Effects of Intensive Plasmapheresis on Normal Blood Donors

By Allan Kliman, Paul P. Carbone, Lawrence A. Gaydos and Emil J. Freireich

IN 1914 Abel described the technic of plasmapheresis in animals and demonstrated that very large amounts of plasma could be withdrawn if the red cells were re-transfused. Since that time, the technic of plasmapheresis has been used extensively in the research laboratory for the study of protein metabolism and more recently has been introduced as an alternate form of blood donation in humans. It has been shown repeatedly that plasmapheresis, by returning the red cells to the donor, allows the individual donor to produce far larger amounts of plasma than would be the case if ordinary means of whole blood donation were used.

While plasma itself is a valuable biological product, plasmapheresis is also useful for producing platelets. The resultant platelet-rich plasma is a useful product for replacement transfusion therapy of thrombocytopenia. It is anticipated that plasmapheresis will be used increasingly as a source of supply for plasma and platelets.

At present, two systems of plasmapheresis are in use in the United States, one which uses the Cohn ADL Blood Fractionator, and the other which uses simple containers and ordinary centrifuges. Another method utilizing magnetic fields to separate red cells from plasma may soon be introduced but has not yet been reported in routine operation. The use of complex biomechanical equipment is not within the scope of ordinary blood banks and for at least the immediate future the use of simple equipment for plasmapheresis will most likely remain the most widespread of the methods currently feasible.

Despite the growing use of plasmapheresis in humans, precise limits to plasma withdrawal have not yet been defined and opinion has varied among those engaged in collecting plasma concerning the safety of intensive or prolonged plasmapheresis. Previous reports have emphasized that even with frequent donations of plasma, little depletion is seen, although there is certainly no reason to believe that plasma production is limitless. The present report concerns observations on normal blood donors subjected to intensive and prolonged plasmapheresis by means of simple equipment. A successful attempt to approach depletion levels of plasma substances was made in normal individuals and data was obtained concerning maximal withdrawal of plasma proteins and formed elements.

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MATERIALS AND METHODS

Apparantly healthy blood donors of both sexes were accepted for plasmapheresis if they had an initial serum protein concentration of 6.3 Gm. per cent or higher (biuret method), a platelet count above 150,000 per cu. mm. and were otherwise suitable for blood donation according to the usual history and physical examination given to prospective donors of whole blood.

To perform plasmapheresis, a "Double Plasmapheresis, Double Blood Pack" was used. This equipment consisted of two 500 ml. whole blood bags connected in series with a Y connection so that the phlebotomy needle could be kept open between bleedings with a slow saline infusion. As described in a previous communication, the donor was bled into the first bag, this container disconnected, and the phlebotomy needle kept open while the centrifugation and separation of plasma were accomplished. The red cells were returned by replacement of the saline bottle with the red cell container, but as soon as the first red cell transfusion had been completed, the tourniquet was reapplied to the donor's arm and a second unit was taken with return of the red cells as before. When more than two units were taken, a blood recipient set made with a second "Y" connection was used, and additional units of plasma were obtained by connecting additional plastic blood containers.

Separation of plasma from red cells was accomplished by accelerating the blood in an International PR 2 centrifuge to 2500 rpm and centrifuging for just 3 minutes at room temperature. The total time required for plasmapheresis was approximately 35 minutes per unit of plasma produced. Each donor received replacement of plasma removed with an equal amount of isotonic saline which was given as a steady drip to keep the phlebotomy needle open during the time of centrifugation, and to minimize any change in plasma volume brought about by the plasma withdrawal itself.

