Technical methods

A simple method for the isolation of blood platelets

P. GANGULY AND W. J. SONNICHSEN *From the Laboratory of Haematology, St Jude Children's Research Hospital, Memphis, Tennessee, USA*

Investigations of the physiological and biochemical properties of blood platelets *in vitro* have been severely hindered due to the lack of a suitable method for their isolation. The platelets are fragile, they can be easily stimulated by a wide variety of agents leading to the release reaction and aggregation, also there are several plasma proteins loosely associated with the membrane. An ideal method for isolation should remove all foreign materials which are not an integral part of the platelet without causing any damage. Unfortunately, the conventional washing procedure causes gross and uncontrolled alteration of the platelets leading to variable results. The objective of this communication is to present a simple, economical, and reproducible method for isolating the platelets.

This method is based on density gradient separation of platelets using sodium metrizoate as a cushion. Sodium metrizoate (Hypaque, Nyegaard Co, Oslo, Norway) combined with Ficoll is extensively used for the density gradient isolation of lymphocytes (Boldt, Skinner, and Kornfeld, 1972; Lopes, Nachbar, Zucker-Franklin, and Silber, 1973).

Siliconized glass or plastic materials were used throughout this study. Normal blood from volunteer donors was collected in acid-citrate-dextrose (NIH formula A) in plastic bags and the red cells were removed by differential centrifugation. The platelet-rich plasma (PRP) was recovered in a plastic bag in less than one hour after collection.

Sodium metrizoate (32.8%), a clear, sterile liquid with a density of 1.2, is supplied in glass ampoules (Nyegaard Co, Oslo, Norway). This material was diluted with distilled water to a density of 1.1. In a typical experiment, about 35 ml of PRP was slowly layered on top of 10 ml of diluted metrizoate in a 50-ml polycarbonate centrifuge tube. The interface between the PRP and metrizoate was gently disturbed with a siliconized stirring rod made from a pasteur pipette to produce a density gradient approximately 1 cm wide (fig 1a). The tubes were

Received for publication 21 May 1973.

Fig. 1 Density gradient separation of platelets using sodium metrizoate. (a) 35 ml of PRP was layered over 10 ml of metrizoate and the interface was gently disturbed to produce a gradient. (b) After centrifugation the platelets separated into a band while the contaminating red cells went down to the bottom of the tube. (c) The platelets in Tyrode's solution after centrifugation.

centrifuged in an IEC centrifuge at $2500 \times g$ using a swing-out rotor for 15 minutes at room temperature. The platelets sedimented and formed a band in a region with an average density of 1.05 (fig 1b). The contaminating cells passed through the metrizoate and were pelleted at the bottom. The platelet band was removed, diluted with a modified Tyrode's solution free of calcium, and the density gradient separation was repeated with centrifugation for 10 minutes (fig 1c). This washing can be carried out as many times as desired. Sodium metrizoate reversibly inhibits thrombin and ADP-induced aggregation of the platelets. This property of metrizoate can be usefully exploited for the density gradient separation of the platelets without aggregates with an average yield of 70%.

The final preparation of the washed platelets was collected and diluted with autologous platelet-poor plasma (PPP), sufficient to restore the platelet concentration to approximately that of the original platelet-rich plasma. The residual metrizoate was rapidly (two hr) removed by dialysis against a large

Fig. 2 Aggregation curves of control PRP platelets (left column) and washed platelets (right column) with ADP and thrombin. (a) $5 \times 10^{-6} M$ ADP final concentration, (b) $10^{-5} M$ ADP, and (c) 0.5μ thrombin. The platelet concentration for the control and the washed platelets was 325 000 and 375 000/ μl respectively.

Technical methods

volume (1:200) of Tyrode's solution free of calcium. A control sample of PRP was handled in the same manner.

Under the phase microscope, the washed platelets retained their disc-like appearance and were indistinguishable from platelets in the PRP control. The washed platelets were virtually free of aggregates.

