

Partial Plasma Exchange Using Albumin Replacement: Removal and Recovery of Normal Plasma Constituents

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Using albumin and crystalloid as the only replacement fluids, the effect of partial plasma exchange on the removal and recovery of normal plasma constituents was studied. The results of 30 procedures on 10 individuals were evaluated. Four patterns of removal are described; reduction in the concentration of fibrinogen and C₃ were greater than would be expected based upon the extent of the exchange, while IgG, IgM, cholesterol, alkaline phosphatase and SGPT were removed as expected. Reduction of serum glutamicoxalacetic transaminase (SGOT), lactate dehydrogenase (LDH), amylase, and creatine phosphokinase (CPK) averaged 17% less, and uric acid, calcium and K⁺ averaged 53% less than expected. Concentrations of HCO₃⁻ and glucose did not change. The mean recovery for all constituents except fibrinogen, C₃, cholesterol, IgG and IgM was near 100% at 48–72 hr postpheresis. The 72-hr recovery of fibrinogen and complement was 66% and 60%, respectively. Cholesterol recovery was also slow, requiring a minimum of 1 wk to reach prepheresis levels. Measured at a time when quantitative IgM levels were still reduced, alloantibody agglutinating activity (anti-A and anti-B) in a postpheresis sample exceeded prepheresis agglutinating activity. These data demonstrated that, depending upon quantity and frequency of pheresis, partial plasma exchange using albumin replacement may cause progressive marked reduction in concentrations of immunoglobulin, complement, fibrinogen, and cholesterol. Furthermore, newly synthesized antibody may have increased biologic activity.

THERAPEUTIC partial plasma exchange has been applied to the treatment of a variety of diseases. The success of this mode of therapy in most cases is presumed to be dependent upon the removal from the circulation of soluble factors that contribute to the pathologic process. Current routine techniques do not allow for the removal of selected plasma components. Depletion of normal plasma constituents, therefore, accompanies removal of pathogenic factors. The extent to which normal plasma constituents are removed, and the rate at which they return to prepheresis levels, may influence decisions regarding the type of replacement fluid and the frequency or duration of plasma exchange therapy. In this report, the kinetics of removal and subsequent recovery of a variety of normal plasma constituents are presented. Included are measurements of IgG, IgM, C₃, fibrinogen, calcium, uric acid, sodium (Na⁺), potassium (K⁺), chloride (Cl⁻), bicarbonate (HCO₃⁻), alkaline phosphatase (alk. phos.), LDH, SGOT, serum glutamicpyruvic transaminase (SGPT), CPK, amylase, and cholesterol. In one normal subject, the prothrombin time (PT) and partial thromboplastin time (PTT), factors VIII, IX, X, and XI were also measured during the exchange procedure and subsequently to assess recovery. In two normal subjects, anti-A and anti-B antibody activity were measured by manual titration and automated agglutination for 2 wk after the exchange procedures.

MATERIALS AND METHODS

Eight patients, 6 with neuromuscular disease, and 2 with systemic lupus erythematosus (SLE), were studied. Five patients with neuromuscular disease were begun on a course of azathioprim (2 mg/kg) 2–3 wk prior to initiation of partial plasma exchange therapy. Patients with SLE and a single patient with myasthenia gravis had been on prednisone (approximately 1 mg/kg) for variable lengths of time. Two normal subjects were also studied. Partial plasma

exchange was performed on a Haemonetics Model 30 cell separator using a double venipuncture technique. Anticoagulation was achieved with acid citrate dextrose (ACD) (Formula A) in a ratio of 8:1. Removed plasma was replaced with 5% normal serum albumin in equivalent volumes. Chemical determinations were performed in the clinical chemistry laboratory. Measurements of IgG, IgM, and C₃ were made with a Beckman Immunochemistry Analyzer. The PT, PTT, fibrinogen, factors VIII, IX, X, and XI were performed using standard methods and commercial reagents. Manual titres measuring agglutinating activity of anti-A and anti-B were performed after a 1-hr room temperature incubation, included an antiglobulin phase, and were read macroscopically. Tube dilutions were adjusted to reflect approximately 10% changes in antibody concentration. All cells and antisera were obtained commercially. Automated quantitative tests of agglutinating activity were performed in a Bromelin-PVP system.¹ Manual titres and automated quantitative agglutination were done on the same day against the same test cells using sera previously frozen and stored at –30°C. Dithiothreitol (DTT) treatment of sera was performed using standard methodology.² DTT treated sera was inactive in the automated system at all dilutions.