Calculations:
1. Actual protein loss (Gm.) = volume of plasma removed (cc.) x protein concentration (Gm./cc.).
2. Initial protein pool (Gm.) = plasma volume (liters) x initial concentration protein (Gm./liter). Weight (Kg.) x .081 + x (1-hematocrit) = plasma volume (liters).
3. Final protein pool (Gm.) = plasma volume (liters) x final concentration protein (Gm./liters).
4. Apparent protein loss (Gm.) = initial protein pool (Gm.) - final protein pool (Gm.).
5. Net protein replacement (Gm.) = actual loss (Gm.) - apparent loss (Gm.).
6. Per cent recovery = net protein replacement actual protein loss

A. Immediate Effects of Plasmapheresis

The effects of removal of 1000 ml. of plasma at one venipuncture in a period of 2 hours were studied in four donors on their initial occasion of plasmapheresis. Hemoglobin, hematocrit, white blood cell count, reticulocyte count, platelets, total protein and paper electrophoretic protein patterns were obtained immediately before and immediately after the procedure of plasmapheresis. The laboratory methods for the blood counts and protein determinations were the same as listed in previous publications.1,8

B. Effects of Chronic Plasmapheresis

Twenty-one donors were plasmapheresed repeatedly at varying rates for periods up to 162 days. The chronic effects of plasmapheresis were studied by determining hemoglobin,
EFFECTS OF INTENSIVE PLASMAPHERESIS

Table 1.—Blood Counts and Total Protein Values Before and After Donating 1000 ml. of Plasma by Means of Plasmapheresis

<table>
<thead>
<tr>
<th>Donor</th>
<th>HB Gm. %</th>
<th>Hct.</th>
<th>WBC/cu. mm.</th>
<th>Platelets/cu. mm.</th>
<th>Retic.</th>
<th>Total Protein Gm. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. K. before</td>
<td>14.4</td>
<td>46</td>
<td>7900</td>
<td>170,000</td>
<td>0.3</td>
<td>6.5</td>
</tr>
<tr>
<td>after</td>
<td>15.5</td>
<td>48</td>
<td>7700</td>
<td>190,000</td>
<td>0.4</td>
<td>5.9</td>
</tr>
<tr>
<td>R. C. before</td>
<td>15.4</td>
<td>44</td>
<td>10,700</td>
<td>155,000</td>
<td>0.2</td>
<td>6.5</td>
</tr>
<tr>
<td>after</td>
<td>16.8</td>
<td>47</td>
<td>8100</td>
<td>180,000</td>
<td>0.5</td>
<td>6.0</td>
</tr>
<tr>
<td>L. S. before</td>
<td>16.2</td>
<td>49</td>
<td>8700</td>
<td>290,000</td>
<td>0.7</td>
<td>7.4</td>
</tr>
<tr>
<td>after</td>
<td>17.0</td>
<td>52</td>
<td>6100</td>
<td>153,000</td>
<td>0.2</td>
<td>6.4</td>
</tr>
</tbody>
</table>

C. Effects of Intensive Plasmapheresis

Five donors were subjected to a 5-day period of plasmapheresis during which 1 liter of plasma was removed daily. At the end of the 5-day period, plasmapheresis was terminated and the donors were observed for at least 3 months by means of the blood determinations listed above (A and B). Net protein replacement for the 5-day period was calculated in the same manner as in the study of the immediate effects of plasmapheresis. Platelet and white blood cell counts were performed on all plasmas removed and the total amount of these elements withdrawn was calculated for each donor.

RESULTS

A. Immediate Effects of Plasmapheresis

The acute withdrawal of 1000 ml. of plasma was studied in four donors (see table 1.) The hemogram was not altered except for a slight rise in hematocrit. Serum protein concentration did show an abrupt fall in each case which amounted to 1.0 Gm. per cent on the average. When net protein replacement was calculated (see table 2), this ranged from 11 to 54 per cent. Serum electrophoretic patterns obtained after plasmapheresis showed a slight diminution in all elements as compared with preplasmapheresis patterns, but no change in relative proportions was noted.

