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The Role of Replacement Fluids in Plasma Exchange and Therapeutic Applications of Apheresis

by

© Rhona Watkins

Submitted for the degree of M.D. in the Faculty of Medicine, University of Glasgow.

This work was performed in the Department of Haematology, Royal Infirmary, Glasgow.

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DECLARATION

The planning and initiation of all the investigations in this thesis are entirely my own work, as was the co-ordination of laboratory data and the analysis of results. All specimen collection and supervision of all patients undergoing plasma exchange was carried out by myself.

The majority of the laboratory techniques utilised were of a routine nature and I have received the willing co-operation and assistance of my colleagues. The extent of my personal contribution in relation to this technical assistance can be outlined as follows:-

1. Coagulation Screening

All coagulation screening was carried out by me. Individual Factor Assays of all patients were carried out by me. The remainder of the haematological investigation, namely a routine full blood count was carried out by the technical staff using a Coulter 'S' Counter.

2. Clinical Response

The clinical response to plasma exchange was determined by me. I personally examined all patients before and after plasma exchange and carried out any further investigations as required.

3. Statistical Methods

I personally carried out the statistical tests.

SUMMARY

By 1981 it had become apparent that a new technique, plasmapheresis, offered considerable potential in the treatment of an ever increasing number of diseases by its ability to remove an affecting component from the patient's blood. Plasmapheresis involved the selective removal of a small volume of plasma which was not replaced, whereas plasma exchange involved the removal of a large volume of plasma which was replaced by an almost equal volume of appropriate fluid.

A blood cell separator (IBM Type 2997) was acquired by the Department of Haematology, Glasgow Royal Infirmary, and during the period 1981-1985, 2685 plasma exchange procedures were carried out on 201 female and 125 male patients being treated for a range of clinical disorders. During this period I carried out a number of individual studies which form the basis of this thesis.

The thesis first reviews the literature relating to the application of therapeutic plasma exchange in the treatment of selected disorders. It outlines the types of exchange procedures and the methods of component removal based on physical and chemical means. Some continuous flow and discontinuous flow centrifugation equipments and membrane filtration equipments are described and a comparison made of centrifugation and filtration procedures. The potential offered by chemical separation procedures is indicated. The practical considerations, particularly those of vascular access, anticoagulation and the choice of replacement fluids, are discussed. The rationale for treatment by plasma exchange, evidence of its efficacy and the implications of such treatment are considered. Some applications of plasma exchange in the treatment of

selected disorders are described. Whilst exchange procedures are relatively safe they are not without risk and some of the adverse reactions and hazards are indicated.

The thesis has three basic objectives.

1. To assess the effectiveness of three selected replacement fluids used in the plasma exchange procedures carried out on three groups, each with patients being treated for myasthenia gravis, Guillain-Barré Syndrome, chronic polyneuropathy and polymyositis.
2. To assess the effectiveness of plasma exchange procedures in the treatment of patients with cold haemagglutinin disease, Waldenstrom's macroglobulinaemia and hyperviscosity due to multiple myeloma, immune haemolytic anaemia, Factor VIII inhibitors, polyneuropathy, myasthenia gravis and Eaton-Lambert syndrome, Guillain-Barré syndrome, glomerulonephritis and Goodpasture's disease, aplastic anaemia together with pure red cell aplasia and polymyositis.
3. To undertake a preliminary assessment of the potential offered by leukapheresis as an adjunct to conventional chemotherapy in the treatment of patients with acute myeloid and lymphatic leukaemias and with chronic myeloid and lymphatic leukaemias.

In the first study, three groups of patients with selected disorders were treated by plasma exchange procedures in which their plasma was replaced by Plasma Protein solution (2 litres 5% albumin plus 0.5 litre normal physiological saline), Polygeline 1 (1 litre 5% albumin plus 1 litre Haemaccel plus 0.5 litre normal physiological saline), and Polygeline II (2 litres Haemaccel plus 0.5 litre normal physiological saline) respectively. The haematological response, as assessed by

changes in standard full blood count, white blood count, platelet count, coagulation screening and quantitative assays of coagulation factors; the biochemical response, as assessed by changes in urea and electrolyte values, liver function evaluation, plasma protein evaluation and serum immunoglobulin assays; and the clinical response of each patient was continuously monitored throughout the sequences of exchange procedures. The mean value of each measured haematological and biochemical parameter was calculated for all the patients in each of the three groups. The number of patients was sufficiently large to permit a statistical comparison to be made of the changes in mean values of each parameter, throughout the exchange procedure. Significant differences in values were thus a direct consequence of the choice of replacement fluid used by each group in the plasma exchange procedure.

In the remaining studies specific haematological and biochemical parameters only were measured as required. Changes in parameter values and in the clinical response to treatment were monitored continuously throughout the procedure. Patients treated for the different disorders and those involved in the leukapheresis procedures varied greatly in numbers, but were not sufficiently numerous at any one time to permit the setting up of controlled trials.

From this study I have obtained the following results and conclusions.

1. For each of the three groups of patients, the mean values of the full blood counts, platelet counts, prothrombin time, Kaolin cephalin clotting time, thrombin time, assays of coagulation factors I,II,VIII, IX and X; of urea, sodium,potassium, chloride, creatinine, calcium, magnesium, phosphate, serum bilirubin, asparate transaminase, alanine transaminase, alkaline phosphatase, total protein, albumin, globulin,

serum iron, total iron binding capacity and serum immunoglobulins IgG, IgM and IgA pre and post-plasma exchange differed only slightly. The change in mean values at intervals post-plasma exchange exhibited similar trends although the absolute values of change were slightly different in the three groups. It was noted that in the case of some individual parameter measurements the differences in the mean values were statistically significant, and these occurred randomly within the three groups.

In the treatment of myasthenia gravis, Guillain-Barré syndrome, polyneuropathy and polymyositis the three groups of patients exhibited similar clinical response to the exchange of plasma with the three replacement fluids, namely plasma protein solution, polygeline I and polygeline II. The incidence of side effects arising from the use of polygeline II was marginally greater than the incidence of side effects observed in patients exchanged with plasma protein solution and polygeline I. On the basis of the haematological, biochemical and clinical assessments it is concluded that the three selected fluids are equally effective in the replacement of plasma in the plasma exchange procedures.

The data obtained in this study and the conclusions drawn are in general agreement with those noted in other studies of different clinical disorders treated by plasma exchange using similar replacement fluids but involving considerably fewer patients.

2. Plasma exchange in the treatment of patients with cold haemagglutinin disease produced a significant increase in haemaglobin and a decrease in the cold antibody titre. Four patients required to

be transfused during the period of exchange, one patient relapsed and died from a myocardial infarction. In general, only transient responses to plasma exchange have been obtained, the antibody titres returning to their pre-exchange levels in only seven days.

Plasma exchange in the treatment of patients with Waldenstrom's macroglobulinaemia and hyperviscosity due to IgA and IgG myeloma produced significant reductions in the whole blood viscosity at all three shear rates and in plasma viscosity immediately after plasma exchange. The value of serum immunoglobulin IgM also decreased in patients with Waldenstrom's macroglobulinaemia and all but one patient noticed a marked improvement in clinical status. All but two patients with hyperviscosity due to IgA and IgG myeloma showed improvement in clinical status.

In the treatment of patients with immune haemolytic anaemia, plasma exchange resulted in a significant increase in haemoglobin associated with a decrease in warm antibody titre. This permitted temporary control of haemolysis and a measure of patient stability, giving other modalities time to act.

In the treatment of patients with Factor VIII inhibitors values of prothrombin time, thrombin clotting time and Factor VIII Rg were not significantly altered by plasma exchange. The values of Kaolin cephalin clotting time and inhibitor screen were reduced and the Factor VIII C concentration was markedly increased. The patients being treated for a range of disorders reacted favourably to the procedure.

Half of the patients with chronic inflammatory polyneuropathy improved

after treatment by plasma exchange. No change was discernable in the others and one patient unfortunately died.

In patients with myasthenia gravis, plasma exchange resulted in a significant reduction in Anti-AchR values and a corresponding increase in vital capacity. The clinical status of three patients was unchanged by the procedure but in three patients with high initial vaues of Anti-AchR a marked improvement was seen.

Patients with Eaton-Lambert syndrome reacted similarly to treatment by plasma exchange.

In the treatment of patients with the Guillain-Barré syndrome an improvement in clinical status of one grade was observed in seven patients, of two grades in seventeen patients, and of three grades in three patients in whom the duration of the symptoms ranged from nine to twelve days and for whom the procedure was initiated one day after diagnosis of the disorder.

The serum creatinine concentrations in patients being treated for glomerulonephritis were markedly lowered as a result of plasma exchange. Regular plasma exchange appeared to prevent progression to renal failure in nine out of twelve patients with mesangiocapillary Type 1 GN, for a mean period of eighteen months. In one patient the procedure was stopped following a rise in serum creatinine. Four patients with mesangiocapillary Type II GN progressed to renal failure despite continuing plasma exchange, whilst two patients did not progress to end stage renal failure. Three patients with mesangial IgA disease had virtually stable serum creatinine during nine months of procedures.

Two patients with idiopathic membranous nephropathy progressed to end stage renal failure despite plasma exchange. Pulmonary haemorrhage occurred in ten patients and was rapidly controlled in four. Long term follow up revealed that eight patients were still alive, five with functioning allografts and three maintained on dialysis.

Both serum creatinine and anti IgM Ab (% binding) were significantly lowered by plasma exchange in the treatment of patients with Goodpasture's syndrome. Improvement in clinical status was noted in six patients, four patients proceeded to dialysis and two ultimately died. In both disorders recovery of renal function was clearly related to the severity of glomerular damage. The prognosis for patients presenting with creatinine of 550 μ mol/1 or less was significantly better than for those with creatinine levels of 600 μ mol/1 or greater.

Plasma exchange in the treatment of patients with red cell aplasia and aplastic anaemia produced a marked increase in the numbers of CFU-C units. Values of creatinine kinase, asparate transaminase, alanine transaminase and lactate dehydrogenase were all reduced by plasma exchange in the treatment of patients with polymyositis. The clinical status of three patients showed an improvement of one grade whilst that of seven patients was improved by two grades.

Short term side effects were observed during the treatment of the above disorders, some of which could be attributed to the choice of replacement fluid and others to the exchange procedure itself. The longer term effects embraced the hypercoagulable state, bacterial infection and viral infection. No patient developed side effects which have had deleterious consequences on health.

In some disorders the clinical effect of plasma exchange could be subjectively correlated with the level of specific disease marker. In other disorders correlation between the serological findings and the disease activity was poor and the assessment of the therapeutic value of the procedure was based on clinical evaluation. Where improvements in clinical status were observed these were generally due to relief of clinical manifestations rather than to any effect on the underlying disease itself. The favourable response of many disorders to plasma exchange was short term and this limits its value to therapy for acute conditions.

3. Leukapheresis provided a relatively rapid means of safely reducing the circulating white cell count and resulted in clinical improvement. It did not provide a superior form of treatment for chronic myeloid leukaemia and did not appear to delay blastic transformation. In the treatment of chronic lymphathic leukaemia, leukapheresis did reduce the lymphocyte count, spleen size, lymph node size, bone marrow infiltration and liver size , but it should be used primarily for those patients failing other forms of treatment.

The application of Blood Cell separators and plasma exchange procedures will be justified by their contribution to the improvement of the clinical management of disorders by cellular or plasma depletion or by plasma replacement. Hopefully, they may add to the understanding of the separation characteristics and the kinetics of cell population which should lead to an improvement in the rationale of treatments based on apheresis procedures. There is however, a need for a procedure that will provide the expeditous return of the patient's own plasma from which the offending component(s) have been selectively removed in the course of the exchange.

INTRODUCTION

Blood cell separators were initially introduced and subsequently developed to provide granulocyte transfusions for patients, with acute leukaemia, who become profoundly neutropoenic secondary to induction therapy and then subsequently infected. Such separators also proved to be highly efficient in reducing the numbers of circulating blast cells and thereby decreasing the complications of leukostasis, namely the incidence of pulmonary and cerebral insufficiency.

Cell separators were later developed for the exchange of large volumes of plasma and this procedure was performed on small numbers of patients with diverse pathological conditions where there were known, or presumed, abnormal plasma factors contributing to the aetiology, or pathogenesis, of the particular conditions. Whilst beneficial results were reported, the absence of well-controlled randomised studies made if difficult to assess the efficacy of the procedures.

It was established that some 75% of a patient's plasma could be removed safely in one to two hours provided that an appropriate replacement fluid was substituted to maintain colloid osmotic pressure and thus prevent formation of peripheral oedema. At the time no information was available as to which replacement fluid would be of optimum benefit to the patient. The choice was determined primarily by the replacement fluid's ability to:

1 restore volume and oncotic pressure

2 replace the constituents removed during plasma exchange, namely, coagulation factors, immunoglobulins and fibronectin.

At Glasgow Royal Infirmary, plasma exchange has provided a basis for the treatment of selected patients with:

1. Antibody Mediated Diseases

   a. Myasthenia Gravis, b. Guillain-Barré Syndrome, c. Polymyositis and d. Factor VIII Inhibitors.

2. Immune Complex Diseases

   a. Rapidly progressive Glomerulonephritis, b. Goodpasture's Syndrome, c. Systemic Lupus Erythematosus, d. Rheumatoid Arthritis, e. Dermatomyositis.

3. Non-Immune Mediated Diseases

   a. Hyperviscosity Syndrome (Waldenstrom's Macroglobulinaemia)
   b. Raynaud's Phenomenon.

Over the period 1980-1985, I carried out 2685 therapeutic plasma exchanges on an IBM 2997 cell separator. Circulatory access was achieved by venepuncture of the antecubital veins with 19 Gauge cannulae. Those patients with renal disorders had either Scribner shunts, or latterly, arteriovenous fistulae. Each patient underwent a minimum of five daily plasma exchanges with 2.5 litres of plasma being removed during each procedure. Heparin was used as anticoagulant, 5000 IU being given to the patient at the start of the procedure and 3000-5000 IU during the procedure.

The main replacement fluids used were plasma protein fraction and albumin 5% and, very occasionally, fresh frozen plasma. With albumin 5% and plasma protein fraction there was no risk of transmitting hepatitis and no matching for ABO incompatibility required, factors which had to be taken into consideration if fresh frozen plasma was used.

During the period 1980-1982 the consumption of plasma products was as shown in Table 1.

Table 1

Consumption of Plasma Products

Year	1980	1981	1982
Number of Plasma Exchange procedures Undertaken	332	222	237
Albumin 5% (litres)	200	285	300
Plasma Protein Fraction (litres)	100	98	75

The consumption of albumin 5% was a large percentage of the hospital's total allocation and alternative replacement media were sought. It was known that dextrans, gelatins and hydroxyethyl starch (HES) were widely accepted where rapid expansion of plasma volume was required and that synthetics were generally more efficient than albumin in the immediate restitution of blood volume e.g., dextran has a colloid osmotic pressure approximately twice that of albumin and therefore pulls more water out of tissue into the vasculature. Whilst gelatins have been in use for a considerable period they are known to have three major drawbacks namely:

1 Alteration of the haemostatic mechanism due to defects in platelet function

2 Transient effects on renal function

3 Incidence of anaphylactoid reactions

Polygeline (Haemaccel) was known to have a low incidence of adverse reactions and was introduced as the main replacement fluid in 1983. Since then the consumption of albumin 5% used in plasma exchange procedures has decreased dramatically as shown in Table 2.

Table 2

Consumption of Albumin 5%

Year	1983	1984	1985
Number of Plasma Exchange procedures Undertaken	276	286	295
Albumin 5% (litres) used	310	201	175

Since the introduction of polygeline it has not been necessary for the Infirmary to use commercial albumin as a replacement fluid.

At that time, data on the efficacy of polygeline as a replacement fluid in plasma exchange were limited. Results had been published[1] of a study in which polygeline had been compared with plasma protein fraction as the sole replacement fluid in a sequence of four to six plasma exchange procedures undertaken in the treatment of six patients with various types of glomerulonephritis and deteriorating renal function. Measurements had been made of full blood count, blood concentrations of urea, electrolytes, proteins, immunoglobulins, complement and coagulation factors before and at one hour and fifteen hour post-exchange. Creatinine clearances were measured between exchanges which were undertaken at four to seven day intervals. There was no evidence of

hypoalbuminaemic hypovolaemia and no postural hypotension occurred; but it was thought that low albumin concentrations could be a problem if multiple procedures were carried out at very short intervals.

Also available were the findings of studies undertaken to establish the effect of plasma exchange on fibrinogen and platelet levels[2] and on serum immunoglobulins and complement components[3]. Plasma protein solution was used as the replacement fluid but the studies included data for a limited number of exchanges (37 in all) carried out on five patients using plasma protein solution supplemented by Haemaccel. No significant effect attributable to the addition of Haemaccel was detected.

There was clearly a need for more detailed information of the effect, on the routine haematological, biochemical and coagulation screening of patients, of replacing plasma wholly, or partially, by polygeline during plasma exchange in the treatment of various disorders and of any consequential adverse effects of the procedure. It was also desirable that a datum for comparison should be provided by a similar evaluation of the effect of replacing plasma by plasma protein solution.

In the course of further studies, in which albumin 5% was also used as a replacement fluid, it was possible to assess the efficacy of plasma exchange in the treatment of patients with:

1. Cold haemagglutinin disease
2. Waldenstrom's macroglobulinaemia and hyperviscosity due to multiple myeloma.
3. Immune haemolytic anaemia
4. Factor VIII inhibitors
5. Chronic polyneuropathy

6. Myasthenia gravis and Eaton-Lambert syndrome
7. Guillain-Barré syndrome
8. Glomerulonephritis and Goodpasture's disease
9. Aplastic anaemia together with pure red cell aplasia
10. Polymyositis

Experience was gained in the use of leukapheresis in the treatment of patients with acute and chronic leukaemias ,both myeloid and lymphatic, as an adjunct to conventional chemotherapy. A preliminary study was made of the factors involved in the conduct of such procedures.

This thesis is based on the above studies of plasma exchange and leukapheresis procedures carried out over a four year period 1981-1985 during the tenure of my post as Registrar in Haematology at the Glasgow Royal Infirmary.

The clinical indications for therapeutic plasma exchange, the efficiacy of fluid replacement and the nature of concomitant side effects have been critically analysed and the results of these studies are presented and discussed.

1. REVIEW OF THE LITERATURE

1.1 The Basis of Apheresis

The history of contemporary apheresis predates Hippocrates. Apheresis[4,5] means 'taking off' and is applied to the individual components of whole blood, plasma, platelets and white blood cells. It will be of benefit if the circulating product is pathological and is no longer being introduced, synthesised, or accumulating. In the case of an immune disorder, the offending antibody (or cellular agent) is being synthesised continuously. The end-organ, whether it be nerve, muscle junction, synovial membrane, or renal glomerulus can repair itself at some finite rate, provided the disease is of long duration and the end-organ's ability to repair has suffered no, or only limited, damage. If, following the effective removal of a circulating antibody, repair can occur quickly, then improvement will be visible rapidly. If there is no repair, or it occurs only slowly, improvement will be limited, or the condition will remain stable. If the circulating antibody is re-synthesised rapidly then only limited benefits will be derived from its removal, even in the presence of a rapidly repairing end-organ[5].

The extent to which the re-synthesis, or re-introduction, of the offending factor occurs and to which the end-organ can repair itself, will markedly influence the benefit gained from apheresis and the length of time over which the procedure may have to be applied. If the medication given to reduce re-synthesis acts slowly the apheresis procedure may have to be repeated over a

period of time. This intermittent mechanical removal of the factor could however be preferred to long-term medication with cumulative risks. If the rate of repair of the end-organ is slow the apheresis procedure may be of no more value than the agents which only reduce re-synthesis[5].

Clearly, effective treatment of a disorder by an apheresis procedure requires the causative role of the postulated circulating factor to be specifically established. This could be achieved by measuring the removal of the factor which is thought to be pathogenic, studying the effects of its removal, the duration of any observed effect on both clinical and laboratory measurements and the influence of removal of a factor of known laboratory markers of disease activity. If the removal of a factor correlates strongly with clinical improvement or stability, coupled with changes towards normality in other laboratory parameters of disease activity, then that circulating factor may be presumed pathogenic[5].

1.2 Types of Procedures

Therapeutic apheresis now includes a range of procedures to remove one or more circulating products pathogenic to a given end-organ and which are re-synthesised at a variable rate.

1. Plateletpheresis

Marked elevations of the platelet count to levels greater than $1000 \times 10^9/1$ can be seen in all the myeloproliferative disorders, as a reactive thrombocytosis, in post-surgical

patients (particularly post-splenectomy), and in patients with a variety of malignancies, or infections[6].

Plateletpheresis provides means for the safe and rapid removal of large numbers of platelets from patients[7,8]. Depending on the initial platelet count it is possible to remove as many as 5-10 x 10^{12} platelets (equivalent to more than 100 units of platelet concentrate) in a single donation. Whilst this results in a rapid lowering of the platelet count, the effect is temporary and the count rises again within the next 24-48 hours. The procedure may be repeated until conventional therapy has time to become effective. e.g.,before chemotherapy becomes operative.

In-vitro abnormalities of platelet function can sometimes be corrected following plateletpheresis[7]. Depending on the cell separator used and the centrifugation setting, it is sometimes possible to remove sub-populations of platelets, particularly larger platelets, more selectively. It is possible that circulating platelet aggregates of differing size are more effectively removed.

Immediate plateletpheresis appears to be indicated in patients with a significant elevation in platelet count and signs or symptoms attributable to thrombocytosis. It is not clear whether plateletpheresis is also indicated as a prophylactic measure in the asymptomatic patient and if so, at what level of platelet count it is indicated.

2 Erythrocytapheresis

The removal of red blood cells from patients with polycythaemia is easily accomplished using phlebotomy techniques and there is no advantage in the use of blood cell separator for this purpose[9]. However, exchange transfusions have been used, but infrequently, in the treatment of sickle cell disease[10,11]. Indications have included priapism, unrelenting painful crises, pregnancy and preparation for surgery. The rationale is to remove erythrocytes containing haemoglobin S and replace them with normal red cells containing haemoglobin A. The introduction of cell separators has enabled large volume exchanges to be undertaken with the possibility of achieving higher concentrations of haemoglobin A, generally in excess of 90% in a 3-4 hours procedure.

3 Stem Cell Collection

Mononuclear cell fractions which can be efficiently collected by all the continuous-and intermittent-flow centrifugation devices have been shown to contain a variable number of CFU-GM (myeloid), BFU-E (erythroid) and megakaryocytic precursors[12,13].

It is likely that harvesting peripheral blood stem cells by leukapheresis will be evaluated more fully for the management of patients with other forms of malignant neoplasms even in cases where peripheral blood leukocyte counts are normal. Precursor cells as measured by CFU-C assay can be collected in quantities that approach the theoretical number required for bone marrow re-population if

five to ten procedures are performed. Furthermore, it has been found that after a single plateletpheresis, wherein 30% of peripheral blood progenitor cells are collected concomitantly, there occurs a doubling of both erythroid and myeloid precursors in the donor's peripheral blood 48-72 hours later. Thus cell separator technology may be used as an instrument for facilitating autologous marrow reconstitution after chemotherapy.

4 Lymphocytapheresis

Intensive lymphocytapheresis can result in lymphocytopenia comparable to that achieved by thoracic duct drainage[14]. Lymphocytopenia is due predominantly to a loss of circulating T-lymphocytes which in turn can be reflected functionally in a decrease of in-vitro response to T-cell mutogens. Repeated aphereses are necessary.

Lymphopheresis has been used mainly in the treatment of patients with systemic lupus erythematosus and rheumatoid arthritis[15-17].

5 Leukapheresis

Leukocytes are less deformable than red blood cells with primitive blasts considerably less distensible than mature myeloid cells. With increasing numbers of blast cells ($100 \times 10^9/1$) in acute and chronic myeloid leukaemia the blood flow in the micro-circulation can be impeded by plugs of poorly deformable blasts[5]. Local hypoxaemia may be exaccerbated by the high metabolic activity of the continuously dividing blasts with endothelial damage and

haemorrhage. The situation can be further exaccerbated by red blood cell transfusions that rapidly increase whole blood viscosity in the presence of high circulating white cell count, thereby further compromising local blood flow[18].

Although pathological evidence of leukostasis can be found in most organs in patients with extremely high white cell counts, clinical symptomatology is usually related to central nervous system and pulmonary involvement[19]. Occasionally pulmonary symptomatology with worsening hypoxaemia can occur with therapy and lysis of entrapped leukaemic cells.

Should the white cell count reach 100 x 10^9/1 it may constitute a medical emergency and the count must be reduced. Often it takes a period of time for chemotherapy to work and because of metabolic problems it may well be contra indicated for 48-72 hours. There are numerous reports of intensive leukapheresis leading to improvement of pulmonary and haematological symptoms[19-21]. However, there may be life-threatening side effects. These patients tend to be extremely ill with marked pulmonary vascular insufficiency characterised by tachycardia, dyspnoea and hypoxia. Vascular insufficiency can lead to priapism and necrosis of the finger tips. Due to central nervous system effects, patients may become delirious, stuporose or dizzy. Leukapheresis itself may worsen these problems.

Leukapheresis is thought to be of short-term value in the treatment of chronic myeloid leukaemia[22]. It will provide

symptomatic relief only and there is no evidence that it will increase overall survival. Hyperleukocytosis is rarely a problem in chronic lymphatic leukaemia. Leukapheresis may produce improvements in spleen size, lymph node size and bone marrow infiltration but will not increase haematocrit or platelet count[19]. There is no evidence that intensive leukapheresis will provide clinical improvement or control of the peripheral blood eosinophil count[5]. There may have been a few reports of favourable thereapeutic effects of leukapheresis in patients with hairy cell leukaemia despite low yields of hairy cells[6,23,24].

6 Plasmapheresis

In plasmapheresis a limited volume of blood is collected by venesection and the plasma separated from the red cells by centrifugation and removed[25-29]. The red cells are returned subsequently to the donor after re-suspension in isotonic saline. In plasma exchange however, large volumes of plasma may be removed rapidly from the circulation and simultaneously replaced with red cells by an equivalent volume of suitable substitute fluid. The development of cell separators has made large volume exchange possible and allowed the expansion of therapeutic applications. As a result, automated plasma exchange has been attempted in treating every condition in which there is a known or presumed abnormal plasma factor contributing to the aetiology or pathogenesis of that disease.

Originally plasma exchange was thought to be a benign procedure, thus encouraging the tremendous growth of interest in its use. The increasing need for blood components (plasma, albumin, etc)

as replacement fluids provided further impetus directed towards removal of specific pathogenic substance.

This considerable activity has masked the fact that, in the vast majority of cases, the efficacy of plasma exchange has not been critically evaluated[28]. This and other considerations, such as cost and the recognition that plasma exchange can be a potentially dangerous therapy, have finally prompted a more careful evaluation of the role, if any, of plasma exchange in those diseases where proof of efficacy is lacking[30].

The terms, plasmapheresis, and plasma exchange tend to be used indiscriminately but this present study is concerned with plasma exchange.

1.3 Methods of Component Removal

Plasma exchange requires that the removed plasma be replaced. The use of the patient's own plasma is a more physiologic, less costly and often more efficient replacement, provided the plasma constituents deemed to be harmful can be selectively removed[31]. This can be accomplished by physical, chemical, or immunological means, in either on-line, or off-line circuits.

1.3.1 Physical Separation of Components

In theory, any physical property of the blood cells can be used to permit selective separation and collection of components and particularly properties such as density, volume, surface charge, adhesiveness and membrane receptors.

Cell separation has been achieved by gravitational forces in gravity leukapheresis[32,33] and by filtration in the collection of granulocytes[34,35]. In practice, however, differences in density provide the most convenient means of separating the cellular components[36-38].

Granulocytes are lighter than red cells, but are much heavier than plasma, lymphocytes and platelets. When granulocytes are subjected to centrifugal forces they become concentrated in a layer 'inside' the red cells and 'outside' the plasma, lymphocytes and platelets. At low G forces, with sedimenting agents the layer can be preferentially removed and the granulocytes collected whilst the majority of platelets are left free in the plasma. This provides optimal collection of granulocytes and adequate, but less than optimal, yields of platelets. To obtain sufficient granulocytes a continuous flow circuit, broadly analogous to that used in dialysis, is necessary. As the G forces are increased, granulocytes are replaced by mononuclear cells in the leukocyte-rich interface and are extracted with the buffy coat. That increases platelet yield but sacrifices yield of neutrophils[39].

The donor or patient forms part of a closed loop circuit coupled with the centrifugation equipment, wherein the patient's total blood volume becomes available for processing, extracting or replacing specific cellular constituents of the cellular fraction.

The early types of cell separator were cumbersome to use and a

number of parts required sterilisation before each operation. The development of more sophisticated machines with disposable items such as bowls, collection bags, ancillary containers, tubing etc has greatly facilitated the procedure.

The discovery that granulocytes in the presence of calcium (heparinised blood) will adhere to scrubbed nylon wool fibres led to the development of filtration procedures[40]. The granulocytes collected on the fibres can be eluted with citrated plasma. Whilst more granulocytes can be harvested (per litre of blood processed) by filtration than by centrifugation method, the granulocyte concentrates obtained are associated with a higher incidence of febrile reactions as a result of membrane changes caused by the adherence-elution process[41]. Severe priapism has also been experienced, which required surgical decompression and whilst a direct cause and effect has not been established, the same unusual complication has been found in patients undergoing renal haemodialysis[42].

1. Centrifugation Methods

A wide variety of commercial equipments based on either discontinuous or continuous centrifugation cell separation is now available[43]. These include:

a) Discontinuous Flow Machines

i) Haemonetics Model 30S, Model V 50

ii) Dideco Progress BT 970

b) Continuous Flow Machines

i) IBM Cell Separator Series 2990, Series 2997.

ii) Fenwal Celltrifuge II, Series CS 3000

iii) Dideco Viva BT 790

A) Discontinuous Flow Machines

In cell separators of the discontinuous flow type[37], the blood components are separated within the bowl and removed through a centrally placed channel starting with the lightest components, namely the plasma and ending with the heaviest, the red cells. At the end of each cycle the bowl is temporarily filled with air so that the process is essentially discontinuous or intermittent. The apparatus is relatively cheap and easy to operate and is particularly useful for the collection of platelets and granulocytes as required. The donor's fluid balance is disturbed more than during continuous flow centrifugation and the volume of blood that can be processed in unit time is somewhat less.

Haemonetics Cell Separator V 50

The Haemonetics V50 cell separator consists of a desk type console, the lower part of which contains the rotating bowl, its motor drive unit and speed control. (Figure 1). The upper part of the console carries two peristaltic pumps together with the control panel and alpha-numeric display unit. Different extracorporeal volumes are accommodated by centrifuge bowls of various sizes which are coupled to pre-connected sterile disposable sets which provide the external fluid pathway. The array of collapsible sterile containers complete the assembly with the minimum of set up time.

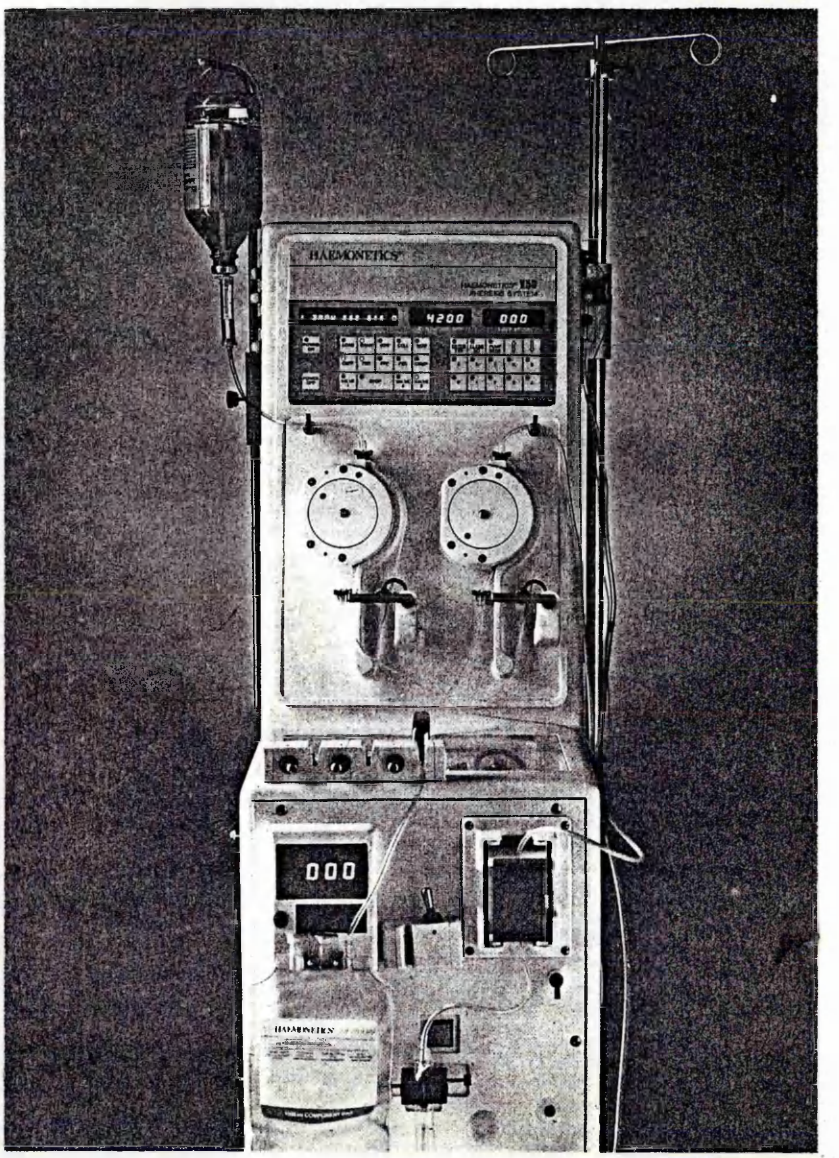
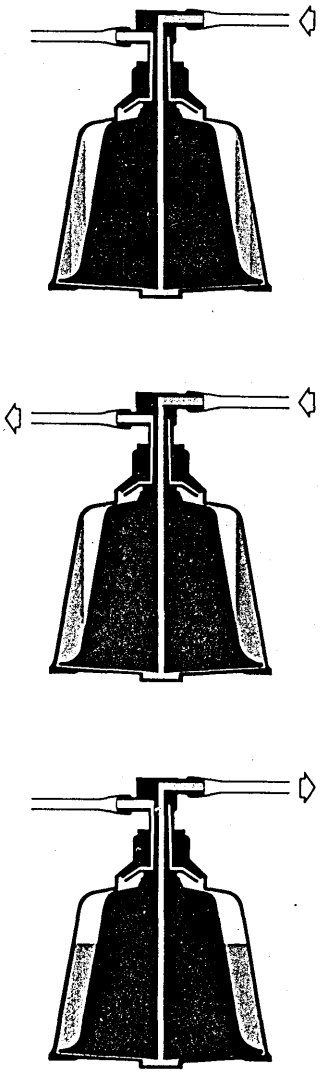


Figure 1. Haemonetics V50 Cell Separator

The machine automatically draws anticoagulated whole blood which is pumped to the rotating bowl and plasma separation begins. As the bowl fills, component bands appear, plasma (the lightest component), platelets and white cells are sequentially expelled through a centrally located channel in the rotating seal. (Figure 2). When the bowl is full of red cells (the heaviest component), the centrifuge stops and the uncollected components pumped to a container bag for for gravity reinfusion. At the end of each cycle, the bowl is temporarily filled with air, so that the procedure is discontinuous or intermittent. When a preset volume of plasma has been collected (as determined by weight) the procedure is automatically terminated.

During the procedure the display panels continuously indicate the volume of plasma removed, the pump speed, the processing cycle and other data. A special programmed display up-dates the operator on the states of the instruments and prompts the operator through the procedure. Flow indicator lights confirm adequate flow. In the event of a decrease in flow a monitoring system slows or stops the pumps until adequate flow is achieved. The presence of air in the circuit is detected ultrasonically. Many variables are pre-programmed but the automatic protocols can be manually over-ridden at the time.

This instrument collects therapeutic quantities of platelets and granulocytes, either simultaneously or separately. Other products collected include cell-poor plasma and



Anticoagulated blood is pumped to the rotating bowl and plasma separation begins. As the bowl fills, component bands appear. Plasma, platelets and white cells are sequentially expelled. When the bowl is full of red cells, the centrifuge stops, and uncollected elements are pumped to a bag for gravity reinfusion.

Figure 2. Plasma Separation in Rotating Haemonetics Bowl

lymphocytes. Procedures have been developed for plasma exchange, lymphocytapheresis, leukapheresis, plateletpheresis and erythrocytapheresis. A single or double-venepuncture technique may be used.

B) Continuous Flow Machines

In continuous flow cell separators[44-46], whole blood is drawn from a vein in one arm and returned to a vein in the other arm of the donor. From the donor the anticoagulated blood passes to the centrifuge bowl, or rotor, in the machine. As it enters the channel and flows in a clockwise direction, parallel to the axis of rotation, towards the collection chamber, the blood separates into three principal fractions, namely plasma, granulocytes (buffy coat) and packed red cells. The interface position between fractions and collection are under the direct control of the operator, who must vary the extraction rates of packed red cells and plasma to obtain the desired component which is removed through the lid of the separator bowl. The packed red cells and plasma are then recombined and returned to the donor while the buffy coat is fed into a collection bag. A special seal permits the continuous transfer of whole blood from the stationary to rotating portions of the bowl and the simultaneous removal of the separated components in the reverse direction throughout the procedure.

The centrifugal separation which allows the leukocytes to be collected, can be expressed[47] in terms of a packing factor P_F defined as

$$P_F = \frac{Gts}{d}$$

Where G = separation acceleration, t = time of separation, s = sedimentation rate of blood, and d = distance through which sedimentation takes place for the channel used. The separation time t is expressed as

$$t = \frac{AL}{Q}$$

Where A = channel area, L = channel length, and Q = mean flow through the channel.

Thus $$P_F = \frac{G}{Q} \cdot \left[\frac{AL}{d}\right] \cdot S$$

The channel area A, length L and distance d through which which sediment takes place, are geometrical properties of the system and are fixed. Sediment (S) is variable and not directly controllable. Thus the degree of separation in the single-stage channel can be characterised by the ratio of separation acceleration G to flow rate Q through the channel. If this ratio is held constant, similar separation should be obtained at different values of G and Q. Using channels of different geometric design, acceptable yields of 3.0×10^9 cells/litre of blood processed were found at a packing factor equivalent to 580 rpm/40 ml/min flow rate. For the same constant ratio, comparable yields would also have been obtained at flow rates of 650 rpm/50 ml/min, 720 rpm/60 ml/min and 820 rpm / 80 ml/min.

The continuous flow cell separator can also be used with a single site of vascular access if fitted with a device that intermittently reverses the direction of flow through the

cannula every few seconds[48].