Serum samples were obtained immediately prior to and following the procedure in all cases. In the one instance where removal data was obtained during the exchange, samples were obtained following each pass of the fourteen pass procedure. Postpheresis samples were obtained 48 hr postpheresis and, in some instances, at longer intervals.

The predicted removal of plasma components was derived using the formula $R = [(V - S)/V]^n$ where R is the percentage of the original plasma factor remaining following plasma exchange, V is the plasma volume (calculated on the basis of weight and hematocrit), S is the quantity of plasma removed per pass (corrected for anticoagulant admixture) and n is the number of passes. This formula has been previously published³ and assumes that no synthesis takes place and no equilibration occurs between intravascular and extravascular compartments during the course of the procedure.

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The comparison of the actual removal to the predicted removal was made using the formula

$$D = \frac{R - \left[\frac{\text{postpheresis}}{\text{prepheresis}} \right]}{R}$$

Brackets denote measured concentration of plasma constituents. D is the percent difference from predicted. The recovery of serum concentrations following an exchange procedure is expressed as a percentage of the quantity removed during that exchange. Thus, percent recovery at 48 hr was calculated by the formula:

$$\% \text{ Recovery} = \frac{\left[\frac{48 \text{ hr postpheresis}}{\text{prepheresis}} \right] - \left[\frac{\text{immediate postpheresis}}{\text{prepheresis}} \right]}{\left[\frac{48 \text{ hr postpheresis}}{\text{prepheresis}} \right] - \left[\frac{\text{immediate postpheresis}}{\text{prepheresis}} \right]} \times 100.$$

RESULTS

Removal

In one plasma exchange of a normal subject, reduction in plasma concentration was determined after each pass of a fourteen pass procedure. A similar reduction was achieved for IgM, IgG, C₃, CH₅₀, fibrinogen, SGPT, alk. phos., cholesterol, and factor XI. Their logarithmic removal could be described by the line $\log y = -.0544x + 1.9555$ ($r = -.95$), where y is the percentage remaining and x is the number of passes (350 ml/pass). The predicted reduction for the exchange was 81%, very close to the 84% calculated from the equation. In contrast, the reduction of SGOT, LDH, CPK, amylase, and factor X ($\log y = -.0375x + 1.9679$, $r = -.97$) was less than predicted, 71% rather than 81%. Concentration of calcium, uric acid, and K⁺ ($\log y = -.0067x + 1.9909$, $r = -.74$) was reduced even less effectively, 21% versus 81%. The concentrations of HCO₃⁻ and glucose did not fluctuate during the course of the procedure. In Fig. 1, the changes in the PT, PTT, fibrinogen, factor VIII, and factor IX activities for

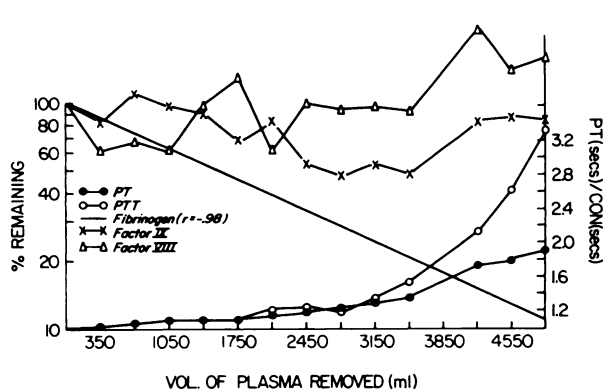


Fig. 1. Changes in the PT, PTT and concentrations of factors VIII and IX during a single partial plasma exchange with albumin replacement. Individual points represent actual measurements. The Fibrinogen curve is based upon a least squares regression analysis of 13 data points.

this exchange are shown. The PT and PTT became increasingly prolonged as the volume of the exchanged plasma increased. Removal of fibrinogen is shown and measured values decreased to approximately 25% (40 mg%) of the prepheresis level. Factor VIII and factor IX activity remained above 50% of the prepheresis activity throughout the course of the exchange. At the end of the procedure, both were within normal limits.