B. Effects of Chronic Plasmapheresis

In a period of 1 year, 21 donors were included in this portion of the study by undergoing repeated plasmapheresis, i.e., plasmapheresis on more than three occasions. Maximal rates of plasmapheresis varied from 0.5 to 5.0 liters of plasma removed per week and the periods of plasmapheresis varied from 1 to 23 weeks. Table 3 details the amount of plasma donated by each donor and gives the lowest serum protein value observed at any time during the period of plasmapheresis. At maximal rates under 1.0 liters per week (less than 60 Gm. of protein removed per week), no obvious effect on serum protein levels was seen. At rates up to 2.5 liters per week, comparison of lowest serum protein with initial serum protein also failed to show unequivo-
Table 2.—Net Protein Replacement during Removal of 1000 ml. of Plasma by Plasmapheresis

<table>
<thead>
<tr>
<th>Donor</th>
<th>R. C.</th>
<th>J. K.</th>
<th>L. S.</th>
<th>D. S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein withdrawn (Gm.)</td>
<td>66</td>
<td>63</td>
<td>55</td>
<td>69</td>
</tr>
<tr>
<td>Initial protein pool (Gm.)</td>
<td>228</td>
<td>230</td>
<td>213</td>
<td>230</td>
</tr>
<tr>
<td>Final protein pool (Gm.)</td>
<td>181</td>
<td>201</td>
<td>164</td>
<td>187</td>
</tr>
<tr>
<td>Apparent protein loss (Gm.)</td>
<td>47</td>
<td>29</td>
<td>49</td>
<td>43</td>
</tr>
<tr>
<td>Net protein loss (Gm.)</td>
<td>19</td>
<td>34</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>Per cent replacement</td>
<td>29</td>
<td>54</td>
<td>1</td>
<td>37</td>
</tr>
</tbody>
</table>

Average replacement = 33%.

Table 3.—Effect of Chronic Plasmapheresis on Serum Protein Level

<table>
<thead>
<tr>
<th>Maximal Rate of Plasmapheresis per Week</th>
<th>Duration of Plasmapheresis (weeks)</th>
<th>Amount of Plasma Donated (liters)</th>
<th>Initial Serum Protein (Gm. %)</th>
<th>Lowest Serum Protein (Gm. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>6</td>
<td>3.3</td>
<td>7.7</td>
<td>6.8</td>
</tr>
<tr>
<td>0.5</td>
<td>4</td>
<td>3.0</td>
<td>7.3</td>
<td>6.7</td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>1.5</td>
<td>7.5</td>
<td>6.5</td>
</tr>
<tr>
<td>0.5</td>
<td>15</td>
<td>1.5</td>
<td>7.3</td>
<td>6.3</td>
</tr>
<tr>
<td>0.5</td>
<td>16</td>
<td>3.5</td>
<td>6.7</td>
<td>6.0</td>
</tr>
<tr>
<td>0.7</td>
<td>9</td>
<td>1.8</td>
<td>7.6</td>
<td>7.1</td>
</tr>
<tr>
<td>0.7</td>
<td>17</td>
<td>2.3</td>
<td>6.9</td>
<td>7.0</td>
</tr>
<tr>
<td>1.0</td>
<td>9</td>
<td>5.0</td>
<td>7.4</td>
<td>6.9</td>
</tr>
<tr>
<td>1.0</td>
<td>4</td>
<td>3.5</td>
<td>7.0</td>
<td>6.5</td>
</tr>
<tr>
<td>1.0</td>
<td>17</td>
<td>4.5</td>
<td>6.6</td>
<td>6.5</td>
</tr>
<tr>
<td>1.0</td>
<td>23</td>
<td>12.5</td>
<td>6.6</td>
<td>6.5</td>
</tr>
<tr>
<td>1.0</td>
<td>4</td>
<td>4.5</td>
<td>7.2</td>
<td>6.4</td>
</tr>
<tr>
<td>1.0</td>
<td>4</td>
<td>3.5</td>
<td>8.0</td>
<td>5.7</td>
</tr>
<tr>
<td>1.0</td>
<td>20</td>
<td>5.5</td>
<td>6.3</td>
<td>5.6</td>
</tr>
<tr>
<td>1.5</td>
<td>1</td>
<td>1.5</td>
<td>7.4</td>
<td>6.5</td>
</tr>
<tr>
<td>1.5</td>
<td>1</td>
<td>1.5</td>
<td>6.6</td>
<td>6.5</td>
</tr>
<tr>
<td>1.5</td>
<td>8</td>
<td>6.5</td>
<td>7.5</td>
<td>6.0</td>
</tr>
<tr>
<td>2.5</td>
<td>1</td>
<td>2.5</td>
<td>6.8</td>
<td>6.0</td>
</tr>
<tr>
<td>5.0</td>
<td>11</td>
<td>16.0</td>
<td>7.6</td>
<td>4.9</td>
</tr>
<tr>
<td>5.0</td>
<td>14</td>
<td>15.3</td>
<td>7.4</td>
<td>4.6</td>
</tr>
<tr>
<td>5.0</td>
<td>21</td>
<td>19.0</td>
<td>7.3</td>
<td>4.4</td>
</tr>
</tbody>
</table>