The IBM 2997 Cell Separator

The IBM 2997 cell separator[44] consists of a desk-type unit which carries the centrifuge bowl with its motor drive unit, the colour-coded peristaltic pumps and control box (Figure 3). The latter contains the digital flow rate counters for all input and output pumps and a comprehensive system of fault detectors which immediately stop the pumps if air is detected in the donating, or return tubes or, if they become blocked. Simultaneously the operator is warned by both audible and visible signals. The rotational speed of the centrifuge bowl can be varied by controls located in the desk itself. The equipment is completed by sets of appropriate tubing, delivery containers, collection bags and circular separation channel which fits into the centrifuge bowl. All the tubing and the separation channel itself is sterile pyrogen-free and disposable. It is supplied in two packs with one sample sterile connector, needing only to be placed in position and run through the colour-coded, peristaltic, collection pumps. The essential components and tubing circuits are shown in Figure 4.

Blood is drawn through a central rotating seal (Figure 5a) into the separation channel at one side of a sealed bulkhead and flows around it as the centrifuge rotates. By the time the blood arrives at the collection chamber on the other side of the bulkhead, it has been separated by centrifugal action into red blood cells on the outside, plasma on the inside, and the

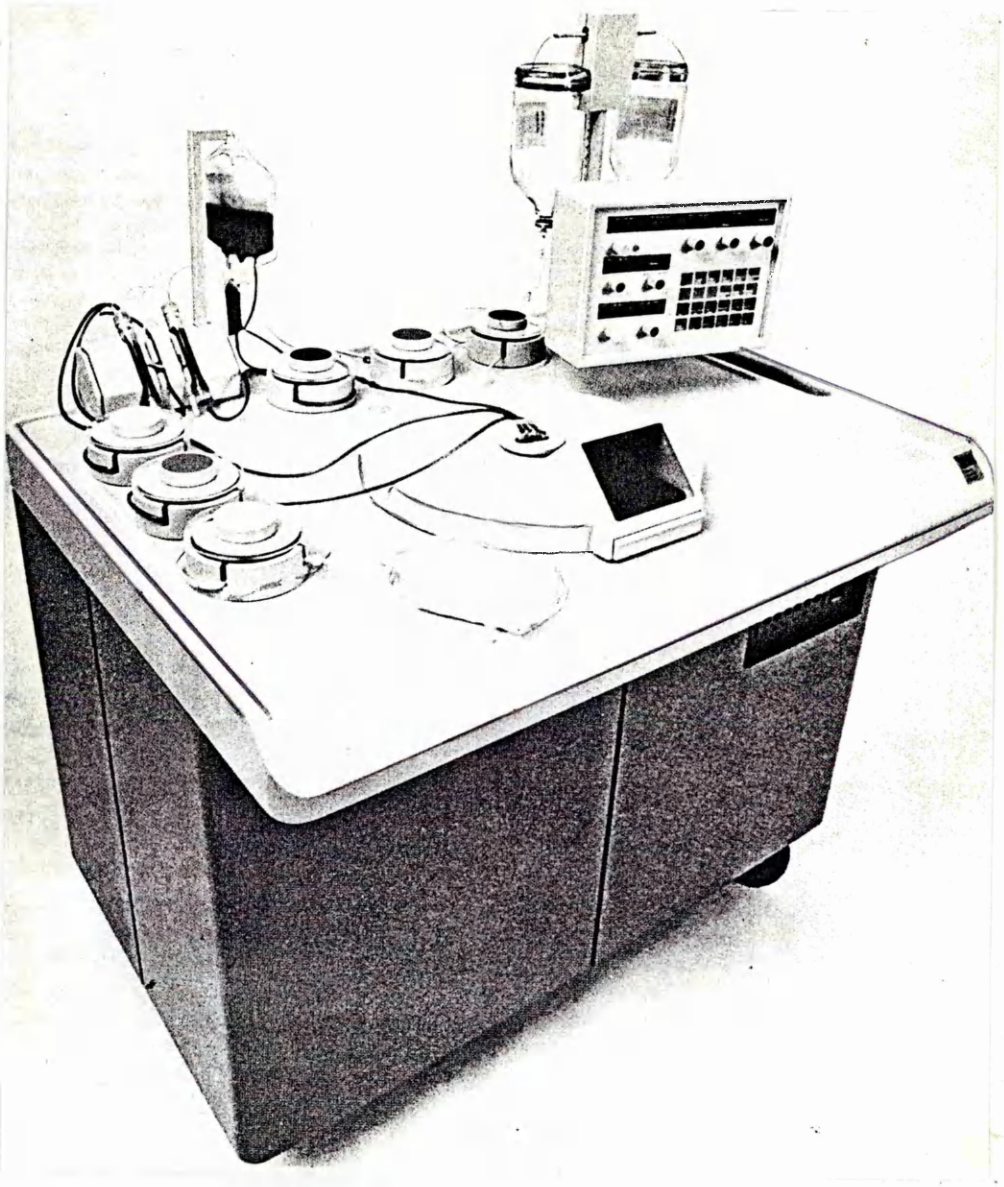


Figure 3. IBM Type 2997 Cell Separator

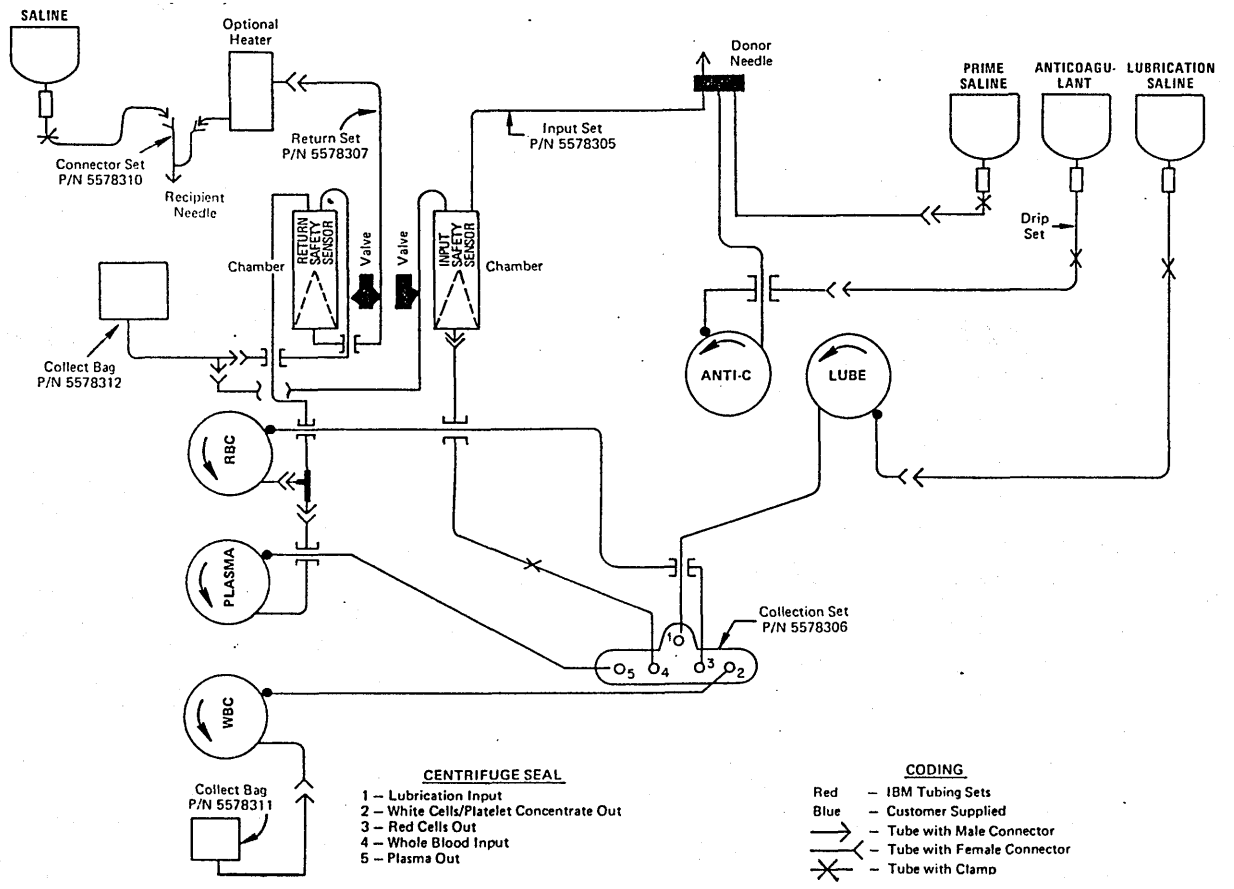


Figure 4. Circuit arrangement of IBM type 2997 Cell Separator.

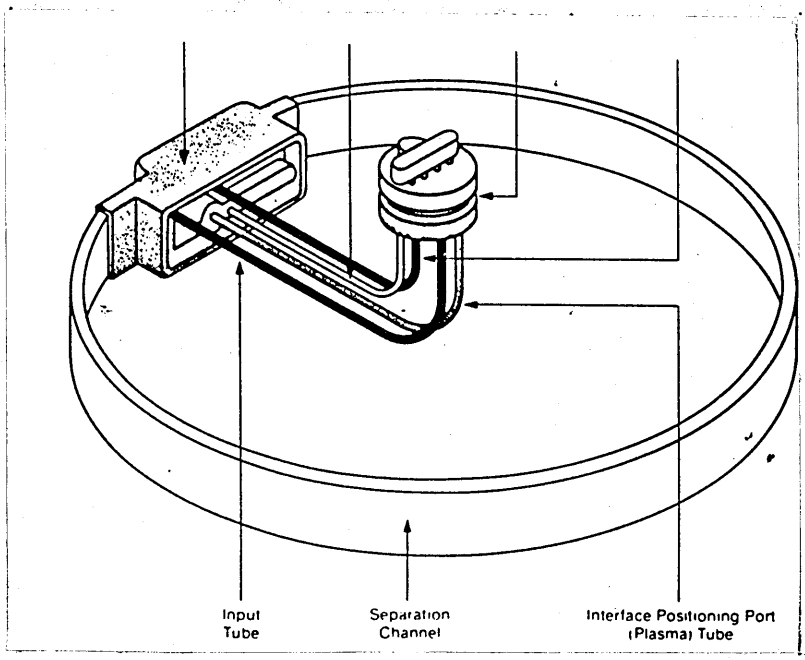


Figure 5. (a) Separation Channel of IBM Cell Separator

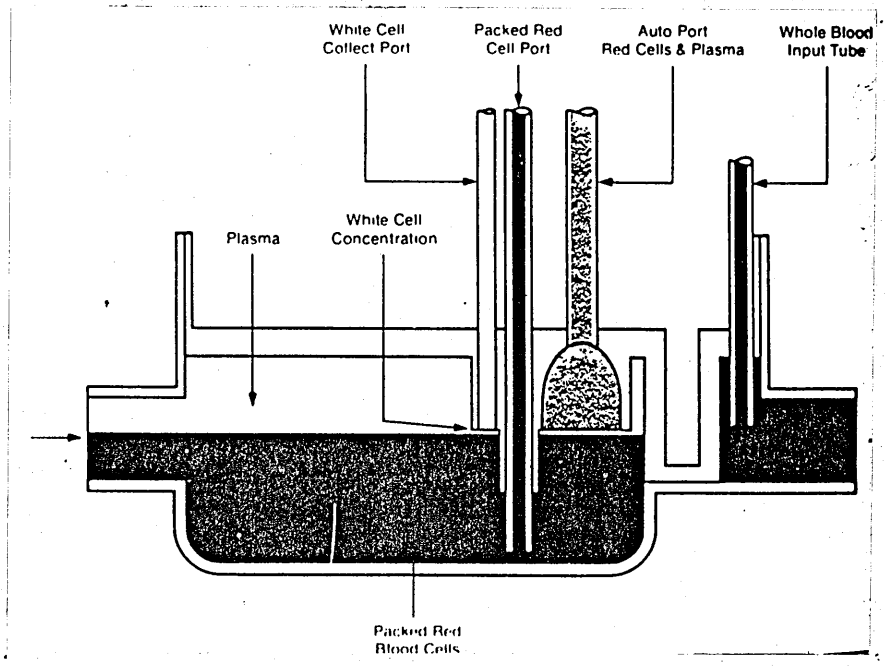


Figure 5. (b) Port Arrangement of IBM Cell Separator

buffy coat of platelets and white cells in between. Three tubes run radially from the collection chamber through their individual channels in the rotating ceramic seal and out through the peristaltic pumps to the point at which the blood components are recombined. One tube, which penetrates almost to the outside of the collection chamber, is the exit port for the packed cells (Figure 5 b). Another tube, penetrating roughly half way up is the white cell port. This and the interface positioning (third) port are radially located to be on the centre-line of the channel. They are separated from each other by a barrier that extends from the top to the bottom of the chamber. Plasma can pass around the barrier along the inner wall and the red cells flow around the barrier along the centre wall of the chamber. Downstream from the barrier the red blood cells and plasma are recombined and extracted by the third port. Alterations in the pumping rate of the pump that controls the extraction from the third exit port will adjust the radial position of the red cell/plasma interface. The adjustment of the interface position relative to the fixed tube port by varying the pumping rates allows the white cell port to draw off from the buffy coat, either a mixture rich in platelets or rich in white cells. The continuous centrifuge technique allows a steady extra-corporeal blood averaging only 280 ml total volume to be maintained and permits donors and patients to be balanced under safe equilibrium conditions. A relatively low amount of anticoagulant is used, the ratio of which relative to the replacement fluid, can be raised. Clear LED display of the actual ratio allows positive and uniquely sensitive control of this potentially hazardous element.

The machine is accurate enough to deliver white cell concentrates, preferentially rich in lymphocytes or granulocytes. Not only can undesirable plasma or unwanted blood cells be removed, but concentrates of platelets or granulocytes can be collected for therapeutic infusion. Similarly lymphocytes can be collected for immunological studies or the production of the transfer factor.

Typical procedures possible on the IBM 2997 blood cell separator and data on performance are given in Tables 3 and 4. New procedures may be easily developed to meet specific situations. The equipment is already being used routinely in combination with a number of different affinity chromatography columns for plasma modification as an alternative to plasma exchange.

TABLE 3

Some of the Protocols Possible on the IBM 2997 Blood Cell Separator

Exchanges	
Plasma Exchange (Platelet Poor)	Red Cell/Plasma Exchange
Plasma/Red Cell Exchange	Plasma Modification (using affinity chromatography columns)
Red Cell/Red Cell Exchange	
Depletions	
Platelets	Granulocytes
Lymphocytes (Including simultaneous Plasma Exchange)	Red Cells (Including old cells)
Harvest	
Plasma (from high titre donors)	Granulocytes
Platelets (Including Leukocyte and Red Cell poor)	Red Cells (Including Neocytes, young cells)
Lymphocytes/Monocytes	

TABLE 4

Performance Data for IBM 2997 Blood Cell Separator

Plasma Exchange	
Volume Exchanged	2.6 - 4.0 litres
Procedure Time	62 - 120 minutes
RBC Exchange	
Volume of Packed Red Cells	6 - 8 units
Procedure Time	90 minutes
Cytapheresis - Leukocytes	
Patients - children (5 years, 44 lb)	
Post WBC counts decreased average of 54.7%	
Granulocyte Collection	
Non-stimulated donors	
PMN Yield	13.9×10^9
Procedure Time	138 minutes
Stimulated donors	
PMN Yield	$19.3 - 31.8 \times 10^9$
Procedure Time	138 - 168 minutes
Granulocyte Platelet Collection	
Granulocyte Yield	24.1×10^9
Platelet Yield	3.8×10^{11}
Procedure Time	126 minutes
Lymphocyte Collection	
Lymphocyte Yield	11.0×10^9
Procedure Time	150 - 180 minutes
Platelet Collection	
Dual Stage (Leuko-RBC Poor)	
Platelet Yield	4.8×10^{11}
WBC	0.31×10^9
Haematocrit	Not measurable
Procedure Time	90 minutes
Bleeding Time	Corrected
Single Stage	
Platelet Yield	$4.3 - 6.6 \times 10^{11}$
WBC	$3.2 - 7.0 \times 10^9$
Haematocrit (%)	1.0 - 2.0 %
Procedure Time	90 - 130 minutes
^{51}Cr Life Span	9.3 days

Celltrifuge II Cell Separator

The rotating seal is a potential source of trouble and a continuous flow cell separator has been produced in which the rotating seal has been replaced by a loop of tubes twirling like a skipping rope in the opposite direction to the rotation of the centrifuge and which is based on work[49] on a flow through centrifuge.

Other Developments

The increasing clinical demand for fresh plasma and platelet concentrates cannot always be met from conventionally collected whole blood donations. The first generation cell separator equipments yield products heavily contaminated with leukocytes and whilst concentrates may be collected with 0 'haematocrit' there is still some red cell contamination. Further equipment development has permitted the harvesting of platelet concentrates from single donors with maximal yield and minimum white blood cell contamination[50,51].

In the Haemonetics V50 cell separator this is achieved by incorporating a surge pump. Once the buffy coat shows optimal separation during the first cycle, autologous plasma is reinjected at high speed into the spinnning centrifuge bowl and the platelets are separated from the white blood cells and the red blood cells. The optimal buffy coat position depends on the haematocrit, plasma viscosity, draw speed and platelet shape, consequently the procedure was neither automatic nor reproducible. New platelet programmes have been developed to overcome these difficulties[51].

Similarly, in the IBM type 2997 cell separator, a dual stage channel has been incorporated which combines a two-step

centrifugation procedure into a continuous method to collect platelet concentrates which are virtually free of leukocyte and red blood cell contamination[52].

Haemonetics V50 Cell Separation with Surge Pump

The Haemonetics V50 Cell Separator is connected up as shown in Figure 6. Blood is drawn from the donor, mixed with anti-coagulant and is pumped at 55 to 65 ml/min. into the centrifuge bowl spinning at 4800 rpm, where it is separated into plasma components. When the buffy coat reaches the shoulder of the bowl (i.e. about 1.5 cm from the top) it is optically detected. The filling of the bowl with whole blood from the donor is automatically stopped and the surge pump is activated. The plasma accumulated in the plasma air bag during the centrifugation is reinjected back into the centrifuge bowl at high speed 200 ml/min. This results in resolution of the platelets from white blood and red blood cells (the surging effect). The valve to the collection bag then opens to collect the surged platelets. As soon as the platelet band disappears the surge stops before red blood cells are removed. Three main interdependent operating variables control the surge procedure and determine the quality of the final platelet product; namely, the volume off-set which determines the point where the surge of plasma from the air/plasma bag begins, the collect delay volume, which is that occupied during the time interval from the initiation of the surge to the opening of the collection bag for platelets and the platelet/white cell factor, which determines the end point of the surge. Whilst there are recommended settings, optimal values are usually established by trial.

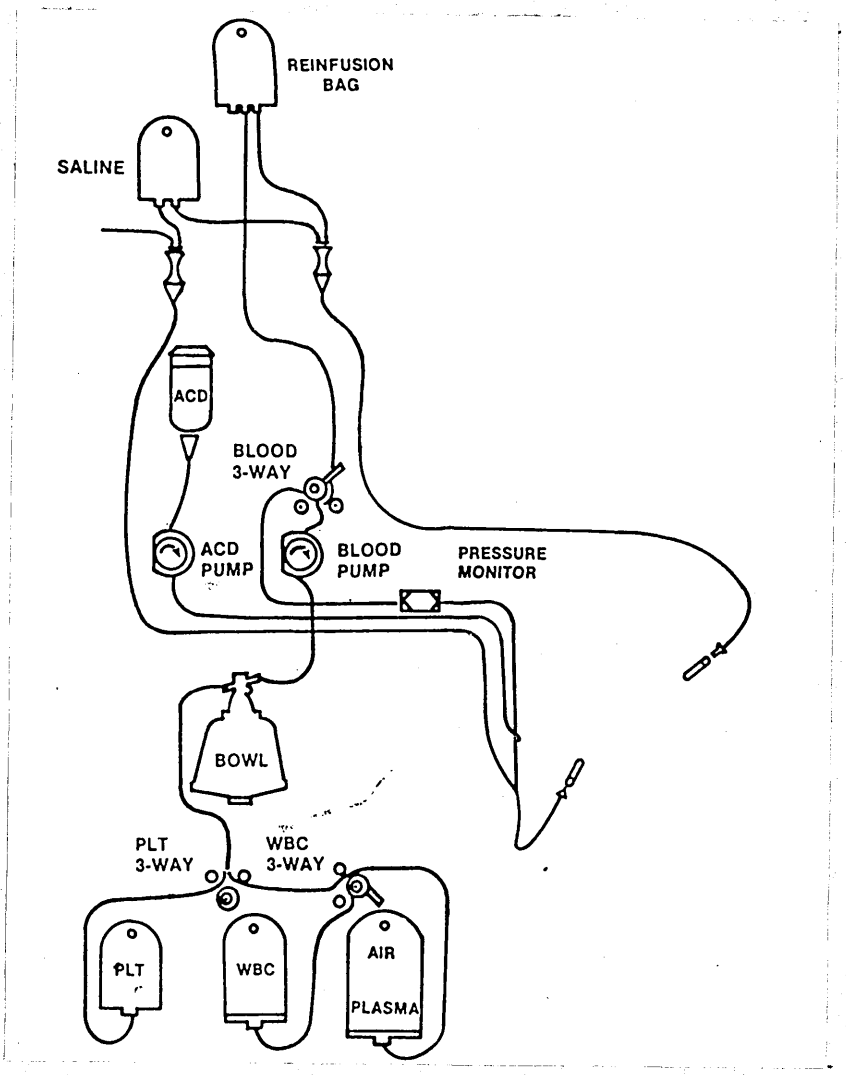


Figure 6. Haemonetics V50 Cell Separator with Surge Pump

The automated cell separator has also been used for the combined procurement of platelet-poor plasma and platelet concentrate, the two products being obtained from the one collection harness without compromising the yield of factor VIII in platelet-poor plasma[53,54].

IBM Type 2997 Cell Separator with Dual Stage Channel

The dual-stage channel consists of an extruded semi-rigid plastic tube of rectangular cross-section, 25 mm x 6 mm, which has a transitional area about one-third of the way down its length where it measures 30 mm x 1.5 mm (Figure 7). This tube is attached at both ends to the input/collection chamber and forms a closed loop. The larger cross-section area portion of the tube is formed in a circular arc. The transition region curves inward so that the thin portion of the channel can form a spiral. Four tubes from the face seal assembly attached to the chamber,provide passages for whole blood, packed red cells, platelet-poor plasma, and platelet concentrate. The chamber is shaped so that two separated regions of radial extent are formed. The shorter region contains the input port and the packed red cell port which are at the inner and outer radial extremes of the chamber respectively. The longer region, which also has ports at both radial extremes allows collection of platelet-poor plasma from the inside port and platelet concentrate from the outside port.

The channel assembly is mounted in the centrifuge with the axis of rotation in the centre of the loop. It is supported by the centrifuge assembly containing a removable plastic insert. The

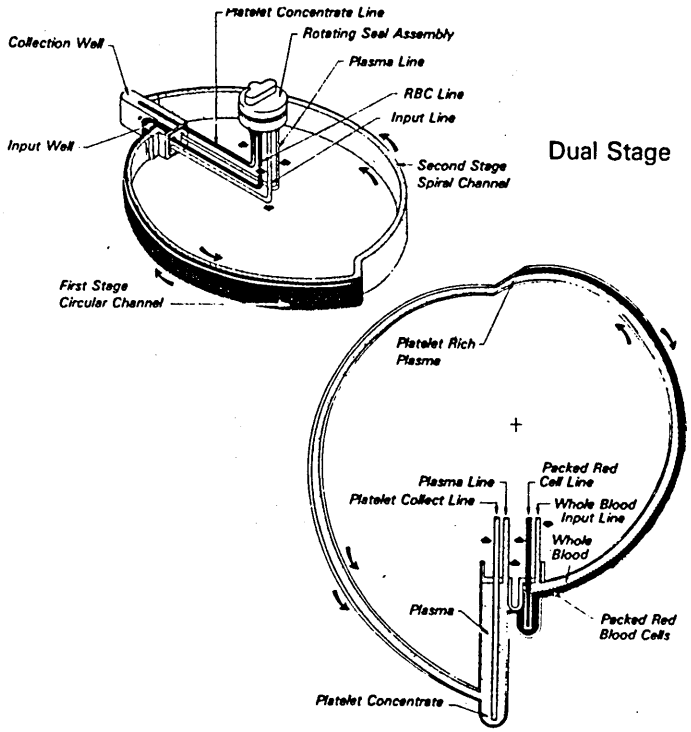


Figure 7. IBM Type 2997 Cell Separator with Dual Stage Pump

channel is spun at 2400 rpm, and experiences internal pressures of up to 7 bar. The centrifugal acceleration experienced by the blood components is in the range of 530 to 1000 G. The channel volume is 70 ml.

Anticoagulated blood is drawn into the channel by initiating flow with the plasma pump. As the channel fills with blood, the plasma port extracts saline first, then mixture of saline plus plasma, and finally plasma alone. Red cells accumulate in the first stage of the channel and are extracted through the red cell port when the interface nears the inside channel wall in the transition zone. The relative flow rates of the plasma and red cells are a function of donor haematocrit. The total blood flow rate through the channel is predetermined by donor blood volume estimates and relate to expected ionized calcium depletion from the ACD-A anticoagulant used in processing.

Platelet rich plasma enters the spiral stage of the channel, platelets are concentrated along the outer wall of the channel, and are extracted into a sterile transfer bag through the collect line. As in all continuous flow systems, the remaining donor red cells, leukocytes, plasma and platelets are continuously recombined and returned to the donor.

Two factors, ACD-A flow rate (calcium binding) and AC:WB dilution (appropriate pH) determine the processing rate per minute, the procedure time and therefore the total blood processed.

Membrane Filtration Methods

In recent years membrane filtration has been increasingly used in apheresis procedures[55-57]. Plasmapheresis or plasma separation involves the use of filters which produce a plasma filtrate from whole blood. Plasma fractionation involves the use of a second and tighter filter (i.e. cascade filtration) which yields a globulin-depleted solution of albumin for return to the patient and a globulin-rich retinate.

Equipments and filtration systems now available include:

Fenwal PS 400

Cobe Centry TPE System [58]

Dideco Filtra BT 810 [59], Separa BT 796

Organon Teknika Curesis

AGB Semca Media Micro labo 85

Kuraray System KM 8500

Plasauto 1000

NIPRO DP-1

Also Hemascience Autopheresis C [60] in which filtration is combined with centrifugation.

Principle of Operation

The various equipments are similar in function but differ in the general layout of the pumps, disposable circuit tubing and containers, pressure sensors, flow sensors, electronic control/ programming for various operating procedures, safety devices and the use of either single or double-vascular sites.

In all equipments, blood is removed from the donor, citrated,

pumped through a membrane filtration unit and returned to the donor. The plasma and red cells are separated in the filtration chamber as dictated by the operating characteristics of the filter.

Dideco Filtra BT 810

The equipment consists of a single, compact, L-shaped console which can be placed on a table (Figure 8). The flat-topped horizontal module contains the three pumps, whilst the vertical module contains the electrical control equipment, measuring instruments, stands for the disposable circuitry/container bags and for the vertical mounting of the filter. The filter contains hollow propylene fibres of 0.5 μm mean bore size having an active surface of 0.1 m^2, 160 mm total length, steam sterilized and without surfactant addition. Using a single needle system, blood is drawn from the donor's vein and after the addition of an antocoagulant, is delivered by the draw pump to the filter while the reinfusion pump is stopped (Figure 9). This produces a pressure increase in the filter. When the pressure reaches a maximum value pre-set by the operator, the draw pump is automatically stopped and the reinfusion pump is started. The blood is returned to the donor through the same needle and this produces a pressure decrease in the filter. When a value, pre-set by the operator is reached, reinfusion automatically stops and a new draw phase begins. Thus, the donor blood flow is discontinuous whilst the flow through the filter is continuous. The flow rate of the filtered plasma is about 12-17 ml/min. and is dependent on the blood flow rate, hematocrit and blood lipid content. The extra-corporeal blood

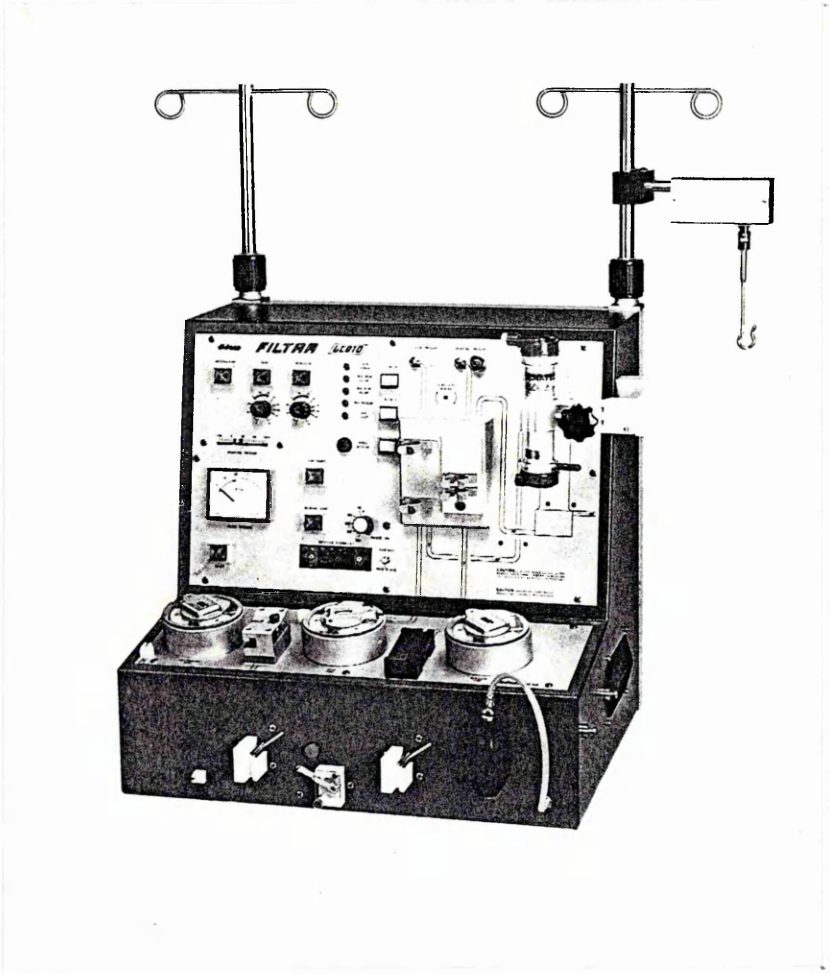


Figure 8. Dideco Filtra BT 810 Cell Separator

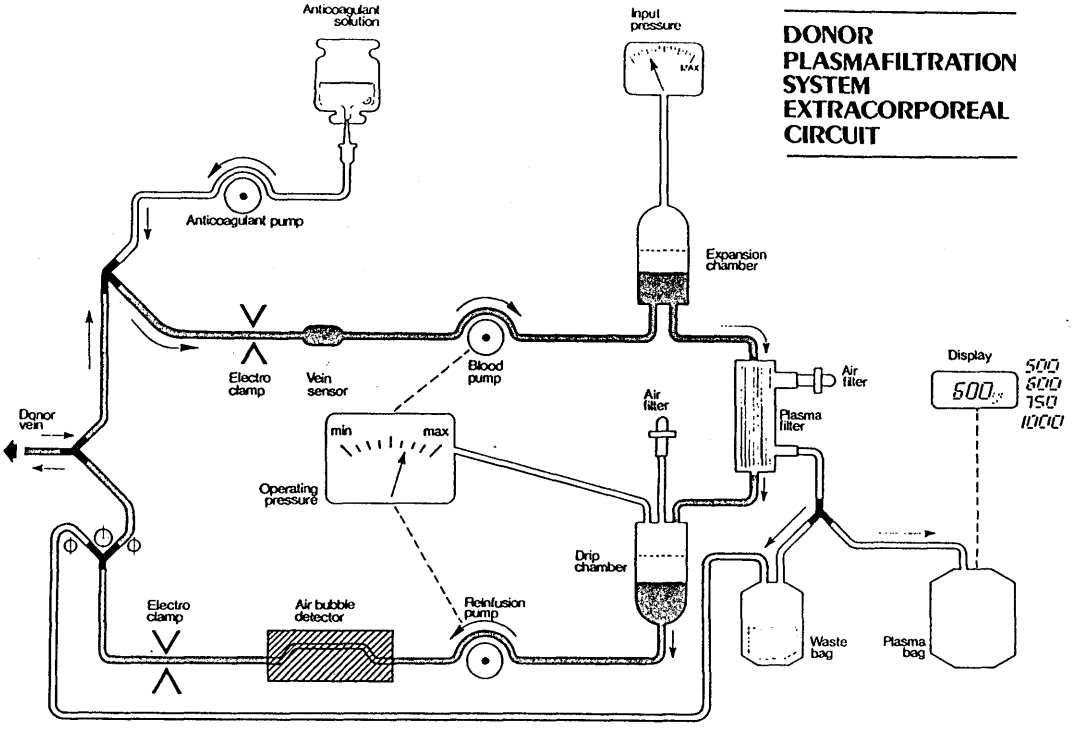


Figure 9. Circuit Arrangement of Dideco Filtra BT 810

volume is about 70 ml, whilst the amount of blood processed during a draw/reinfusion cycle is 12-15 ml. A vein sensor on the draw line automatically stops the pumps when donor blood flow is inadequate.

A deep, chamber-level, sensor and a bubble detector in the reinfusion line prevents infusion of air to the donor. 500 ml plasma is collected per procedure, in about 40 min with an average blood flow of 55 to 60 ml/min. Approximately 160 ml of anticoagulant is used per procedure, about half of which is collected with the filtrate.

Factors in the Filtration Procedure

The effectiveness of the equipment and procedure depends on the capacity of the filter to remove defined substances from the blood. Unfortunately, there is no standardized method of valuating capacity, nor are there precise definitions of various test parameters used[61]. Performance has been assessed on a number of criteria which make comparative valuations very difficult. Thus, coagulation and fibrinolysis parameters have been used to assess plasma protein removal and coagulation and fibrinolysis activation. Platelet number and granule content have been used to study platelet loss and platelet activation. The sieving coefficient of membranes has been evaluated by studying von Willebrand factor multipliers[62].

The operating characteristics of a filter depend on the membrane material, its geometric form and physical arrangement, on the rate of blood flow and on the transmembrane pressure[63]. The

filter systems are of two kinds, one employing a flat bed filter[58], the geometry of which may be varied and one employing fixed geometrical arrangements of hollow fibres[64,65]. Scouring efficacy and platelet loss of both types of filters appear to be similar but platelet activation is less for the flat bed filter than the capillary filter[62]. The physical arrangement of membrane material and the conditions under which it operates does influence the degree of blood trauma and the level of plasma extraction[63].

The filtration rate in plasmapheresis is not a linear function of filter area and applied transmembrane pressure and cannot be uniquely evaluated (i.e. a fixed value of permeability). Filtration rate initially increases with applied transmembrane pressure up to a plateau value beyond which no further increase in rate occurs. Filtration rate also increases with blood flow rate in a complex manner. At the plateau level of transmembrane pressure a maximum value of filtration rate could be ascribed for a given blood flow rate but unfortunately this value decreases during the course of a plasma filtration procedure[61].

The contact surface between blood and membrane can result in haematological and biochemical changes[63]. Material from the blood may be deposited on the membrane surface as a consequence of net passage of filtrate and this reduces the overall membrane permeability (i.e. a progressive plugging of the membrane occurs)[66]. Material deposition is particularly influenced by the composition of the membrane material and to

a lesser extent by the membrane geometry, flow rate and transmembrane pressure[63]. Further membrane material greatly influences complement activation; significant activation of the anaphylatoxins C3a and C5a is found with cellulose diacetate membranes but not with polycarbonate membrane which correlates with a drop followed by an overshoot of both leukocytes and lymphocytes. Both membranes generate significant amounts of anaphylatoxins in the filtrates, reflecting high intermembrane activation[67].

The progressive loss of filter efficiency during the procedure can be countered by increasing the transmembrane pressure, but in a whole plasma volume exchange the pressure, would rise beyond practical limits and the procedure would have to be terminated[66]. Further, membrane plasma separation may be limited by the onset of hemolysis which is generally associated with a high mean transmembrane pressure[68]. However, very high transmembrane pressures can be used without hemolysis if applied for only a short time, as with pulsatile blood flows at frequency of 2 Hz, and this can increase the filtration rate by 30 to 50%. Increasing transmembrane pressure leads to a greater deposition of material on the membrane. Membrane plugging can be prevented by wetting the membrane surface with a surfactant but this raises ethical issues[63].

The selective removal of pathologic macromolecules with cascade membrane (double-filtration) systems is primarily dependent on the transport characteristics of the second filter which will be exposed to increasing protein concentrations during treatments[69].

The plasma filtrate in the re-circulation circuit will contain various substances, depending on the patient's disorder, that can interact directly with the membrane and this makes prediction of membrane performance more difficult. Assessments based on sieving coefficients of the cascade filter or on direct analysis of the retentate are open to doubt[70]. The former are misleading because the separations are highly irreversible and the latter is unreliable because non-permeable proteins are often aggregated, de-natured or bound to membranes. Indirect methods have been used to measure the removal of known marker molecules, (e.g. albumin, IgG, IgM and B-lipoprotein). The need for substitution fluids is greatly reduced with cascade filtration compared with plasmapheresis but the removal of IgG and IgM is less efficient[71]. The amount of C3a and C5a returned to the patient is significantly higher with cascade as opposed to single filtration procedures[67].

The high cost of plasma filters is a limiting factor in their use for plasma exchange and the possibility of reusing filters is economically attractive. Filters have been used at monoclonal gammapathies of the IgA and IgM types up to four times provided adequate rinsing is undertaken after each application[72].

1.3.2 Chemical Separation of Components

The plasma constituents can be removed by perfusion of whole blood (haemoperfusion) or plasmaperfusion[73-75]. Alternately some constituents of plasma or whole blood can be precipitated

or absorbed from blood or plasma which is subsequently transfused back to the patient.

Haemoperfusion

Haemoperfusion involves the direct passage of anticoagulated blood over activated charcoal. Its role has been limited because of potentially life-threatening thrombocytopenia and charcoal embolisation.

Plasma Perfusion

Plasma is separated from the formed elements of blood by centrifugation or filtration, and then pumped over an affinity column or a filter with an immobilised sorbent. The effluent of column or filter is re-constituted with the formed elements of blood and returned to the patient after passage through a blood filter. Thrombocytopenia is avoided and sorbents can be used that need not be bio-compatible with the formed elements of blood. The bio-compatibility of the column is improved when plasma only is passed through the column and the by-passed cellular elements are returned directly to the patient. This removes the problem of platelet depletion and it allows a wider choice of type and concentration of anticoagulant[76]. It permits the use of smaller bead packing and adsorption is therefore faster and more efficient. The patient's own plasma is returned without the need for artificial plasma expanders or plasma products. This eliminates one of the major hazards of apheresis and should reduce the cost of the procedure.