Removal characteristics of the entire study group were then analyzed. Thirty exchanges were performed, and each constituent was measured a minimum of twenty-two times, both for removal and recovery. The actual percent reduction of each of the measured parameters at the end of any single plasma exchange was compared to the predicted percent reduction (see Materials and Methods). The results are shown in Fig. 2. Shaded areas represent a confidence limit of 95%, using Student's 2-tailed t distribution. Shaded areas above zero indicate a removal greater than predicted. Shaded areas below zero indicate removal less than predicted. Four groups are distinguishable by their removal characteristics. Fibrinogen and C₃ were removed to a greater extent than would be expected based upon their plasma concentration and the volume of the plasma exchanged. IgG, IgM, cholesterol, alkaline phosphatase, and SGPT were removed as predicted. The average removal was

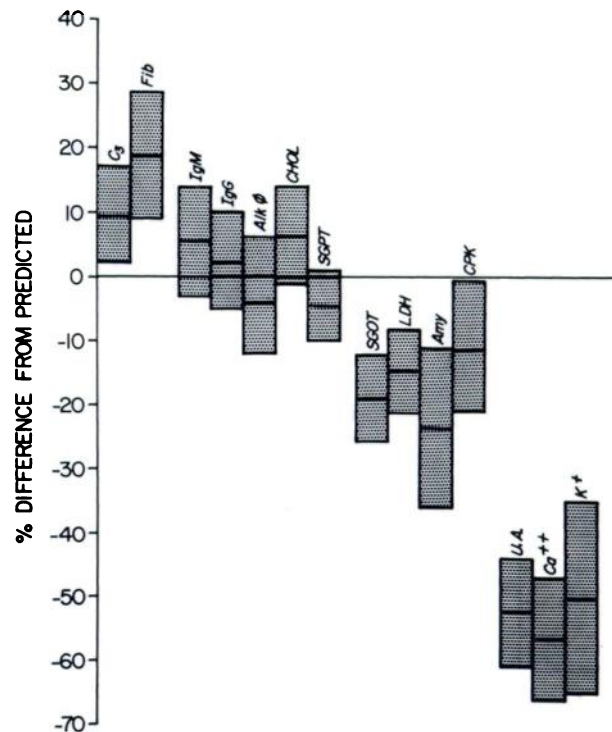


Fig. 2. Removal characteristics of normal plasma constituents. Shaded areas above "0" represent greater removal than predicted (See Materials and Methods). Shaded areas below "0" represent less removal than predicted.

within 1.6% of the predicted value for these parameters. SGOT, LDH, amylase, and CPK were removed an average of 17% less than predicted. Furthermore, the predicted removal was not included within the 95% confidence range for these parameters. Uric acid, calcium, and K^+ were removed an average of 53% less than predicted. The differences among the removal characteristics of the four groups are statistically significant ($p < 0.001$). Not shown are the measurements of HCO_3^- and glucose. The serum concentration of these was not affected by plasma exchange. Na^+ and Cl^- concentrations did not change either, but these were included in the replacement fluid.

Recovery

The mean recovery (± 1 SD) at 48 hr following plasma exchange, expressed as a percentage of the quantity removed during the procedure, is shown in Fig. 3. Recovery of SGPT, SGOT, amylase, alkaline phosphatase, and CPK was highly variable. Recovery

