Repeated Plasmapheresis of Blood Donors
As a Source of Platelets

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FRESH WHOLE BLOOD has been widely used as an emergency means of platelet transfusion and does have a definite but limited effect on thrombocytopenic hemorrhage.1 The red cell content of whole blood imposes a volume limitation to this form of platelet therapy and restricts its use mainly to patients with serious bleeding where chances of a successful outcome are small. Platelet-rich plasma and platelet concentrates represent a possible form of platelet replacement but practical considerations have so far prevented their full exploitation for the long-term treatment of thrombocytopenic patients. The difficulties associated with platelet-rich materials have recently been discussed by Gardner2 and revolve about the problems of finding and bleeding very large numbers of blood donors, preparing the platelets for transfusion within four hours of bleeding and avoiding the unhappy results of blood wastage.

The development of plastic phlebotomy equipment has made the procedure of plasmapheresis feasible as an alternate form of blood donation.3 By returning the red cells to the donor, plasmapheresis allows the normal individual to donate plasma repeatedly and has been suggested as a means of overcoming the problem of supply when large quantities of plasma are required for any purpose. In order to study the usefulness of repeated platelet transfusions in treating thrombocytopenic patients with leukemia, the technic of plasmapheresis was adapted to produce large amounts of platelet-rich plasma conveniently from a small group of donors. This report describes the use of plasmapheresis as a source of platelet transfusion and details the effects of intensive plasmapheresis on the normal blood donor.

MATERIALS AND METHODS

Normal blood donors were considered acceptable for plasmapheresis if they were in good general health, had an initial hemoglobin concentration of 12.5 Gm. per cent or above, a platelet count of 150,000 mm.3.4 and a total protein concentration above 6.5 Gm. per cent (biuret method). Six such donors were chosen and agreed to provide plasma for leukemic children as required. Donors were subjected to plasmapheresis as often as the recipient required platelet transfusions but in no case was any donor used more than twice in one week. The donors were studied by determining hematocrit, hemoglobin, reticulocyte count,5 white blood cell count,6 platelet count3 and total protein (biuret) concentration at the start of each plasmapheresis, at the end of each procedure and after termination of a period of plasmapheresis. Isohemagglutinin titers7 were performed periodically on the donors' sera to obtain a practical measurement of antibody activity during and after the period of plasmapheresis. In addition, the donors were observed by a physician during the...
procedure and were also asked to report all subjective reactions and any change in general health or well-being.

Plasmapheresis was performed using the plastic equipment illustrated in fig. 1. This was a "Double-Blood-Pack Double Plasmapheresis Set (Fenwal #Y-2086, Fenwal Laboratories, Inc., Morton Grove, Ill.) which was connected to a standard blood recipient set. The donor was bled into the container nearest the phlebotomy needle and this first unit was then detached for centrifugation while the phlebotomy needle was kept open with a slow infusion of .02 per cent heparin in isotonic saline. To obtain platelet-rich-plasma, the whole blood was centrifuged at 22 C. in a PR-2 International Centrifuge at 1100 r.p.m. for 15 minutes. After expressing the plasma into the satellite plasma bag, the red cells were returned to the donor. As soon as retransfusion was completed, a second 500 ml. whole blood unit was collected using the second blood container, and the process of centrifugation, plasma separation and red cell retransfusion was repeated. With this sequence, a total of 500 ml. of platelet-rich-plasma could be collected for transfusion while the packed red cells were returned to the donor. Each donor received a total of 10 mg. of heparin and 500 ml. of isotonic saline during the procedure. Platelet and white blood cell counts were performed on the platelet-rich-plasmas to determine the effect of centrifugation.

RESULTS

The immediate effects of removing 500 ml. of platelet-rich-plasma were studied in all six donors. Comparison of hemogram before and after showed
no change beyond the limits of individual variation so far as cellular blood components were concerned. On 19 occasions of plasmapheresis we were able to collect before and after determinations of serum protein levels. Total serum protein concentrations fell an average of 0.6 Gm. per cent but never fell below 5.7 Gm. per cent. Table 1 illustrates the immediate effects of plasmapheresis on one of the six donors and shows the paucity of changes in blood components observed when 500 ml. of plasma were taken.