Column Technology

The technique is based on the disposable (or reusable) column[73]. It includes the container, its sterile packing, connectors and associated reusable apparatus. The latter is based on haemodialysis and haemoperfusion machines with added anticoagulant pumps, temperature controls, flow reversal and regeneration mechanisms, pH and optical detectors.

A volume of 300 ml is used to avoid excessive haemodilution, plasma proteins, the red cells are returned directly to the patient. The plasma flow rate of 60 ml/min is based on an assumed maximum venous blood flow of 100 ml/min and a maximum procedure time of 3-6 hours for treatment of patients or 1-2 hours for donors. Because of the limited performance of peristaltic pumps a maximum back pressure of 0.7 bar is specified, but ideally the pressure is less than this. The absence of most cellular elements allows a wider choice of pressure and shear than is the case with blood perfusion with rigid packing materials. Typically, spherical beads of 50 μm diameter are used with a plasma flow of 50 ml/min, a back pressure of 0.7 bar, a temperature of 37° C (which almost halves the viscosity of plasma compared with that at 15° C), and a packing geometry 6 cm diameter and 6 cm high. Semi-rigid beads are generally larger, around 70 μm diamter whilst the lower limit of particle size for gels is 150 μm diameter. The smallest size of bead is chosen in the interest of efficiency, speed, capacity and cost.

Because of the clogging effect of even small amounts of

platelets which are inevitably left in the plasma (typically $2 \times 10^9/1$), the back pressure will increase with time of perfusion and the longer treatment sessions could become painful. Thus, it is desirable that 99% of the beads should be within the range 40-60 μm. Because of the dilution of the returned plasma the reduction in any particular component follows an exponential law so that treatment times of 2,4, and 6 hours could, at best, produce final concentrations of 30, 10 and 3% of starting levels. Column adsorption efficiencies of 80% and extra-vascular pools will significantly reduce such performance.

More recent treatments differ from charcoal haemoperfusion on renal dialysis in that the molecular size of the material to be removed is many orders of magnitude greater. Since adsorptive capacity is dependent on total surface area whose capacity for protein is typically 1-5 mg/m^2, several hundred square metres are needed to remove mg/g of substance. Both static (steric hindrance) and dynamic (non-equilibrium with flow) factors reduce the efficiency of any given absorbent and the reduction in potential adsorptive capacity can be alleviated by using tandem columns operated alternately with regeneration of the resting column. This approach is limited and it is more effective to ensure that the surface area and the porosity are sufficient for species to be removed.

The properties of the column are such that a space twice the size of a molecule allows access to, on average, only 5% of absorptive area. It is not until the cavity is 10-20 times

larger than the molecule that efficiency of access reaches 80-90%. Thus immunoglobulins and Ag/Ab complexes which may readily exceed 10 nm in stokes radius (functional radius in solution) would need cavities of around 400 nm diameter for 90% efficiency of access. Larger species such as very low density lipoprotein (30 nm radius) and viruses (50-100 nm radius) would need cavities of around 1200-4000 nm diameter. These molecular dimensions are also approached by asymmetric molecules in solution such as DNA and Factor VIII so that large cavities are likely to be a common requirement for both therapeutic and procurement procedures.

The surfaces exposed to plasma and the ligands (or affinity reagents) immobilised thereon should not initiate coagulation, or excessive aggregation of platelets, or of other cells, such that flow ceases, or capacity within beads is severely reduced. The correct choice of solid phase chemistry and anticoagulant should minimise this problem. Where solid phases are not inherently non-thrombogenic (and considering the relatively short treatment time compared to, for example indwelling prostheses) this is unlikely to be a major problem. When a surface coating needs to be applied to improve bio-compatibility, coating procedures that are satisfactory for charcoal haemoperfusion are unlikely to work with these larger molecules. The pores in cellulose nitrate, cellulose acetate and polyhydroxyethylmethacrylate coatings are approximately 3 nm and are inadequate to permit free access of larger molecules. Thus coatings should be grafted onto skeletal molecular structure of the bead prior to subsequent grafting of the

affinity ligand[77].

The particulate material may be shed during manufacture, transport and use. This can be serious with rigid bead materials like charcoal, silica or other metalic oxides because it impairs column performance by reducing flow and embolism can occur in the patient's vasculature causing long-term toxicity. This may be alleviated by careful handling and washing during manufacture and by the choice of spherical particles and smooth surfaces (reducing fragmentation sites). A number of groups have successfully used agarose as a bio-compatible macroporous coating[78]. The leakage of soluble material from beads, coatings and ligands is a possibility that has to be considered.

Only a limited number of substances make useful beads which have adequate pore size, internal surface area and strength. These include the natural polymers agarose and cellulose and synthetic and semi-synthetic polymers such as polyhydroxyethylmethacrylate, allyl-dextran and poly-vinyl alcohol. Silica has found widespread application in beaded form as a porous solid phase because of its ease of fabrication, manipulation of pore size and strength. It is biocompatible with plasma only if coated. Alumina can be used for phosphate removal, zirconia for oxalate/glyoxalate removal[79] and ion exchange resin for bilirubin removal.

It is difficult to produce affinity ligands with multiple purpose applications. Monoclonal antibodies are limited to a

single epitope and unless this is widely distributed, they will always suffer from limited areas of application.

Group specific ligands such as heparin, being both non-immunogenic and having several specificities of binding have had some success. Both synthetic and semi-synthetic polymers and dyestuffs can be expected to have 'accidental' group specific binding effects. They are also attractive being non-immunogenic and low in cost compared to proteins. Polymers such as gelatin and collagen can be useful for binding fibronectin and coagulation FVIII.

Specific antibodies can be removed by immobilising the appropriate antigen (where this is known). Methods of Ab removal include lectins such as concavlin A, which has been used to remove IgM, IgA, IgD and IgE myeloma proteins[80] and the bacterial cell-wall protein A from Staphylococculus Aureus which displays specificity for the Fc region of many mammalian IgG molecules and may be useful as a universal affinity system for procurement of IgG antibodies from immunised volunteer donors.

Enzymes may also be immobilised and used, though few biochemical applications have been reported.

The largest number of applications involve protein A which not only has the useful property of removing IgG subclasses 1,2 and 4 but can also reactivate a suppressed immune system either by removing blocking Ab or by stimulating the cellular system[81].

The treatment of colonic cancer and the effects on the immune system of adsorption on Staphylococculus Aureus ex-vivo have been described. Canine mammary adenocarcinoma has been treated and a system is being developed for treating human patients in an off-line system[82]. A direct immobilised Fab antibody technique for the treatment of a patient with hypernephroma has been described. The application of protein A is not limited to soluble immunoglobulins for cells bearing IgG on their surface may be adsorbed provided the size of beads in the column is increased to 300 μm to allow free passage of cells through the interstices.

The use of totally synthetic blood group antigens A and B for removal of AB prior to marrow transplantation has been described[83]. These commercially available adsorbents were rendered biocompatible by cellulose citrate/albumin coating.

There has been much interest in the removal of cholesterol and/or low density lipoprotein for the treatment of hyperlipoproteinaemia. Heparin agarose has been used, also a sheep polyclonal to apolipoprotein B, and a combination of both[84].

Plasma perfusion over charcoal has been tried for the removal of bile acids in the treatment of systemic lupus erythematosus. Inhibitors to Factor IX have been removed prior to surgery by using protein A - sepharose.

1.3.3 Other Procedures

Several schemes to recycle the plasma in a closed loop circuit have been proposed and are in various stages of development and application. Cascade filtration systems have been examined[85-87], but the selective removal of macromolecules from blood can only partially be achieved. Currently available second filters do not give optimal selectively between albumin recovery and macromolecule removal. Whilst existing systems are clinically acceptable and cheaper than plasma filtration, further refinement of cascade filtration is necessary[71].

Electroplasmapheresis[88] combines a flow membrane separation and fluid phase electrophoreses. Plasma is separated into two fractions; the bottom fraction contains 80% albumin input and is returned to the patient with a sterile electrolyte solution. The top fraction contains 70% gamma globulin and is discarded.

Cryoglobulins precipitate readily at freezing and subfreezing temperatures to form a dense floccular material that can be removed by filtration, sedimentation or centrifugation[89]. Cryoglobulin-poor plasma can be frozen and stored for subsequent autologous use after an initial plasma exchange in which conventional replacement is used. This procedure results in substantial savings for the patients thus treated[90]. When heparin, dextran and magnesium chloride are added to heparinized, hyperlipoproteinemic plasma, a visible lipoprotein precipitate develops rapidly. The precipitate, containing 97% of the

original content of Low Density Lipoprotein and Very Low Density Lipoprotein can be removed totally by centrifugation or filtration to produce a lipoprotein-depleted plasma. Zinc diglycinate specifically precipitates immunoglobulins (92.4% of IgG, 44.4% of IgA and 25% of IgM) from plasma, which are then readily removed by centrifugation[74]. It also precipitates immune complexes and anti-acetylcholine receptor antibodies. The precipitate however does not contain albumin, haptoglobin, complement or coagulation factors VIII and X or prothrombin. The residual zinc must be reduced to non-toxic levels from treated plasma by dialysis or exchange resins.

Macromolecules have been separated by cryogelation and filtration from plasma previously separated by filtration from the formed elements of blood[91]. A membrane filter, with a molecular cut-off of about 100,000 daltons, permits the passage of albumin but excludes gammaglobulins. The effluent of the cryoprecipitation circuit and macromolecule filter is reconstituted with the mainstream blood flow before it is returned to the patient. This system is capable of substantially reducing circulating immune complexes. It also appears to be simple and safe, but expensive and there are high non-specific losses of all plasma components. Red cell antibodies have been removed from maternal plasma of patients undergoing plasma exchange for treatment of haemolytic disease of the newborn and the absorbed plasma used for autologous replacement of subsequent exchanges. An anti-M antibody was greatly reduced in titre by incubation of the plasma with M-positive red cells; plasma thus treated was then reinfused

back to the patient[92]. Anti-D and Anti-C antibody levels have been reduced substantially by absorbing plasma containing the antibodies with red cells that possess the corresponding antigens. The absorbed autologous plasma was reinfused back to the patient. The recipient, unlike the patient with Anti-M, had a dramatic and unexpected rise of antibody levels, suggesting that she may have received Rh-positive material with the absorbed plasma[93].

No procedure proposed for closed loop plasmapheresis (cryofiltration, cascade filtration, fractional precipitation, electrophoresis, selective adsorption) is capable of complete separation[70]. Some useful proteins will always be lost and some toxins will always be returned to the patient.

1.3.4 Comparison of Centrifugation and Filtration Procedures

There are a number of possible criteria on which an assessment of the advantages and disadvantages of various equipments and procedures may be based. These include safety of separation, initial equipment and procedural costs, the complexity of and the time for setting up a procedure, the efficiency of equipment and procedure,, the type of vascular access required (i.e. one or two sites), the reliability of equipment and associated disposable ancillaries, the maintenance of plasma volume, especially in sick people, etc. In practice, comparisons of procedures are made on the basis of one or a limited number of criteria.

Alternatively, the equipments and procedures may be used in studies which permit a comparison of their performance in essentially similar applications, as in the production of leukocyte concentrates, in the collection of platelets and in therapeutic treatment of a range of disorders.

Considerable data now exist on the comparative performances, in similar procedures, of various centrifugation equipments, e.g. Haemonetics Model 30, Bellco Progress (intermittent flow) versus IBM Type 2997 and Fenwal CS 3000 (continuous flow) in the production of leukocytes[94]; Dideco Vivacell, versus IBM Type 2997, versus Fenwal CS 3000 in plateletpheresis[46]; Haemonetics V50 with surge pump, versus Fenwal CS 3000, versus manual Fenwal quadruple triple-blood packs for the collection of platelet and granulocyte concentrates[95]; Haemonetics 30, versus Haemonetics V50 with surge pump, versus Fenwal CS 3000, versus IBM 2997 Single Channel, versus IBM 2997 Dual Channel in plateletpheresis[96]; of filtration systems, e.g. COBE (PVC membrane), versus Organon Teknika (polypropylene membrane), versus Kuraray (PVA membrane) in plasmapheresis[97]; Plasauto 1000 using a single filter cascade filter, versus Plasauto 1000 using a single filter, versus Dialysetichnik with single filter in plasmapheresis[71]; and of centrifugation and filtration equipments, e.g. Haemonetics V50, versus Dideco Filtra BT 810 in plasmapheresis[98]; Haemonetics PCS, versus Dideco Filtra (Hemaplex), versus Organon Teknika (Plasmapur), versus Hemascience (Autopheresis-C) in plasmapheresis[99]; Organon Teknika Curesis, versus Fenwal CPS 10, versus IBM Type 2997, versus Haemonetics V50 in plasmapheresis[100].

Typical data obtained in such studies are shown in Tables 5 and 6. No overall comparison of all equipments and procedures mentioned is possible because data were collected under different operating conditions. All systems were safe and relatively efficient. All procedures were well tolerated by donors, some were more prone to adverse technical incidents than others.

Theoretical modelling has also been suggested as a basis for comparison of equipments but clearly conclusions drawn will be dependent on the validity of the selected model[101-104].

1.4 Plasma Exchange - Practical Considerations

Codes of practice for the clinical use of cell separators and the automated plasmaphereses of volunteer donors have been issued by the Department of Health and Social Security. These documents, however, say little about therapeutic plasma exchange[105-106]. In general, patients are accepted for a trial of plasma exchange only when more conventional therapy has been instituted and has failed and when the procedure is judged to be beneficial for the patient. A typical plasma exchange procedure necessitates the patient to be supported on a bed inclined at 30° to facilitate the circulation[107]. Procedures are carried out by an experienced medical staff with the help of a technician.

TABLE 5

Performance of Centrifugation Equipments in Plateletpheresis[96]

Protocol	A	B	C	D	E
Separator	Haemonetics H-30	IBM 2997 Single Channel	IBM 2997 Dual Channel	Fenwal CS-3000	Haemonetics V50 Surge Pump
Total Platelets ($\times 10^{11}$)	5.46 ± 2.29	6.01 ± 2.13	4.46 ± 1.87	3.83 ± 1.1	3.47 ± 1.5
Percentage Recovery	77.46 ± 16.1	52.27 ± 15.1	50.51 ± 15.8	43.02 ± 11.0	47.61 ± 18.4
Time (Minutes)	161 ± 28	119 ± 28	135 ± 18	136 ± 18	155 ± 20

TABLE 6

Performance Characteristics of Four Equipments in Plasmapheresis[99]

System	Centrifugation	Filtration	Filtration	Combined Centrifugation Filtration
Equipment / Component	Haemonetics PCS	Dideco Filtra BT 810	Organon Teknika Plasmapur	Hemascience Autopheresis-C Plasmacell
Total protein g/l	61.54 ± 3.66	60.27 ± 5.1	53.5 ± 5.13	60.2 ± 3.58
Albumin g %	4.01 ± 2.69	3.85 ± 0.41	3.58 ± 3.97	3.94 ± 2.28
IgG mg %	915 ± 232	846 ± 238	796 ± 134	894 ± 152
IgA mg %	179 ± 72	175 ± 52	149 ± 59	170 ± 50
IgM mg %	134 ± 82	113 ± 63	122 ± 51	115 ± 83
F VIII:C IU/ml	0.86 ± 0.3	0.87 ± 0.3	0.88 ± 0.4	0.85 ± 0.3
F V IU/ml	0.74 ± 0.24	0.76 ± 0.24	0.70 ± 0.22	0.75 ± 0.27
F I g/l	2.19 ± 0.47	2.14 ± 0.38	2.01 ± 0.57	2.27 ± 0.33
IgM Sieving coefficient 10 min	99.5 ± 2.1	98.7 ± 3	98.2 ± 2.5	99.3 ± 2.7
IgM Sieving Coefficient 30 min	98.5 ± 3.7	98.2 ± 2.7	97.9 ± 3.6	96.3 ± 3.8

TABLE 6 (Cont)

Performance Characteristics of Four Equipments in Plasmapheresis

System	Centrifugation	Filtration	Filtration	Combined Centrifugation Filtration
Component \ Equipment	Haemonetics PCS	Dideco Filtra BT 810	Organon Teknika Plasmapur	Hemascience Autopheresis-C Plasmacell
Cholesterol Sieving Coefficient 10 min	99.3 ± 2.5	99 ± 3.7	99 ± 2.7	99.1 ± 2.1
Cholesterol Sieving Coefficient 30 min	98 ± 4	97.8 ± 3.5	98.1 ± 2.6	98.6 ± 3.6
Plasma Volume Collected ml	580.1 ± 32.4	578 ± 88.4	579.5 ± 84.02	584.5 ± 56.72
Install set time sec	105	220	220	216
Total procedure time min	44	60	57	40
Priming time sec	15	210	180	60
Citrate level in Plasma mg/l	332 ± 22	310 ± 10	379 ± 8	337 ± 14
Citrate level in donor after plasmapheresis mg/l	147 ± 95	110 ± 70	162 ± 90	130 ± 60

1.4.1. Vascular Access

Problems with vascular access have been common in the management of patients with chronic renal failure by haemodialysis. This experience can be applied to plasma exchange which requires lower blood flow rates than those used in dialysis. Whilst venepuncture of antecubital veins are adequate in many patients, alternative techniques are available[108].

1. Antecubital Venepuncture

This is the method selected for most patients managed by intermittent therapy, i.e., patients with mixed cryoglobulinaemia or cutaneous vasculitis[109]. When more intensive exchange is needed as in cases of Goodpasture's Syndrome, arm veins are not always adequate[110].

The antecubital fossae are cannulated with approximately 16 gauge needles. Flow rates of 60-80 ml/min. can often be achieved, but when necessary the procedure can be performed with lower flows of 20-40 ml/min. Approximately 66% of patients are cannulated via antecubital veins. Of these some 10% will require other forms of access. In cases of severe systemic disease other forms of access will be used.

2. Femoral Vein Catheterisation

This method of vascular access was widely used for haemodialysis[111,112], especially in cases of acute renal failure. It is particularly suitable for patients requiring

prolonged intensive plasma, e.g., those with Goodpasture's Syndrome, who require daily exchanges for more than two weeks. It has now been largely superseded by arteriovenous fistulas.

Flow rates in excess of 100 ml/min. can be achieved and with continuous flow centrifugal system an automatic alternating flow device can be used[47], which allows rapid exchange with minimal recirculation. Membrane plasma filtration can also be operated using a single femoral vein catheter and automated alternating flow, as has been described for subclavian cannulae[113]. With membrane filtration a dual lumen catheter has been used. Flow rates of up to 70 mm/min can be achieved.

Extremely low incidence of complications have been experienced with femoral catheters. There have been well documented episodes of clinical infection, venous thrombosis, external haemorrhage and retroperitoneal haemorrhage. This contrasts with a high incidence of infection at arteriovenous shunt sites[114], which proved a major problem in immunosuppressed patients.

3. Subclavian Vein Catheterisation

The catheterisation of the subclavian vein may be used in patients with unsuitable femoral vessels[115]. Subclavian catheters are used rarely because of the greater risk of complications including pneumothorax and cardiac arrhythmias[116]. Cardiac complications may be related to the use of citrate as an anticoagulant with the subsequent risk of hypocalcaemia. The catheter is situated however in a more hygenic site and obviously allows the patient greater mobility.

4. Arteriovenous Shunts

The use of surgically implanted shunts for vascular access was a major advance in haemodialysis, although a recent review[117] shows that infection and thrombosis remain considerable problems. The method was accompanied by an unacceptable incidence of infection at the shunt site. Many patients were on immunosuppressive drugs rendering them more susceptible to infection, and such infections led to an increase in disease activity in both Goodpasture's Syndrome[118] and Wegener's Granulomatosis[119].

5. Arteriovenous Fistulae

Surgically created subcutaneous fistulae are the method of choice of vascular access in chronic haemodialysis. The radio-cephalic fistula is most commonly used but many variations have been described[120]. The long-term survival of these fistulae makes them suitable for some patients whose underlying disease predisposes them to complications including thrombosis and gangrene of the hand, e.g., those diseases in which vasculitis or severe Raynaud's phenomena are major features, namely SLE and scleroderma[121].

A wide range of arteriovenous grafts, both biological and synthetic are available for patients in whom all other vascular access has failed[122]. A patient who has had multiple operations for access over six years currently receives intermittent plasma exchange via a femoro-popliteal Dacron graft.

6. Complications of Access

The commonest cause of fistula or graft failure among these patients is thrombosis. Other complications such as infection, haemorrhage and aneurysm formation are much less common.

A recent survey[123] of 86 arteriovenous graft insertions for haemodialysis performed over a five year period showed that 8 out of 29 biological grafts and 20 of 57 synthetic grafts became thrombosed, while there were no major surgical septic complications which resulted in graft loss. Arteriovenous grafts were performed only in patients whose arteriovenous fistulae had thrombosed and these constituted 11.5% of the haemodialysis pool. Patients with renal failure have a haemorrhagic diathesis and in patients with normal haemostasis the risk of thrombosis is likely to be even higher. In patients with immune complex disease, vasculitis will further increase the risk of thrombosis and some of these patients will suffer from recurrent thrombophlebitis. Cryoglobulins and circulating anticoagulants, if present, are likely to add to the problem. Thrombotic phenomena have occasionally been observed in patients undergoing plasma exchange, and it has been suggested that reduced levels of Antithrombin III might lead to a hypercoagulable state[124]. Subsequent work[125], has not substantiated that patients undergoing plasma exchange and receiving corticosteroids and cytotoxic drugs are particularly liable to infection. The consequence of infection were particularly severe and led to amputation. This complication has also been reported in a patient with scleroderma[126].

Whilst the morbidity associated with the construction of AV fistulae and grafts appears to be high, other procedures used to gain access to the circulation are not without complications. Superior vena-caval cannulation appears to be associated with a high incidence of cardiac arrhythmias, probably because citrated plasma is returned direct to the right atrium[116]. In addition the risk of traumatic complications is significant and in one series of 160 SVC cannulations major traumatic complications occurred in 6 cases - one perforation of the subclavian vein, one perforation of the subclavian artery, one innominate vein thrombosis and three cases of pneumothorax[122]. Femoral vein cannulation may be complicated by thrombosis, sepsis, accidental arterial puncture, etc, but the incidence of such complications appears to be low even in emergency situations. Whilst thrombosis occurred shortly after femoral vein cannulation in one patient, no complications were reported in one series of 350 femoral vein cannulations, and this method is now preferred[127].

1.4.2 Anticoagulation

In cell separator procedures heparin and citrate are normally used for anticoagulation[128]. Side effects may be encountered with both drugs.

1. Heparin

Heparin is widely used in filtration leukapheresis, initially in the pre-anticoagulation of the donor and subsequently when added slowly to the extracorporeal circulation to prevent

coagulation during the procedure. Recommended doses have ranged from 15,000-20,000 units over periods of 2-4 hours[129] and are well above those used to prevent thromboses in abdominal or thoracic procedures. These doses also exceed those used to prevent progression of venous thrombosis and are at the upper limit of normal for the initial treatment of patients with pulmonary thromboembolism[130]. Thus, for a short period, there is a risk to the patient of potential bleeding although this is rarely seen in practice, except as haematoma around the venepuncture site.

Adequate anticoagulation in a leukapheresis procedure over a 2-3 hour period is provided by a loading dose of 2500 units followed by a further 9000-10,000 units at a rate of 1 unit/ml of exchange. Coagulation in-vitro is prevented by a single intravenous injection of 10,000 units followed by 1 unit/ml. This is equivalent to a concentration of 3 units/ml in a patient of 70 kg weight.

Filtration leukapheresis is now being used to lesser extent because of the side effects produced in donors and recipients arising from suspected complement activation during filtration[131].

In plasma exchange, the most widely used dosage schedule involves a bolus of 5000 units immediately prior to the procedure, followed by an infusion of 2 units of heparin for each ml of blood processed.

A combination of heparin and citrate has been used for both

centrifugal leukapheresis and plasma exchange. The donor is provided with an initial anticoagulant dose of 5000-6000 units of heparin followed by additional extracorporeal anticoagulation using ACD-A1:20[132]. This produces less severe citrate toxicity than ACD-A alone and reduces the risk of serious reactions to heparin.

2. Citrate

The anticoagulant effect of citrate is achieved by the complexing of ionised calcium in plasma to a level which will not support the proteolytic activities of factors IXa, Xa, VIIIa even in the presence of extrinsic stimuli. In practice a ratio of 10 mol citrate to 1 mol calcium is an effective extracorporeal anticoagulant. In plasma containing 14 nmol/1 citrate, ionised calcium is not detectable by calcium electrodes. Below 14 nmol/1 citrate, variable low levels of ionised calcium are found and occasional small clots are seen.

The symptoms of citrate toxicity due to hypocalcaemia and possibly hypomagnesaemia begin with circumoral of peripheral paraesthesia, increasing intensity and duration and spreading to the face and trunk[133]. More severe reactions are characterised by chills, painful pressure in the chest, generalised neuromuscular tension, nausea and abdominal cramps.

A reduced ionised calcium is associated with prolongation of the QT interval and there is good correlation with the plasma citrate level[134]. Symptoms rapidly cease when the infusion rate is reduced but often reappear as the rate is increased. Symptoms

are less frequent on the discontinuous flow separator because there is a break in the infusion during the bleed cycle and the citrate build-up is less marked.

The occurrence of citrate toxicity is more common in leukapheresis, plateletpheresis and plasma exchange, all of which involve the processing of large volumes of blood and citrated plasma is returned together with cells at rates often in excess of 60 ml/min. Obviously slowing the flow rate over the whole procedure would be counter-productive and cause the patient discomfort.

A reduction of the citrate load would reduce the degree of hypocalcaemia and its associated effects and can be achieved either by reducing the anticoagulant ratio or by using an anticoagulant with a lower citrate concentration[135]. Decreases in ionised calcium of 32% have been reported for ACD-A compared to 16% with half-strength ACD-A and of 22% compared to 13% with ACD-B respectively. Plasma protein fraction (PPF) or albumin (which have low citrate concentrations) in place of fresh frozen plasma (FFP) for plasma exchange reduces the citrate load but unfortunately presents other problems when used extensively[136].

The fall in ionised calcium has also been reduced by prophylactic addition of calcium gluconate to irrigation solutions in discontinuous flow leukapheresis and replacement PPF in plasma exchange, instead of using bolus injections of calcium when symptoms occur[137]. The addition to an extracorporeal circulation

must be made with care to ensure that it is not added in excess or at a point where coagulation could be induced[116]. Further, hypercalcaemia is as dangerous as hypocalcaemia and death from over-correction of citrate-induced hypocalcaemia following cardiac surgery has been reported. The occurrence of hypocalcaemia cannot be reliably predicted from the weight of donor, or patient and the infusion rate of the citrate, nor is the reporting of symptoms by the patient reliable[116]. Several patients receiving 60-80 mg/kg/hour citrate without prophylactic calcium showed severe hypocalcaemia without reporting symptoms. The ionised calcium levels were 0.6-0.7 mmol/1 and plasma citrate 20-24 mmol/1 which is on the borderline of the range reputed to cause cardiac depression[138].

The citrate anticoagulant also alters and buffers plasma pH to optimise conditions for the collection and storage of blood components. Thus, despite adequate anticoagulation, platelets have a tendency to aggregate during centrifugation at a pH value above 7.2 and become non-viable in storage if the pH value falls below 6.0. Because the collection of blood components by cell separator is being increasingly used to augment routine blood bank products, anticoagulant systems must be developed which will permit long-term storage of components, whilst avoiding undesirable donor side effects.

Continued use of ACD-A at a ratio of 1:7 for leukapheresis or plateletpheresis or the use of FFP for plasma exchange without calcium supplementation will produce paraesthesia in the majority of cases and constant interruption of the exchange

flow would be necessary to prevent more serious symptoms. This can be avoided by using ACD-A at a larger ratio where possible, or by changing to ACD-B where the ratio is fixed. ACD-A at half strength could result in wider coagulation in some instances, especially in conjunction with a low haematocrit. For plasma exchange the use of PPF, or albumin, possibly in conjunction with FFP, will by reducing the citrate load, lower the incidence of hypocalcaemia. Since plasmapheresis involves minimal return of anticoagulant, the ratio may be chosen to best suit the conditions for optimal preservation of the plasma components. In routine transfusion practice ACD solutions have now been superseded by CPD. It would then seem logical to use CPD in cell separator procedures where appropriate, with adjustments in pH and citrate content to provide optimal condition.

1.4.3 Replacement Fluids

1. Consequences of Plasma Removal

Therapeutic plasmapheresis is of three forms, namely:

1. Partial plasma exchange involving the removal of small quantities of plasma.
2. Total plasma exchange involving the removal of almost total effective plasma volume. Depending on the patient's size and weight this usually involves the removal of some 3 litres of plasma.
3. Selective plasma exchange in which a specific antigen, antibody complex, or toxin is removed from the plasma with the return of the denuded plasma to the patient.

The removal of 0.5 litres of plasma, twice a week, from healthy donors will result, over a number of years, in a reduction of immunoglobulins within the normal range[139]. Without replacement with crystalloid solutions this approaches the limit for the resynthesis of albumin 30-40 g/day. In the large volume plasma exchanges, required for the removal of pathogenic factors, there is a coincidental removal of normal constituents, e.g. albumin, immunoglobulins, complement and the coagulation factors[2,3,140-144]. The removal of more than one litre of plasma will result in haemodilution, with the formation of peripheral oedema because of the reduction in colloid osmotic pressure, if albumin is not replaced with colloid. The duration of the effect on protein depletion depends on the portion that is intravascular, the extent and rate of redistribution and the fractional catabolic rate, because soluble factors of the cell micro-environment appear to be necessary for the development and regulation of the immune system. The removal of the plasma fluid for therapeutic purposes may therefore decrease humoral components of immunoglobulins, circulatory immune complexes, complement components, cleavage products, prostaglandins and neurotransmitter[145].

It seems unlikely that the removal of plasma interferes in the sophisticated micro-environment system of immunoregulatory cytokines where minute amounts are involved in the triggering of cell membrane.

The removal of larger humoral components, such as immunoglobulins or circulatory immune complexes, may alter cellular functions. The rebound phenomenon observed after plasma exchange in some

autoimmune diseases, (e.g. Goodpasture's Syndrome, Bullous pemphigoid) obviously implicates the immunoregulatory circuits of the idiotypic network and subsequently the mediators of the T-B interaction. Similarly, extraction of circulating immune complexes may not only restore the physiological clearance capacity of the mononuclear phagocyte system, but also reverse functions such as antibody-dependent cytotoxicity, antibody formation and lymphokine production. These limited plasma exchange-dependent changes in T-subsets and con-A induced suppressor activity remain to be confirmed and explained[145]. The simultaneous taking of immunosuppressive drugs by most patients markedly increased the difficulties of such studies[146].

The physiological and haemostatic modifications induced in the donor or patient during and for selected periods of time after a single or multiple plasma exchanges have been widely studied over the past fifteen years and considerable data are now available.

Changes in blood pressure are commonly present during the course of plasma exchange, possibly the consequence of variations in blood volume or in hormonal parameters. Measurements of blood pressure, plasma renin activity, aldosterone, plasma catecholamines, natriuesis and blood before and after plasma exchange showed that in normotensive patients significant changes occurred during and immediately after PE but they are not related to changes in blood volume or to hormonal changes[147]. Blood volume and natiuresis values remained unchanged.

Changes in plasma renin activity, antidiuretic hormone and insulin have been determined in patients with a spectrum of immunologically related diseases in order to assess the response of the releasing cells to removal from plasma of their product of secretion[148]. The response kinetics differed for the three peptides. Compensation was fast and nearly total (91.4%) for antidiuretic hormone, whereas for plasma renin activity and insulin compensation had not occurred even at the end of the exchange procedure. Plasma volume and plasma osmolality were not changed significantly.

Plasmapheresis influences the composition of plasma electrolytes and hormones which in turn may influence cardiac contractility[149]. The function of the left ventricular pump has been assessed by time-motion echocardiography before and after plasmapheresis in patients with systemic lupus erythematosus, progressive glomerulonephritis and Graves Disease. No changes in blood pressure and heartrate were found. The left ventricular dimensions were comparable before and after plasmapheresis and no change was detected in the fractional fibre shortening ratio. Ionised calcium levels decreased significantly during treatment.

There is now a wealth of information on the effect of plasma exchange on serum immunoglobulin concentrations, complement concentrations and coagulation parameters during individual and successive procedures using coagulation factor-free replacement fluids[2, 3, 150-153].

Analyses have shown that platelet counts are lowered to 50% of

their pre-exchange levels after the first exchange to 21% after the fifth exchange and 23% after the tenth exchange[142]. The time needed for recovery was dependent mainly on the intensity of the previous exchange programme, but only 2 - 3 days were needed for platelets to return to normal, regardless of the actual level[2, 142].

The concentrations of the serum immunoglobulins IgG, IgA and IgM were reduced to 45%, 47% and 38% of the pre-exchange values after a single procedure. The times for recovery of immunoglobulin concentrations to pre-exchange values have been variously reported as 24-48 hours and in excess of two weeks[3, 141, 143]. With consecutive daily plasma exchanges the cumulative percentage reductions in immunoglobulins concentrations continued to decrease, e.g. to approximately 20%, 23% and 21% for IgG, IgA and IgM respectively after the fifth procedure. The decreases are maintained when the period between procedures does not exceed two days. With longer intervals between procedures the concentrations of serum immunoglobulins begin to increase again.

If immunosuppression is used, the plateau level may be below 2g/l and remain low for several months. This may result in complicating infections in immunocompromised patients (particularly in renal patients)[143] and the replacement of some immunoglobulin may be desirable[153]. When the IgG level is low its functional catabolic rate decreases and this may prolong the half-life of a pathological antibody. Rapid synthesis during an immune response may reduce the efficiency of the pathological antibody removal particularly in immune diseases and in haemolytic diseases of the newborn[93].

Most coagulation proteins have a short half-life with synthesis rates of hours and rapid re-equilibrium occurs between the intra- and extra-vascular compartments. Measurements[142,143,150] have been made on groups of patients, of the circulating levels of coagulation factors I (fibrinogen), II, V, VII, VIII, IX and X, and antithrombin III during, and in a 24 hour period after a single plasma exchange, after consecutive daily exchanges and after exchanges at intervals of two days or more. The circulating levels of all factors were reduced by differing amounts after a single exchange using coagulation factor-free replacement.

The reduction for each individual factor expressed as a percentage of the pre-exchange level did not differ for the various patients, nor were they significantly influenced by the interval between exchanges. Further, the extent of the percentage reduction during exchange was not influenced by the pre-existing level of the coagulation factors. After a single exchange, Factor I (fibrinogen) and Factor II were reduced to approximately 30%, Factors V and X to approximately 38%, Factors VII and VIII to approximately 45% and Factor IX to around 60% of the pre-exchange levels[143].

The cumulative effects of a course of five plasma exchanges on a daily or two-daily basis showed that Factors I, II and X were removed more efficiently than Factors V, VII and IX. All factors exhibited a rapid recovery in the four-hour period after exchange, but thereafter the levels increased more slowly. Factors V, VII, VIII and IX had returned to their normal values within 24 hours but Factors I, II and X had not achieved normality even after 48 hours. Thus, exchange on a daily or two-daily basis without

plasma replacement will gradually deplete levels below those thought to be necessary to maintain haemostasis. Provided the platelet levels are maintained there is little danger of spontaneous haemorrhage[143]. However replacement of plasma may be desirable if surgical procedures are to be undertaken[153]. Alternatively, normality can be restored by endogenous replacement if the exchange is stopped 48 hours beforehand. Coagulation inhibitors such as ATIII also exhibited a fall in the level of activity after a single procedure. The drop to approximately 40% of its pre-exchange value is thought to be partly due to consumption as well as physical removal. Levels are restored to normality in 2 to 3 days. Reports of the occurrence of spontaneous thrombosis are conflicting and most patients tolerate this transient coagulopathy without haemorrhage or thrombosis unless there is a pre-disposing factor, or the exchange is repeated in less than 48 hours. Immediately after one plasma exchange with plasma protein fraction there is a significant decrease in plasma fibronectin levels which are restored to pre-exchange levels within 48 hours presumably by redistribution between compartments and increased synthesis[144]. Daily plasma exchange produces progressive depletion of plasma fibronectin, whereas levels were maintained close to the initial value with longer intervals between exchanges, except in the more severly ill patients. Reduction of plasma fibronectin by exchange may increase susceptibility to infection and reduce the efficacy of the procedure if used to reverse reticuloendothelial blockade.

Factor VIIIC and VIIIRAg and ristocetin Co Factor are significantly depressed immediately after plasma exchange (to 50%, 35% and 40%

activity levels respectively) but recover to pre-exchange values within 24 hours[142]. The prothrombin time (PT), kaolin cephalin clotting time (KCCT) and thrombin time (TT) are significantly prolonged (by 60%, 100% and 300% respectively) immediately after plasma exchange. KCCT and TT return to within the normal range after four hours and all return to pre-exchange values after 24 hours[143].

Further studies have involved measurements of the total haemolytic complement (CH50) and the fractions C3, C4, B, C3d before and immediately after plasma exchange and thereafter at specified intervals[3,141,152]. After a single exchange, concentrations of CH50, C3, C4 were lowered to 50%, 45% and 38% of their pre-exchange values respectively. Resynthesis is rapid and levels return to normality within 24 to 48 hours. During periods of consecutive daily exchanges the concentration levels of C4 and particularly of CH50 fall much less rapidly than that of C3. The substitution of Ig during plasma exchange with 5% human albumin will reduce the extent of the fall in serum levels of C3 and C4[153].

Complement activation and C3a, C5a generation is found in the extracorporeal circuit during membrane plasmapheresis[154]. This has been accounted for in neutropenia and pulmonary leukostasis which may occur under such conditions. The observed increase in C3a production is attributed to the filtration circuit and the possibility of massive infusion of active C3a should be a matter of concern when fresh frozen plasma is used as the replacement fluid.

64% of plasma cholinesterase is removed in a single whole volume plasma exchange using plasma protein fraction as the replacement fluid[155]. A series of daily plasma exchanges almost completely removes circulating cholinesterase, particularly in those patients with initially low levels and suxamethonium apnoea may occur during induction anaesthesia due to raised sensitivity to the drug.

The kinetics of removal and subsequent recovery have been investigated for other plasma constituents namely calcium, uric acid, sodium, potassium, chloride, bicarbonate, aklaline phosphatase, lactate dehydrogenase, transaminases, creatinine phosphokinase, amylase and cholesterol[143].

Although the selective removal and modification of blood components offer considerable potential for the treatment of many disorders, there remains a major requirement for the replacement of products withdrawn by apheresis. The replacement fluid should be selected on the basis of the clinical condition of the patient and the rationale for the exchange. With the possible exception of plasma replacement in thrombotic thrombocytopenic purpura, in some forms of SLE, and where there is a bleeding tendency there are no therapeutic data on which to assess the relative merits of one form of replacement over another.