cal depression. However, with the three donors who underwent not only prolonged but intensive plasmapheresis at 5.0 liters per week, depletion of serum proteins was readily apparent, as can be seen from the serum proteins listed in table 3.

Moreover, while hemoglobin, platelet and white blood cell counts showed no change in the groups plasmapheresed at lower rates, the three donors who received the most intensive plasmapheresis showed mild thrombocytopenia toward the end of the week of maximal plasmapheresis. Initial platelet counts for these three donors were 170,000, 218,000, and 198,000 per cu. mm., but during the time of most intensive plasmapheresis, platelet counts of 76,000, 120,000 and 104,000 per cu. mm. respectively were recorded. Paper strip electrophoresis of serum proteins showed no change in the patterns of the
EFFECTS OF INTENSIVE PLASMAPHERESIS

Table 4.—Effects of Removal of 5000 ml. of Plasma during a 5-Day Period by Means of Plasmapheresis

<table>
<thead>
<tr>
<th>Donor</th>
<th>Hb</th>
<th>Hct</th>
<th>WBC</th>
<th>Platelets</th>
<th>Retic</th>
<th>Total Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. C.</td>
<td>15.4</td>
<td>45</td>
<td>5200</td>
<td>195,000</td>
<td>0.1</td>
<td>7.3</td>
</tr>
<tr>
<td>final</td>
<td>13.0</td>
<td>39</td>
<td>5680</td>
<td>133,000</td>
<td>0.5</td>
<td>4.6</td>
</tr>
<tr>
<td>J. K.</td>
<td>14.4</td>
<td>48</td>
<td>7900</td>
<td>170,000</td>
<td>0.3</td>
<td>6.5</td>
</tr>
<tr>
<td>final</td>
<td>14.7</td>
<td>43</td>
<td>6700</td>
<td>61,000</td>
<td>0.1</td>
<td>4.6</td>
</tr>
<tr>
<td>L. S.</td>
<td>14.4</td>
<td>48</td>
<td>10,700</td>
<td>155,000</td>
<td>0.2</td>
<td>6.5</td>
</tr>
<tr>
<td>final</td>
<td>15.1</td>
<td>44</td>
<td>11,600</td>
<td>108,000</td>
<td>0.5</td>
<td>5.0</td>
</tr>
<tr>
<td>F. K.</td>
<td>15.8</td>
<td>48</td>
<td>4100</td>
<td>278,000</td>
<td>0.2</td>
<td>6.7</td>
</tr>
<tr>
<td>final</td>
<td>15.7</td>
<td>47</td>
<td>4100</td>
<td>158,000</td>
<td>0.1</td>
<td>5.2</td>
</tr>
<tr>
<td>D. S.</td>
<td>15.7</td>
<td>48</td>
<td>8700</td>
<td>185,000</td>
<td>0.7</td>
<td>7.0</td>
</tr>
<tr>
<td>final</td>
<td>15.1</td>
<td>48</td>
<td>10,600</td>
<td>125,000</td>
<td>1.3</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Table 5.—Net Protein Replacement During 5 Days of Plasmapheresis with Removal of 1000 ml. of Plasma Per Day