Plasmapheresis was performed at rates varying from 250 ml. to 1000 ml. per week and for periods ranging from 10 to 95 days. Table 2 describes the effects of repeated plasmapheresis on hemogram and protein concentration in the six donors. Despite intensive plasmapheresis with removal of up to two liters of plasma in 10 days, change in serum protein concentration was minimal. Plasmapheresis repeated over a period as long as 95 days was well tolerated and abrupt cessation of plasmapheresis produced no recognizable effect. Serial determinations of hemoglobin, white blood cells and reticulocytes as well as hematocrit, platelets and total protein revealed no significant depletion of these blood components. While there was some individual variation in hematocrit and hemoglobin, no reticulocyte response was observed at any time and only one hemoglobin determination was ever below 12.5 Gm. per cent. In four donors where follow-up determinations were obtained after abruptly terminating plasmapheresis, serum protein determinations and blood cell counts averaged the same as the initial values obtained on those donors. Isoagglutinin titers before, during and after the intervals of plasmapheresis provided one index of protein activity to compare with protein concentration. Although there was a trend of the titers to rise by one tube dilution during plasmapheresis, this variation is not considered significant for the method employed. No significant change in isoagglutinin titer was seen even with intensive plasmapheresis.

Table 3 gives information on the material transfused to the thrombocyto-

| Table 1.—Immediate Effects of Donating 500 ml. of Plasma |
|----------------------------------|-----------------|----|-----|----------|---------|--------|
|                                  | Hemoglobin Gm. % | White blood cells per mm. | Hematocrit % | Platelet count x 10^11 per mm. | Total protein Gm. % |
|                                  | median | range | median | range | median | range | median | range | median | range |
| Preplasmapheresis                | 13.8   | 15.1  | 4700   | 3600  | 42     | 40     | 185    | 152    | 6.1    | 6.1   |
| Donor T. E.                      |         |       |        |       |        |        |        |        |        |       |
| Postplasmapheresis               | 13.9   | 13.3  | 5000   | 4200  | 42     | 39     | 183    | 138    | 6.1    | 5.7   |

Donor T. E.: The medians given above represent at least seven separate occasions of plasmapheresis.

| Table 2.—Effects of Repeated Plasmapheresis on Normal Blood Donors |
|---------------------------------------------------------------|-------------|-----------------|---------------|-----------------|-------------|-----------------|---------------|-----------------|---------------|
| Donor             | Plasma produced (ml.) | Duration of plasmapheresis (in days) | Hematocrit % | Platelet count x 10^11 per mm. | Total protein Gm. % |
|                  |                      |                               | median | range | median | range | median | range | median | range |
| L. T.            | 2000                 | 10                            | 41     | 40-41 | 210    |       | 155-255|       | 6.8    | 6.5-7.3 |
| E. S.            | 2000                 | 13                            | 43     | 41-45 | 273    |       | 225-305|       | 6.4    | 6.2-7.0 |
| C. B.            | 2500                 | 20                            | 41     | 39-42 | 288    |       | 220-380|       | 6.7    | 6.5-7.0 |
| E. K.            | 9500                 | 95                            | 42     | 38-44 | 263    |       | 168-313|       | 6.7    | 6.1-7.5 |
| T. E.            | 7000                 | 52                            | 41     | 39-45 | 185    |       | 105-268|       | 6.7    | 6.2-7.1 |
| F. K.            | 3500                 | 40                            | 49     | 48-49 | 290    |       | 188-248|       | 7.1    | 6.6-7.5 |
penicillin recipients. The centrifugation method used produced approximately 250 ml. of plasma in every case although the red cells were only loosely packed. The platelet counts on the plasma were always higher than those on the donor's whole blood, often by a factor of 2. Presumably, the platelets were relatively unmoved by the centrifugation and remained in the supernatant plasma. Despite the fact that a platelet-rich product was consistently obtained, no significant change in platelet count was noted in any donor during or after plasmapheresis.

**Discussion**

The procedure of plasmapheresis to obtain 500 ml. of platelet-rich-plasma at a time from a single donor was developed as a research procedure to provide a uniform supply of platelets for studies of platelet antigenicity. It soon became apparent that the procedure was not only well tolerated by the donor but economical in terms of providing a reliable source of platelets without the difficulties encountered in procuring fresh whole blood. For the transfusionist, the plasma required less routine laboratory work, since the processing and crossmatching could be done once for each donor and not repeated. This is in contrast to the situation with multiple whole blood units which require processing and crossmatching each time a transfusion is prepared. With proficiency, the time required for the procedure of obtaining 500 ml. of plasma by plasmapheresis was under two hours and frequently only one hour and thirty minutes were needed. This contrasted favorably with the time to produce plasma from multiple whole blood units. It had the advantage for the recipient that after the initial study of the donor, the fresh platelet-rich plasma could be transfused as soon as prepared.