The nature and volume of fluid replaced will depend on the volume of fluid removed, the physiological needs for replacement and the therapeutic objective. Additionally the availability, convenience and cost of the replacement fluid must also merit attention.

2. Available Replacement Products

1. Patient's Own Plasma

The best replacement fluid would seem to be the patient's own plasma minus the offending component. Only in cryoglobulinaemia is this readily accomplished by precipitation of the abnormal protein from plasma stored at 4°C.

The removal of the offending component from the patient's own plasma, either by secondary separation, or by the combination of apheresis procedures with column techniques has already been discussed. Clearly, there are considerable problems to be overcome before the preferential elimination of identified components becomes a routine cost effective procedure.

2. Fresh Frozen Plasma

Acid Citrate Dextrose (ACD) or Citrate-Phosphate Dextrose (CPD) plasma separated from red cells within a few hours of donation, frozen rapidly and stored at -30°C has a coagulation factor content which will remain at near normal levels for up to 12 months. If kept at -20°C it should be stored for not more than 6 months. (See Table 7).

Fresh frozen plasma requires to be thawed rapdily at 37°C with continuous agitation and transfused within 30 minutes to avoid rapid post-thaw deterioration of coagulation factors. The volume varies from about 200 ml from random

Table 7

PLASMA PRODUCTS [139]

FRESH FROZEN PLASMA

Volume:	200 ml $\pm$ 10% - random donor 500ml $\pm$ 10% - selected donor
Storage:	In frozen state at below -30°C for 1 year
Administration:	Must be thawed (30 mins) and transfused as soon as possible (within 2 hrs) Must be given ABO group specific (occasionally Rh(D) specific).
Potential advantages:	Normal levels of: coagulation factor (not cryo-supernatant) antithrombin III and other inhibitors immunoglobulins complement enzymes and carrier proteins Iso-oncotic and isotonic

donors to 400-600 ml for apheresis donors. About 40-50 ml of the original citrate anticoagulant will be present in FFP and if infused at rates above 50 ml/min symptoms of hypocalcaemia are observed.

FFP should be given to ABO compatible recipients. It should contain all the non-cellular coagulation factors. As such it can be used to replace any of them. The volume which a patient can receive without being overloaded is limited and this determines the amount by which a particular factor can be raised in a patient's circulation. This is particularly true of FVIII,which has a short in-vivo half life.

The incidence of allergic reactions is greater with plasma than with any other replacement fluid and is particularly common if used as sole replacement fluid. The majority of reactions are mild urticarial reactions, but occasionally bronchospasm, tachycardia, hypotension and anaphylasis occur.

FFP is not, in many cases, a practical replacement fluid to use in plasma exchange by virtue of its relative inaccessibility, the problems of grouping and the incidence of allergic reaction. In conditions where a source complement is required it is the ideal fluid replacement.

3. Cryoprecipitate

When plasma is frozen and then thawed the small amount of protein precipitated is rich in Factor VIII. After decanting

almost all the supernatant plasma, the protein can be redissolved by warming to give a small volume of solution containing about 50% of the Factor VIII activity of the original unit. Cryoprecipitate can be stored at -20°C for at least 2 years with full preservation of Factor VIII activity. Only a small number of donors are used and thus the risk of transmission of viral hepatitis is decreased[156]. It contains all the plasma proteins in normal amounts, except for fibrinogen, Factor VIII and fibronectin.

4. Albumin Solutions

Human albumin is a highly soluble, symmetrical, slightly heterogeneous protein which weighs about 67,000 daltons. It is responsible for 80% of the colloid osmotic pressure of plasma.

Three manufactured albumin products are available:

1. Albumin 5%
2. Plasma Protein Fraction
3. Albumin 25%

Plasma protein fraction yields somewhat more useful protein per litre of starting plasma in the fractionating process. The non albumin proteins are quickly removed from the circulation because of heat denaturation. Shelf-life depends on storage temperatures; 3 years are permitted at below 37°C and 5 years at 2 - 10°C. (See Table 8).

Table 8

PLASMA PRODUCTS[139]

ALBUMINOID SOLUTIONS

Volume:	Variable, but 100 ml and 500 ml sizes common
Storage:	In liquid state at ambient temperature (5-25°C) for 3-5 years (depends on manufacturer).
Administration:	Ready for immediate use
	No blood group restriction
	Can be supplemented with electrolytes (K^+ or Ca^{2+}) if required
Potential advantages:	No hepatitis risk
	No fibrinogen or complement (inflammatory mediators)
	Minimal allergic reactions
	4-5% solution is essentially isoosmotic and isotonic
Potential Disadvantages:	Expensive
	No coagulation factors
	No immunoglobulins
	No trace plasma proteins (e.g. enzymes)

Albumin 5%

Albumin may be produced commercially and non-commercially, the final product containing not less than 95% albumin. It is available not only in a 5% solution but also in a more concentrated form. The solution contains approximately 130-160 mmol/1 of Na and not more than 1 mmol/1 of K. The product is heated to 60° C for ten hours to inactivate the hepatitis B virus.

Plasma Protein Fraction (PPF)

PPF is a liquid preparation containing about 45 g of protein per litre. By itself this would be a hypotonic solution, so 130-160 mmol of sodium chloride is added per litre. The protein in solution is Cohn Fraction V precipitate and consists of 90% or more albumin with not more than 10% alpha and beta globulins and is stabilised with sodium caprylate. Albumin in this concentration can be heated to 60° C for ten hours without causing undue aggregation of the protein. This inactivates Hepatitis B virus and hence the final product is non-icterogenic. This was one of the major reasons for introducing PPF but since then the risk of transmitting hepatitis with freeze dried plasma itself has been dramatically reduced as a result of modern methods of testing donations for the presence of the hepatitis B antigen.

Albumin 20-25%

This is sometimes called salt-poor albumin. It is a solution in water of human albumin, containing a low proportion of salt. It is clear liquid prepared from the plasma of suitable human donors whose transaminase levels are constantly checked and whose donations are HBsAg negative.

It contains 20% of protein, of which 97% is albumin, the rest being thermostable alpha and beta globulins.

20% albumin is hyperoncotic and is not suitable for plasma exchange unless diluted to 4-5% with an electrolyte solution. Caution is indicated in administering albumin; a careful watch must be kept for the possible development of pulmonary oedema. If it occurs the infusion must be stopped immediately.

Commercial albumin solution may be a potential source of intravenously administered aluminium, the long term effects of which remain to be assessed.

Adverse Effects of Albumin

Albumin products are inherently safe. Chronic adverse effects are:

1. Bacterial contamination - Occurs rarely. Occasional febrile reactions, transient bacteraemia, shock and occasional sepsis have occurred.

2. Pyrogenic Reactions - 75% of all reported reactions are characterised by chills and fever. They are rarely severe.

3. Hepatitis - When properly heated and protected from subsequent contamination, albumin and PPF are unable to transmit hepatitis. The screening for hepatitis B surface antigen HBsAg and elimination of plasmas that are positive has resulted in a sharp decline in the amount of antigen detectable.

4. Hypotension - It has been known for some time that rapid administration of PPF is frequently associated with transient hypotension. It has also occurred less commonly with albumin products. This has now been attributed to the Kallikrein Activator. This activates the recipient's own kinin system to produce bradykinin.

Both albumin and PPF have been extensively used in plasma exchange, despite the fact that they contain no coagulation factors, immunoglobulins or plasma proteins.

Caution should be exercised when albumin is infused repeatedly, particularly in patients with renal insufficiency. In such patients' plasma aluminium levels should be monitored regularly and any clinical signs and symptoms compatible with aluminium toxicity should be evaluated promptly[157].

5. Plasma Volume Expanders

Human blood and blood products are costly fluids, in monetary terms and in personal inconvenience to the donor. Thus there are a number of plasma substitutes used as plasma volume expanders usually in surgical and trauma patients[1,158,159] None has significant advantages over human albumin products. A requirement of artificial colloid is that they may be eliminanted from the body either by excretion or by metabolism. At the same time they should remain in the circulation sufficiently long to maintain the plasma colloid osmotic pressure while regeneration of plasma protein occurs. Plasma expanders are therefore required to imitate the function of plasma proteins particularly albumin, and should ideally be neutral to the body, non-toxic and non-antigenic. Three major problems are experienced with the available colloid volume expanders, namely a) tissue storage and elimination from the body, b) allergic and anaphylactoid reactions,c) production of an acquired bleeding tendency. Gelatins, dextrans and hydroxyethyl starch are all associated with these side effects but not to the same extent[159].

All artificial colliods lack carrier function and remain in the recipient's circulation for a much shorter time than albumin. Dextrans and hydroxyethyl starch interfere, in varying degrees, with haemostasis which limits the total amount that can be given to 1-1.5 litres. The problem is more pronounced with dextrans and the bleeding defect induced can be corrected only by transfusion. The major disadvantage of hydroxyethyl starch is the possible occurrence of hypersensitivity reactions.

Gelatins

Gelatins are macromolecular substances with average molecular weight of 30-25,000 daltons which are slowly metabolised, the intra-vascular half -life being 4-6 hours. Gelatin is an insoluble animal collagen (from skin, tendon or bone) which is hydrolysed and modified to give a stable fluid material. The three commercial types are:

1. Gelofusine (oxypolygelatin) - gelatin 4%, sodium chloride 0.9%.
2. Plasmagel - modified fluid gelatin
3. Haemaccel (urea linked gelatin) - gelatin 35 g/1, Na 145 mmol/1, K 5.1 mmol/1, Ca 6.26 mmol/1, C1 145 mmol/1.

The gelatins may be used to expand and maintain blood volume in shock arising from conditions such as burns or septicaemia. Typically 1 litre of gelatin and 3 litres of PPF may replace 4 litres of plasma without adverse effects on albumin levels or on blood volume. They are excreted rapidly in the kidneys without renal impairment, with only a small percentage being metabolised.

There is no effect on the coagulation or fibrinolytic systems except by simple dilution.

Gelatin's shelf life is 8 years at room temperature and is unaffected by wide temperature fluctuations.

Electrolytes

These have no carrier function but can expand the plasma volume if used in sufficient amounts (about 3 times equivalent of plasma). The duration of the effect is short because of the shift into the interstitial fluid space. They temporarily lower the plasma colloid osmotic pressure and should be used with caution where patients have pre-existing hypoproteinaemia, i.e. with albumin below 30 g/1 and total protein below 60 g/1.

Electrolytes alone should not be used to replace more than 500 ml of plasma. However much larger volumes could be used during the initial part of a 3-4 litre plasma exchange (of the order of 75% crystalloid followed by 25% of colloid). The replacement fluid given early in the procedure will be partially removed by the end so that where a protein (e.g. albumin) has to be replaced, it is more logical and cost effective to give this at the end of the exchange. The onset of hypovolaemia may necessitate the earlier use of colloid. FFP should also be given where there is a need for protein which cannot be provided in any other way (e.g. serum cholinesterase) and generally, in exchanges of larger volumes 4-5 litres.

The relevant features of various plasma substitutes are compared in Table 9.

Table 9

COMPARISON OF PLASMA SUBSTITUTES

	ELECTROLYTE SOLUTIONS	PLASMA PROTEIN FRACTION	HAEMACCEL
pH	5.5 - 6.5	6.7 - 7.3 buffering capacity	7.2 - 7.3 buffering capacity
Oncotic pressure compared with plasma	Hypo-oncotic	Iso-oncotic	Iso-oncotic
Interference with subsequent blood-typing and cross matching	none	none	none
Interference with coagulation haemostasis	none	dilution	dilution
Renal function	improved in shock	improved in shock	improved in shock
Intravascular interstitial fluid balance	oedema	rehydration	rehydration
Effective T½	short	several days	4-6 hours
Cardiovascular overload	unlikely	less likely than	whole blood
Possible reactions	unlikely	histamine release anaphylactic	histamine release
Transmission of disease	none	unlikely	none
Shelf life	3 years	3-5 years	8 years
Storage	Room temperature	2 - 30°C	Room temperature

1.5 Applications of Plasmapheresis

1.5.1 Rationale for Treatment

Any change produced by plasmapheresis is governed by the pathophysiology of the disease. It is reasonable to assume that in disease characterised by circulating toxic factors, humoral mediators, altered viscosity or abnormal haemostasis, the use of intensive plasmapheresis is logical.

The serological abnormalities may be favourably influenced by a change of the "milieu interior". When factors responsible for the clinical syndrome are unknown or the serological markers are poorly associated with the activity of the disease, it is difficult to justify and interpret the results of plasma exchange. Not surprisingly the majority of diseases are associated with immunological and serological difficulties.

The possible mechanisms[28] by which plasma exchange could be of benefit to patients are suggested in Table 10. The postulated mechanism is correct only for some of the examples listed.

1.5.2 Protocols for Treatment

Ideally, criteria should exist for the intensity, frequency and duration of the procedure, for the choice of replacement fluids, the serological parameters to be followed and the clinical evaluation of the effects of the procedures. Existing protocols for plasma exchange are empirical and largely influenced by

TABLE 10

Possible Mechanisms of Action in Therapeutic Plasma Exchange[28]

MECHANISM OF ACTION	DISORDER
1. Removal of Autoantibody	Myasthenia Gravis
2. Removal of Alloantibody	Rh alloimmunisation in Pregnancy
3. Removal of immune complexes	Systemic Lupus Erythematosus
4. Removal of Monoclonal Protein	Hyperviscosity Syndrome
5. Removal of Toxin	Mushroom Poisoning
6. Replenishment of specific plasma Factor	Thrombotic Thrombocytopenic purpura
7. Placebo effect	Rheumatoid Arthritis

factors such as vascular access and scheduling. They will remain so until the metabolism and pathogenicity of the abnormal plasma constituents removed by the plasma exchange are established.

There is, however, evidence that the period in which plasma exchange is initiated may be one of the most crucial decisions[160]. Direct measurement of the effector cells would provide meaningful information of the necessary frequency and duration of exchange therapy. The patients' response to the therapy and to the disease process will also influence protocol design[160]. Procedures, in which the volume exchanged approaches the patient's plasma volume, remove approximately 50-60% of an intravascular substance[28]. Four or five such exchanges over a period of 7-10 days are generally thought to be adequate for short term therapy. If catabolism balanced synthesis and intravascular shift throughout this time, the final concentration of a substance would be less than 10% of its initial concentration. However because a sustained balance is unlikely, this value is rarely achieved.

In disorders where plasma exchange can be considered an established therapy, serial clinical assessment and measurement of the pathogenic plasma factor permit the assessment of the volume and frequency of exchanges , even if the distribution and metabolism of the plasma factor are not completely understood[28].

1.5.3 Evidence of Efficacy

The benefit of plasma exchange can be confirmed in two ways[28] -

namely, (a), demonstrable removal (or replenishment) by plasma exchange of an abnormal plasma component (or a deficiency of a normal component) in the pathogenesis of the disease or one of its components, (b) conclusive evidence of patient benefit from a properly designed randomised controlled clinical trial.

Diseases which are associated with excessive or abnormal proteins, lipids, toxic substances, circulating immune complexes or antibodies are monitored by sequential quantifications of these substances. In such cases the clinical effect of plasmapheresis may be correlated with the level of specific disease marker[160]. Unfortunately the levels of many plasma factors are decreased by the exchange and a demonstration that the removal of particular factor caused the disease is difficult[28]. Moreover, even if a plasma factor is known to be pathogenic, its reduction by plasma exchange is not necessarily associated with a clinical response, e.g. in maternal Rh allo-immunisation, it is not yet known whether plasma exchange is of benefit to the foetus, despite the decrease in Rh antibody concentration that can be effected by the procedure. There is little argument that plasma exchange can alter the level of a pathogenic plasma factor in each of the disorders listed in Table 11, but it should not be considered established therapy in all these disorders. When there is poor correlation between the serological findings and the disease activity or when no discrete markers exist, therapeutic assessment becomes a function of clinical evaluation. Even in diseases which unquestionably benefit from plasma exchange, the improvement is usually due to relief of clinical manifestations rather than

Table 11

Disorders in which the Efficacy of Therapeutic Plasma Exchange can be Monitored by Measurement of a Pathogenic Substance in Plasma[28]

Disorders	Pathogenic Substance
Hyperviscosity syndrome	Monoclonal immunoglobulin
Cryoglobulinaemia	Cryoglobulin
Cold antibody-type autoimmune haemolytic anaemia	Red-cell autoantibody
Factor VIII deficiency unresponsive to factor VIII	Antibody to factor VIII
Post-transfusion purpura	Platelet antibody (Anti-$P1^{A1}$)
Preparation for ABO-incompatible marrow transplantation	Antibody to a or b antigen
Maternal alloimmunization to Rh(D) antibody	Antibody to Rh(D) antigen
Familial hypercholesterolaemia	Low-density lipoproteins and cholesterol
Poisoning	Drug
Myasthenia gravis	Autoantibody to acetylcholine receptor
Fabry's disease	Ceramide trihexoside
Refsum's disease	Phytanic acid
Goodpasture's syndrome	Antibody to basement membrane
Pemphigus	Autoantibody to epidermal cell-membrane glycoproteins

to any effect on the underlying disease itself. The short term improvement that is characteristic of the response of many disorders to plasma exchange, frequently limits its value to therapy for acute conditions.[28]

1.5.4 Implications for Treatment

The economic and social implications of plasma exchanging are far-ranging. Both cell separator and procedure are expensive. Equipment costs are extremely high (although this may be reduced by the advent of newer membrane therapy). Each procedure itself is expensive including staff time, replacement fluids used and disposable items. The cost effectiveness of plasma exchange has yet to be established, whilst the feasibility of large scale application could pose problems of plasma replacement supply. It has been calculated,[161] that if plasma exchange had been proven successful in the treatment of rheumatoid arthritis and made available to all potential sufferers, then not only would the health care budget have to be increased enormously, but so also would the number of blood donations necessary to supply the amount of albumin needed as replacement fluid. Further, if the report, that disseminated cancer might regress with plasma exchange was confirmed, the cost and supply problems would be almost unsurmountable.[162]

These economic and social implications provided considerable impetus for controlled trials to establish the efficacy of the treatment and to establish a stricter evaluation.[163] The diagnosis of many disorders is based partially, or totally,

upon a set of criteria rather than specific diagnostic tests. Thus assessment of disease activity and therapeutic responses rests largely on subjective evaluation of a complex number of parameters by patient and doctor. The situation is further compromised by the fact that many therapeutic modalities can be associated with improvement of varying consistency and duration indicating a strong placebo effect.

The vast majority of trials are uncontrolled and report a much higher response rate to the exchange procedure than had been observed in the few studies based on a control group (Table 12). Many deficiencies have been noted in reports of randomised controlled trials of plasma exchange, e.g. inadequate description of patient selection, inadequate information on patients rejected and withdrawn, and effectiveness of randomisation[164].

The design of a clinical trial involving plasma exchange is controversial. Efficacy should be established by using a trial in which the assignment of patients to treatments is controlled[165]. The experimental groups of patients must be representative of the general patient population with the disease under consideration and should be identical in every way except for the intervention being evaluated[166,167]. This must include all compounding variables (factors other than the treatment that may influence outcome, e.g. age, sex) and all aspects of ancillary care that may affect response to treatment during the trial[167,168]. Thus, to assess the effect of removing plasma or cells during plasma exchange on selected groups of patients, the control must replicate the procedure except for the irreversible extraction of plasma and cells.

TABLE 12

Controlled and Uncontrolled Trials in Therapeutic Plasmapheresis[163]

Year	Uncontrolled Trials			Controlled Trials		
	Prospective Trial	Multiple Case Report	Single Case Report	Randomised	'Sham'	Other
Before 1976	-	6	12	-	-	-
1976–1980	18	23	27	-	-	5
1981	6	9	8	3	-	-
1982	10	8	5	-	1	-
1983	2	9	10	2	3	1
1984	1	6	2	4	-	2
Response to PE %	100	91	80	56	25	67

There are several methods for selecting the control group. Ideally control group should be randomly selected from the study population[167]. Much has been written about the ethics and legal implications of the randomised clinical trail[165,166,169]. The process of randomisation, i.e., allocating patients to treatment groups by a chance procedure[167,170], clearly strains the traditional doctor/patient relationship. The physician is unable to give the patient what he may feel to be the best possible care and both lose the freedom to chose a particular therapeutic option. The design and management of a randomised trial pose ethical questions, namely the facts that standard treatment may be withheld from the experimental group, the possibility that the control patients are denied a potentially beneficial new treatment and the concern that patients are randomly assigned to therapies with different risks (if these are known).[165,167] This conflict between individual patient ethics and the collective ethics of the patient population with the disease being studied must be resolved by both referring and participating physicians if the trial is to be successfully completed[165,166,169]. The rights of the individual patient must to a certain extent be sacrified in a properly controlled clinical trial and this may affect patient referral[169]. As a result the aim of the randomised clinical trial may not be achieved[169]. If patients enrolled in the trial are for ethical or other reasons, not representative of the patient population for whom the study treatment is being evaluated the significance of the study's conclusions may be questionable.

Implicit in the process of randomisation is the concept that patient and/or physician preference for a type of therapy is being removed[169]. Unfortunately, however patient bias may still affect

the interpretation of results and may contribute to the high response rate reported in controlled studies of plasma exchange. The placebo effect must be accounted for in the assessment of any treatment results.

This effect could be overcome by using a placebo in the control group[167,170]. If patients are unaware of their assigned treatment group then patient attitudes to the trial should be the same in both the placebo and the experimental groups. Any differences in response between these groups should then be real. For some therapies the appropriate placebo may not be available[171]. This applies to invasive procedure, where the risks to the control patients may be unacceptable and even greater than the potential benefit of the experimental therapy[166, 167]. The ethics of intentionally deceiving patients also deserve careful consideration.

Double blind studies, where neither patient nor observer know which treatment is being administered may avoid bias and ensure that there is no disparty between the groups[166]. Double blinding will however further complicate the design and implementation of a study.

For those disorders where the definition of response to plasma exchange involves the measurement of specific end-points, e.g. renal function in rapidly progressive glomerulonephritis; platelet count in immune thrombocytopenia purpura or foetal outcome in Rh alloimmunisation, the blinding of patients is not necessary[164]. For disorders where such objective assessments are more difficult, e.g. multiple sclerosis or rheumatoid

arthritis, it has been suggested that the patients should undergo a sham plasma exchange procedure[28]. This is identical to a true exchange except that, without the patient's knowledge, the separated blood components are reinfused without any plasma replacement. This procedure is difficult to justify bearing in mind the potential complications[172]. It has also been argued, however, that a sham procedure which entails a temporary extraction of plasma or cells and extracorporeal manipulations may in itself be therapeutic and capable of inducing measurable changes in the blood[163,173]. The irreversible extraction of plasma, or cells, has been shown to be therapeutically more effective than the temporary removal[174,175].

1.5.5 Typical Applications of Plasma Exchange

The technique has been applied to a wide range of diseases as in Table 13[176]. The earlier invesigations[160] were exploratory assessments of the therapeutic potential of the procedure and that much of the controlled testing necessary to establish the efficacy of the technique remained to be undertaken. This deficiency is still being remedied[177]. The present review is restricted to those disorders under treatment at the Glasgow Royal Infirmary.

Hyperviscosity States.

i. Paraproteinaemias

Paraproteins are large abnormal proteins with a high intrinsic viscosity. They tend to precipitate on cooling and have the ability to interact with other proteins. These properties account for the symptoms they produce in malignant and non-

Table 13

List of Diseases in which Plasmapheresis has been used[176]

Haematological:

Macroglobulinaemia
Myeloma
Immune thrombocytopenia
Haemophilia with inhibitors
Post transfusion purpura
Acquired Von Willebrand's disease
Pruritis of myeloproliferative disease
Acute leukaemia
Bone marrow transplants
Haemolytic disease of the newborn
Autiommune haemolytic anaemia
Cold haemagglutininin syndrome
Paroxysmal cold haemoglobinuria
Paroxysmal nocturanal haemoglobinuria
Sickle cell crisis
Pure red cell aplasia
Aplastic anaemia

Renal:

Goodpasture's syndrome
Crescentic glomerulonephritis
Light chain nephritiis
Haemolytic uraemic syndrome
Henoch Schonlein nephritiis
Mesangiocapillary glomerulonephritis
Renal transplant rejection

Neurological:

Myasthenia gravis
Lambert Eaton Syndrome
Amyotrophic lateral sclerosis
Schizophrenia
Guillain-Barre syndrome
Multiple sclerosis
Parkinson's disease

Endocrinological:

Thyroid storm
Exophthalmos and pretibial myxoedema
Insulin resistant diabetes

Table 13 (continued)

Toxins and Drugs:

Ammanita phalloides poisoning
Phenylbutazone overdose
Digoxin overdose
Paraquat poisoning
Methyl parathion poisoning
Aluminium encephalopathy
Sodium Chlorate poisoning

Malignant Disease:

Disseminated carcinoma
Melanoma
Immunotherapy blocking factor
Neuroblastoma
Hyperhephroma
Sweats and pruritus of malignant disease

Respiratory Disease:

Idiopathic pulmonary haemosiderosis
Idiopathic pulmonary fibrosis
Asthma

Miscellaneous Disorders

Familial hyperlipidaemia
Refsum's syndrome
Fabry's disease
Immunodeficiency syndrome
Thrombotic thrombocytopenic purpura
Eclampsia
Uncontrolled hypertension
Cyclical angioneurotic oedema
Antibodies to HLA

Dermatological:

Pemphigus
Pemphigoid
Herpes gestationis
Porphyria cutanea tarda
Necrotising vasculitis
Psoriasis
Autoerythrocyte sensitisation
Scleromyxoedema

Table 13 (continued)

Connective Tissue Disorders:

Systemic lupus erythematosus
Rheumatoid arthritiis
Dermatomyositis
Still's disease
Scleroderma
Behcet's syndrome
Rheumatic fever
Polymyositis
Polyarteritis nodosa
Wegener's granulomatosis
Raynaud's syndrome
Cryoglobulinaemia
Mixed connective tissue disease
Sjogren's syndrome

Infections:

Subacute bacterial endocarditis
Meningococcal septicaemia
Staphylococcal septicaemia
Leptospirosis
Leprosy

Gastrointestinal:

Hepatic coma
Pruritis of cholestasis
Crohn's disease

malignant conditions.

It has been shown [178,179,180] that plasma exchange can be extremely effective at removing these paraproteins, the effectivness depending on the distribution within the vascular compartments.

ii) Waldenstrom's Macroglobulinaemia

This disorder[181-183] is due to the proliferation of an IgM producing clone. It is characterised by many unpleasant and occasional life-threatening complications due to hyperviscosity, of which blindness, deafness, coma and retinal haemorrhage are a few. Conventional treatment consists of chemotherapy - Chlorambucil being the drug of choice. It can, however, be many weeks before it begins to decrease the production of IgM. One single plasma exchange, on the other hand, can lower the serum IgM by 50%, because 75% of the IgM antibodies responsible for the condition circulate in the intravascular compartment. There is also an immediate improvement in the clinical condition. Some patients can be maintained symptom-free by two to three-monthly plasma exchanges without chemotherapy.

iii) Multiple Myeloma

This condition may present in a variety of ways, one of which is acute renal failure[184]. Multiple pathogenic factors contribute to the problem and it is associated with a poor prognosis[185]. One factor known to be associated is the effect of Bence-Jones proteins which form obstructive casts in the distal nephrons. Intensive plasma exchange may remove 50% of these circulating proteins in patients unresponsive to more conventional therapy, e.g. diuretics, urinary alkalinisation and chemotherapy. The

effectiveness of plasma exchange on the long-term management and prognosis of these patients is presently unknown, but it may well be a very useful short term measure until cytotoxic therapy, namely, Melphalan in combination with Prednisolone takes effect.

Hyperviscosity is also associated with Multiple Myeloma[186,187] due to increased production of IgG and IgA. These molecules tend to have a higher extravascular distribution than IgM, but this larger extravascular load is offset by a slower re-accumulation rate of immunoglobulins. IgG and IgA have lower molecular weights than IgM, making hyperviscosity a less frequent occurrence than Waldenstrom's Macroglobulinaemia. Often single plasma exchanges lead to considerable clinical improvement during the lag period before established chemo-therapy reduces production of the offending immunoglobulin.

iv) Cryoglobulinaemia

Cryoglobulins are paraproteins which tend to precipitate on cooling. Their presence is associated with many disturbing effects namely hyperviscosity, vasculitis and Raynaud's Phenomenon[188,189]. Gangrene and necrotic ulcers are common consequences.

Therapy [190,191] is aimed at the underlying disease process, e.g. collagen disorders, infectious mononucleosis and cytomegalovirus. In the acute situation plasma exchange is the treatment of choice. The response is often quite dramatic with long-term remissions being achieved.

Immune Based Haematological Disorders

i) Autoimmune Haemolytic Anaemia

The Haemolytic anaemias characterised as autoimmune are identified by the presence of autoantibodies against red cells. More often than not, a specific aetiological basis is not identified and treatment with steroids, splenectomy or immunosuppressive agents aims at decreasing the distruction of antibody-coated red cells and reducing antibody production[192-195]. Some patients fail to respond to such conventional therapy and present with severe anaemia. In such cases it is often impossible to obtain compatible blood and treatment is thus difficult.

It has been shown that plasma exchange has a potential role in the treatment of both warm and cold antibody-mediated haemolytic anaemia. It causes a temporary reduction in cytotoxic antibody levels. The fall in titre of antibodies allows prolongation of survival of transfused cells and immunises the destruction of autologous red cells. The majority of patients will not require such treatment. However, where conventional therapy fails, a trial of plasma is recommended, especially in the setting of fulminant haemolysis.

ii) Idiopathic Thrombocytopenic Purpura

IgG and IgM platelet antibodies are present in the majority of patients with I.T.P. and are implicated in platelet destruction. Conventional therapy for I.T.P. includes the use of steroids which are thought to block the macrophages in the spleen thus decreasing platelet sequestration and destruction[196]. If this fails reduction of autoantibody production is attempted by

the use of cytotoxic agents - vincristine and cyclophosphamide, with or without splenectomy, the spleen being the major source of antibody production. Conventional therapy fails to produce a substantial response in 30% of patients, with 7% having severe continuous bleeding problems[197].

Plasma exchange [192,198] can remove a predictable amount of circulating immunoglobulin.

It has been demonstrated that a single plasma exchange can bring about a 50% decline in anti-platelet activity, with an associated increase in platelet counts within hours of procedure, resulting in an immediate improvement in haemostatic function. On available data, therapeutic plasma exchange has a potential role in acute I.T.P. whenever splenectomy or immunosuppressive agents are contra-indicated. It is also useful in preparing patients for surgical procedure.

The possibility that plasma exchange will result in an anti-coagulation effect with increased risk of bleeding should also be considered.

iii) Thrombotic Thrombocytopenic Purpura

The disorder, of unknown pathophysiology, is characterised by microangiopathic haemolytic anaemia, thrombocytopenia, central nervous system dysfunction and renal impairment. The clinical manifestations are due to the formation of platelet thrombi in the microcirculation.

Many therapies[199,200] have been used for I.T.P. - namely transfusion, corticosteroids, antiplatelet agents and splenectomy.

In spite of such treatment, mortality rates range from 72% at 3 months to 93% at 1 year.

Plasma exchange[201,202] is known to bring about a rapid improvement, whether by removing toxic factors or by adding normal stimulatory substances. It may well be that it provides a missing plasma factor which has a stimulatory effect on vascular endothelium, producing prostacycline PG12, a potent inhibitor of platelet aggregation. Absence of the factor leads to platelet consumption, which in turn triggers the syndrome. Since plasma exchange has been incorporated into the therapeutic regime, recovery has occurred in 70% of cases.

iv) Factor VIII Inhibitors

Antibodies to FVIII, occur in a variety of haematological and non-haematological diseases and may be present with life threatening bleeding. Therapeutic plasma exchange[203-205] is now used increasingly in the treatment of patients with this disorder. Combination therapy, namely immunosuppression (corticosteroids) and plasma exchange may decrease the concentration of Factor VIII inhibitors by 85%. It is now the first line treatment to rapidly remove the inhibitor, allowing standard chemotherapy to take effect in the ensuing three weeks.

Alloimmunisation

i) Rhesus Haemolytic Disease

There are still a considerable number of alloimmunised women of

child-bearing age despite the advent of immune globulin. Full-term pregnancy for these women can still be a major problem[93,206].

It has been shown[207,208] that plasma exchange, commenced early in pregnancy, can achieve a more successful outcome in a significant number of cases. Plasma exchange is known to remove circulating proteins, e.g. antibodies. In some cases of Rhesus disease the antibody concentration may be lowered by 80%. Sufficient amounts of the offending immunoglobulin may be removed and accordingly lead to a dramatic decrease in foetal wastage. Its greatest potential is achieved when haemolytic disease begins after the 30th week of gestation.

Antibody Mediated Non-Haematological Disorders

i) Myasthenia Gravis

The antibody to the acetylcholine receptor is basic to the pathogenesis of myasthenia gravis. Amongst different patients the correlation between the level of acetylcholine receptor antibody and the severity of myasthenic symptoms is not strong; changes in antibody level in the individual patient may parallel changes in disease activity. Acetylcholine receptor antibody that is transferred from mother to foetus is associated with neonatal myasthenia gravis[209]. In this situation plasma exchange may ameliorate acute myasthenia weakness[210].

Plasma exchange is often used in the preparation of an individual for thymectomy respiratory muscle weakness. In many cases, it may be useful to relieve weakness before immuno-suppressive therapy has become effective[210-212].

ii) Goodpasture's Syndrome

This syndrome is characterised by rapidly progressive glomerulonephritis and antibody to glomerular basement membrane. Plasma exchange reduces the level of this antibody, whilst immunosuppression inhibits its further synthesis[213-215]. It is not been established that the exchange improves patient survival, nor has a comparison been made of immunosuppression therapy alone with immunosuppression combined with plasma exchange. It is also possible that the duration of the disease may significantly affect the outcome of the treatment because of irreversible damage to the lungs and kidneys by high levels of antibody.

iii) Refsum's Disease

This rare inherited disorder is characterised by chronic polyneuropathy, ichythosis, deafness and retinitis pigmentosa associated with accumulation of phytanic acid in tissues[216]. Plasma exchange has been used successfully to complement diet in the restriction of phytanic acid and in an effort to reduce levels in blood and tissue [217,218].

iv) Guillain Barré Syndrome

The mechanism by which plasma exchange may benefit patients with acute Guillain Barré syndrome is unclear[219-222]. The rationale is provided by the evidence that the serum of patients affected may contain demyelinating factors, antibodies to peripheral nerve tissue of immune complexes. The role of these factors in causing the syndrome is unknown.

Plasma exchange is of value in the treatment of the syndrome if commenced early and aggressively enough, i.e. within 10 days of

of the onset of muscular symptoms and removing a minimum of 15 litres of plasma.

The median time that patients are on a respirator will be decreased by some 10 days, and the time to make a functional recovery is shortened by a mean of 73 days.

v) Aplastic Anaemia

The role of immune mechanisms in bone marrow failure associated with aplastic anaemia suggests that the application of plasmapheresis could be beneficial in the treatment of the disorder[223,224].

The mechanisms responsible for the bone marrow failure in aplastic anaemias have long been studied. In some patients, serum inhibitory immunoglobulin has been shown to be directed against marrow CFU-C. Thus, in patients in whom antibodies were shown to be responsible for the inhibition of in-vitro CFU-C formation, plasma exchange could well be beneficial.

The potential side-effects of plasma exchange in this situation must be considered. There may be a rebound humoral immune response and plasma exchange should be followed by immunosuppressive drug therapy[225,226].

It is suggested that plasma exchange is limited to selected patients with active life-threatening disease to provide an acute, although temporary, therapeutic option.

vi) Neoplasia

Studies of plasma exchange in the treatment of patients with cancer have been largely confined to solid tumours. The theoretical

basis for plasma exchange as a specific treatment, rather than as supportive care, is controversial and involves the manipulation of the patient's immune system into a more favourable state, perhaps by removing immune complexes that may act as 'blocking factors'. Anti-tumour responses have been reported after plasma exchange in a variety of patients with solid tumours, but improvements have been mostly temporary[227-230].

Partial responses have been reported in patients with heavily treated, chemotherapy-resistant lymphoma after a series of plasma exchanges.

Patients with acute myeloid and lymphatic leukaemia have been treated with plasma exchange before and during induction therapy. Those subjected to plasma exchange and chemotherapy exhibited more complete and partial remissions than those patients subjected to chemotherapy alone. However, other prognostic factors such as age, state of infection, etc., influenced the more favourable results and few conclusions can be drawn[22,229,230].

Treatment by allogeneic bone marrow transplant is favoured for aplastic anaemia and certain forms of leukaemia[231,232]. Donor and recipient are usually matched by human leukocyte antigen (HLA), but in approximately 20% of cases, a major ABO incompatibility exists, i.e., the marrow recipient has anti-A or anti-B antibody directed against donor-type red cells.

Marrow has the same red cell content as whole blood and measures must be taken to reduce the possibility of a haemolytic trans-

fusion reaction at the time of the marrow infusion. Haemolysis, at the time of infusion, may be avoided by the removal of red cells from the marrow using continuous and intermittent flow centrifugation, hydroxyethyl starch, gravity sedimentation and separation on fresh gradients.

These methods eliminate the target cell because early precursor red cells do not express ABO blood group antigens. Whilst effective, these methods may deplete the marrow of haemopoietic stem cells. This may be critical in some transplant situations, such as aplastic anaemia, where the cell dose is known to be important for engraftment.

Large volume plasma exchange to remove the undesired haemagglutinins from the marrow receipient will avoid marrow manipulation. Patients treated in this way for ABO incompatibility have been found to engraft as rapidly as patients who are ABO matched. The incidence of graft versus host disease and rejection is also reduced.

Plasma Exchange in Children

Plasma exchanges have been used in paediatric nephrology and include renal failure in association with crescentric nephritis due to Goodpasture's Syndrome, Henoch-Schonlein Syndrome and idiopathic nephritis. Other conditions include systemic lupus erythematosus, haemolytic uraemic syndrome and systemic vasculitic disorders such as polyarteritis. Some of the patients required peritoneal dialysis at the same time as plasma exchange[233-235]. The majority of patients have been exchanged on the continuous

flow IBM 2997 cell separator; although a few of the earlier patients were exchanged using a Haemonetics 30 with a paediatric bowl. In general, the continuous cell separator has provided greater haemodynamic stability, as well as faster exchanges. Plasma exchange is not a substitute for dialysis and it is essential that all patients are stable haemodynamically before exchange is commenced.

i) Preparation for Plasma Exchange

Investigations prior to plasma exchange should include full blood count, coagulation screen, Hepatitis B SAg, electrolytes, total protein and creatinine clearance when indicated. The child's body weight and resting blood pressure should be recorded. All patients are cross-matched and whole blood is used to prime the cell separator for the child's first exchange and subsequent exchanges if the daily pre-exchange haemoglobin is below 9 g/dl. The machine should be primed through to the return line with blood or plasma in children < 30 kg weight (if under 20 kg, whole blood should be used). The exchange is performed using plasma protein fraction 80-120 ml/kg. Fresh frozen plasma is given at the end of the exchange from the third day to help replace depleted clotting factors.