<table>
<thead>
<tr>
<th>Donor</th>
<th>R. C.</th>
<th>J. K.</th>
<th>L. S.</th>
<th>D. S.</th>
<th>F. K.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein withdrawn (Gm.)</td>
<td>307</td>
<td>290</td>
<td>282</td>
<td>290</td>
<td>312</td>
</tr>
<tr>
<td>Initial protein pool (Gm.)</td>
<td>228</td>
<td>222</td>
<td>197</td>
<td>221</td>
<td>243</td>
</tr>
<tr>
<td>Final protein pool (Gm.)</td>
<td>159</td>
<td>172</td>
<td>164</td>
<td>171</td>
<td>192</td>
</tr>
<tr>
<td>Apparent protein loss (Gm.)</td>
<td>69</td>
<td>50</td>
<td>33</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>Net protein replacement (Gm.)</td>
<td>238</td>
<td>239</td>
<td>249</td>
<td>240</td>
<td>261</td>
</tr>
</tbody>
</table>

Average replacement = 245 Gm. in 5 days (49 Gm./day).

donors plasmapheresed at lower rates, but three donors subjected to the highest rates showed relative depletion of gamma globulin similar to that described below in donors subjected to 5 days of intensive plasma withdrawal.

C. Effects of Intensive Plasmapheresis

Five donors were subjected to plasma withdrawal of 1 liter per day for 5 days and observations were made during and after termination of the period of plasmapheresis. While this intensive plasmapheresis did not affect hemoglobin or white blood cell counts, platelets and especially serum protein levels were affected (see table 4). Net protein replacement for the 5-day period was calculated, and amounted to an average of 245 Gm. of protein (see table 5). Serial serum electrophoretic patterns showed progressive depletion of all elements, but with greater percentage decreases of gamma globulin than of any other element (see figure 1).

Data on the recovery period was obtained in four of the five donors. Recovery of total protein to pre-plasmapheresis levels was achieved within 2 to 4 weeks after terminating plasmapheresis and there was no "rebound" to levels of total protein higher than those initially observed. Gamma globulin recovered more slowly and was not back to preplasmapheresis levels for 26 to 90 days (see figure 1).

As pointed out above, platelets and white cells were also removed and the total amounts withdrawn in the 5 days are given in table 6. There was
Fig. 1.—Effect of removal of 5 liters of plasma in 5 days on serum proteins of four normal donors. The shaded area represents the period of plasmapheresis.

no significant change in white cell count in this group. The platelets did drop significantly in two of the five donors and a trend toward lowered platelet counts was observed in the remaining donors. Recovery of platelet count was prompt, occurring within 3 days, but no thrombocytosis was observed in the 3 months of observation thereafter.

The donors were observed closely by physicians during the period of depletion and recovery; no ill effects were noted clinically. The donors reported no change in appetite, activity or sense of well being and there was no peripheral edema or purpura at any time.

**DISCUSSION**

In the present study, chronic and intensive plasmapheresis was well tolerated by normal blood donors in the sense that no subjective changes were reported. In addition, laboratory study showed that no donor developed anemia or reticulocytosis during the study even when intensive plasmapheresis was performed. This is in accord with previous reports concerning plasmapheresis.
The removal of protein from the animal organism has been studied extensively in dogs and other animals. Until now, data on protein synthesis in the human has been limited mainly to turnover studies of isotopically labeled protein and to information provided by protein losing diseases. Such observations may not be applicable to unlabeled proteins or to normal individuals. Our observations of protein turnover under conditions of plasmapheresis add to the available data on protein turnover and suggest methods for further study of the normal.
The observations on removal of 1000 ml. of plasma allow us to make some conclusions concerning readily available protein reserves. If we compare the net protein replacement to the total protein withdrawn in the study of the immediate effects of plasmapheresis, we find that the net protein replacement approximates 33 per cent of the protein removed. If we assume that protein replacement is provided by diffusion from an extravascular pool in ready equilibrium (an assumption warranted by the short time taken by the procedure of withdrawal) rather than by synthesis of new protein, the size of this extravascular pool must be somewhat less than the intravascular one to account for only 33 per cent replacement. Our estimate for the size of the extravascular protein pool thus is smaller than the estimates arrived at by isotopic methods.18,19