Since only one venipuncture was necessary at a time and scrupulous care of veins was maintained, no donor had to be discontinued because of expenditure of suitable veins. No vasovagal reactions were encountered in this series but since the procedure does not differ from ordinary whole blood donation in this potential, we would expect to see such reactions in any larger series.

The method described may have certain advantages when compared to the Cohn Blood Fractionator technic of plasmapheresis in use by others. The equipment we used is simple in design, completely disposable for each donor and does not require a large investment in biomechanical apparatus. The technic described produces twice as much plasma per phlebotomy as
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does the Cohn Blood Fractionator and can be readily adapted to produce any amount of plasma from a single individual.

Although the rates of plasmapheresis described here were more intensive than any previously reported for normal blood donors, no significant depletion of blood components was observed and it seems clear that the limits of plasmapheresis for normal blood donors were not passed. The only parameter that was distinctly affected was the serum protein level. Considering the ability of the bone marrow to increase cellular production, plasma protein level rather than the blood cells might eventually prove the limiting factor to routine plasmapheresis.

While protein production seems the only physiologic limitation to plasmapheresis, other studies indicate that the production of one liter of plasma per week is well within normal capabilities. Whipple16 long ago noted in dogs that even the most intensive plasmapheresis could not render them hypoproteinemic unless a protein deficient diet was used as well. Studies in human diseases states11,12 have tended to confirm the experience in dogs that plasma protein production is far less limited than is red cell protein production. Until the present study plasmapheresis of normal human blood donors had not been attempted at rates beyond 500 ml. of plasma per week. From the data presented, it may be surmised that the practical limitation to plasma donation currently is related to donor motivation and efficiency of equipment rather to any physiologic effects of plasmapheresis.

In this series, six donors produced 26.5 liters of plasma, an amount which would have required the phlebotomy of 106 separate donors had conventional means been followed. This fact emphasizes the enormous expansion of plasma supplies that plasmapheresis affords, but it should be pointed out that we were guided by the requirements of the patient rather than the capabilities of the donor. The donors were not pushed to the limits of plasma production and in fact they might have safely donated more plasma than we actually required them to produce. The data obtained indicates that production of one liter of plasma per week is not attended by significant depletion even if continued for many weeks. Since whole blood donation presently is limited by the American Red Cross to less than 500 ml. every eight weeks,13 the technic of plasmapheresis is capable of increasing the nation's potential supply of plasma by at least a factor of 32.

The platelet response aspects of this study deserve separate discussion and have been reported in detail elsewhere.14 However, it should be apparent from the data given here that plasmapheresis is especially convenient for studying and controlling the various factors which may affect platelet transfusion. Since the donor can be kept constant for a given recipient, evaluation of platelet infusions can be critical in examining the effect of time, temperature, immunity, concentration and preservatives on platelet response and platelet survival time in the thrombocytopenic recipient.

SUMMARY

Repeated plasmapheresis with simple plastic equipment was performed on six normal donors for the purpose of obtaining platelet-rich-plasma. Plasma-
Pheresis was performed at rates of up to 1000 ml. of plasma per week for periods up to three months. The six donors produced a total of 26.5 liters of platelet-rich-plasma, an amount which would have required 106 separate blood donations had conventional means been used.

No significant changes in hemoglobin, platelets or white cells were observed in the donors and serum protein depletion was minimal. No reactions to intensive plasmapheresis were encountered even when the procedure was abruptly terminated. Repeated donor plasmapheresis is a convenient method for producing large amounts of platelet-rich-plasma and affords a practical means of controlling donor factors in platelet transfusion studies.

**SUMMARIO IN INTERLINGUA**

Repetite plasmaphereses, con le uso de un simple equipamento plastic, esseva effectuate in sex normal donatores con le objectivo de obtener plasma ric in plachettas. Plasmapherese esseva effectuate con un rendimento de usque a 1000 ml de plasma per septimana durante periodos de usque a tres menses. Le sex donatores produceva un total de 26.5 litros de plasma ric in plachettas. Iste quantitate haherea requirite 106 separate donationes de sanguine si methodos conventional habeva essite empleate.

Nulle significative alterationes de hemoglobina, plachettas, o leucocytos esseva observate in le donatores. e le depletion del proteina serial esseva minime. Nulle reactiones a ille intense programma de plasmapherese esseva incontrate, mesmo quando le procedimento esseva terminate abruptemente. Repetite plasmaphereses ab donatores particular es un convenibile metodo pro producer grande quantitates de plasma ric in plachettas e provide un metodo practic pro le controlo de factores connectite con le donator in studios de transfusion de plachettas.

**REFERENCES**

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