Adequate venous access is vital to the success of plasma exchange in children and needs to be planned before exchange. In the majority of children plasma exchange is a short term procedure undertaken in the acute illness and needs to be considered against the complications of the more invasive forms of vascular access.

ii) Management During Exchange

Patients are given intravenous hydrocortisone and anti-histamine immediately before exchange to reduce the risk of allergic reactions. Heparin is the anticoagulant of choice at a dosage of 100 IU/kg to a maximum of 2,000 units as a bolus pre-exchange with 40 IU/min as maintainance (i.e. 20,000 IU in 500 ml of normal saline at 1 ml/min). This may be reduced under certain clinical conditions, e.g. post-surgery or with coagulation abnormalities. At the beginning of the exchange the child should be supine and providing the observations of blood pressure, pulse and general clinical state remain stable, the initial flow rate through peripheral lines is 30 ml/min. Hyper- or hypovolaemia may occur. The former is particularly common in the patient with renal failure. Hypovolaemia may present in children with irritability, restlessness, yawning, drowsiness or cramps which later progress to abdominal pain, vomiting and collapse. Cessation of the exchange and correction of the hypovolaemia by the plasma infusion is usually adequate.

iii) Post Exchange

Once the full exchange has been completed, it may be necessary to wash back the remaining blood contained in the centrifuge if the child remains anaemic, or the circuit was not primed with blood. Many patients may be haemodynamically unstable post-exchange and on return to the ward they need to be monitored accordingly. The haemoglobin is checked at the end of the exchange.

iv) Complications

There have been 3 reported cases of septicaemia, one of which

was fatal. This occurred in a patient with a long line in-situ. A haemothorax and subsequent empyema occurred in relation to malinsertion of subclavian line. One patient with severe vasculitiis developed a hemiplegia 12 hours following a second exchange. Subsequent CAT scan showed no evidence of haemorrhage, but the patient deteriorated and died.

Thus, the procedure in children is not without hazard but its careful application will minimise the risks and may be of some therapeutic value in otherwise serious disease.

1.6 Side Effect of Plasmapheresis

Initially, it was generally believed that the plasma exchange procedure was both easy and harmless. This attitude was based on a simplistic view of the procedure, perhaps compounded by a mistaken extrapolation to it of the experience with healthy blood donors. In fact, whilst major complications are few and far between, minor complications are surprisingly common[236].

1.6.1 Vascular Problems

In many cases patients have already been under extensive medical care and venous access for purposes of therapeutic exchange has already been jeopardised via peripheral routes[116]. Repeated trauma through multiple venepuncture can result in thrombosis and sclerosis.

In such cases access is only possible by indwelling catheters in, e.g., subclavian, or femoral vein, or fistula. Once again this may result in complications. Fatal perforations, thrombosis, sepsis and circulatory interference may occur[114].

1.6.2 Procedural

1) Circulatory Effects

Even in healthy donors the procedure can cause tiredness, vague malaise and vasovagal reactions. These effects, presumably caused by extracoporeal circulation and shifts in body fluid balance, are obviously more pronounced in sick patients undergoing plasma exchange[236]. Vasovagal reactions may be accompanied by pallor, sweating and occasional hyperventilation and are relatively common, particularly in intermittent flow centrifugation[237-238]. They tend to be aggravated by psychological factors and can be minimised by reducing the patient's state of anxiety, whilst simultaneously correcting hypovolaemia[236]. Hypotension may occur, although it usually does so in patients undergoing plasma exchange for hyperviscosity from Waldenstrom's disease, due to inability of the patient's blood vessels to compensate rapidly to changes in the intravascular plasma volume. Patients who suffer from hyperviscosity or cryoglobulinaemia may develop severe hypotensive episodes.

Fluid overload can occur in the elderly towards the end of a procedure when the blood outflow has been stopped and the return of fluid is continuing. Cardiac failure may be precipitated.

2) Anticoagulant Effects

There is an occasional occurrence of cardiac arrhythmia which has been responsible for some deaths and may be related in part to a citrate effect. It appears to occur more often when subclavian lines are used, perhaps because of the delivery of citrated plasma to the great veins close to the heart. Bradycardia, atrial, nodal and ventricular ectopics, atrial fibrillation and supraventricular tachycardias have all been reported, but only when ACD has been the anticoagulant used. These are due to a marked decrease in the amount of ionisable calcium because of the chelating effect of the citrate. Syncope during exchange is associated with severe sinus bradycardia and hypotension mostly due to hypocalcaemia. Other manifestations of hypocalcaemia, namely circumoral paraesthesia, increased muscle tension, nausea, vomiting and overt tetany can occur but may be avoided by giving prophylactic calcium during exchange. It would seem that if citrate is used as anticoagulant then calcium and potassium supplements should be given.

Failure to obtain a homogeneous concentration of anti-coagulant has been found to result in massive extra-corporeal blood clotting. These manifestations are thought to be a consequence of a thrombotic trend during the procedure[239]. Actual contact activiation of the fibrinolytic system induces a thrombotic state during plasma exchange in which anticoagulation is achieved by ACD alone. It is also known that, following intensive plasma exchange, significant decreases in AT III levels occur inducing a hypercoagulable state which may persist for some 72 hours or more[124].

3) Effects of Replacement Media

There are potential disadvantages associated with each of the available replacement media[137,240,241].

Plasma Products

There is usually a delay in the administration of such products which are in relatively infrequent supply. The main disadvantage however is in the transmission of disease - hepatitis B and A non - B Hepatitis. This occurs despite screening all donors for Hepatitis B.

The incidence of allergic reactions is higher and tachycardia, hypotension, bronchospasm and even anaphylactic shock may occur.

Albumin Solutions

These contain no coagulation factors or immunoglobulins which may be a drawback. Occasionally anaphylactoid reactions or hypotension may occur[242,243].

Gelatins

Gelatins may be associated with very severe allergic reactions which may be fatal. They will also cause a reduction in albumin levels which may result in peripheral oedema.

4) Hypothermia

Many patients feel cold and shivery during plasma exchange because their blood is in an extra-corporeal circuit at room temperature. This occurs in exchanges of long duration (4-6 hours) and is due to the cooling of blood in the centrifugal

bowl at 26°C ± 2°C[236]. The chilling may also be part of the manifestation of the citrate effect or may aggravate the latter. The problem can be overcome by using a thermostatically controlled blood warmer in the returning line which increases the infusion fluid temperature to 35°C ± 2°C.

5) Haemolysis

Red cell haemolysis[236] is possible whenever blood comes into contact with foreign surfaces or is moved by pumps against resistance, i.e., as a result of cessation of blood flow through the system and the continuation of pump rotation around a stationary column of red cells. Input and output sensors are normally provided on equipments to detect an appropriate blood flow and automatically stop pump rotation. If attempts are made to process more than venous access will permit and the pump is stopped by the occluded vein sensor, then negative pressure may arise on the input side. Restarting the pump rotation manually may result in red cell haemolysis within the channel. Further, if the centrifuge is spinning in the absence of flow, the seal hold-down exerts pressure on the seal surface and haemolysis of red cells may occur in the area of the seal. In intermittent flow centrifugation procedures, the presence of a kink in the tubing leading to the reinfusion bag can cause haemolysis[244]. This will seldom occur in continuous flow centrifugation systems.

The red cells may be more damaged than the degree of visible haemolysis would appear to indicate. Haemolysis is less likely to occur if priming saline is opened to alleviate negative pressure while attempting to reinstate blood flow. If flow is

stopped because of venous access and new bleeding or return sites are needed, the centrifuge may be safely turned off for procedures using the single-stage channel. For dual-stage procedures, reducing the centrifuge speed from 1400-1200 rpm usually prevents haemolysis and red cell spill over into the spiral channel, so that platelet collection may continue when flow is resumed.

6) Loss of Medication

Obviously the removal of a patient's plasma will also remove any medications that may at the time be carried in the plasma[236]. This can be deleterious in a patient whose stable clinical state is dependent on a level of medication in the blood, e.g. anti-convulsant therapy. Supplemental medication may be needed during or immediately after a procedure.

1.6.3 Mortality

It is estimated that about 10,000 procedures are carried out annually in the UK and no deaths due to the procedure have been reported[236]. In the USA 23,000 procedures are undertaken annually and 16 deaths have been reported over 3 years. Most of the respiratory and cardiac deaths were associated with the use of plasma, which is more commonly used outwith the UK (Table 14). The typical cardiac death usually is preceded by an arrhythmia followed by cardiac arrest. It is thought to be due to a reduction in serum ionised calcium levels produced by infusion of citrated plasma. Respiratory deaths are usually preceded with development of signs and symptoms of acute noncardiogenic pulmonary oedema.

TABLE 14

Deaths Associated with Therapeutic Plasmaphereses[236]

			Country			Replacement Fluid		
Cause of Death	Total Nos.	USA	France	Canada	Other	Albumin/PPF	FFD	Both
Cardiovascular	12	3	8	1	0	0	11	0
Respiratory	13	4	8	0	1	0	7	6
Anaphylactic	3	3	0	0	0	1	2	0
Pulmonary Thromboembolism	2	0	2	0	0	0	0	1
Vascular Perforation	3	2	1	0	2	2	0	0
Hepatitis	2	1	1	0	0	0	2	0
Haemorrhage	2	1	0	1	1	1	1	0
Sepsis	2	1	0	0	2	2	0	0
Data incomplete	3	1	0	1	0	0	0	7
Total	42	16	20	3	6	6	23	7

2. SCOPE OF THESIS

Over the period 1981 to 1985, 2,685 plasma exchange procedures were carried out on 201 female and 125 male patients in the course of treatment for a range of clinical disorders. The procedures were all performed on an IBM 2997 Cell Separator (continuous flow). During this period of time a number of individual studies were undertaken. These involved an evaluation of

(1) the efficiency of the selected replacement fluids used in the treatment of 3 groups of patients

(2) the effectiveness of the procedures in the treatment of:

1. Cold Haemagglutinin Disease
2. Waldenstrom's Macroglobulinaemia and Hyperviscosity due to Multiple Myeloma
3. Immune Haemolytic Anaemia
4. Factor VIII Inhibitors
5. Polyneuropathy
6. Myasthenia Gravis and Eaton Lambert Syndrome
7. Guillain Barré Syndrome
8. Glomerulonephritis and Goodpasture's Disease.
9. Aplastic anaemia together with pure red cell aplasia.
10. Polymyositis

(3) Leukapheresis in the management of patients with acute and chronic myeloid and lymphatic leukaemia.

A complete record of each patient's clinical response to treatment was kept throughout the period of the individual studies.

2.1 Details of Patients and Exchange Procedure

2.1.1 Study of selected replacement fluids

One hundred and forty patients with Myasthenia Gravis, Guillain-Barré Syndrome, chronic relapsing polyneuropathy and polymyositis were studied in 3 groups as detailed in Table 15. No patient had an intercurrent disease likely to affect the parameters measured.

Three fluids, namely 5% albumin, plasma protein fraction and gelatins (Haemaccel) were used to replace plasma withdrawn during the exchange. The efficacy of selected types of replacement fluids was studied by examining the reactions to plasma exchange of the 3 groups of patients, as below.

Group 1:- 60 patients, who underwent 300 exchanges
each patient received as replacement fluid,
Polygeline I, consisting of

1. 1 litre of 5% albumin
2. 1 litre of Haemaccel
3. 0.5 litres of normal physiological saline

Group 2:- 60 patient, who underwent 300 exchanges
each patient received as replacement fluid,
Plasma protein solution consisting of

1. 2 litres of 5% albumin
2. 0.5 litres of normal physiological saline

TABLE 15

Clinical Conditions of Patients Treated By Plasma Exchange Using Selected Replacement Fluids

Group	Replacement Fluid Used	Patient Disorder	Number Of Patients	Total Number Of Patients In Group
Group 1	Polygeline I	Myasthenia Gravis	30	60
		Guillain-Barré Syndrome	17	
		Chronic Relapsing Polyneuropathy	10	
		Polymyositis	3	
Group 2	Plasma Protein Solution	Myasthenia Gravis	28	60
		Guillain-Barré Syndrome	19	
		Chronic Relapsing Polyneuropathy	10	
		Polymyositis	3	
Group 3	Polygeline II	Myasthenia Gravis	5	20
		Guillain-Barré Syndrome	5	
		Chronic Relapsing Polyneuropathy	5	
		Polymyositis	5	

Group 3:- 20 patients, who underwent 100 exchanges each patient received as replacement fluid, Polygeline II, consisting of

1. 2 litres of Haemaccel
2. 0.5 litres of normal physiological saline

For the majority of patients venous access for plasma exchange was via an antecubital vein, but arteriovenous shunts or fistula were used in 49 cases.

Approximately 2.5 litres of plasma was exchanged during each procedure, undertaken daily for 5 consecutive days (i.e. a total of 12.5 litres of plasma was exchanged in each patient). Heparin was used as anticoagulant, each patient being given a loading dose of 5,000 units followed by a further 3,000 to 5,000 units during the procedure. On 5 occasions only, protamine sulphate was used to reverse the effect of Heparin.

2.1.2 Study of Selected Disorders

The patients were aged between 18 and 65 years with a mean age of 42 years and were treated for a variety of clinical disorders as indicated in Table 16 and below:

1. Cold Haemagglutinin Syndrome

Eight patients received treatment, 4 were known to have a lymphoma, 1 to have an adenocarcinoma of the lung and 3 had proven viral illness. All patients were anaemic, some profoundly so. All had failed to respond to conventional chemotherapy.

Table 16

Age and Sex Distribution of 186 patients treated for Various Disorders by Plasma Exchange at the Glasgow Royal Infirmary over the period 1981 - 1985

DISEASES	Number of Patients	Sex		Age Group					No. of Ex-changes
		M	F	20 30	30 40	40 50	50 60	60 70	
Cold haemagglutinin Syndrome	8	6	2	0	2	3	2	1	36
Macroglobulinaemia	18	10	8	0	2	6	8	2	171
Myeloma with Hyperviscosity	12	7	5	0	1	2	9	0	60
Immune Haemolytic Anaemia	3	0	3	0	1	1	1	0	12
Factor VIII Inhibitors	8	1	7	3	3	0	2	0	40
Chronic Polyneuropathy	15	5	10	8	5	2	0	0	50
Myasthenia Gravis	18	6	12	3	6	5	4	0	80
Eaton Lambert Syndrome	5	2	3	0	0	2	3	0	20
Guillain Barré Syndrome	27	14	13	14	10	2	1	0	162
Glomerulonephritis/ Goodpasture's Syndrome	36	13	23	18	15	3	0	0	252
Pure red cell aplasia	4	3	1	0	1	2	1	0	16
Aplastic Anaemia	2	2	0	2	0	0	0	0	6
Polymyositis	10	7	3	6	4	0	0	0	35

A total of 36 exchanges were undertaken using plasma protein solution as replacement fluid, 86 litres of plasma were exchanged in total.

2. Waldenstrom's Macroglobulinaemia

Eighteen patients with hyperviscosity syndrome each received treatment by means of plasma exchange. 65% of patients exhibited neurological symptoms, 55% suffered from general malaise, 50% suffered from bleeding and 15% from non-specific pain. Patients were aged 35 - 79 years. The mean age on the onset of symptoms was 55 years and on diagnosis of disorder was 60 years.

A total of 171 plasma exchange procedures were carried out over a 3 year period 1982 - 1985. The mean value of plasma exchanged during each procedure was 2.5 litres. The replacement fluid was generally plasma protein solution but this was, on occasions, partially replaced by Haemaccel (some 15 procedures).

A further seven male and five female patients with hyperviscosity due to multiple myeloma underwent sixty exchanges. They were aged between 39 and 58, with a mean age of 49 years. They were each receiving haemodialysis.

3. Immune Haemolytic Anaemia

Three female patients received treatment. One had Evans' Syndrome, one was post-infectious and one had a malignant lymphoma. All had severe haemolysis and had failed to respond to steroid and immuno-suppressive therapy. One patient had had a splenectomy.

A total of 12 procedures was carried out. The mean value of plasma exchanged during each procedure was 2.5 litres. The replacement fluid was primarily plasma protein solution, but this was, on occasions, partially replaced by Haemaccel or fresh frozen plasma. All patients had simultaneous immunosuppressive therapy. None received a blood transfusion during the period of exchange.

4. Factor VIII Inhibitors

Eight patients aged between 22-58 years received treatment. Two patients suffered from systemic lupus erythematosus, two were post-partum, one from rheumatoid arthritis, one from ulcerative colitis, one from dermatitis herpetiformis and one had recently received penicillin (i.e. drug-induced).

Five daily plasma exchanges were carried out in which 2.5 litres of plasma was replaced on a daily basis by 1.5 litres of plasma protein solution and one litre of fresh frozen plasma. A reduced dose of 2,500 units of heparin was used as anti-coagulant.

5. Chronic Polyneuropathy

Five male and ten female patients with chronic inflammatory polyneuropathy, aged between 25 and 45 years (mean age 35 years) underwent a total of 50 plasma procedures in each of which 2.5 litres of plasma protein solution and 500 ml of physiological saline were exchanged. All patients received concurrent treatment with immunosuppressive therapy, initially using prednisolone 60 mg/day, and when no response was obtained, azathioprine 150 mg/day, or cyclophosphamide 90 mg/day was added.

If there was no response to corticosteroids and one cytotoxic agent, then the other cytotoxic agent was also added, i.e. triple therapy.

6. Myasthenia Gravis and Eaton Lambert Syndrome

Twelve female and six male patients with ages ranging from 22 to 58 years (mean age 40 years) underwent 80 plasma exchanges. In the majority of cases plasma protein solution was the replacement fluid used with a small quantity of Haemaccel.

All patients were receiving anticholinesterase drugs, namely, neostigmine or pyridostigmine and immunosuppressive therapy, namely, corticosteroids or azathioprine tapered according to the patient's clinical condition.

Five patients with Eaton Lambert Syndrome aged between 48 and 66 years underwent 20 plasma exchanges. Of the 3 males and two females, 4 patients had histologically proven carcinoma of the bronchus, and 1 patient had histologically proven carcinoma of the breast.

They differed from the patients with Myasthenia Gravis in that they presented with marked proximal limb weakness, with less frequent involvement of the ocular muscles. They were receiving no concurrent medication.

In all exchanges plasma protein solution was the replacement fluid of choice.

7. Guillain Barre Syndrome

Fourteen male and thirteen female patients aged between 14 and 56 years (mean age 35 years) underwent 162 plasma exchanges using plasma protein solution and Haemaccel as replacement fluids in equal quantities. The patients satisfied the following criteria:

1. the duration of symptoms was less than 30 days.
2. there was no clinical evidence of improvement in limb function at the time of assessment.
3. no previous treatment with prednisolone or immuno-suppressive agents.
4. no other concurrent disease.

All patients had developed a progressive flaccid quadriparesis and all required ventilation. The mean duration between the onset of symptoms and the start of plasma exchange was 8 days.

8. Glomerulonephritis and Goodpasture's Syndrome

Thirteen male and 23 female patients aged between 24 and 45 years (mean age 33) were treated. Twelve patients had mesangiocapillary glomerulonephritis MCGN Type 1, six patients had Type II MCGN. Four patients had membranous nephropathy and three patients had mesangial IgA disease. The remaining eleven patients had Goodpasture's Syndrome; 10 showed evidence of nephritis and pulmonary haemorrhage and 1 had nephritis alone. A renal biopsy was carried out on all patients. Immuno-fluorescence showed linear IgG along the GBM in all biopsies. Circulating anti-GBM antibody was detected in all patients.

All patients received azathioprine and cyclophosphamide for periods of 6 - 8 weeks. Prednisolone was commenced at a dose of 60 mg/day and reduced gradually. Patients underwent 252 plasma exchanges over periods ranging from 6 months to 7 years prior to plasma exchange and thus served as control patients.

9. Aplastic Anaemia and Red Cell Aplasia

Three male and one female patient had a diagnosis of pure red cell aplasia. They had severe anaemia for some 8 - 32 months, (mean period 20 months). Two male patients had aplastic anaemia of unknown aetiology and had had severe pancytopenia for 6 months. None of the patients had a matched bone marrow donor and they were all steroid and androgen therapeutic failures. The patients underwent 22 plasma exchanges, each exchange removing 2.5 litres of plasma which was replaced by 5% albumin.

10. Polymyositis

Ten patients, seven male and three female, ages ranging from 23-38, mean 31 years with florid polymyositis underwent 35 plasma exchanges of 2.5 litres of plasma, replaced by 5% Albumin (85%) and Haemaccel (15%). All were concurrently treated with corticosteroid and azathioprine.

2.1.3 Study of Leukapheresis

Of 58 patients aged between 22 and 71 years treated by leukapheresis, 5 had acute lymphatic leukaemia, 14 acute myeloid leukaemia, 3 acute myelomonocytic leukaemia and 25 chronic myeloid leukaemia and 11 chronic lymphatic leukaemia.

In each leukapheresis procedure 3 litres of blood (mean value 2.5 litres) was processed through the IBM type 2997 cell separator in times ranging from 60 to 150 minutes. Heparin was used as anti-coagulant.

Fluid replacement consisted of 5% Albumin, physiological saline, and very occasionally Plasma Protein Solution. Platelet concentrate was transfused post-pheresis in severely thrombocytopenic patients.

2.2 Parameters Examined

2.2.1 Study of Selected Fluids

2.2.1.1 Sampling

Haematological, biochemical and clinical response of each patient was continuously monitored throughout the sequence of exchange procedures. The haematological and biochemical base-line investigations were made:-

1. immediately pre-plasma exchange
2. immediately post-plasma exchange
3. 12 hours post-plasma exchange
4. 24 hours post-plasma exchange
5. 3 days post-plasma exchange
6. 5 days post-plasma exchange

The only pre-requisites before plasma exchange were that each patient was HBsAG negative and had normal base-line coagulation screen.

All samples were collected into the appropriate containers through a 16 gauge cannula without venous stasis and without frothing. Blood for coagulation studies was collected in 1/100 volume of 40% sodium citrate. Plasma was separated after centri-

fugation at 1800 G at 4 °C for 15 minutes. All coagulation studies were performed immediately or the sample was frozen at -30°C and studies carried out at a later date. The prothrombin time (PT) the Kaolin Cephalin Clotting time (KCCT) and Thrombin Clotting Time (TCT) were carried out immediately at 30 minutes, 4 hours and 24 hours post-exchange respectively. The platelet counts were performed on blood collected in ethylene diamine tetra-acetic acid.

2.2.1.2 Haematological Parameters

1. Standard full bood counts

   Haemoglobin estimation,packed cell volume (haematocrit) and white blood cell count were determined using Coulter Counter Model 'S'.

2. Platelet Count

   Platelet counts were determined using routine Coulter S analysis.

3. Coagulation Screening

   a) Prothrombin time

   Prothrombin time was evaluated by a one stage method[245] in which 0.1. ml of plasma was placed in the bottom of a 75 x 10 ml tube in a water bath at 37°C and 0.1. ml of rein-suspension added to it after 60 seconds. 0.1 ml of warmed 0.025 mmol/l calcium chloride was added and the contents mixed. The time taken for the development of a fibrin clot marking the end point was measured. The normal range is 10 - 14 seconds.

b) Kaolin Cephalin Clotting Time

The Kaolin Cephalin Clotting Time was evaluated[246] by incubating 0.1 ml of plasma with 0.2 ml of a well-shaken Kaolin phospholipid mixture which allows activation of contact factors. Platelet activity is replaced by phospholipid. Ten minutes was allowed for maturation of incubation at 37°C at which point 0.1 ml of pre-warmed calcium chloride is added. The time taken for a clot to form is noted. The normal range is 35 - 45 seconds.

c) Thrombin Clotting Time

Thrombin clotting time was measured by placing 0.2 ml of normal plasma in a standard glass tube at 37°C with 0.1 ml of thrombin and noting the time for a clot to form. The normal range is 6 - 9 seconds.

4. Quantitative Assays of Coagulation Factors

a) Fibrinogen

This was estimated by a modified quantitative method[247], which measures fibrinogen as Thrombin clottable protein. The fibrinogen in plasma is converted into fibrin by the action of thrombin and fibrin is collected on glass beads, washed, boiled with sodium hydroxide and its tiozine content estimated. Normal values 2 - 4 g/l.

b) Prothrombin

This was carried out using a two-stage method[248] with Taipan Snake venom which converts prothrombin directly to thrombin in the absence of any other non-clotting factor.

A dilution of plasma is mixed with venom in the presence of phospholipid and calcium chloride. The amount of thrombin generated is estimated by adding Fibrinogen and determining the clotting time. Normal range 50-100 IU/dl.

c) Factor VIII

Estimation is based on the partial thromboplastin time test[249] performed on mixtures of Factor VIII deficient plasma and various dilutions of both standard and test plasma. A standard curve is prepared by plotting the clotting time of each mixture against the calculated concentration of Factor VIII. The relative concentration of Factor VIII in the patient's plasma is extrapolated from this graph by testing similar dilutions of unknown plasma. Normal range 50 - 100 IU/dl.

d) Factor IX

Assays of Factor IX activity are effectively determined using the KCCT method[250]. This is performed in exactly the same way and has the same limitations as that for Factor VIII assays, except that known congenitally Factor IX-deficient plasma is used in place of Factor VIII-deficient substrate. Normal range 50-150 IU/dl.

e) Factor X

Specific assays of Factor X are performed using substrate plasma for a patient who is known to be congenitally devoid of Factor X[251]. Such substrate can be used in an intrinsic assay system using the KCCT test. Normal range 50-150 IU/dl.

2.2.1.3 Biochemical Investigations

1. Urea and Electrolytes

These parameters were estimated on the Technicon Auto-analyser[252].

Normal range
Sodium 135-145 mmol/l
Potassium 3.5 - 5.0 mmol/l
Chloride 97-107 mmol/l
CO^2 content 23-30 mmol/l
Urea 2.5 - 8 mmol/l
Creatinine 40-130 µmol/l

2. Liver Function Tests

Serum bilirubin, serum asparate transaminase (AsT), alanine transaminase (A1T) and serum alkaline phosphatase were estimated on the Technicon Auto-analyser. Serum bilirubin was estimated using the DPD method, asparate transaminase, alanine transaminase and alkaline phosphatase were estimated by established methods[253,254,255].

Normal range		
	Asparate transaminase	12 - 24 units/1
	Alanine transaminase	3 - 55 units/1
	Alkaline phosphatase	88 -280 units/1

3. Plasma Proteins

The proteins were measured by the Biuret method[256] and albumin concentration determined by the Brilliant Crystal Green technique. The globulin factor was calculated by subtraction of the albumin value from the total protein value.

Normal range	total proteins	62-82 g/l
	albumins	35-55 g/l
	globulins	23-33 g/l

4. Serum Calcium and Phosphate

The parameters were measured on the Technicon Auto-analyser[257].

Normal range	calcium	2.2 - 2.6 mmol/l
	phosphate	0.7 - 1.4 mmol/l

5. Serum Iron and Total Iron Binding Capacity

Values were determined by a routine method on the Auto-analyser[258,259].

Normal range	iron (males)	9 - 30 mmol/l
	iron (females)	6 - 25 mmol/l
	total iron binding capacity	45 - 75 mmol/l

6. Serum Immunoglobulins

These components were measured by an immunonephelometric method using a Technicon Auto-analyser.

Normal range	IgM	0.4 - 3.0 g/l
	IgA	0.8 - 4.5 g/l
	IgG	6.0 -15.0 g/l

2.2.1.4 Statistical Analysis

The numerical data was subjected to statistical analysis[260,261] as under:

1) Standard parameters, mean X, variance, standard

deviation (S.D.) and standard error of the mean values were calculated by an electronic calculator.

2) Significance Tests

Student's t tests were used for comparison of the means of two samples. For non-paired comparisons the appropriate t test was used and the degrees of freedom calculated according to whether or not there was a significant difference between the variances to the two samples. The significance of all probabilities was determined from statistical tables using the 'two-tailed' criterion.

2.2.2 Study of Selected Disorders

Tests were carried out on patients treated for the selected disorders as under:

1. Cold Haemagglutinin Syndrome

   Haemoglobin and serological parameters were measured pre- and post- plasma exchange. The antibody concentrations were determined.

2. Waldenstrom's Macroglobulinaemia and Hyperviscosity due to Multiple Myeloma

   Serum immunoglobulins and plasma viscosity were measured pre-plasma exchange and post-plasma exchange. Serum immunoglobulins were measured as before using a Technicon Autoanalyser. Whole blood viscosity was measured at three shear rates using a Contraves LS 30 Viscometer, and plasma viscosity was measured using the Harkness Capillary Viscometer.

3. Immune Haemolytic Anaemia

The haemoglobin and serological parameters were measured pre-and post- plasma exchange. Quantification of the antibody was graded on a scale of 1 to 5 (i.e. + low titre increasing to +++++ high titre).

4. Factor VIII Inhibitors

The antibodies were detected by incubating test plasma at 37°C with a source of Factor VIII C and then performing serial coagulation tests. The results were expressed in Bethesda Units. Coagulation screens including one-stage prothrombin time, Kaolin Cephalin Clotting time and Thrombin time were estimated pre- and post-exchange.

5. Chronic Polyneuropathy

Full neurological examinations were carried out on each patient and the response to treatment was measured pre- and post-plasma exchange by the noted improvement in the level of function, which is graded 1 - 6[262] as under:

0 = Healthy

1 = Minor symptoms or signs

2 = Able to walk 5 meters without assistance

3 = Able to walk 5 meters with assistance

4 = Chair bound

5 = Requiring assisted ventilation for at least part of the day or night

6 = Dead

The neurological examination included records of muscular strengths for 8 movements in each arm and 6 in each leg in accordance with the scale indicated below:

F = normal

E = movement against gravity and resistance

D = complete movement against gravity

C = complete movement in the absence of gravity

B = flicker

A = no movement

The following variables were assessed:

1 Time to onset of improvement in muscles spasm.

2 Time to onset of change in functional grade

3 Change in functional grade after 2 weeks,4 weeks, 3 months, 6 months and one year

6. Myasthenia Gravis and Eaton Lambert Syndrome

i) The response to treatment was also assessed by an improvement in the level of function, graded 1 - 6 as previously defined.

ii) The levels of acetylcholinesterase antibody activity were determined pre- and post- exchange.

iii) Vital capacity pre-and post- exchange, was measured by analysis of a single forced expiration and used as a means of monitoring respiratory function.

7. Guillain-Barré Syndrome

The clinical status and amelioration of symptoms of each patient was measured pre- and post- exchange, and graded on a scale of 1 - 5, as previously defined for chronic polyneuropathies.

8. Glomerulonephritis and Goodpasture's Syndrome

Urea and electrolytes, routine biochemical parameters, were measured pre- and post- plasma exchange, and the progression of renal failure assessed. The anti-GBM antibody titre was also measured pre- and post- plasma exchange in those patients with Goodpasture's syndrome.

9. Aplastic Anaemia

Haemoglobin, and serological parameters were measured pre- and post- plasma exchange. A white cell precursors CFU-C assay (colony forming unit in culture) was undertaken to evaluate serum inhibitory factors. The effects of incubating bone marrow at 37°C for 60 minutes with a small quantity of each patient's, or control, serum was determined in the presence and absence of fresh guinea pig serum. If the serum was found to inhibit CFU-C this sufficed to prove that the inhibitory factor was immunoglobulin. The immunoglobulin class responsible for the suppression was characterised using a Technicon Auto-Analyser.

10. Polymyositis

Biochemical parameters were measured pre- and post- plasma exchange. An improvement in clinical status was also estimated on a scale of 1 - 6.

2.2.3 Study of Leukapheresis

Haematological changes were measured pre- and post- leukapheresis in all patients. The patient was continuously monitored for changes in white blood count (WBC), platelets and haematocrit using routine Coulter S analysis. The efficiency[21] of leukapheresis in harvesting white blood cells was assessed as under:

$$\text{Efficiency \%} = \left[\frac{\text{WBC's Yield}}{\text{WBC's entering centrifuge}}\right] \times 100$$

The number of white cells entering the centrifuge was estimated from the volume processed (flow rate x time) and the average WBC. The average WBC is defined by:

$$\text{WBC's entering} = \left[\frac{\text{Pre + mid + post WBC}}{3}\right] \times \text{volume processed}$$

$$= \text{mean WBC's/mm}^3 \times \text{flow rate} \times \text{run time.}$$

A close watch was maintained over the patient at each exchange procedure and any adverse reactions were noted. The condition of the patient was carefully monitored over the course of each study.

The response of all patients to initial and subsequent leukapheresis procedures was studied in the short-term and in the long-term. Each patient was clinically assessed with particular reference to the size of lymphadenopathy and hepatosplenomegaly and regular haematological monitoring was carried out weekly after the last leukapheresis. As all patients received chemotherapy at the time of, or after, leukapheresis no comparison between leukapheresis and chemotherapy was possible.

3. RESULTS

3.1 STUDY OF SELECTED REPLACEMENT FLUIDS

Details of the statistical comparisons of the haematological and biochemical data obtained during tests on the three groups of patients are given in Appendix A.

3.1.1 HAEMATOLOGICAL PARAMETERS RESPONSE TO PLASMA EXCHANGE

STANDARD FULL BLOOD COUNTS

Values of Haemoglobin estimation (Hb), white blood cell count (WBC) and haematocrit (HCT) for patients in Groups 1, 2,3 prior to plasma exchange in day 1 and post-exchange in day 5 are given in Table 17. The values on the 5th day are marginally lower than the pre-exchange values on the first day of all 3 groups of patients.

The values of haemoglobin and white blood cell count in Group 1 patients, pre- and post- plasma exchange were not significantly different ($p > 0.05$) from those of patients in Group 2 and Group 3. The values of haematocrit in Group 1 patients, pre- and post- plasma exchange were not significantly different ($p > 0.05$) from those in Group 2 and Group 3 patients, but the values of haematocrit in Group 2 patients differed from those in Group 3 patients pre-plasma exchange ($0.05 > p > 0.02$) and post-plasma exchange ($0.02 > p > 0.01$).

TABLE 17

Variations In Full Blood Count During Plasma Exchange

	TIME OF ASSAY	GROUP 1 PATIENTS			GROUP 2 PATIENTS			GROUP 3 PATIENTS		
		POLYGELINE I			PLASMA PROTEIN SOLUTION			POLYGELINE II		
		Mean	Range	S.D.	Mean	Range	S.D.	Mean	Range	S.D.
Haemoglobin Hb g/dl	Pre-Plasma Exchange Day 1	12.1	10.6-14.5	± 2.0	12.6	10.6-12.9	± 1.7	11.9	11.0-13.0	± 1.0
	Post-Plasma Exchange Day 5	11.9	10.2-14.2	± 2.0	12.1	11.0-13.0	± 1.0	11.6	10.8-12.8	± 1.0
White Blood Cell $x\ 10^9/l$	Pre-Plasma Exchange Day 1	4.9	3.9- 7.7	± 1.9	5.4	3.9- 6.3	± 1.2	4.8	4.0- 6.5	± 1.3
	Post-Plasma Exchange Day 5	4.6	3.3- 6.9	± 1.8	4.8	4.0- 6.3	± 1.7	4.4	3.5- 6.5	± 1.5
Haematocrit %	Pre-Plasma Exchange Day 1	36	32-43	± 5.5	37	31-38	± 3.5	35	33-39	± 3.0
	Post-Plasma Exchange Day 5	35	31-42	± 5.5	36	33-39	± 3.0	34	32-38	± 3.0

PLATELET COUNT

Values of the platelet counts prior to plasma exchange and at selected intervals post-plasma exchange are given in Table 18 and 19 respectively and in Figure 10.

Platelet counts for the 3 groups of patients fell precipitously after the first exchange procedure to almost half the pre-plasma exchange level in the first 12 hour period of post-exchange. Thereafter the values decreased marginally with each succeeding procedure to a quarter of the pre-plasma exchange level after the fifth procedure. This downward trend in the platelet counts of Group 1 patients differed significantly ($p < 0.001$, $0.05 > p > 0.02$, $0.01 > p > 0.002$) from those of Groups 2 and Group 3 patients over the first three days, but this difference was not significant ($p > 0.05$) on the fifth day. The downward trend in the platelet counts of Group 2 patients was not significantly different ($p > 0.05$) from that of Group 3 patients over the 12 hour period but thereafter the difference became significant ($p < 0.001$, $0.05 > p > 0.02$).

COAGULATION SCREENING

Values of prothrombin time (PT), Kaolin Cephalin Clotting Time (KCCT) and thrombin clotting time (TT) prior to plasma exchange and at selected intervals post-plasma exchange are given in Table 20 and in Figure 11.