The aggregation properties of the platelets were studied in a Chronolog aggregometer under standard conditions. Figure 2 shows representative aggregation curves of the washed platelets and the PRP control produced by two different concentrations of ADP and one concentration of thrombin. The aggregation patterns are not significantly different. In contrast, conventionally prepared platelets washed by differential centrifugation show little response to ADP (Walsh, 1972).

The importance of obtaining a platelet preparation free of contaminating cellular elements and plasma proteins for physiological and biochemical studies has long been recognized. The initial efforts were directed towards modifying and improving the wash medium. However, it became apparent that methods based on differential centrifugation are not satisfactory and that a different approach was necessary (Mannucci, 1972). Tangen *et al* devised a method of isolation of the platelets based on gel filtration through Sepharose 2B while Walsh developed the density gradient method using a 45% albumin solution (Tangen, Berman, and Marfey, 1971;

Walsh, 1972). Both methods are superior to conventional differential centrifugation methods. However, these methods are costly, time consuming, and require a certain degree of experience. These drawbacks are magnified when large quantities of platelets must be processed. The metrizoate density gradient method provides a suitable alternative. Some changes in the platelets do occur in the isolation process; however, these alterations are insignificant compared with the conventional method of differential centrifugation and are perhaps unavoidable due to the fragility of the platelets. The method is economical, simple, reproducible, and can easily be applied to processing large quantities of platelets.

This work was supported in part by grant HL15090 from NIH and by ALSAC.

References

- Boldt, D., Skinner, A. M., and Kornfeld, S. (1972). Studies of two subpopulations of human lymphocytes differing in responsiveness to concanavalin A. *J. clin. Invest.*, **51**, 3225-3234.
- Lopes, J., Nachbar, M., Zucker-Franklin, D., and Silber, R. (1973). Lymphocyte plasma membranes: analysis of proteins and glycoproteins by SDS-gel electrophoresis. *Blood*, **41**, 131-140.
- Mannucci, P. M. (1972). Methods for the preparation of washed platelet suspensions. In *Platelet Function and Thrombosis: A Review of Methods*, edited by P. M. Mannucci and S. Gorin, p. 57. Plenum Press, New York and London.
- Tangen, O., Berman, H. J., and Marfey, P. (1971). Gel filtration: a new technique for separation of blood platelets from plasma. *Thrombos. Diathes. haemorrh. (Stuttg.)*, **25**, 268-278.
- Walsh, P. (1972). Albumin density gradient separation and washing of platelets and the study of platelet coagulant activities. *Brit. J. Haemat.*, **22**, 205-217.

Letter to the Editor

Recruitment of Pathologists for Medical Schools and Hospitals in the Tropics

During the last six months I have received over one dozen letters from universities and large hospitals in various parts of the tropics asking if I could find them staff. In many cases the shortage of pathologists is having a very adverse effect upon the medical schools and hospitals. It is increasingly difficult for younger members of the profession to go to these areas because of uncertainty about the future and because of the increasing specialization that is now part of modern pathology.

It seems, however, that there may be a real opportunity for pathologists reaching retiring age to contribute in some of these

places, even for a short period of time. If anyone is interested, perhaps they could get in touch with me personally so that I could give them some more information about these possibilities.

M. S. R. HUTT

*Department of Pathology,
St Thomas's Hospital Medical School,
London SE1*

Book reviews

Immunology, Immunopathology, and Immunity By Stewart Sell. (Pp. vii + 277 illustrated. \$12.95.) New York, Evanston, San Francisco and London: Harper and Row. 1972.

There is an amazing wealth of up-to-date information in this publication. It covers a wide field and a variety of immunological problems, both basic and applied, with particularly good chapters on the delayed hypersensitivity reactions, immunodeficiency diseases, and anaphylactic reactions.

The handling of the subject is, as the author quite rightly states, unorthodox and I think it is an interesting approach. The idea of combining basic immunology with immunopathology and immunity