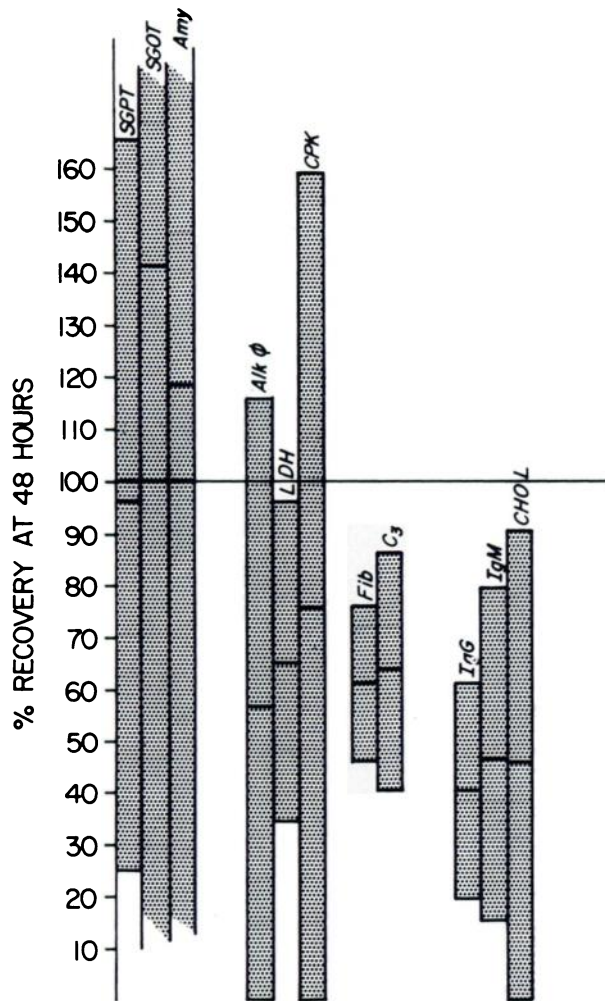


Fig. 3. Recovery of normal serum constituents 48 hr following partial plasma exchange. Recovery is expressed as a percentage of the amount removed by the exchange procedure.

of LDH, fibrinogen, C_3 , IgG, IgM, and cholesterol was more consistent. Despite the large amount of variation, differences in recovery characteristics can be discerned. The mean recovery of SGPT, SGOT and amylase, Group I, was 111%, and was significantly different ($p < 0.05$) from the mean recovery of group II, alkaline phosphatase, LDH and CPK, 60%; and the mean recovery of group III, fibrinogen and C_3 , 63%. The mean recovery, 44%, of IgG, IgM and cholesterol, group IV, was significantly different from groups II and III ($p < 0.05$). Furthermore, when considered together, the mean recovery of groups III and IV was significantly less than groups I and II ($p < 0.001$). Concentrations of group II parameters usually returned to prepheresis levels by 72 hr. Fibrinogen and complement occasionally required more time. In those cases where recovery data at 72 hr were available, mean recovery was 66% and 60%, respectively. IgG, IgM and cholesterol were not at prepheresis levels by 72 hr, and serial measurements in the two normal individuals indicated that recovery of these constituents was more prolonged. Cholesterol recovery, measured in the two normal subjects, was only 63% and 72% complete at 1 wk. At 2 wk postpheresis, quantitative IgG determinations showed only 67% and 75% recovery, respectively. Manual antiglobulin titres of DTT treated serum were consistent with the quantitative measurements. Recovery characteristics of IgM are shown in Fig. 4. In addition to the quantitative IgM levels determined by nephelometry, specific antibody activity (means and ranges) measured as anti-A or anti-B by manual and automated quantitative