TABLE 18

Values of Platelet Counts Pre-Plasma Exchange

	Normal Range
Platelets	150-400 x 10^9/l

TABLE 19

Values of Platelet Counts Post-Plasma Exchange
(Results expressed as percentage of initial count)

	Percentage Value		
	GROUP 1	GROUP 2	GROUP 3
	Polygeline I	Plasma Protein Solution	Polygeline II
Pre-Exchange	Normal	Normal	Normal
30 min Post-Exchange	60 ± 2.5	57 ± 4.6	58 ± 2.5
12 hours " "	57 ± 2.5	51 ± 3.8	52 ± 2.6
24 hours " "	45 ± 2.8	44 ± 2.5	48 ± 2.7
3 days " "	33 ± 2.7	36 ± 2.1	39 ± 2.6
5 days " "	25 ± 2.4	28 ± 2.05	27 ± 2.5

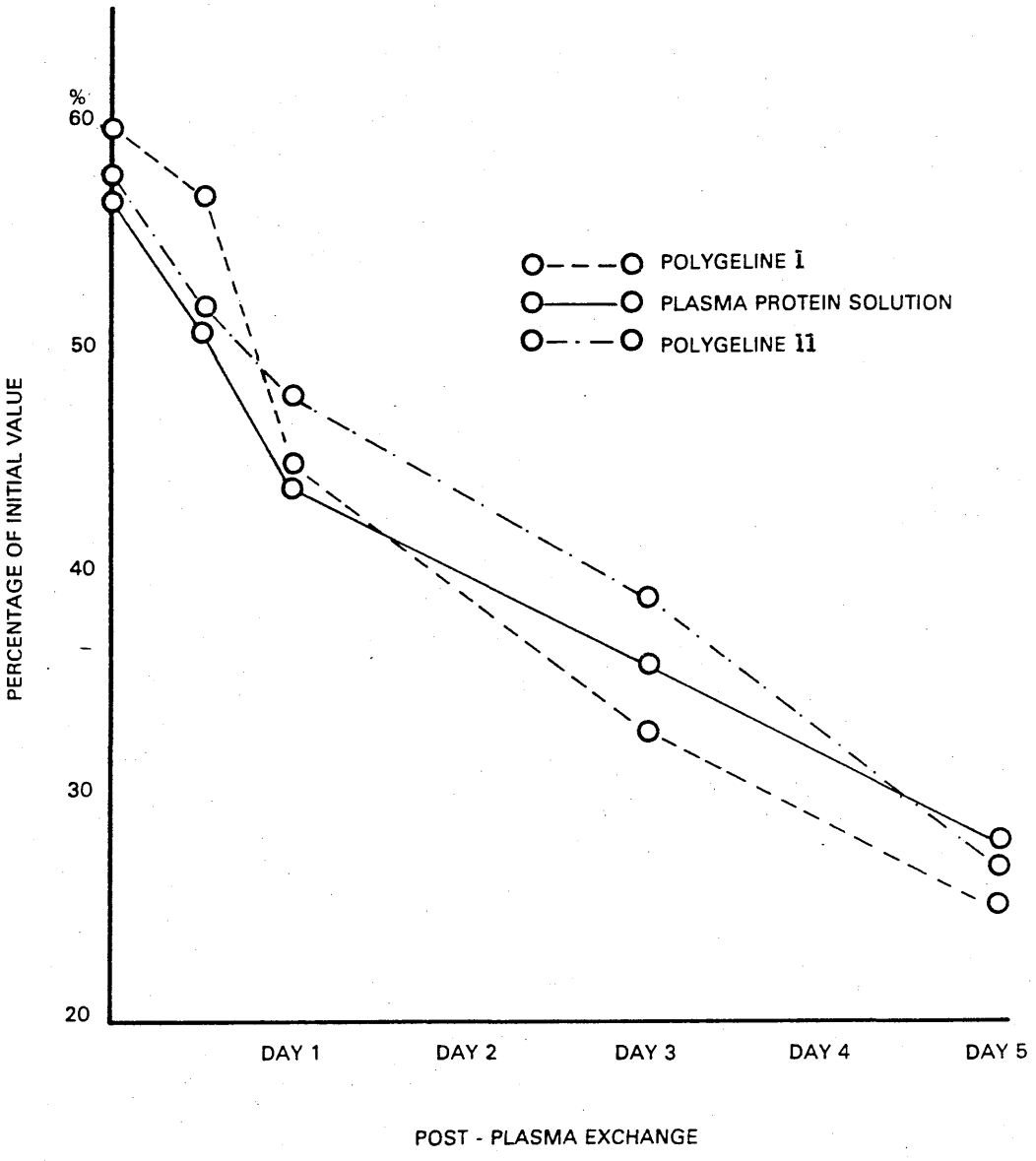


Figure 10. Effect of Replacement Fluid on Value of Platelet Counts post-plasma exchange.

TABLE 20

Values of Coagulation Screening Pre- and Post-Plasma Exchange

	Time of Test	MEAN VALUES (seconds)		
		GROUP 1 PATIENTS	GROUP 2 PATIENTS	GROUP 3 PATIENTS
Prothrombin Time	Pre-Exchange	14 ± 2	13 ± 1	14 ± 1
	Post-Exchange 30 minutes	19 ± 1	18 ± 2	20 ± 2
	Post-Exchange 6 hours	16 ± 2	16 ± 1	16 ± 1
	Post-Exchange 24 hours	13 ± 2	13 ± 1	13 ± 1
Kaolin Cephalin Clotting Time	Pre-Exchange	38 ± 3	40 ± 4	40 ± 4
	Post-Exchange 30 minutes	66 ± 4	68 ± 5	63 ± 5
	Post-Exchange 6 hours	43 ± 4	43 ± 3	42 ± 3
	Post-Exchange 24 hours	39 ± 3	39 ± 2	39 ± 3
Thrombin Clotting Time	Pre-Exchange	7 ± 1	7 ± 1	7 ± 1
	Post-Exchange 30 minutes	16 ± 1	14 ± 1	15 ± 1
	Post-Exchange 6 hours	9 ± 1	10 ± 1	10 ± 1
	Post-Exchange 24 hours	7 ± 1	7 ± 1	7 ± 1

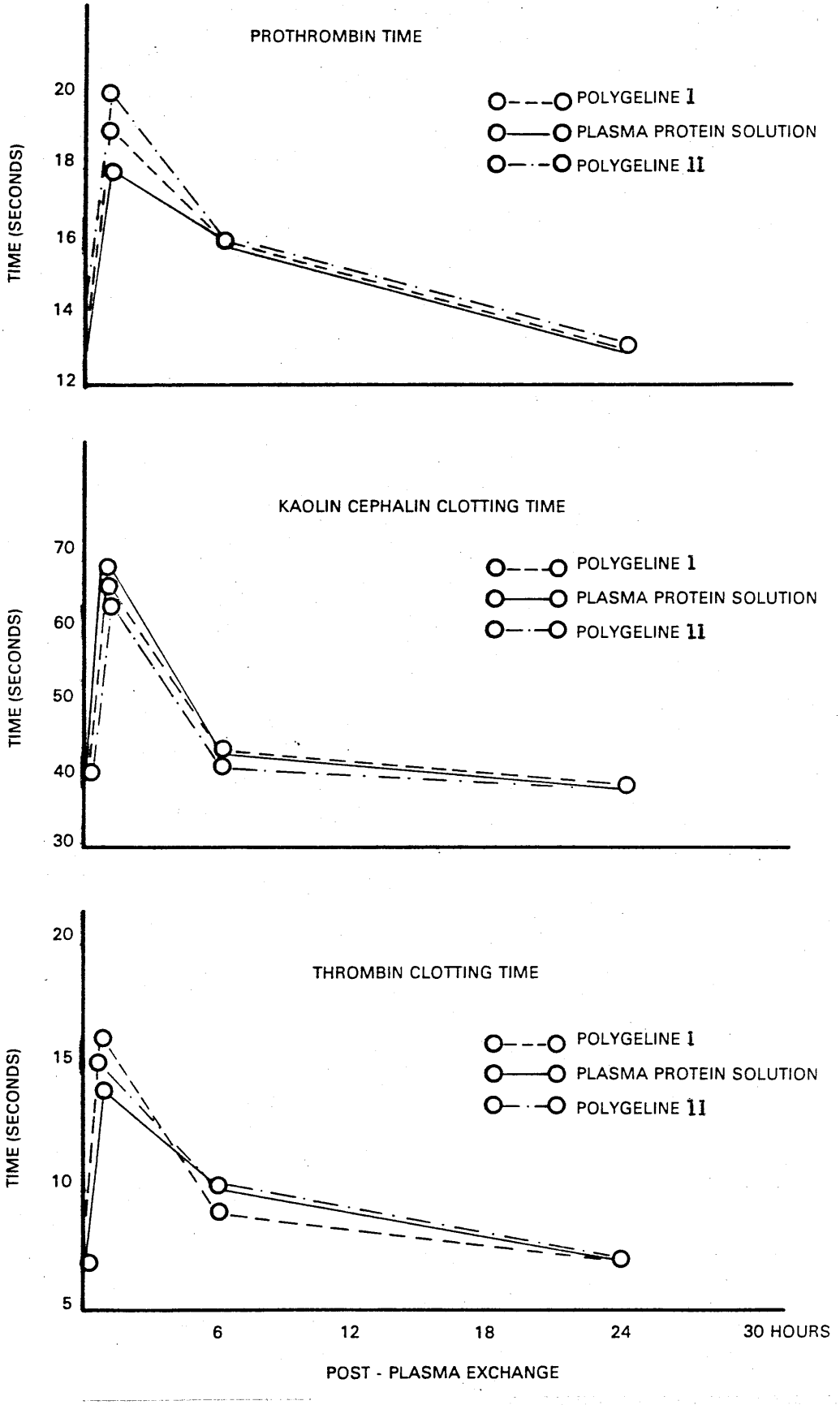


Figure 11. Effect of Replacement Fluid on Values of Coagulation Screening Post-Plasma Exchange

The mean values of PT, KCCT and TT at 30 minute post-first plasma exchange are 40%, 70% and 100% respectively, higher than the pre-plasma exchange values for all 3 groups of patients. These values had returned to almost pre-plasma exchange levels within a period of 24 hours post-exchange. With succeeding daily exchanges the values remained high for the second and third day but thereafter fell on the fourth and fifth day to values which were marginally higher than the initial pre-exchange.

The values of PT for Group 2 patients differed significantly ($p < 0.001$) from those for patients in Groups 1 and 3 over the initial 30 minute period after plasma exchange. Over the remaining 24 hour period the values were not significantly different ($p > 0.05$). Only at 30 minutes post-plasma exchange did the PT values for Group 1 patients differ significantly ($p < 0.001$) from those of Group 3 patients.

The values of KCCT of Group 1 patients differed from those of Group 2 patients ($0.01 > p > 0.002$, $0.02 > p > 0.01$) and of Group 3 patients ($0.05 > p > 0.02$, $0.01 > p > 0.002$) over the initial 30 minute period after plasma exchange, thereafter the difference was not significant ($p>0.05$). The values of KCCT of Group 2 patients differed significantly ($p < 0.001$) at 30 minutes post-plasma exchange.

The difference in values of TT for all three Groups of patients was not significant ($p > 0.05$) pre-plasma exchange and 24 hours post-plasma exchange. After 30 minutes and 6 hours post-plasma exchange the values for each Group were significantly different ($p < 0.001$).

QUANTATATIVE ASSAYS OF COAGULATION FACTORS

Values of Factor 1 (fibrinogen), Factor II (prothrombin), Factor VIII, Factor IX and Factor X pre-plasma exchange and at selected intervals post-plasma exchange are given in Table 21, Table 22 and Figure 12, Table 23 and Figure 13, Table 24 and Figure 14, Table 25 and Figure 15 and Table 26 and Figure 16 respectively.

All values fell relatively sharply in the 12 hour period immediately following the first plasma exchange procedure to approximately 30%, 35%, 40%, 30%, and 30% of the initial pre-plasma exchange value. Thereafter values continued to decrease but to a much lesser extent in succeeding daily procedures. After the fifth procedure, values had been reduced to 15%, 25%, 20%, 25% and 25% respectively of the initial pre-exchange levels.

Over the five day period the values of the percentage reduction in Factor I and Factor II levels in Group 1 patients were significantly different ($p < 0.001$, $0.01 > p > 0.002$) from

those in patients of Groups 2 and 3. The values of the percentage reduction in Factor 1 levels in Group 2 patients were significantly different ($p < 0.001$) from those of Group 3 only at 24 hours post-exchange ($0.05 > p > 0.02$) and at 3 days post-exchange, whilst the values of the percentage reduction in Factor II levels in Group 2 patients differed significantly from those in Group 3 patients only at 30 minutes ($p < 0.001$) and at 3 days ($0.02 > p > 0.01$) post-plasma exchange. The values of the percentage reduction in Factor VIII levels in Group 1 patients were significantly different ($p < 0.001$) from those in Group 2 patients over the five day period. The values of the percentage reduction in levels in Group 3 patients differed significantly ($p < 0.001$) from those in Group 1 and Group 2 patients at 30 minutes and 3 days post-plasma exchange.

Over the five day period the percentage reductions in the levels of Factor IX in Group 2 patients were significantly different ($p < 0.001$) from those in Group I and Group 3 patients. The values of the percentage reduction in levels of Factor IX in Group I patients differed significantly from those in Group 3 patients at 30 minutes, 3 days ($p < 0.001$) and 5 days ($0.01 > p > 0.002$). The percentage reduction in levels of Factor X in Group I patients differed significantly ($p < 0.001$) from those in Group 2 and Group 3 patients except at 12 hours post-plasma exchange. The percentage reduction in Factor X levels in Group 2 patients differed significantly ($p < 0.001$) from those in Group 3 patients except at 30 minutes and 5 days post-plasma exchange.

TABLE 21

Quantitative Assays of Coagulation Factors Pre-Plasma Exchange

FACTOR	NORMAL RANGE
Fibrinogen g/l	2.0 - 4.0
Prothrombin u/l	50 - 150
Factor VIII u/l	50 - 150
Factor IX u/l	50 - 150
Factor X u/l	50 upwards

TABLE 22

Quantitative Assays of Coagulation Factors Post-Plasma Exchange

Factor I Fibrinogen
(Results expressed as a percentage of Initial Value)

Time of Assay	PERCENTAGE VALUE		
	GROUP 1 Patients	GROUP 2 Patients	GROUP 3 Patients
	Polygeline I	Plasma Protein Solution	Polygeline II
Pre-Exchange	Normal	Normal	Normal
Post-Exchange 30 minutes	34 ± 1.8	30 ± 1.7	30 ± 1.2
Post-Exchange 12 hours	27 ± 1.6	28 ± 1.5	28 ± 2.1
Post-Exchange 24 hours	27 ± 1.3	25 ± 1.6	26 ± 1.1
Post-Exchange 3 days	24 ± 1.5	23 ± 1.4	25 ± 2.0
Post-Exchange 5 days	18 ± 1.2	15 ± 1.5	16 ± 1.1

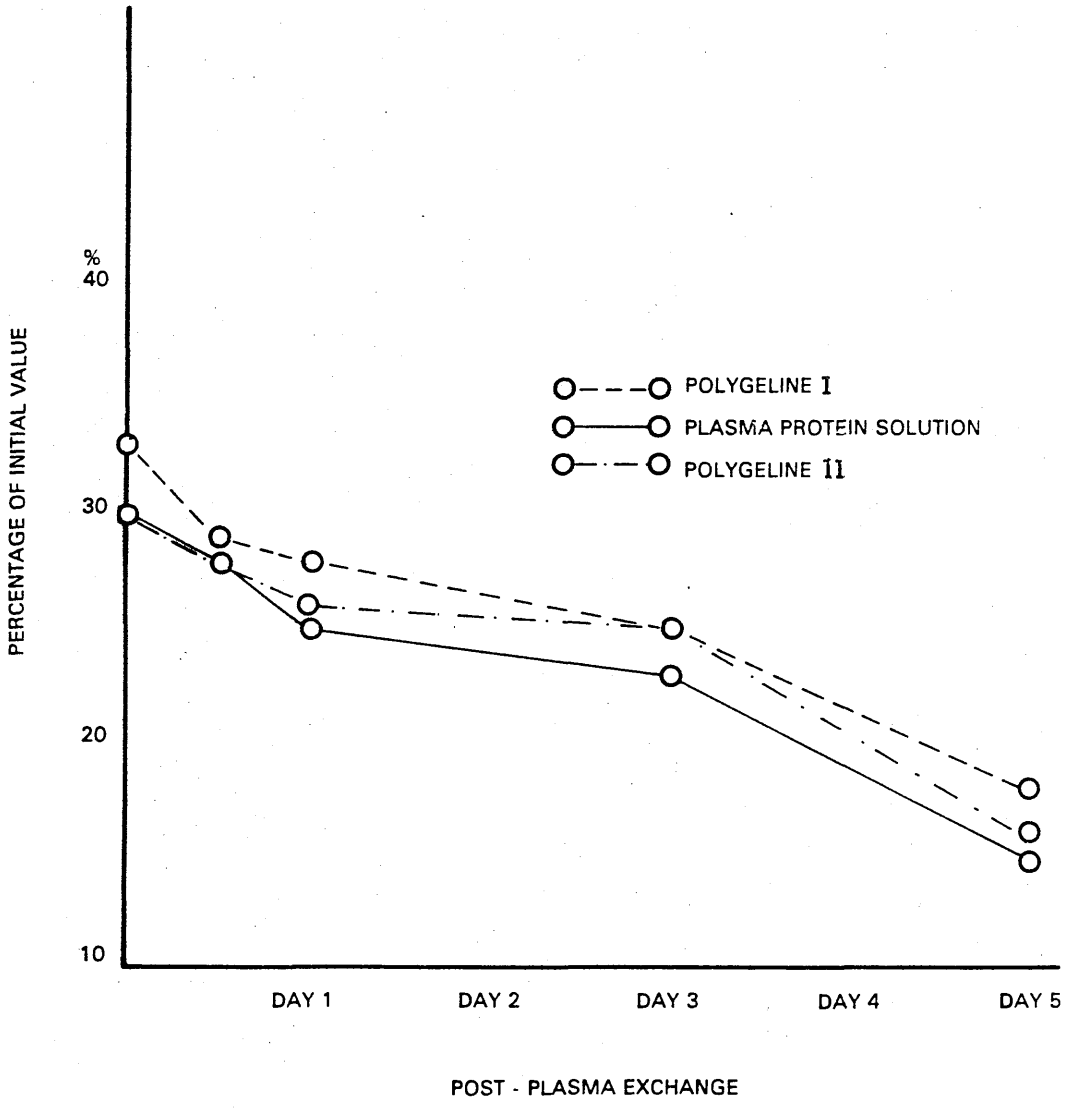


Figure 12. Effect of Replacement Fluid on Coagulation Factor 1 Post-Plasma Exchange

TABLE 23

Quantitative Assays of Coagulation Factors Post-Plasma Exchange

Factor II
(Results expressed as a percentage of initial value)

Time of Assay	PERCENTAGE VALUE		
	GROUP 1 Patients	GROUP 2 Patients	GROUP 3 Patients
	Polygeline I	Plasma Protein Solution	Polygeline II
Pre-Exchange	Normal	Normal	Normal
Post-Exchange 30 minutes	40 ± 2.5	39 ± 1.9	43 ± 2.2
Post-Exchange 12 hours	33 ± 2.4	36 ± 2.3	36 ± 3.0
Post-Exchange 24 hours	28 ± 1.7	30 ± 2.3	30 ± 2.0
Post-Exchange 3 days	26 ± 1.8	28 ± 1.9	27 ± 2.4
Post-Exchange 5 days	25 ± 2.1	26 ± 2.0	27 ± 2.6

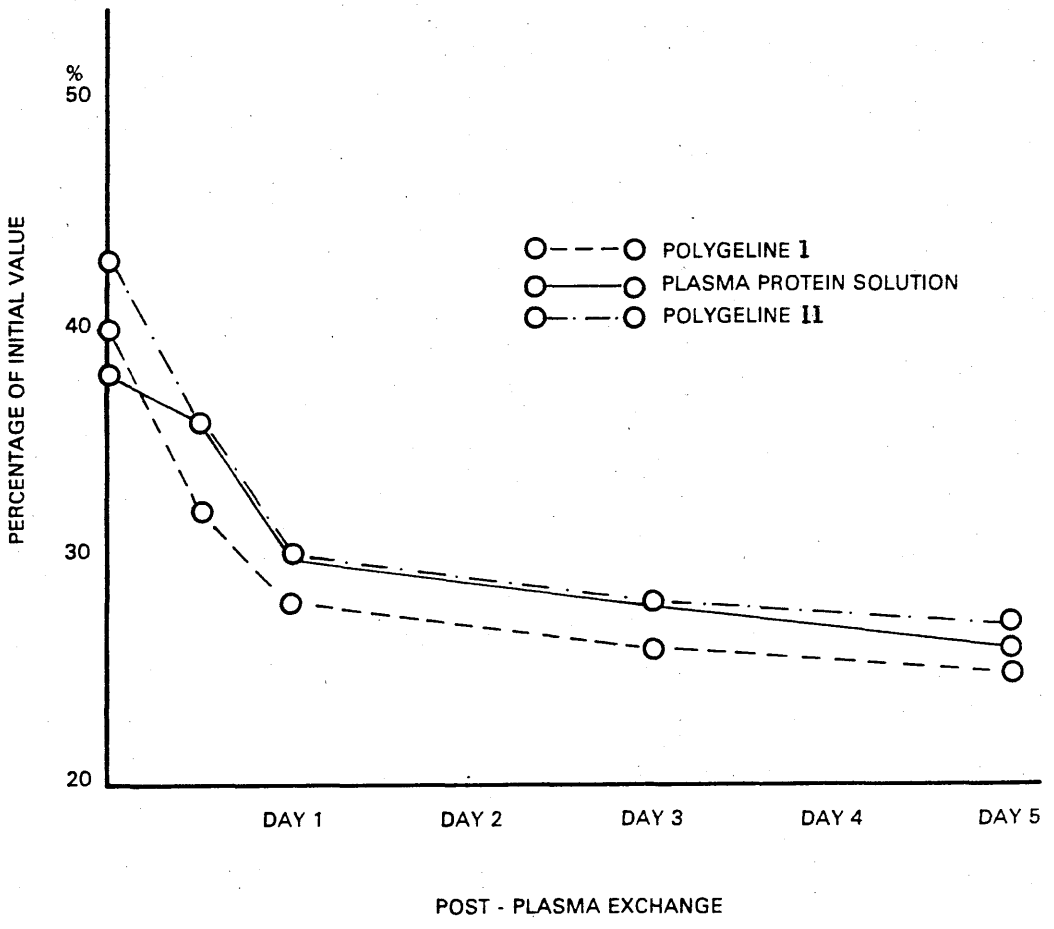


Figure 13 Effect of Replacement Fluid on Coagulation Factor II Post-Plasma Exchange

TABLE 24

Quantitative Assays of Coagulation Factors Post-Plasma Exchange

Factor VIII C
(Results Expressed as a Percentage of Initial Value)

Time of Assay	PERCENTAGE VALUE		
	GROUP 1 Patients	GROUP 2 Patients	GROUP 3 Patients
	Polygeline I	Plasma Protein Solution	Polygeline II
Pre-Exchange	Normal	Normal	Normal
Post-Exchange 30 minutes	47 ± 2.9	51 ± 1.5	45 ± 2.5
Post-Exchange 12 hours	38 ± 2.0	39 ± 1.7	38 ± 1.4
Post-Exchange 24 hours	36 ± 1.7	37 ± 2.2	36 ± 0.9
Post-Exchange 3 days	33 ± 2.5	34 ± 1.4	31 ± 1.5
Post-Exchange 5 days	19 ± 2.4	20 ± 2.1	18 ± 2.5

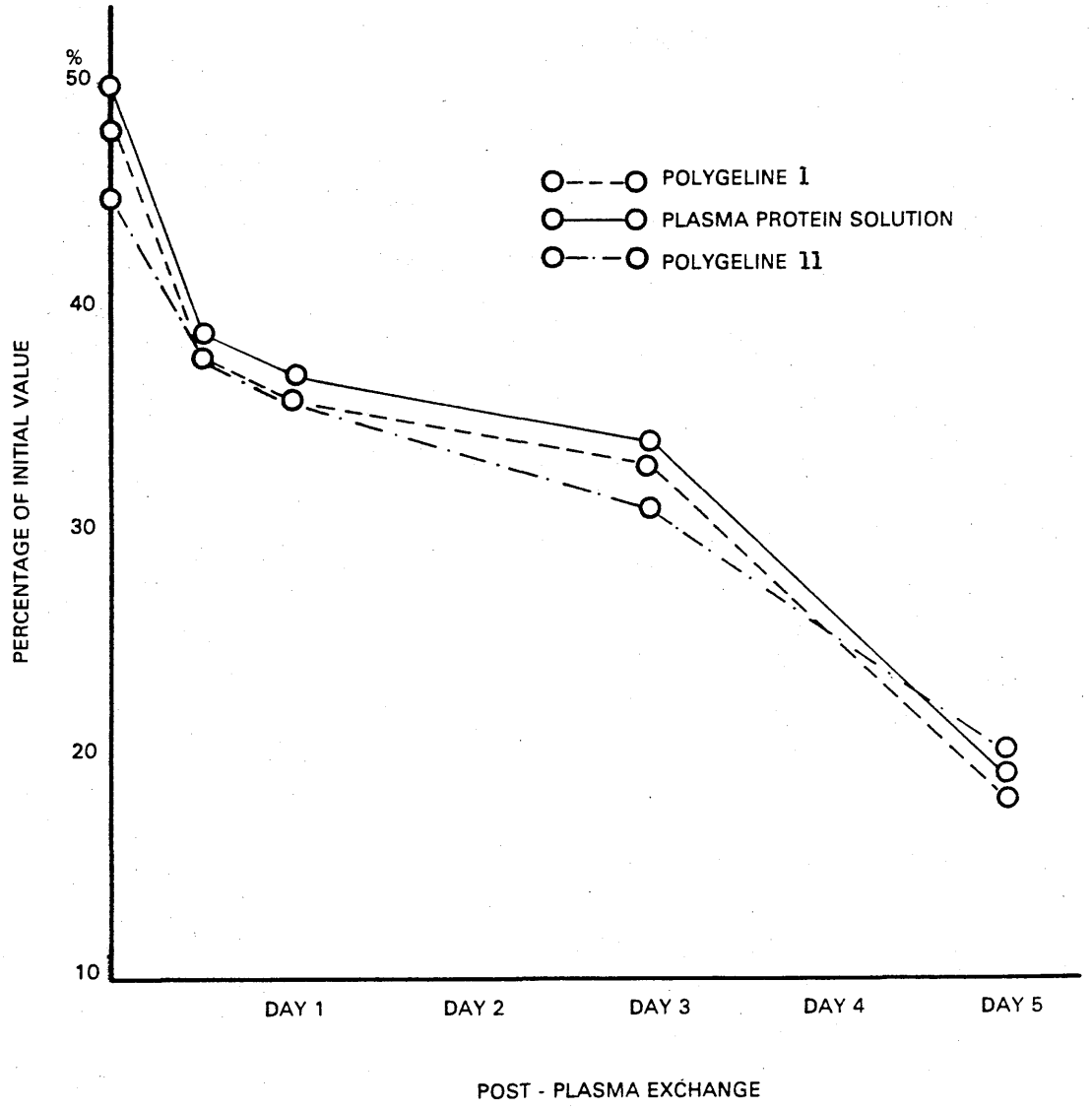


Figure 14. Effect of Replacement Fluid on Coagulation Factor VIIIC Post-Plasma Exchange

TABLE 25

Quantitative Assays of Coagulation Factors Post-Plasma Exchange

FACTOR IX
(Results Expressed as a Percentage of Initial Value)

Time of Assay	PERCENTAGE VALUE		
	GROUP 1 Patients	GROUP 2 Patients	GROUP 3 Patients
	Polygeline I	Plasma Protein Solution	Polygeline II
Pre-Exchange	Normal	Normal	Normal
Post-Exchange 30 minutes	40 $\pm$ 2.1	35 $\pm$ 2.3	34 $\pm$ 1.2
Post-Exchange 12 hours	30 $\pm$ 2.3	33 $\pm$ 1.9	30 $\pm$ 1.2
Post-Exchange 24 hours	28 $\pm$ 1.8	31 $\pm$ 1.6	28 $\pm$ 2.2
Post-Exchange 3 days	25 $\pm$ 2.2	30 $\pm$ 1.7	27 $\pm$ 1.3
Post-Exchange 5 days	25 $\pm$ 2.4	28 $\pm$ 1.3	26 $\pm$ 1.0

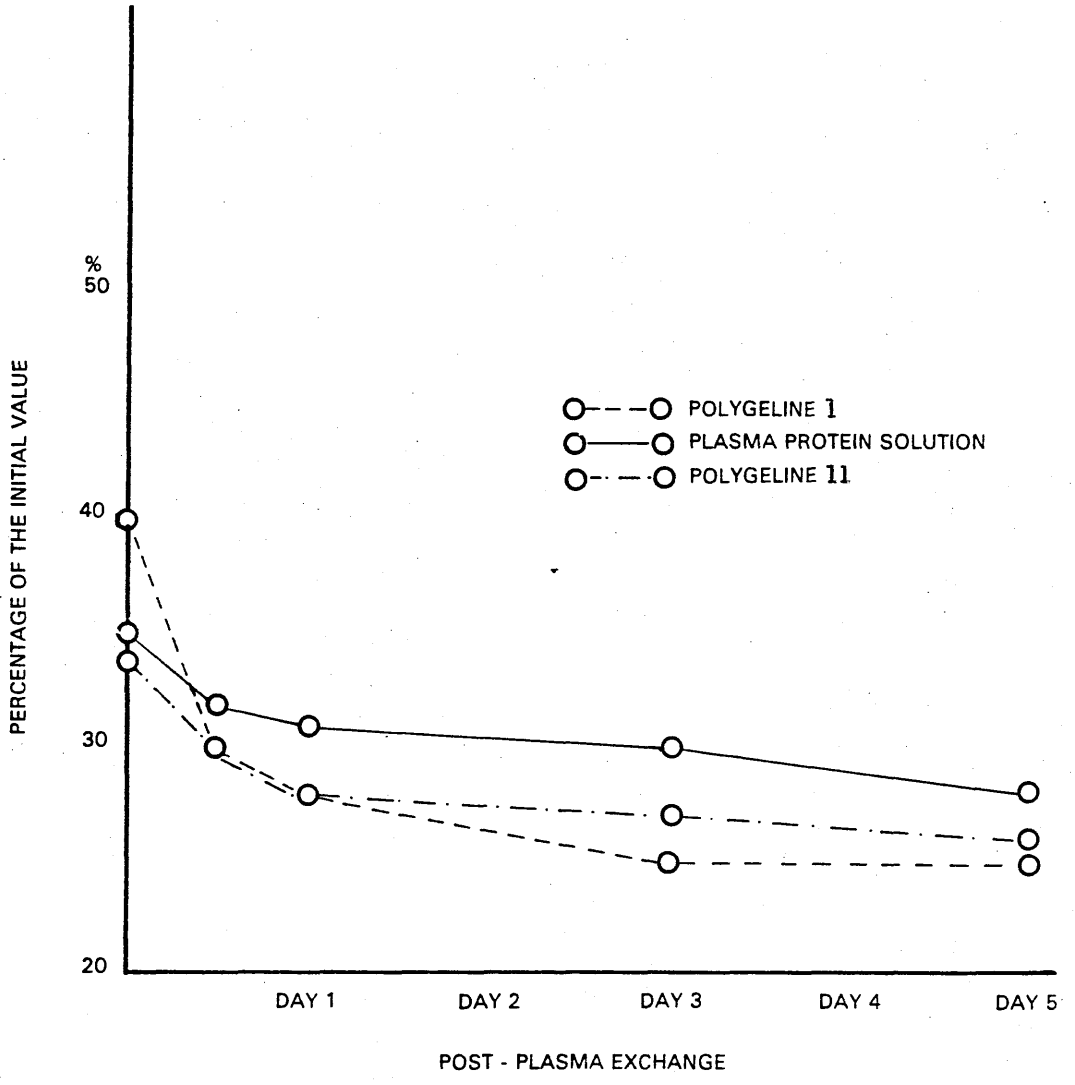


Figure 15. Effect of Replacement Fluid on Coagulation Factor IX Post-Plasma Exchange

TABLE 26

Quantitative Assays of Coagulation Factors Post-Plasma Exchange

FACTOR X
(Results expressed as percentage of initial value)

Time of Assay	PERCENTAGE VALUE		
	GROUP 1 Patients	GROUP 2 Patients	GROUP 3 Patients
	Polygeline I	Plasma Protein Solution	Polygeline II
Pre-Exchange	Normal	Normal	Normal
Post-Exchange 30 minutes	35 $\pm$ 1.5	33 $\pm$ 1.6	32 $\pm$ 1.5
Post-Exchange 12 hours	31 $\pm$ 1.4	30 $\pm$ 1.5	29 $\pm$ 1.8
Post-Exchange 24 hours	29 $\pm$ 1.6	28 $\pm$ 1.4	27 $\pm$ 1.6
Post-exchange 3 days	26 $\pm$ 1.6	27 $\pm$ 1.8	25 $\pm$ 1.8
Post-Exchange 5 days	22 $\pm$ 1.3	24 $\pm$ 1.2	23 $\pm$ 1.4

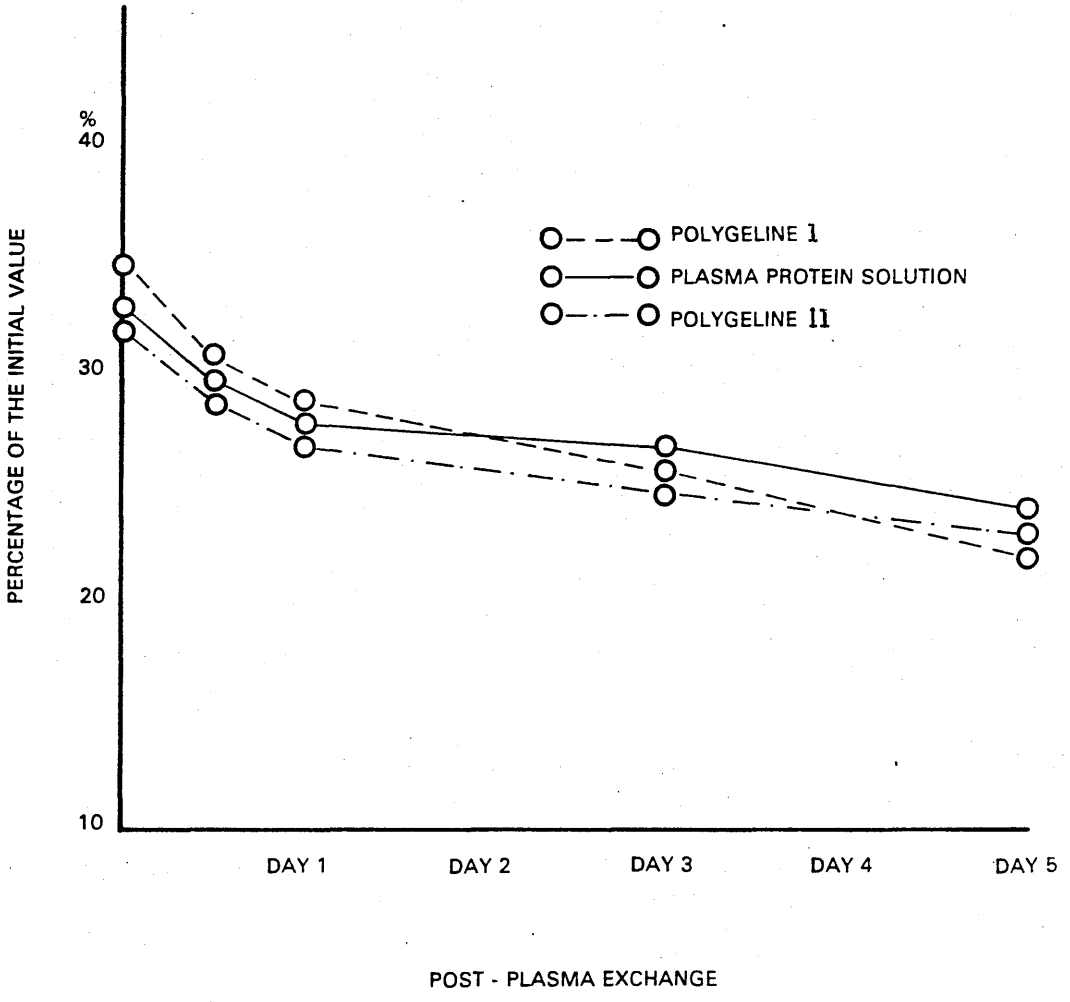


Figure 16. Effect of Replacement Fluid on Coagulation Factor X Post-Plasma Exchange

3.1.2 BIOCHEMICAL PARAMETERS

1 Urea and Electrolyte Evaluation

Values of urea and electrolytes pre-plasma exchange on day 1 and post-plasma exchange on day 5 are given in Table 27.

The values of sodium in Group 1 patients were not significantly different from those in Group 2 and Group 3 patients pre- and post- plasma exchange ($p > 0.05$). The sodium levels in Group 2 patients differed significantly from those in Group 3 patients at pre-plasma exchange only ($p \leq 0.001$). The values of potassium in Group 2 patients differed from those in Group 1 and Group 3 patients at pre-plasma exchange ($0.01 > p > 0.002$), but were not significantly different at 5 days post-plasma exchange ($p > 0.05$). The values of potassium in Group 1 patients were not significantly different from those in Group 3 patients pre-plasma exchange ($p > 0.05$) but became different at 5 days post-plasma exchange ($0.05 > p > 0.02$).

The values of calcium in Group 1 patients were not significantly different from those in patients of Groups 2 and 3 pre-plasma exchange ($p > 0.05$), but became increasingly different at 5 days post-plasma exchange in the case of Group 1 and Group 2 patients ($p < 0.001$). The values of calcium in Group 2 patients differed from those in Group 3 patients pre-plasma exchange ($0.05 > p > 0.02$), but the difference was not significant at 5 days post-plasma exchange ($p > 0.05$).

The values of magnesium in Group I patients were not significantly different from those in patients of Groups 2 and 3 pre- and 5 days post-plasma exchange ($p > 0.05$).

The chloride levels in Group 2 patients differed from those in Group 1 patients ($0.01 \geq p \geq 0.002$) and Group 3 patients ($0.05 \geq p \geq 0.02$) pre-plasma exchange only, otherwise the values were not significantly different ($p > 0.05$).

The values of phosphate in Group 1 patients differed significantly from those in Group 2 and Group 3 patients pre- and 5 days post-plasma exchange ($p < 0.001$).

The values of urea in Group 3 patients were not significantly different from those in Group 1 patients pre-plasma exchange and in Group 2 patients 5 days post-plasma exchange ($p > 0.05$). The differences between all other values were significant ($0.01 > p > 0.002$, $p < 0.001$).

The values of creatinine in Group 1 patients were not significantly different from those in patients of Groups 2 and 3 pre-plasma exchange ($p > 0.05$). The values of creatinine in Group 2 patients differed from those in Group 3 patients pre-plasma exchange ($0.01 > p > 0.002$).

TABLE 27

Values of Electrolyte Change During Plasma Exchange

Electrolyte	Time of Test	ELECTROLYTE VALUE								
		Group 1 Patients			Group 2 Patients			Group 3 patients		
		Polygeline I			Plasma Protein Solution			Polygeline II		
		Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
SODIUM mmol/l	Pre-Exchange Day 1	132-140	135	± 4	133-137	134	± 2	133-138	136	± 2.5
	Post-Exchange Day 5	132-140	137	± 4	131-139	136	± 4	132-144	138	± 5
POTASSIUM mmol/l	Pre-Exchange Day 1	3.3-4.0	3.6	±0.35	3.2-4.0	3.4	±0.4	3.2-4.0	3.7	±0.4
	Post-Exchange Day 5	2.8-3.6	3.2	±0.4	2.5-3.5	3.1	±0.5	2.4-3.6	3.0	±0.3
CHLORIDE mmol/l	Pre-Exchange Day 1	97-108	99	± 8	90-111	103	± 6	97-107	100	± 5
	Post-Exchange Day 5	90-105	96	±7.5	90-100	97	± 5	90-111	98	± 5
UREA mmol/l	Pre-Exchange Day 1	6.0 -8.0	6.6	±1.0	5.8-7.0	6.2	±0.6	6.2-7.8	6.8	±0.8
	Post-Exchange Day 5	5.0-7.6	5.9	±1.3	5.9-7.5	6.5	±0.8	5.8-7.8	6.9	±1

TABLE 27 (cont)

Values of Electrolyte Change During Plasma Exchange

Electrolyte	Time of Test	ELECTROLYTE VALUE								
		Group 1 Patients			Group 2 patients			Group 3 patients		
		Polygeline I			Plasma Protein Solution			Polygeline II		
		Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
CREATININE µmol/l	Pre-Exchange Day 1	85–110	105	± 12.5	105–115	108	± 5	99–109	104	±5
	Post-Exchange Day 5	93–120	110	± 13.5	105–118	110	± 6.5	89– 99	95	±5
CALCIUM mmol/l	Pre-Exchange Day 1	2.20–2.35	2.25	±0.075	2.10–2.35	2.2	± 0.2	2.25–2.35	2.3	±0.05
	Post-Exchange Day 5	2.05–2.25	2.15	±0.10	1.95–2.15	2.05	± 0.1	2.05–2.25	2.1	±0.1
MAGNESIUM mmol/l	Pre-Exchange Day 1	0.60–0.95	0.8	±0.175	0.6–1.0	0.8	± 0.2	0.7–1.2	0.7	±0.25
	Post-Exchange Day 5	0.60–0.90	0.7	±0.75	0.5–0.9	0.6	± 0.2	0.5–0.9	0.6	±0.2
PHOSPHATE mmol/l	Pre-Exchange Day 1	0.75–1.45	1.2	±0.35	0.7–1.1	0.8	± 0.2	0.8–1.3	0.7	±0.25
	Post-Exchange Day 5	0.7–1.2	1.0	±0.25	0.6–1.0	0.7	± 0.2	0.6–1.0	0.7	±0.2

Values of serum bilirubin, asparate transaminase, (AsT) alanine transaminase (ALT), and alkaline phosphatase pre-plasma on day one and post-plasma exchange on day 5 are given in Table 28.

