed by filtration through a sintered glass filter and finally the filtrate was passed through fat-free shankskin filter paper. The filtrate was then dried under reduced pressure and nitrogen in an Erlenmeyer flask at 30°C. It was found useful to add aldehyde-free absolute ethanol to the flask, which formed a water-ethanol azeotrope, thus aiding in the removal of last traces of water (9). The lipid material was stored under nitrogen in C:M 2:1 for a max of 12 hr at −20°C prior to column chromatography.

**Column Chromatography**

In earlier work (6,10), the procedures of Lea, Rhodes and Stoll (11) were used, as well as those of Hirsch and Ahrens (5). Although the methods themselves were excellent, they did not serve our purpose as well as the procedures later developed by Rouser and associates. The reason for this was that there was an unfortunate overlapping between the ethanolamine and serine phosphoglycerides, and only small amounts of serine phosphoglycerides could be obtained. The clot-promoting properties of almost any combination of PE and PS were quite impressive, but we were mainly interested in the activity of individual phosphatides. Thus, we turned our attention to the techniques of Rouser (12,13) in our later studies. Although we have tried others, Mallinckrodt silicie acid 100 mesh has always appeared to be the most suitable. Usually 60 g of the material was washed as described by Rouser (12). The washed silicie acid was heated for 12–15 hr at 120°C under negative pressure and nitrogen in a