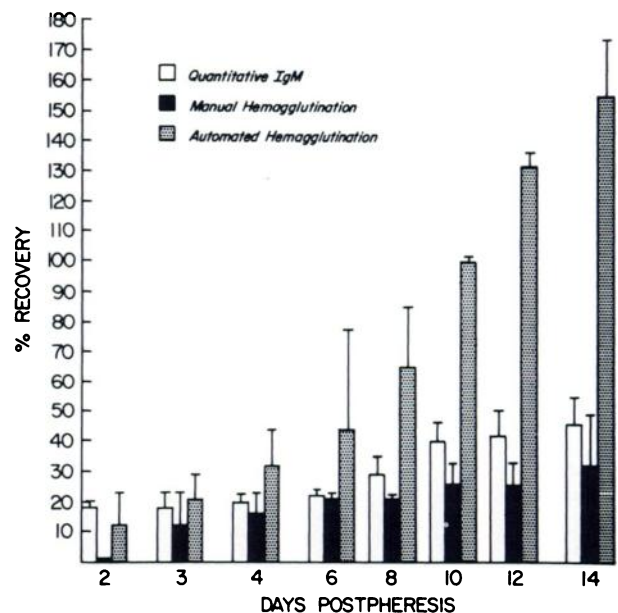


Fig. 4. Comparison of IgM recovery measured by nephelometry, with recovery of specific anti-A or anti-B isoagglutinin activity measured by manual and automated hemagglutination.

methods, is shown. By the sixth postpheresis day, the mean antibody activity, as measured by automated agglutination, was more than twice the mean as measured by manual agglutination titres or nephelometry. By the tenth day, activity as measured by the automated technique achieved the 100% (i.e., prepheresis) level, while quantitative IgM levels and manual agglutination titres showed a recovery of only 40% and 26%, respectively. At 2 wk postpheresis, antibody activity measured in the automated system was 150% of the prepheresis level. Mean activity measured by manual titration and quantitative IgM levels were less than 50%.

DISCUSSION

The application of partial plasma exchange to the treatment of an increasing number of diseases raises many questions regarding replacement fluid therapy, frequency, and volume of exchanges. These questions are particularly important if volume replacement is accomplished with normal serum albumin to avoid the risk of posttransfusion hepatitis. Data is required both on the removal and recovery kinetics of normal plasma constituents to insure patient safety. Recent studies have reported decreased antithrombin III activity following partial plasma exchange⁴ and coincident thromboembolic phenomenon. Cholinesterase levels have also been reported to be decreased⁵ with subsequent difficult anesthesia.

The results of our study indicate that, during the course of a plasma exchange, reductions of most plasma constituents occur logarithmically. That is, a constant percentage of the amount remaining is removed with each pass. In absolute terms, this means that for each aliquot of additional processed volume, the achieved decrement in plasma levels becomes smaller. This relationship holds for nearly all measured constituents. Exceptions are factors VIII and IX (shown in Fig. 1) and HCO_3^- and glucose. Albumin, Na^+ and Cl^- could not be evaluated, since they were in the replacement fluid. The lack of fluctuation in plasma concentrations of glucose and HCO_3^- during the procedure suggests that remarkably exact homeostatic mechanisms are at work. In agreement with a previous report,⁶ factor VIII and factor IX levels were within the normal range at the end of the procedure. They did, however, fluctuate during the course of the exchange. It has been reported⁷ that factor VIII and factor IX are reduced by plasma exchange, but that levels return to normal within 4 hr following the procedure. In the pheresis procedure reported here, the large volume exchange did require excessive time, and therefore may not be in conflict with this report. Even if variability exists in the removal of these two procoagulants, reduction to a level that would impair hemostasis was not apparent.

The prolongation of the PT and PTT appears to be related to the reduction in plasma fibrinogen. Factor X and factor XI were also reduced, but prolongation of the PTT to greater than three times control would not likely be achieved by reduction in the levels of these procoagulants.