The bilirubin concentrations in Group 1 patients were not significantly different from those in patients of Groups 2 and 3 at pre-plasma exchange ($p > 0.05$), but were significantly different at 5 days post-plasma exchange ($0.002 > p > 0.001$, $0.05 > p > 0.02$). The bilirubin concentrations in Group 2 patients were not significantly different from those of Group 3 patients at pre- and 5 days post-plasma exchange.

The asparate concentrations in Group 1 patients differed from those in Group 2 patients at pre-plasma exchange ($0.02 > p > 0.01$) and became significantly different at 5 days post-plasma exchange ($p < 0.001$).

The asparate values in Group 3 patients were not significantly different from those in patients of Groups 1 and 2 at pre-plasma exchange, but became significantly different from those of Group 1 patients at 5 days post-plasma exchange ($0.01 > p > 0.002$).

There were no significant differences in the alanine transaminase concentrations of all three groups of patients at pre- and 5 days post-plasma exchange ($p > 0.05$).

The concentrations of alkaline phosphatase in patients of all three groups were significantly different at pre-plasma exchange ($p < 0.001$). At 5 days post-plasma exchange the

TABLE 28

CHANGES IN LIVER FUNCTION TEST VALUES DURING PLASMA EXCHANGE

Serum	Time of Assay	Group 1 Patients			Group 2 Patients			Group 3 Patients		
		Polygeline I			Plasma Protein Solution			Polygeline II		
		Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
BILIRUBIN mmol/l	Pre-Exchange Day 1	11–19	9	± 4	7–20	10	±6.5	6–14	8	± 4
	Post-Exchange Day 5	9–21	7	± 6	8–16	10	± 4	8–16	10	± 4
ASPARATE u/l	Pre-Exchange Day 1	22–38	26	± 8	22–32	23	± 5	19–30	23	±5.5
	Post-Exchange Day 5	26–36	28	± 5	18–30	24	± 6	18–30	24	± 6
ALANINE TRANSAMINASE u/l	Pre-Exchange Day 1	28–42	33	± 7	32–42	34	± 5	32–38	34	± 3
	Post-Exchange Day 5	34–40	36	± 8	30–40	38	± 5	30–40	38	± 5
ALKALINE PHOSPHATASE u/l	Pre-Exchange Day 1	170–210	180	±20	130–175	160	±22.5	110–140	126	±15
	Post-Exchange Day 5	150–195	165	±22.5	110–150	120	±20	110–150	120	±20

concentrations of alkaline phosphatase in Group 2 patients only were not significantly different from those in Group 3 patients.

3 Plasma Proteins

Values of total protein, albumin and globulin pre-plasma exchange and at selected intervals post-plasma exchange are given in Table 29 and Figure 17.

Values of total protein and albumin concentration exhibited a sharp fall in the first 30 minute period post-plasma exchange. The reduction in values in patients of Groups 1 and 2 was much less than that in patients of Group 3. The calculated globulin concentrations in patients of Groups 1 and 2 showed less variation than those in Group 3 patients. In the case of Group 1 and Group 2 patients values of concentrations showed partial recovery to the initial pre-plasma exchange levels after a period of 12 hours. In the case of Group 3 patients the values of total protein and albumin concentrations continued to fall. All concentrations had returned to their initial pre-plasma exchange levels immediately after plasma exchange on the fifth day.

At pre-plasma exchange, values of the total protein concentration in Group 2 patients were not significantly different from those in patients of Groups 1 and 3 ($p > 0.05$). At 30

minutes post-plasma exchange the values of total protein concentration in Group 1 patients were not significantly different from those in Group 3 patients. All other values of total protein concentration in the patients of Groups 1, 2 and 3 were significantly different ($p < 0.001$) during the period up to 5 days post-plasma exchange.

At pre-plasma exchange, the values of albumin concentration in Group 1 patients were similar to those in Group 3 patients ($p > 0.05$). All other values of the albumin concentration in the three groups of patients were significantly different ($p < 0.001$) during the five day period post-plasma exchange.

The values of the globulin concentration in patients of Group 1 differed significantly from those in patients of Groups 2 and 3 at pre-plasma exchange and throughout the five day period post-plasma exchange ($p < 0.001$), except in the case of patients in Groups 1 and 3 where the concentrations were similar ($p > 0.05$) at 5 days post-plasma exchange. The values of the globulin concentrations in Group 2 patients were not significantly different ($p > 0.05$) from those in Group 3 patients at pre- and 3 days post-plasma exchange. Thereafter the difference between concentrations was significant ($p < 0.001$).

TABLE 29

Mean Values of Serum Plasma Protein Concentration Pre- and Post-Plasma Exchange

Time of Test	Total Protein g/l			Albumin g/l			Globulin g/l		
	GROUP 1 patients	GROUP 2 patients	GROUP 3 patients	GROUP 1 patients	GROUP 2 patients	GROUP 3 patients	GROUP 1 patients	GROUP 2 patients	GROUP 3 patients
	Polygeline I	Plasma Protein Solution	Polygeline II	Polygeline I	Plasma Protein Solution	Polygeline II	Polygeline I	Plasma Protein Solution	Polygeline II
Pre-Exchange	61 ± 3.0	60 ± 2.75	59 ± 2.75	33.5 ± 1.5	36 ± 3.0	34 ± 2.5	27.5 ± 2.75	24 ± 2.0	25 ± 2.0
Post-Exchange	46 ± 2.5	48 ± 2.5	45 ± 2.25	26 ± 1.75	32 ± 3.0	23 ± 2.25	20 ± 2.0	16 ± 1.5	22 ± 2.0
Post-Exchange 12 hours	53 ± 2.5	51 ± 2.5	42 ± 2.5	28 ± 2.5	33 ± 3.0	21 ± 2.25	25 ± 1.5	18 ± 1.5	21 ± 1.5
Post-Exchange 24 hours	57 ± 3.0	53 ± 2.5	43 ± 2.0	30 ± 1.75	33 ± 3.0	22 ± 2.5	27 ± 1.75	20 ± 1.5	21 ± 1.5
Post-Exchange 3 days	57 ± 3.0	56 ± 2.5	48 ± 2.5	31 ± 1.75	34 ± 3.0	26 ± 2.5	26 ± 1.55	22 ± 1.5	22 ± 1.5
Post-Exchange 5 days	60 ± 3.0	58 ± 2.5	56 ± 2.5	33 ± 1.75	36 ± 3.0	29 ± 2.5	27 ± 1.75	22 ± 1.5	27 ± 1.5

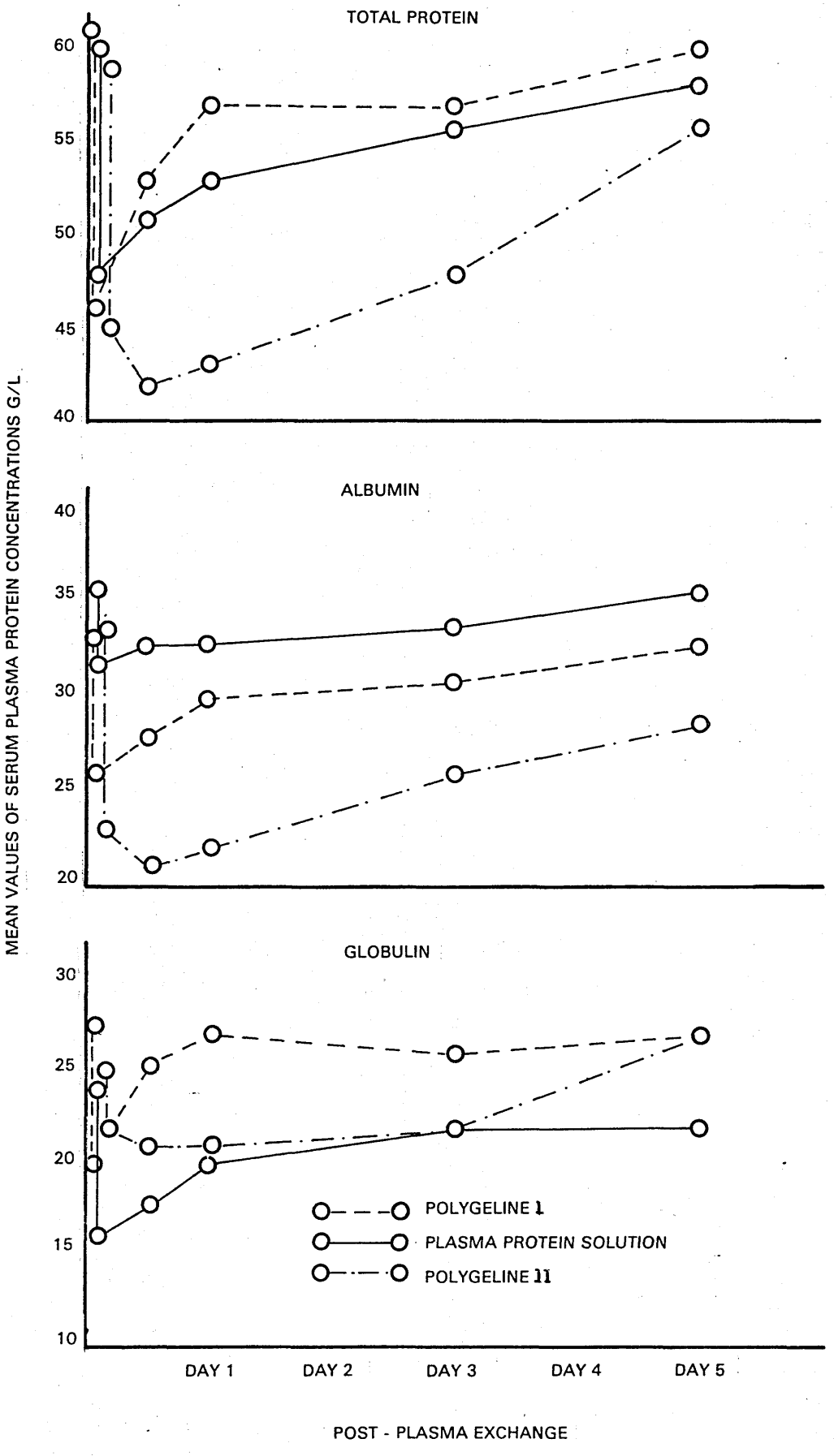


Figure 17. Effect of Replacement Fluid on mean Serum Plasma Protein Concentrations Post-Plasma Exchange

Serum Iron and Total Iron Binding Capacity

Values of serum iron and total iron binding capacity pre-plasma exchange on day 1 and post-plasma exchange on day 5 are given in Table 30.

The concentrations of serum iron in the patients of all three groups were not significantly different at pre-plasma exchange. ($p > 0.05$). At 5 days post-plasma exchange the difference in values between patients of Group 1 and Group 2 and between patients of Group 2 and Group 3 became significant ($p < 0.001$, and $0.01 > p > 0.002$ respectively).

The total iron binding capacities of Group 3 patients were not significantly different from those of Group 1 and Group 2 patients at pre- and 5 days post-plasma exchange. The capacities of Group 1 patients differed from those of patients in Group 2 at pre- and post-plasma exchange ($0.01 > p > 0.002$).

4 Serum Immunoglobulins

Mean values of serum immunoglobulins IgG, IgA and IgM pre-plasma exchange and at selected intervals post-plasma exchange are given in Table 31 and Figure 18. Immediately after the first plasma exchange procedure the levels of all three immunoglobulins fell to around 30% of their pre-plasma exchange level and continued to decrease with each succeeding

TABLE 30

Values of Serum Iron and Total Iron Binding Capacity

	TIME OF ASSAY	GROUP 1 PATIENTS			GROUP 2 PATIENTS			GROUP 3 PATIENTS		
		POLYGELINE I			PLASMA PROTEIN SOLUTION			POLYGELINE II		
		mmol/1			mmol/1			mmol/1		
		Mean	Range	S.D.	Mean	Range	S.D.	Mean	Range	S.D.
Iron	Pre-Plasma Exchange Day 1	16	9-27	± 9	14	10-25	± 12.5	15	12-24	± 6
	Post-Plasma Exchange Day 5	12	10-30	± 10	18	12-26	± 7	13	11-28	± 13.5
Total Iron Binding Capacity	Pre-Plasma Exchange Day 1	52	45-70	± 12.5	46	42-62	± 10	47	39-66	± 13.5
	Post-Plasma Exchange Day 5	44	39-65	± 13	50	38-55	± 8.5	44	50-65	± 22.5

TABLE 31

Mean Values of Serum Immunoglobulins Pre- and Post-Plasma Exchange
(Results expressed as a percentage of initial value)

	PERCENTAGE VALUES								
	IgG			IgA			IgM		
	Group 1 patients	Group 2 patients	Group 3 patients	Group 1 patients	Group 2 patients	Group 3 patients	Group 1 patients	Group 2 patients	Group 3 patients
	Polygeline I	Plasma Protein Solution	Polygeline II	Polygeline I	Plasma Protein Solution	Polygeline II	Polygeline I	Plasma Protein Solution	Polygeline II
Pre-Exchange	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Post-Exchange 30 mins.	32 ± 3	31 ± 3	30 ± 3	33 ± 3	32 ± 3	31 ± 3	34 ± 3	36 ± 3	34 ± 3
Post-Exchange 12 hours	30 ± 4	30 ± 3	28 ± 2	31 ± 3	31 ± 2	29 ± 3	32 ± 3	34 ± 4	31 ± 3
Post-Exchange 24 hours	29 ± 3	28 ± 3	29 ± 2	28 ± 3	30 ± 3	28 ± 2	30 ± 2	32 ± 3	27 ± 2.4
Post-Exchange 3 days	20 ± 3	22 ± 3	25 ± 2	25 ± 2	26 ± 2	23 ± 2	27 ± 2	24 ± 2	24 ± 2
Post-Exchange 5 days	10 ± 2	12 ± 2	12 ± 2	15 ± 2	12 ± 2	16 ± 2	12 ± 1.5	12 ± 1.5	14 ± 1.5

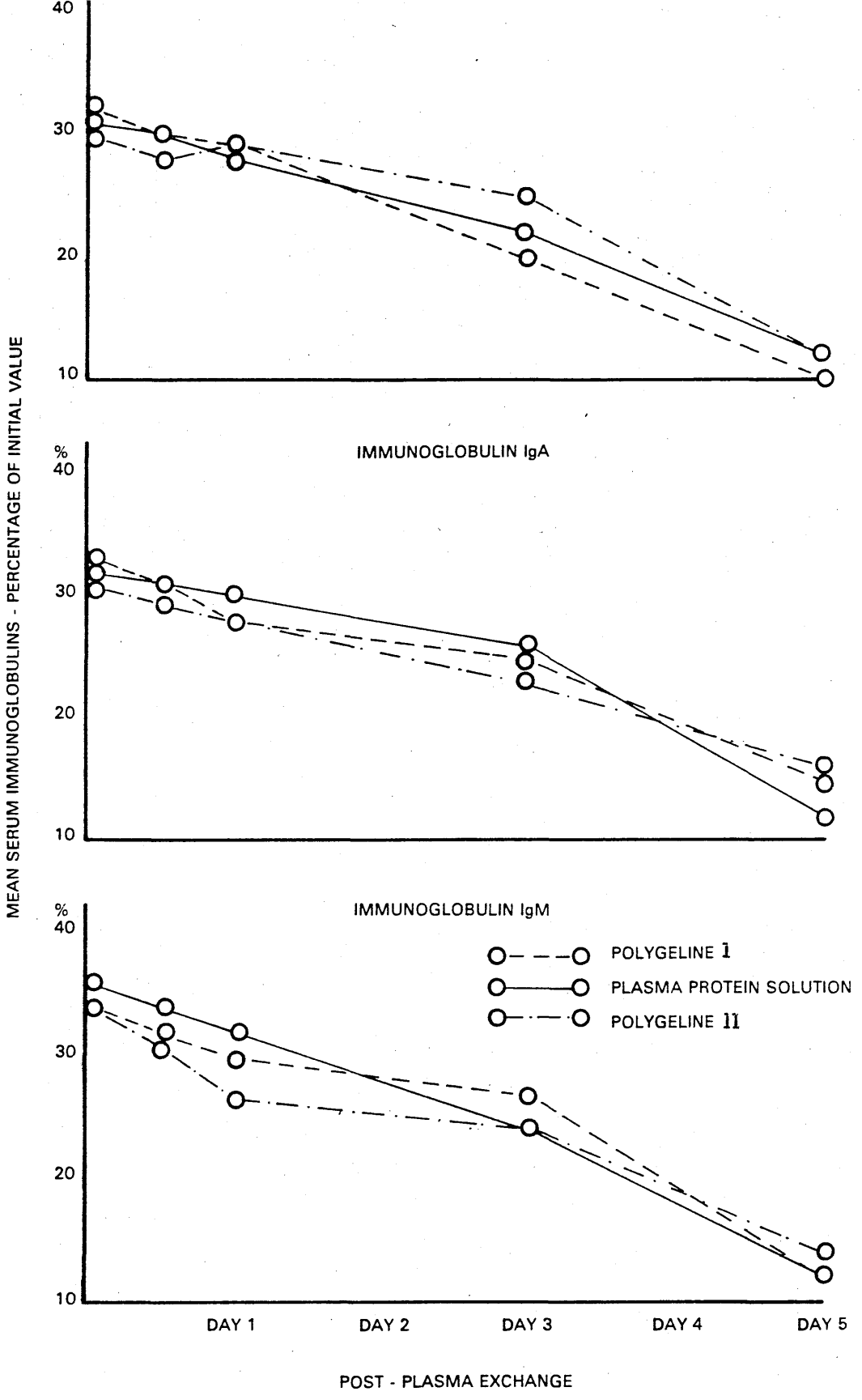


Figure 18. Effect of Replacement Fluid on Mean Serum Immunoglobulins Post-Plasma Exchange.

procedure to around 10 or 15% of their initial value after the fifth exchange.

Over the first 24 hour period post-plasma exchange the values of the percentage reduction of serum immunoglobulin IgG levels in Group 1 patients were not significantly different ($p \geq 0.05$) from those in Group 2 patients but thereafter the difference in values became significant ($p < 0.001$). The values of the percentage reduction of IgG levels in Group 1 patients became increasingly different from those in Group 3 patients ($0.02 > p > 0.01$, $p < 0.001$), except at 24 hours post-plasma exchange when there was no difference ($p > 0.05$). The values of the percentage reduction of IgG levels in Group 2 patients differed from those in Group 3 patients at 12 hours ($0.01 > p > 0.002$) and at 3 days ($p < 0.001$) post-plasma exchange.

Over the first 12 hours period post-plasma exchange the values of the percentage reduction of serum immunoglobulin IgA levels in Group 1 patients did not differ ($p > 0.05$) from those in Group 2 patients, but thereafter the difference became significant. The values of the percentage reduction of IgA levels in Group 1 patients were different from those in Group 3 patients ($0.02 > p > 0.01$, $p < 0.001$) except at 24 hours and at 5 days post-plasma exchange when there was no difference ($p > 0.05$). The values of the percentage reduction of IgA levels in Group 2 patients

were similar to those in Group 3 patients only at 30 minutes post-plasma exchange ($p > 0.05$). Thereafter the difference was significant ($0.002 > p > 0.001$, $p < 0.001$).

The values of the percentage reduction of serum immunoglobulin IgM levels in Group 1 patients were significantly different from those in Group 2 patients ($0.01 > p > 0.002$, $p < 0.001$) pre- and up to 3 days post-plasma exchange. The difference in values was not significant at 5 days post-plasma exchange. After the initial 12 hour post-plasma exchange the values of the percentage reduction in IgM levels in Group 1 patients differed significantly from those in Group 3 patients ($p < 0.001$). The values of the percentage reduction of IgM levels in Group 2 patients were different from those in Group 3 patients ($0.02 > p > 0.01$, $0.01 > p > 0.002$, $p < 0.001$), except at 3 days post-plasma exchange.

3.1.3 CLINICAL RESPONSE

The short-term side effects experienced by some of the 3 groups of patients during treatment by plasma exchange are summarised in Table 32. In the 700 procedures undertaken on 140 patients there were 84 instances of identifiable side effects. More than one side effect was observed in

TABLE 32

SUMMARY OF SHORT TERM SIDE EFFECTS ARISING FROM SELECTED REPLACEMENT FLUIDS USED IN PLASMA EXCHANGE

GROUP	GROUP 1 PATIENTS	GROUP 2 PATIENTS	GROUP 3 PATIENTS
Replacement Fluid	POLYGELINE I	PLASMA PROTEIN SOLUTION	POLYGELINE II
Number of Patients	60	60	20
Number of Exchanges	300	300	100
Total Volume of Fluid Exchanged	750 litres	750 litres	185 litres
Exchanges stopped by difficulties	2	4	2

TABLE 32 (Cont)

GROUP	GROUP 1 PATIENTS		GROUP 2 PATIENTS		GROUP 3 PATIENTS	
SIDE EFFECTS	Number of patients	Percentage of all procedures	Number of patients	Percentage of all procedures	Number of patients	Percentage of all procedures
1 Urticaria	5	1.4	10	3	5	25
2 Rigor and Shivering	4	1.1	8	2.3	5	25
3 Itch	3	0.9	7	2	4	20
4 Hypotension	2	0.6	5	1.5	4	20
5 Nausea and vomiting	2	0.6	5	1.5	3	15
6 Pyrexia	2	0.6	2	0.6	2	10
7 Anaphylaxis	1	0.3	1	0.3	1	5
8 Diarrhoea	1	0.3	1	0.3	1	5

some patients. The incidence of side effects was greatest in Group 3 patients and least in Group 1 patients. Urticaria, rigors and shivering, itch and hypertension were particularly prevalent in Group 3 patients and occurred in around 25% of the procedures.

No side effect was severe and certainly not life threatening. No procedure had to be terminated because of side effects but 8 exchanges were stopped because of other difficulties. One patient exchanged with plasma protein solution and one patient exchanged with polygeline exhibited bronchospasm. Neither patient had a past history of asthma and in neither case was it severe or distressful.

Only one episode of bleeding was encountered.

The response to treatment by plasma exchange of all three groups of patients with myasthenia gravis, Guillain Barré Syndrome, chronic relapsing polyneuropathy and polymyositis is summarised in the consolidated Table 33. This response was not significantly affected by the choice of the 3 replacement fluids investigated, nor was one replacement fluid more effective than another in the treatment of any of the 4 disorders studied.

TABLE 33

Clinical Status of Patients After Plasma Exchange
Using Selected Replacement Fluids

DISORDER	GROUP I PATIENTS				GROUP 2 PATIENTS				GROUP 3 PATIENTS			
	POLYGELINE I				PLASMA PROTEIN SOLUTION				POLYGELINE II			
	NUMBER OF PATIENTS WITH DISORDER	Patient Status After Treatment			NUMBER OF PATIENTS WITH DISORDER	Patient Status After Treatment			NUMBER OF PATIENTS WITH DISORDER	Patient Status After Treatment		
		Condition Improved	Condition No Change	Condition Worsened		Condition Improved	Condition No Change	Condition Worsened		Condition Improved	Condition No Change	Condition Worsened
Myasthenia Gravis	30	20	7	3	28	22	4	2	5	3	2	0
Guillain-Barré Syndrome	17	15	2	0	19	16	2	1	5	4	1	0
Chronic Relapsing Polyneuropathy	10	7	3	0	10	6	4	0	5	3	2	0
Polymyositis	3	2	0	1	3	2	1	0	5	4	0	1

3.2 STUDY OF SELECTED DISORDERS

1. Cold Haemagglutinin Disease

Values of Hb and antibody titres (anti-I) prior to plasma exchange and post-plasma exchange are given in Table 34. In all patients there was a significant increase in the Hb and decrease in the cold antibody titre.

Four patients required to be transfused during the period of exchange, but only required 2 units of packed cells. One patient relapsed 4 days after the fourth exchange with a subsequent high titre cold antibody and unfortunately died from a myocardial infarction.

2. Waldenstrom's Macroglobulinaemia

Values of full blood viscosity at 3 shear rates and of plasma viscosity pre-plasma exchange and post-plasma exchange are given in Figures 19 and 20 respectively. The change in serum immunoglobulin IgM during the course of 3 plasma exchanges is shown in Figure 21.

There is significant reduction in the whole blood viscosity at all 3 shear rates and in plasma viscosity immediately after plasma exchange. The value of serum immunoglobulin IgM decreased with each plasma exchange procedure and had fallen to about half of its pre-exchange value after the third procedure.

TABLE 34

Cold Haemagglutinin Disease

Measurements of Haematological and Serological Parameters
Pre- and Post-Plasma Exchange

	PRE-THERAPY		POST-THERAPY	
	Hb g/dl	Ab Titre Anti I^4 °C	Hb g/dl	Ab Titre Anti I^4 °C
1 Lymphoma	9.5	1/128	12.8	1/64
2 Lymphoma	9.5	1/256	13.0	1/64
3 Carcinoma of Lung	7.8	1/64	10.1	1/16
4 Plasma cell leukaemia	8.6	1/512	10.8	1/128
5 Post-infection	6.4	1/64	8.9	1/16
6 Post-infection	5.5	1/64	8.9	1/16
7 Lymphoma	5.0	1/1204	7.8	1/512
8 Post infection	4.8	1/128	8.6	1/64

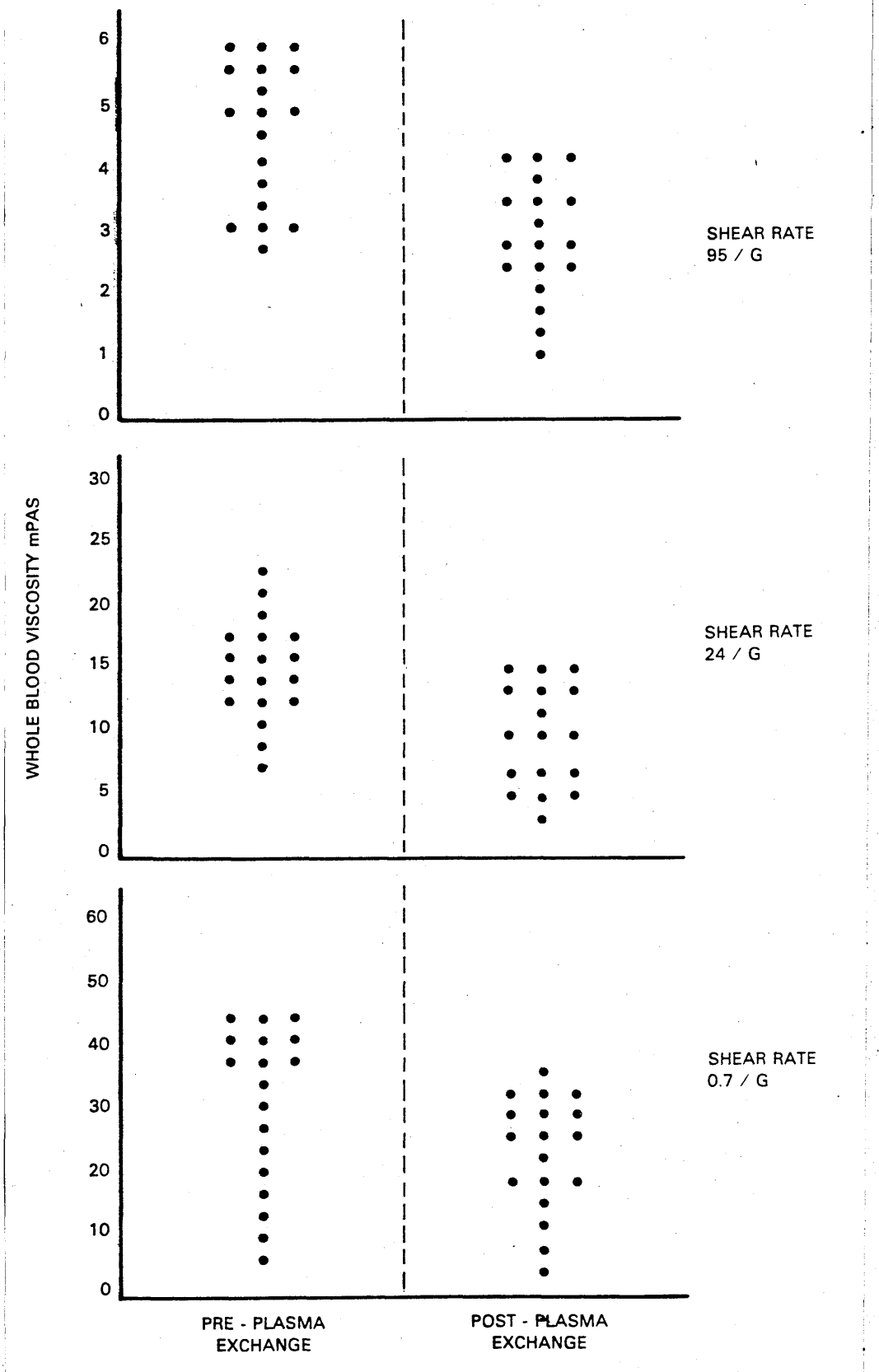


Figure 19. Whole Blood Viscosity at three Shear Rates Pre- and Post-Plasma Exchange. (Waldenstrom's Macroglobulinaemia)

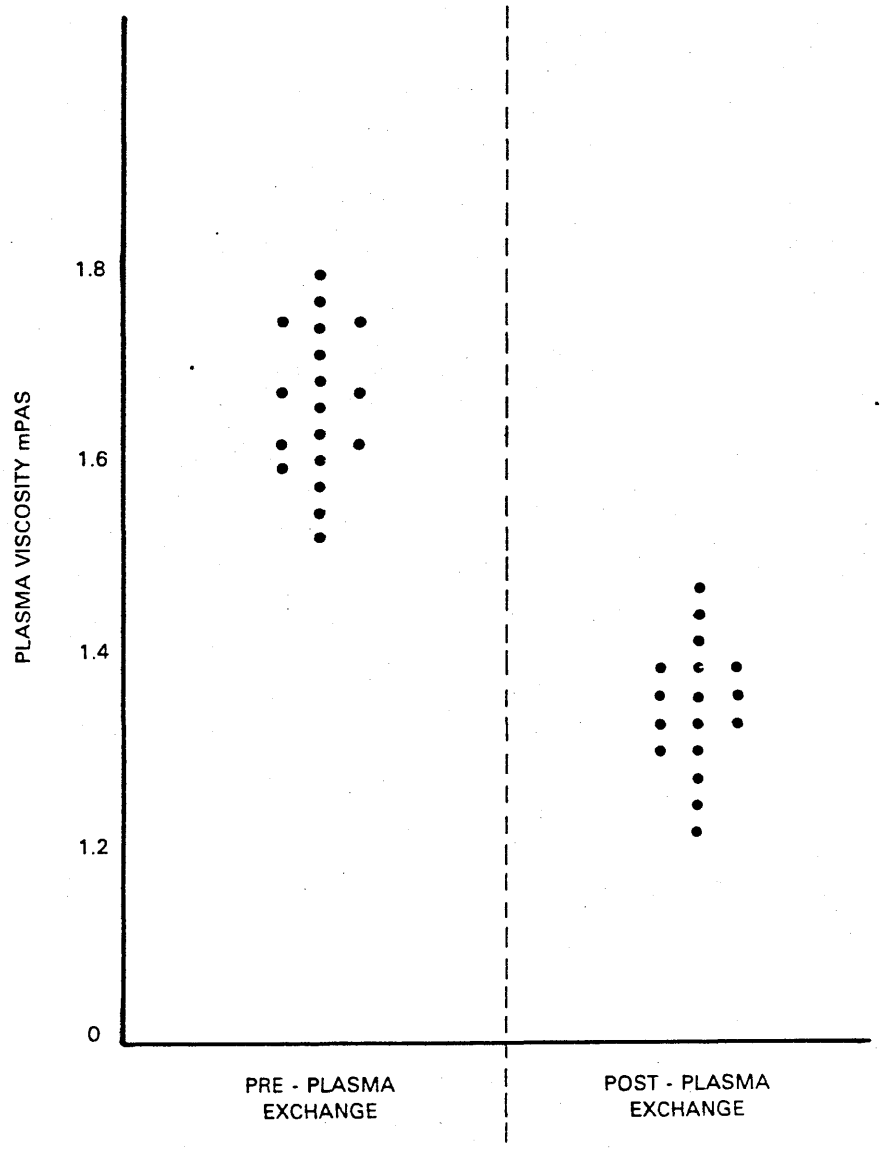


Figure 20. Plasma Viscosity Pre- and Post- Plasma Exchange (Waldenstrom's Macroglobulininaemia)

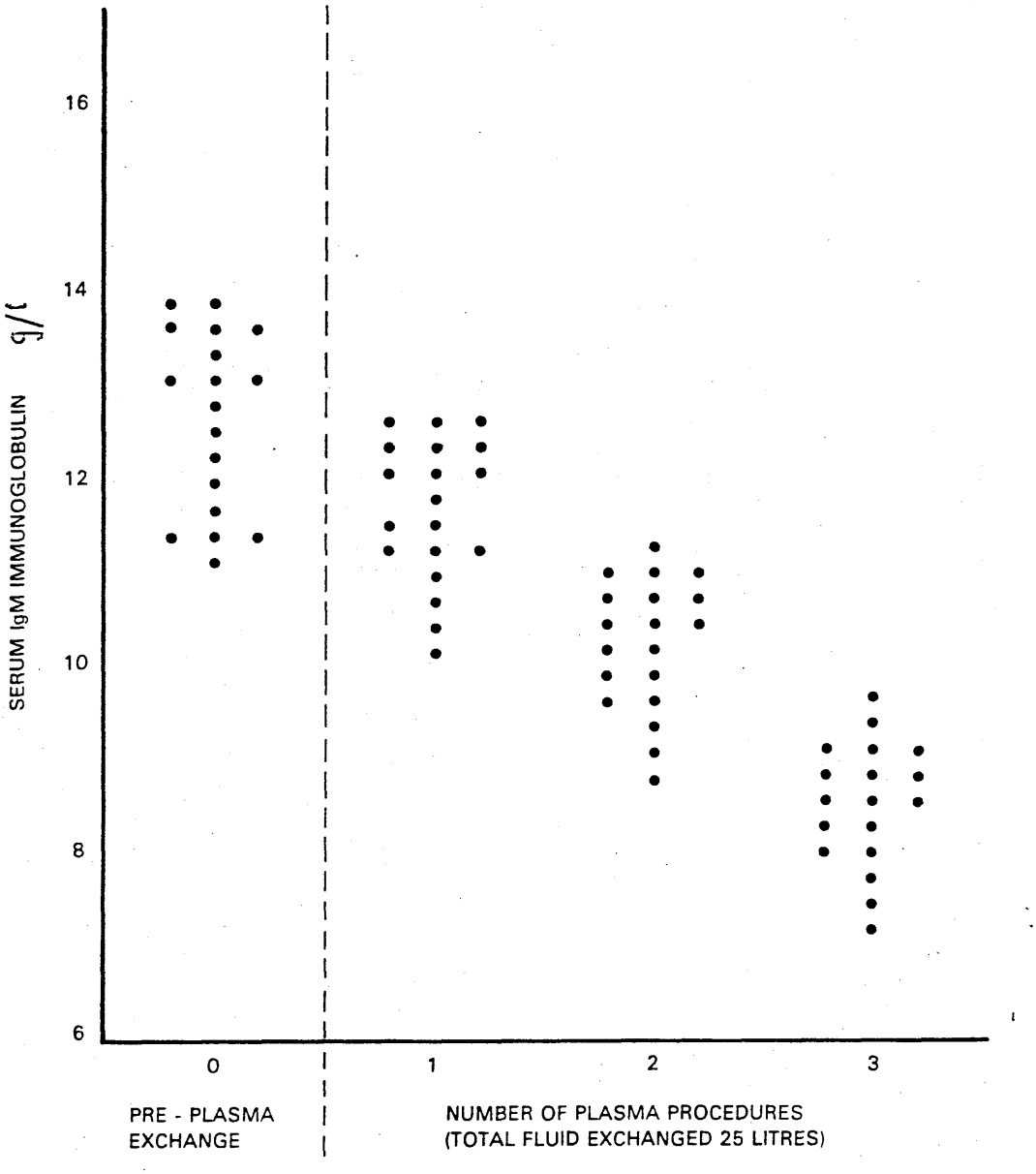


Figure 21. Effect of Number of Plasma Procedures on values of Serum IgM Immunoglobulin. (Waldenstrom's Macroglobulinaemia)

All but one patient noticed a marked improvement in clinical status. 2 patients at pre-coma state were dramatically improved, whilst in one patient both vision and deafness improved dramatically.

Hyperviscosity due to IgA and IgG Myeloma

Values of full blood viscosity at 3 shear rates and of plasma viscosity pre-plasma exchange and post-plasma exchange are given in Figures 22 and 23 respectively.

There is a significant reduction in the whole blood viscosity at three shear rates and in plasma viscosity immediately after plasma exchange. All but two patients noticed a marked improvement in the clinical status and in one patient there was a dramatic improvement in clinical and renal status.

3. Immune Haemolytic Anaemia

Values of Hb and antibody titre pre-plasma exchange and post-plasma exchange are given in Table 35. In all patients there was a significant rise in Hb with an associated decrease in warm antibody titre. In the three cases studied, plasma exchange resulted in, at least, temporary control of haemolysis and patients achieved a measure of stability to allow other modalities time to act.