Our observations on serum proteins during a longer period of protein withdrawal provide further data in the human concerning maximal rates of protein replacement. The five plasmapheresis donors were able to replace an average of 245 Gm. of protein in 5 days or 49 Gm. of protein per day. Protein losses in excess of this amount were apparently not made up and were reflected in decreased levels of circulating serum proteins. Forty-nine Gm. of protein per day corresponds to about three plasma donations (750 cc.) and our data indicates that rates of plasmapheresis must be below this amount if significant donor depletion is to be avoided.

When compared to absolute levels of total serum protein, electrophoretic patterns showed that depletion of individual protein elements does not proceed pari passu but that gamma globulin is replaced at a slower rate than other serum proteins. This observation has practical implications because it points out that total protein determinations alone do not signify the extent to which depletion of gamma globulin is taking place. Thus if plasmapheresis is being performed for the production of large pools of human antibodies, a lower rate of plasmapheresis than that specified above will be necessary to preserve high antibody yields.

Previous reports of plasmapheresis did not record depletion of normal serum proteins in the plasma donors but it is now clear that the rates of plasmapheresis were not intensive enough to cause depletion. Depletion of serum proteins in the donor indeed may be the most serious limiting factor to plasmapheresis, for once depletion occurs, recovery is not immediate, requiring at least 2 to 4 weeks for total protein and as long as 12 weeks for gamma globulin.

Summary

Plasmapheresis was performed in normal blood donors using simple plastic equipment. Removal of plasma in amounts up to 1.0 liter at a single venipuncture proved feasible and produced no major changes in the donor's blood counts and serum protein levels. Chronic plasmapheresis for periods up to 23 weeks with maximal rates not exceeding 1.5 liters of plasma withdrawn per week, produced no changes in formed elements and only minor changes in serum protein levels. Five donors were subjected to acute plasmapheresis with removal of 5 liters of plasma in 5 days. In this group, serum protein
Effects of Intensive Plasmapheresis

Depletion was observed with particular depletion appearing at the end of the 5-day period.

From calculations of protein replacement, the presence of a ready extravascular protein reserve, of a size somewhat less than the intravascular pool, was suggested. An average maximal protein replacement of 49 Gm. per day was observed during the most intensive plasmapheresis, and the data suggest that protein withdrawals should not exceed this amount if serum protein levels are to be maintained. Although no rebound of serum protein was observed, recovery after depletion required at least 2 to 4 weeks.

**SUMMARY IN INTERLINGUA**

Plasmapherese esseva effectuate in normal donatores de sanguine con le uso de un simple equipamento de plastico. Le prisa de quantitates de plasma de usque a 1,0 litros ab un sol venipunctura esseva practicable e produceva nulle major alterationes in le numerationes sanguinee e le nivellos seral de proteina in le donator. Plasmaphereses chronic durante periodos de usque a 23 septimanas con prisas maximal non excedente 1,5 litros per septimana produceva nulle alterationes in le formate elementos e solmente minor alterationes in le nivellos seral de proteina. Cinque donatores esseva subjicite a acute plasmapherese con prisas de 5 litros de plasma in 5 dies. In iste gruppo, depletion del proteina seral esseva observate, con depletion particularmente marcate verso le fin del periodo de 5 dies.

A base de calculationes del reimplaciamento de proteina, le presentia de un preste reserva extravascular de proteina—de un magnitude moderatemente inferior a illo del reserva intravascular—esseva postulate. Un reimplaciamento maximal de proteina de 49 g per die como valor medie esseva observate durante le plus intense plasmapherese, e le datos suggere que le prisas non deberea exceder iste quantitate si le nivellos seral de proteina debe esser mantenite. Ben que nulle resalto del proteina seral esseva observate, restablimento post depletion requireva al minus 2 a 4 septimanas.

**REFERENCES**


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