Although a constant percentage of a given plasma constituent is removed per aliquot of blood processed, this percentage was found to vary. The formula $[(V - S)/V]^n$ predicts removal if no reequilibration or synthesis occur during the course of the partial plasma exchange. The mean removal ± 1 SD of IgM, IgG, alkaline phosphatase, cholesterol and SGPT was $1.6\% \pm 14\%$ of the predicted value. It is of interest that IgG was removed with predictable and expected efficiency. Removal of IgG in patients with multiple myeloma has been reported to be less than expected based upon the amount of plasma removed.⁸ The 55% extravascular distribution of IgG, and presumed rapid equilibration of the intravascular and extravascular compartments during the course of the exchange has been used to explain this observation. Our contrasting finding in nonmyeloma patients may indicate a fundamental difference between transport of pathologic IgG paraprotein and normal IgG. The apparent reduced efficiency seen in patients with abnormal IgG paraproteins could be due to a high intravascular volume. However, if this were the case, IgM removal in these patients would also tend to be less than expected, and this has not been observed. SGOT, LDH, amylase, and CPK are removed less than predicted. Synthesis or equilibration could explain this observation. Uric acid, calcium, and K^+ are only slightly reduced by partial plasma exchange. Their average removal was only 59% of predicted. Thus, a partial plasma exchange that would lead to a 50% reduction in IgG or IgM would only be expected to reduce uric acid, calcium and K^+ measurements by 25%. These are insignificant losses when compared to total body stores. Whether the reduction in calcium is mirrored by a similar reduction in unbound calcium is not known. Fibrinogen and C_3 are removed with a mean efficiency greater than predicted ($p < 0.05$), and suggests consumption of fibrinogen and complement during the course of the procedure. Additional studies are required to substantiate this finding and elucidate the mechanism which explains it.

Most plasma constituents returned to prepheresis levels within 48–72 hr. Recovery of cholesterol was delayed, and 1 wk may be required to obtain prepheresis levels. This slow recovery may explain the success of therapeutic attempts to remove cholesterol.⁹ Mean recovery of fibrinogen and complement for the study group was approximately 65% at 48 hr. Similar considerations apply to IgG and IgM where recovery was also slow. The mean recovery at 48 hr for these

constituents was 44%. In clinical situations this may limit the frequency or extent of partial plasma exchange or may compel the use of plasma for fluid replacement. A previous study¹⁰ reported that, despite marked reduction in concentrations of fibrinogen, clinical bleeding was not a significant problem. The authors concluded that replacement of fibrinogen was unnecessary. IgG and IgM levels measured even 2 wk postpheresis in both normal subjects was still markedly depressed. With regard to recovery data, this is in apparent disagreement with another report.¹¹ The difference, however, probably reflects a difference in the calculation of recovery rather than a difference in actual recovery. Other studies have demonstrated slow immunoglobulin recovery.¹²

Although recovery of quantitative immunoglobulin levels was slow, biologic antibody activity may not correlate with immunoglobulin concentration. Previous reports would suggest this possibility.¹³ Anti-A and anti-B agglutinating activity, as measured in an automated system, was 100% of the prepheresis value at postpheresis day ten, while quantitative immunoglobulin studies suggested only a 40% recovery. Increased synthetic rate for selected antibodies or production of antibodies with greater avidity for antigen could explain this finding. Manual agglutination may be less sensitive to changes in the numbers and avidity of antibody molecules. The automated technique, on the other hand, is exquisitely sensitive to the number of antibody molecules per cell and their avidity.¹⁴ Clinically, following plasma exchange, the titre of pathologic antibody in some cases may remain substantially below prepheresis levels, yet ameliora-

tion of symptoms does not occur.¹³ This observation may be explained by increased biologic activity of newly synthesized antibody in response to antibody removal. Increased and changing antibody avidity has been observed following immunization.¹⁵

Understanding of the kinetics of plasma exchange is required to insure the safety and efficacy of this procedure. These data show that for many plasma constituents, removal and recovery characteristics are predictable. Because of the relatively rapid recovery of most plasma constituents to prepheresis levels following plasma exchange, albumin replacement is adequate where volume and frequency of partial plasma exchange is not excessive. Where the percent of plasma exchanged is large, greater than one plasma volume, or the schedule intensive, more often than every 48 hr, depletion of complement, selected coagulation factors, fibrinogen, factors X and XI, immunoglobulins, and cholesterol is the expected minimum consequence. Replacement with plasma must be considered, but hemorrhage and infection have not been reported or emphasized as complications of the procedure. The mechanisms that control the rate at which plasma constituents return to normal following plasma exchange is not known. Reequilibration may be important for some constituents, but synthetic rates are also of obvious importance. Differences in control mechanisms may exist between normal and abnormal patient populations, particularly those on immunosuppressive drugs, and may affect the applicability of plasma exchange as a therapeutic modality. Further studies are needed to investigate these preliminary observations.

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