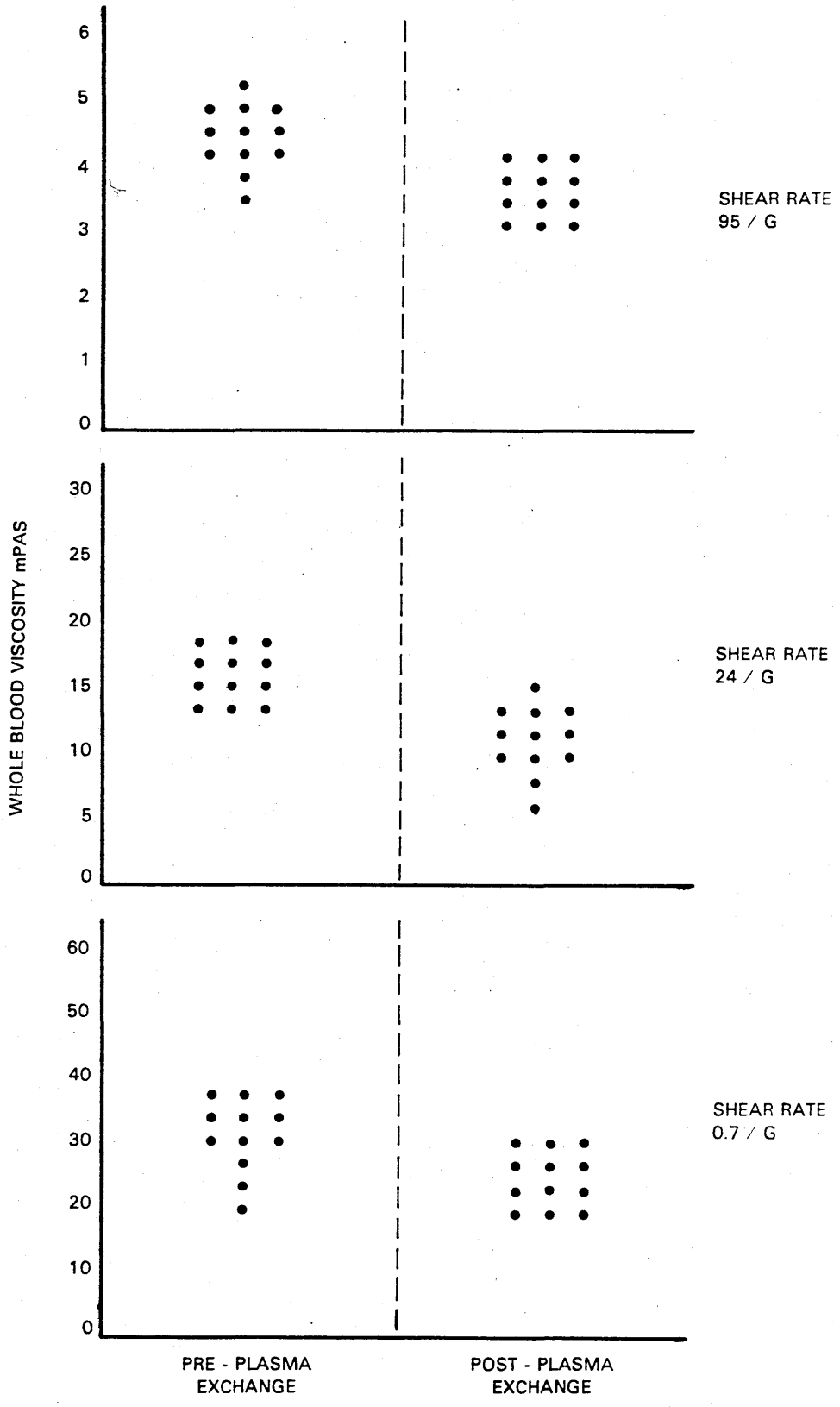


Figure 22. Whole Blood Viscosity at three Shear Rates Pre- and Post-Plasma Exchange in Hyperviscosity due to IgG and IgA Myeloma.

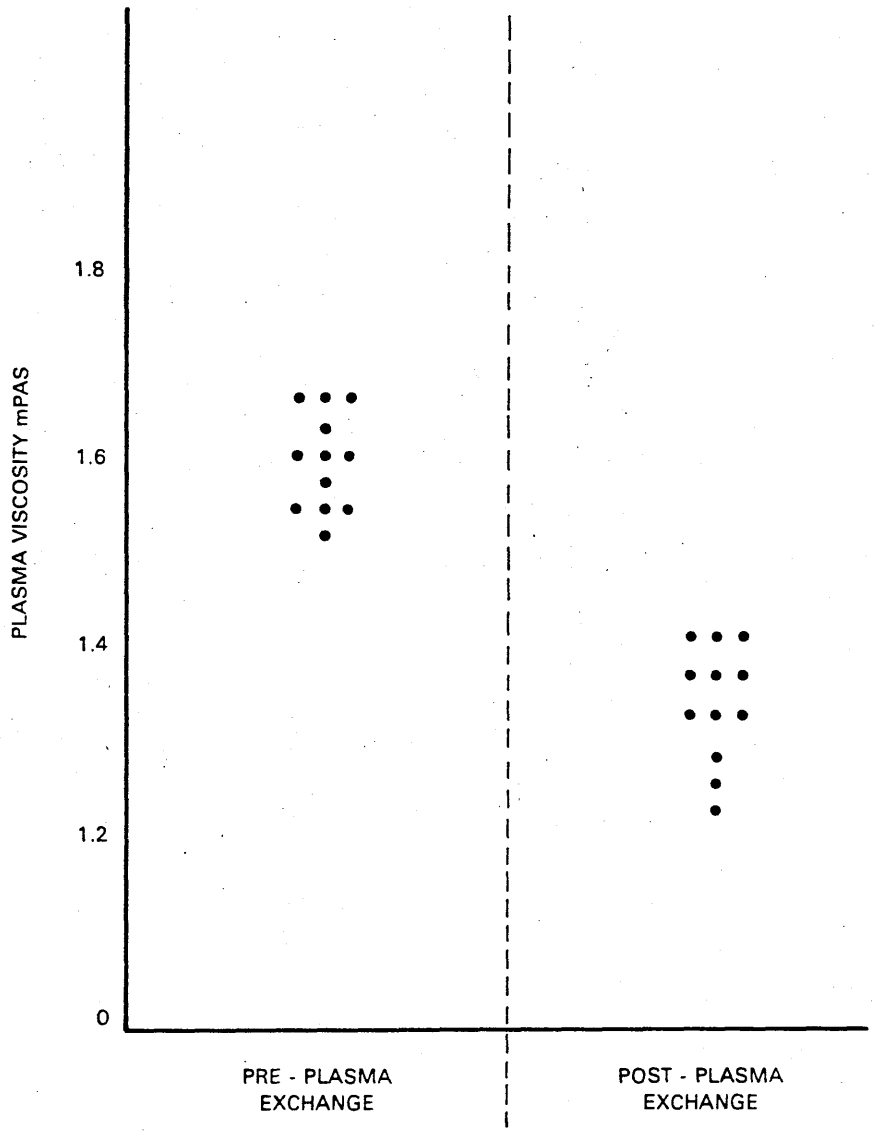


Figure 23. Plasma Viscosity Pre- and Post- Plasma Exchange in Hyperviscosity due to IgA and IgG Myeloma

TABLE 35

Immune Haemolytic Anaemia

CLINICAL FEATURES	PRE-EXCHANGE		POST-EXCHANGE	
	Hb g/dl	Ab titre	Hb g/dl	Ab titre
1 Evans' Syndrome	6	+ + +	9.5	+
2 Post infectious	8	+ + + +	9.8	+ +
3 Malignant Lymphoma	5	+ + + +	9.2	+ +

Ab titre graded from + (low titre) to + + + + + (high titre)

4 Factor VIII inhibitors

Values of Prothrombin time, Kaolin Cephalin Clotting Time, Thrombin Clotting Time, Factor VIIIC, Factor VIII Rag and an inhibitor quantification pre-plasma exchange and post-plasma exchange after the fifth procedure are given in Table 36.

Values of PTT, TT and Factor VIIIRag are not significantly altered by plasma exchange. The values of KCCT and the Inhibitor Sctreen were significantly reduced and the Factor VIII C concentration was markedly increased.

5 Chronic Inflammatory Polyneuropathy

The change in clinical status of the ten patients after plasma exchange may be seen from Table 37.

It will be noted that with the exception of one female and three male patients in whom there was no discernable change, the clinical status of all other patients improved after treatment. One patient unfortunately died.

TABLE 36

Factor VIII Inhibitors
Values of Coagulation Screening Performed Pre- and Post- Plasma Exchange

PATIENT			DIAGNOSIS	TIME OF ASSAY	PT	KCCT	TT	F VIII C	F VIII RAg	INHIBITOR SCREEN
No	Age	Sex			Secs	Secs	Secs	u/dl	u/dl	u/l
1	58	M	RHEUMATOID ARTHRITIS	Pre-Plasma Exchange	15	89	6	1	120	25
				Post-Plasma Exchange	15	49	6	55	120	Nil
2	46	F	SYSTEMIC LUPUS ERYTHEMATOSUS	Pre-Plasma Exchange	14	72	7	1	140	19
				Post-Plasma Exchange	14	60	7	60	140	Nil
3	57	F	PENICILLIN	Pre-Plasma Exchange	13	106	6	<1	110	16
				Post-Plasma Exchange	13	52	6	89	120	Nil
4	25	F	POST PARTUM	Pre-Plasma Exchange	12	89	7			
				Post-Plasma	12	53	7	99	110	Nil

TABLE 36 (Cont'd)

Factor VIII Inhibitors
Values of Coagulation Screening Performed Pre- and Post- Plasma Exchange

PATIENT			DIAGNOSIS	TIME OF ASSAY	PT	KCCT	TT	F VIII C	F VIII RAg	INHIBITOR SCREEN
No	Age	Sex			Secs	Secs	Secs	u/dl	u/dl	u/l
5	32	F	POST PARTUM	Pre-Plasma Exchange	13	95	6	<1	95	12
				Post-Plasma Exchange	14	49	8	110	110	Nil
6	44	F	ULCERATIVE COLITIS	Pre-Plasma Exchange	14	66	7	5	120	15
				Post-Plasma Exchange	15	42	7	120	120	Nil
7	22	F	DERMATITIS HERPETIFORMIS	Pre-Plasma Exchange	14	62	7	5	130	14
				Post-Plasma Exchange	14	48	6	87	140	Nil
8	58	F	SYSTEMIC LUPUS ERYTHEMATOSUS	Pre-Plasma Exchange	14	75	8	5	110	8
				Post-Plasma Exchange	14	45	7	67	120	Nil

TABLE 37

Effect of Plasma Exchange on Clinical Status of Patients with Chronic Inflammatory Polyneuropathy

PATIENT DETAILS			DURATION OF SYMPTOMS MONTHS	CLINICAL STATUS	
No	Age Years	Sex		Pre-Plasma Exchange	Post-Plasma Exchange
1	28	F	6	5	4
2	31	M	9	5	5
3	26	M	12	4	4
4	34	M	8	3	4
5	35	F	18	4	4
6	31	M	12	5	6
7	29	F	6	5	3
8	26	M	8	5	3
9	30	M	6	4	3
10	25	M	5	5	4

Performance Grading

0 - Healthy
1 - Minor Symptoms
2 - Walking without assistance
3 - Walking with assistance
4 - Bed bound
5 - Requiring ventilation
6 - Dead

6 Myasthenia Gravis and Eaton Lambert Syndrome

Measurements of patient Anti-AchR and patient vital capacity, pre- and post-plasma exchange are coupled with the corresponding change in patient status in Table 38.

It will be noted that in all patients there was a significant reduction in the value of patient Anti-AchR and a corresponding increase in the patient vital capacity as a result of plasma exchanges. The clinical status was unchanged three patients. A marked improvement (Grade 4 to Grade 1) was seen in three patients with high initial values of Anti-AchR (86, 68, 75 and 86) respectively.

Eaton Lambert Syndrome

Measurements of clinical status and vital capacity were measured pre- and post exchange as seen in Table 39.

It will be noted that three patients achieved a significant improvement in clinical status and vital capacity and two deteriorated significantly.

7. Guillain Barré Syndrome

The change in clinical status of the 27 patients on the 14th day after plasma exchange is summarised in Table 40.

TABLE 38

Effect of Plasma Exchange on Clinical Status of Patients with Myasthenia Gravis

PATIENT DETAILS			PATIENT STATUS		PATIENT Anti-AchR(u/l)		PATIENT Vital Capacity (Litres)	
No	Age Years	Sex	Pre-Plasma Exchange	Post-Plasma Exchange	Pre-Plasma Exchange	Post-Plasma Exchange	Pre-Plasma Exchange	Post-Plasma Exchange
1	52	M	4	2	45	15	1.55 ± 0.15	2.65 ± 0.12
2	41	F	3	1	35	<10	1.46 ± 0.12	1.85 ± 0.20
3	39	F	2	2	30	<10	1.95 ± 0.16	2.25 ± 0.25
4	36	F	3	1	25	<10	1.75 ± 0.2	2.35 ± 0.25
5	32	F	4	1	86	<10	2.05 ± 0.35	2.55 ± 0.28
6	49	F	3	1	75	<10	1.65 ± 0.15	2.05 ± 0.25
7	51	M	4	2	90	25	1.95 ± 0.25	2.05 ± 0.2
8	31	F	3	3	45	35	1.75 ± 0.18	2.05 ± 0.2
9	47	F	4	1	68	15	1.65 ± 0.16	2.65 ± 0.25
10	30	M	3	1	55	15	1.55 ± 0.14	2.75 ± 0.27
11	56	F	4	1	75	<10	1.85 ± 0.16	2.45 ± 0.25
12	37	F	4	3	68	35	2.05 ± 0.35	2.35 ± 0.25
13	26	F	4	3	75	45	2.05 ± 0.35	2.40 ± 0.2
14	29	M	3	2	60	48	1.65 ± 0.18	2.05 ± 0.18
15	53	M	3	3	48	30	1.65 ± 0.2	2.20 ± 0.16
16	27	F	2	0	25	<10	1.50 ± 0.16	2.05 ± 0.16
17	46	F	3	0	45	<10	1.65 ± 0.2	2.05 ± 0.2
18	42	M	4	1	86	15	1.85 ± 0.21	2.15 ± 0.2

STATUS GRADING

0 - No disability
1 - Minimal disability
2 - Moderate disability
3 - Walking without assistance
4 - Walking with assistance
5 - Requiring ventilation

TABLE 39

Effect of Plasma Exchange on Clinical Status of Patients with Eaton Lambert Syndrome

PATIENT DETAILS			PATIENT STATUS		PATIENT VITAL CAPACITY (Litres)	
No	Age	Sex	Pre-Plasma Exchange	Post-Plasma Exchange	Pre-Plasma Exchange	Post-Plasma Exchange
1	48	F	3	1	2.05 $\pm$ 0.15	2.65 $\pm$ 0.12
2	56	M	3	4	1.95 $\pm$ 0.25	1.45 $\pm$ 0.25
3	61	M	3	1	2.35 $\pm$ 0.35	2.85 $\pm$ 0.25
4	66	F	4	1	2.68 $\pm$ 0.45	2.35 $\pm$ 0.35
5	64	F	3	5	1.85 $\pm$ 0.35	1.45 $\pm$ 0.25

GRADING

1 Minimal disability
2 Moderate disability, but able to walk
3 Walking without assistance
4 Walking only with assistance
5 Bed bound
6 Totally incapacitated

TABLE 40

Effect of Plasma Exchange on Clinical Status of Patients with Guillain Barre Syndrome

PATIENT DETAILS			Duration of Symptoms until Diagnosis. Days	Duration from Diagnosis until Plasma Exchange. Days	CLINICAL STATUS OF PATIENT	
No	Age Years	Sex			Pre-Plasma Exchange	Post-Plasma Exchange 14th day
1	32	M	8	2	5	4
2	22	F	6	1	5	3
3	41	F	7	1	5	3
4	21	M	12	1	5	4
5	36	F	8	1	5	4
6	26	F	10	2	5	4
7	33	M	10	2	5	3
8	28	M	9	1	5	2
9	42	F	7	1	5	3
10	27	M	6	3	5	3
11	25	F	14	2	4	3
12	56	M	12	1	4	3
13	24	F	11	1	5	2
14	35	M	10	1	5	3
15	23	F	12	1	5	3
16	38	M	13	2	5	3
17	26	F	9	1	4	2
18	35	M	6	2	4	2
19	21	M	5	1	5	3
20	31	F	5	1	4	3
21	32	M	7	1	5	3
22	31	M	8	2	5	3
23	26	F	8	1	5	3
24	21	F	10	2	5	3
25	23	F	12	1	5	2
26	22	M	11	2	5	3
27	33	M	10	1	5	3

A total of 162 Plasma Exchanges were carried out (each of 2.5 litres)

STATUS GRADING

0 - No disability
1 - Minimal disability
2 - Moderate disability
3 - Walking without assistance
4 - Walking with assistance
5 - Bed bound

The greatest change was observed in patients 8, 13 and 25 (from Grade 5 to Grade 2) in whom the duration of symptoms ranged from 9 to 12 days and for whom plasma exchange was carried out in one day after diagnosis of disease.

8 Glomerulonephritis and Goodpasture's Syndrome

The change in values of serum creatinine pre- and post-plasma exchange is given in Table 41 for 23 G.N patients. The serum creatinine was significantly lowered by plasma exchange.

Regular plasma exchange appeared to prevent progression to renal failure in 9 out of 12 patients with Mesangio-capillary Type 1 GN (MCGN) for a mean duration of 18 months. In one patient plasma exchange was stopped following a rise in serum creatinine.

In MCGN Type 11 two patients did not progress to renal failure. Despite an initial reduction in serum creatinine, four patients progressed to renal failure although the plasma exchange procedures were continued. Three patients with mesangial IgA disease had virtually stable serum creatinine during 9 months of plasma exchange.

In idiopathic membranous nephropathy two patients progressed to end stage renal failure despite plasma exchange.

TABLE 41

Results of Regular Plasma Exchange in the Progressive glomerulopathies

	Type I GN 12 patients	Type II GN 6 patients	Mesangial IgA 3 patients	Membranous Nephropathy 4 patients
Serum Creatinine (μmol/l 1. Pre-Exchange	890 ± 25	700 ± 36	950 ± 36	1050 ± 36
2. Post-Exchange	360 ± 19	450 ± 22	750 ± 30	690 ± 42
Outcome 1. Improvement	9	4	2	2
2. No response and progression to renal failure	3	2	1	2
3. Death	0	0	0	0

Pulmonary haemorrhage occurred in 10 patients, and was rapidly controlled in 4.

Long-term follow up revealed that 8 patients are still alive, five with functioning allografts and 3 maintained on dialysis.

The changes in values of serum creatinine and anti - IgM Ab (% binding) produced by plasma exchange in the 10 patients with Goodpasture's syndrome are given in Table 42. Both serum creatinine and anti-IgM Ab were significantly lowered by plasma exchange. Improvement in clinical status was noted in 6 patients, 4 patients proceeded to dialysis and 2 ultimately died. In both sets of patients the recovery of renal function was clearly related to the severity of glomerular damage. The prognosis for patients presenting with creatinine of 550 μ mol/l or less was significantly better than for those with creatinine of 600 μ mol/l or greater.

9 Aplastic Anaemia with Pure Red Cell Aplasia

The benefit conferred by plasma exchange on four patients with red cell aplasia and on two patients with aplastic anaemia is seen from Table 43. The increase in the numbers of CFU-C units post-plasma pheresis is significant in all patients.

TABLE 42

Results of Regular Plasma Exchange in Goodpasture's Syndrome

PATIENT'S NUMBER	SERUM CREATININE (μmol/l)		ANTI- IgM Ab (% BINDING)		PATIENT RESPONSE TO PLASMA EXCHANGE		
	Pre-Exchange	Post-Exchange	Pre-Exchange	Post-Exchange	Improved	Dialysis	Death
1	750	750	100	40	No	Yes	Yes
2	400	205	190	30	Yes	No	No
3	450	225	65	25	Yes	No	No
4	520	380	75	35	Yes	No	No
5	690	610	80	35	No	Yes	Yes
6	800	950	135	55	No	Yes	No
7	540	400	70	20	Yes	No	No
8	700	580	100	30	Yes	No	No
9	695	590	95	25	No	Yes	No
10	395	250	55	15	Yes	No	No

TABLE 43

Effect of Plasma Exchange on Pure Red Cell Aplasia and Aplastic Anaemia

Patients	Age/Sex			Nos of CFU-C in Presence of		Control
				Pre-plasma pheresis plasma	Post-plasma pheresis plasma	
PURE RED CELL APLASIA	1	58	M	1 ± 1	15 ± 5	12 ± 3
	2	46	F	2 ± 1	16 ± 3	12 ± 2
	3	41	M	1 ± 1	14 ± 3	9 ± 2
	4	34	M	3 ± 1	12 ± 2	10 ± 2
APLASTIC ANAEMIA	*5	24	M	2 ± 1	8 ± 1	6 ± 1
	*6	21	M	1 ± 1	6 ± 1	4 ± 1

* SLE

* IgM Cryoglobulinaemia

10 Polymyositis

Values of biochemical parameters Creatinine Kinase, Asparate Transaminase, Alanine Transaminase, and Lactate Dehydrogenase pre- and post-plasma exchange in all 10 patients, together with the changes in clinical status, are shown in Table 44. The values of CK, very markedly, AsT, AlT, and LDH were reduced post-plasma exchange. The clinical status of 3 patients improved by 1 grade and 7 patients by 2 grades.

11 Clinical Status of Patients in Study of Selected Disorders

Short term side effects observed in patients during their treatment for the selected disorders are summarised in Table 45 and considered in detail under two groupings, namely, those effects attributable to replacement fluids and those attributable to the exchange procedures.

Side effects attributed to the replacement fluid are :

1. Plasma protein solution (albumin). Undesirable side effects were relatively few, feverish reactions constituting the majority and there were 30 incidents of rigors (3% of all procedures), 16 occurrences of vasovagal attacks (1.6% of all procedures) and 21 cases of minor skin reactions, namely urticaria pruritis and burning sensations. These cases tended to be short lived and usually occurred in patients with well known sensitization

Table 44

Effects of Plasma Exchange on Biochemical Parameters and Clinical Status in Patients with Polymyositis

PATIENT DETAILS			CLINICAL STATUS		BIOCHEMICAL PARAMETERS PRE-PLASMA EXCHANGE				BIOCHEMICAL PARAMETERS POST-PLASMA EXCHANGE			
No	Age	Sex	Pre-Plasma Exchange	Post-Plasma Exchange	CK	AsT	AlT	LDH	CK	AsT	AlT	LDH
						(u/l)				(u/l)		
1	23	M	5	4	580	34	50	520	150	28	42	400
2	32	F	5	3	1200	63	58	816	380	50	42	520
3	34	F	6	4	960	72	39	750	250	42	30	602
4	35	M	5	3	480	86	46	650	100	56	36	600
5	38	M	4	3	2000	70	69	500	100	52	48	357
6	36	M	5	3	1500	65	69	475	480	62	40	529
7	29	F	4	3	1100	58	70	675	250	38	60	479
8	21	M	5	3	750	52	65	820	100	48	48	500
9	22	M	4	2	688	66	75	960	100	52	54	475
10	28	M	4	2	1275	75	89	450	475	50	75	300

GRADING

1 Minimal disability
2 Moderate disability but able to walk
3 Walking without assistance
4 Walking only with assistance
5 Bed bound
6 Totally incapacitated

BIOCHEMICAL PARAMETERS

CK - Creatinine Kinase
AsT - Asparate Transaminase
AlT - Alanine Transaminase
LDH - Lactate Dehydrogenase

Table 45

Short-Term Side Effects observed during Treatment of Selected Disorders

SIDE EFFECTS	Number of Procedures in which side effects were observed	Number of Side Effects Attributed to	
		Plasma Protein Solution	Poly-geline
1. Vasovagal Attack	28	16	12
2. Rigors	36	30	6
3. Urticaria	50	21	29
4. Nausea and vomiting	13	7	6
5. Bronchospasm	2	1	1
6. Anaphylaxis	1	0	1
7. Hypotension	12	10	2
8. Bleeding	1	1	0

Total Number of patients 106
Total Number of procedures 1078

to blood or its derivatives.

2. Haemaccel (Polygeline). The most frequently observed side effects are minor skin reactions, 29 cases of urticaria were noted (2.9% of all the procedures) followed by 12 instances of vasovagal attacks (1.2% of all procedures) and 6 occurrences of rigors (0.6% of all procedures).

   Taking into account that the number of procedures in which Haemaccel was used was considerably fewer than that which plasma protein solution was used, the incidence of side effects with Haemaccel was greater than that with PPS.

3. Fresh Frozen Plasma. Very little fresh frozen plasma (2 litres only) was used in the study and no side effects were noted.

Side effects attributed to the exchange procedure include :

1. Hypotension. Hypotension was the commonest side effect encountered. It tended not to produce symptoms and was noted only because all patients were constantly monitored throughout the procedure. The lowest blood pressure recorded was 80/60 and responded to simple non-invasive measures.

2. Bleeding. Only one episode of bleeding was encountered in the total number of procedures undertaken. Its aetiology was uncertain. Only small doses of Heparin, namely 7,500 IU were used as anticoagulant making it an unlikely contributory aetiological factor.

3. Removal of Medication. Many of the patients treated for myasthenia gravis were given a supplement of medication during and occasionally after plasma exchange to ensure the regular level of medication in the blood necessary to maintain a stable clinical state.

Long-term effects observed in patients during and, in some cases, after their treatment for selected disorders were identified as under :

1. Hypercoagulable state

The studies have confirmed that intensive plasma exchange profoundly influences coagulation parameters and results in a thrombocytopenia, elevation in prothrombin time, kaolin Cephalin clotting time and reptilase time and a reduction in factor level.

In addition reductions in antithrombin III and plasminogen levels with an associated rise in Factor VIII pro-coagulant levels have been observed. This results in a hypercoagulable

state and in fact spontaneous thrombotic episodes. There is also some evidence to suggest that activation during the mechanical stimulus of plasma exchange may also contribute to the hypercoagulable state. In the present study, 2 cases of thrombotic episodes were observed, deep venous thrombosis occurred in one female patient and iliofemoral thrombosis in one male patient. Four patients had proven thrombosis i.e.positive venograms approximately 72 hours after their 5th plasma exchange. Treatment consisted of intravenous heparin followed by oral warfarin therapy for some 3 months. There were no apparent residual side effects and no known pre-disposing features.

2. Bacterial infection

Intrinsic plasma exchange has been shown to reduce a marked depletion of immunoglobulins. This can result in the possibility of complicating infections in patients also receiving immunosuppressive therapy, however it is known that many variables are involved. In the present studies 3 cases of proven bacterial infection were recorded. Esherichia coli was observed in one female patient and staphyloccocus aureus in one female and one male patient. All 3 patients developed septicaemia with positive blood cultures. All were receiving immunosuppressive therapy throughout the period of plasma exchange and were being ventilated in the intensive care unit. The time lag post final exchange to the

development of symptoms was 72 hours. After receiving the appropriate antibiotics all patients recovered without residual problems. No other patients in the unit at that time had any septicaemic episodes.

3. Viral Infection

Information regarding the incidence of viral infection post-plasma exchange was incomplete. Cases of hepatitis A, hepatitis B and non-A non-B hepatitis have been reported.

In the present study 2 cases of hepatitis A and 13 cases of non-A non-B hepatitis were recorded, involving 8 female and 7 male patients. The 13 cases of non-A non-B hepatitis were discovered in follow-up biochemical analysis of patients. All patients were transiently jaundiced, but they were not systemically unwell. The mean period of time from diagnosis to the end of the last plasma exchange was 3.5 months. Long-term follow-up of these patients is continuing, but no patient has more than a modest elevation of transaminases on routine biochemical estimation.

It is difficult to avoid the complication of hepatitis if plasma is used as replacement fluid. The use of fresh frozen plasma as a replacement fluid has resulted in an even higher incidence of hepatitis. The long term follow-up of patients who presented from outlying areas proved very difficult and this aspect of the study is incomplete. As far as is known at present no patient developed side effects which have had

deleterious consequences on his/her health.

Long term effects associated with plasma exchange have been reported, but it is not clear whether these are directly attributable to the procedure. Specifically, no patient has contracted the Human Immuno-Deficiency Virus (HIV) as a result of blood products given during plasma exchange over the period of this study.

The change in clinical status of all patients studied above is summarised in Table 46.

3.3 STUDY OF LEUKAPHERESIS

The results of the study of leukapheresis are shown in Table 47. The volume of buffy coat cells removed during leukapheresis ranged from 300 ml to 1300 ml with a mean of 750 ml. This volume contained a mean yield of 4.50×10^{11} white cells. The calculated efficiency of the procedure was 52%.

The main complication encountered during leukapheresis was maintenance of venous access. Many patients had poor veins and thus obtaining a satisfactory flow rate was a frequent problem. The procedure was safe, efficient and rapid.

In patients with acute leukaemia there was no evidence that leukapheresis provided clinical benefit. There is some evidence to suggest that early death is lower in patients who have

TABLE 46

Clinical Status of Patients with
Selected Disorders after Treatment with Plasma Exchange

DISORDER	Number of Patients with Disorder	PATIENT STATUS AFTER TREATMENT		
		Condition Improved	No Change in Condition	Condition Deteriorated
Waldenstrom's Macroglobulinaemia	18	16	2	0
Multiple Myeloma with Hyperviscosity	12	10	2	0
Cold Haemagglutinin Disease	8	7	1	0
Autoimmune Haemolytic Anaemia	3	1	1	1
Factor VIII Inhibitors	8	8	0	0
Myasthenia Gravis	18	15	3	0
Eaton-Lambert Syndrome	5	3	0	2
Guillain-Barré Syndrome	27	27	0	0
Polyneuropathy	15	10	2	3
Glomerulonephritis	26	19	0	7
Goodpasture's Syndrome	10	6	0	4
Pure Red Cell Aplasia	4	2	0	2
Aplastic Anaemia	2	2	0	0
Polymyositis	10	10	0	0

TABLE 47

Summarised Results of Leukapheresis Study

TYPE	Numbers Of Patients Treated	MEAN WBC x 10^9/1		MEAN Hb x 10^9/1		MEAN DURATION	CLINICAL STATUS		PATIENT SURVIVAL	
		Pre-Exchange	Post-Exchange	Pre-Exchange	Post-Exchange		Improved	No Response	Number	Percentage
Acute Leukaemia										
Lymphatic	5	54.2	43.6	8.8	7.9	3 days	2	3	3	60
Myeloid	14	46.1	38.2	9.6	8.5	5 days	6	8	3	21
Myelomonocytic	3	23.3	14.1	5.8	4.7	5 days	0	3	1	33
Chronic Leukaemia										
Myeloid	25	180	136	9.5	10.8	14 months	20	5	23	92
Lymphatic	11	96	68	11.2	12.3	36 months	7	4	10	99

undergone leukapheresis, but is statistically significant only in patients with acute lymphatic leukaemia.

In those patients with chronic myeloid leukaemia it did not appear to delay blast transformation but it did provide clinical benefit to the patient. The procedure reduced the WBC by at least 40% within 3-5 days of the procedure. It may be continued for some days thereafter. Improvement clinically was noted in 40% of patients, primarily those with neurological dysfunction e.g. blurred vision, confusion and in one case pre-coma. No distinct measurable improvement was noted in other parameters e.g. hepatomegaly, splenomegaly, lymphadenopathy or fever.

4. DISCUSSION OF RESULTS

Rarely have investigations of plasma exchange been conducted under conditions which permit a direct comparison of the results of the many different studies. However, from a number of such studies, characteristic patterns of behaviour, or significant trends in results have emerged despite great differences in procedural and pathological contents. Attention is drawn to correspondence or lack of correspondence between the results of the present studies and established findings of these other studies, where appropriate.

4.1 Study of replacement fluids

The observed values of the haematological and biochemical parameters pre- and post-plasma exchange, and the trends in the change of values where these occur, are in broad agreement with those reported in the literature.

The observed stability of the haemoglobin concentration, white blood cell count and haematocrit value has been reported in other studies.[1,59,98] The sharp decrease in platelet count has also been recorded by others,[2,142] although in other studies[1,59,98] no significant change in platelet count was found.

The investigation has shown that platelet concentration fell only if exchanges were conducted on a daily basis and that the time needed for the recovery of platelets to normal haemostatic levels ($100 \times 10^9/1$) was dependent mainly on the

intensity of the preceding exchange. Regardless of the actual level, it required only an average of two days (maximum of three days) for platelets to return to normal.

The reduction in platelet count must be interpreted in relation to the initial value. Whilst a small number of platelets are lost in the limited clotting which occurs in the centrifuge bowl, the observed decrease in platelets must be attributed to the removal during plasma exchange. The recovery of platelet counts will depend primarily on the state of the bone marrow and particularly on the effects of steroids and cytotoxic drugs. Protamine sulphate can decrease platelet counts but the effect is transient.

Thus, the lowest platelet counts and the greatest risk of bleeding occur during periods of daily plasma exchange when the initial counts are low.

The marked increases observed in prothrombin time, kaolin cephalin clotting time and thrombin clotting time immediately post-exchange are in accord with those reported in other studies,[2,142,143,263] although no such changes were recorded in another study.[59]

However, whilst the trends in behaviour are similar, differences exist in the extent of the changes noted. Increases of 60% and 200% in prothrombin time and thrombin clotting time immediately after the eleventh exchange procedure have been recorded in one study where the exchanged plasma was replaced by an equal volume of albumin 5% or plasma protein fraction

5%. The thrombin time and prothrombin time returned to the initial pre-exchange value in 4 hours and 24 hours respectively, post-exchange. Even greater increases were noted in another study[2] in which plasma protein fraction, plasma protein fraction and Haemaccel (4:1 ratio) and fresh frozen plasma were used as replacement fluids. Mean post-exchange values of patient-control differences in TT and PTTK were found to be 200% and 1000% greater than the mean pre-exchange values immediately after the first exchange, or after a succeeding exchange with an interval between exchanges of more than 5 days. These increases in clotting times became even greater as the interval between exchanges was reduced. Immediately after successive daily exchanges, mean patient-control differences in TT and PTTK of 700% and 3000% of mean pre-exchange values were recorded.

The effect of plasma exchange on coagulation factors has been extensively studied by others[2,142,143] and the reported behavioural trends in post-exchange values are essentially similar to those noted in the present investigation. In general, the percentage reduction in the post-plasma exchange relative to the initial pre-exchange value of each coagulation factor was not significantly influenced by the patient's disorder or by the length of interval between successive exchanges (one day, two day, or more than 2 day periods). The extent to which coagulation factors were removed by plasma exchange did not appear to be influenced by their initial pre-exchange values. A single exchange procedure reduced the post-exchange values of Factor I, II, VIII, IX and X to approx-

imately 30%, 35%, 45%, 60% and 40% respectively of the pre-exchange values.[2,142,143,150,264]

It was noted that the extent to which Factor VIII was removed by plasma exchange, as estimated by biological assay differed significantly from that estimated by immunological assay.

In plasma exchanges undertaken on a daily or two-day basis the observed cumulative reduction of Factors I, II, and X was greater than that of Factor IX and this was in accord with results reported in the literature. Under these conditions there was considerable variation in the extent of reduction for different patients, but the overall reduction patterns were similar. Factors I, II, and X exhibited slower rates of recovery than Factors VIII and IX, but they usually returned to normal values within the 24 hours period after exchange. Recovery was usually rapid during the early stages post-plasma exchange but therefter recovery was more gradual over the period 4 hours to 24 hours post-exchange.

The discrepancy reported by others as existing between biological and immunological levels of Factor VIII in most patients may be due to true preferential removal of one component because Factor VIII coagulant activity and Factor VIII related antigen may reside on separate molecules. Alternatively the excess coagulant activity may arise as a result of protease activation.

Detailed information on electrolyte change during plasma

exchange is sparse and no comparison of the current findings with other data has been possible. With the exception of potassium and calcium the pre- and post-plasma exchange values of the electrolytes are eventually stable. In two studies, where the electrolyte values have been determined, but no data presented, it must be presumed that values remain stable during plasma exchange.[1,97] Where data are presented, one report quotes values of Sodium and Potassium unchanged by plasma exchange and another shows reductions in the values of potassium and calcium after plasma exchange. Renal function between plasma exchanges as measured by serum urea and creatinine concentration did not deteriorate.[59,149]

No published data have been found for changes in liver function parameters, bilirubin, asparate and alanine transaminase. The decrease in the post-plasma exchange values of alkaline phosphatase relative to the pre-exchange values observed in the present investigation is comparable to that quoted in one study.[143]

The changes in the values of total protein and albumin observed in the present investigation, coupled with the stability of the calculated values of globulin following plasma exchange using polygeline as replacement fluid have been noted in two other studies in which plasma was replaced by polygeline (Haemaccel).[1,264] The pattern of changes observed after plasma exchange in which plasma protein solution was used as the replacement fluid, namely the decrease in total protein and calculated globulin coupled with

stable albumin values are similar to those established elsewhere.[1,59,264]

This difference in behaviour, as a consequence of the replacement fluid used, has little clinical significance because total protein and albumin return to near pre-exchange values in 24 hours.

The observed values of serum iron and total iron binding capacity indicate that iron deficiency is unlikely to arise because of the replacement fluid selected. No other data on iron deficiency have been found in the literature and a comparison of findings is not possible.

The variation in immunoglobulin IgA, IgG, and IgM concentrations noted during the present series of plasma exchanges accords with the reported data from an extensive number of studies.[3,97,98,141,143,264] One study reports reductions in IgG, IgA and IgM concentrations to less than 45% of their initial pre-exchange values after the first exchanges, to less than 30% after the fifth consecutive daily exchange and to less than 10% after the tenth consecutive daily exchange. This behaviour was not affected by the plasma volume exchanged nor by the partial replacement of plasma by Haemaccel in the case of two patients only. The patterns of recovery established for serum IgG, IgA and IgM concentrations were similar. Concentrations continued to decrease in plasma exchanges conducted on a daily or two-day basis but increased if the interval between exchanges exceeded two days. The

results from the other studies confirmed these trends.

The mean percentage reductions in these proteins probably reflect differences in their compartmental distribution ratios. The largely intra-vascular IgM is more effectively removed than the more equally distributed IgA.

The effect of immunosuppressive therapy on IgG concentrations has been extensively studied. Both decreased synthesis and increased fractional catabolic rate have been noted in patients receiving steroids and/or cyclophosphamide and azathioprine. The absence of a significant effect of immuno-suppression during daily exchanges, confirmed by the present study, may be explained by the relatively large contribution in the period immediately after plasma exchange of extra-vascular to intra-vascular re-distribution of IgG and IgA. This mechanism which will effectively increase the serum IgG and IgA concentrations is unlikely to be affected by immuno-suppression.

The response of patients to plasma exchange, as assessed by changes in their haematological and biochemical parameters measured before and after the exchange procedures, differed in its detail within the three groups of patients examined. In the comparisons of behaviour of patients from each of the three groups, no significant differences were observed in the values of many of the parameters measured, whilst significant differences were detected between the values of others. No obvious pattern of dissimilar behaviour was apparent in any

series of measurements or in any specific group of patients. In general, there were fewer differences between parameter values which were significant than were not significant. It was concluded that, despite the many random differences between the measured haematological and biochemical parameters before and during the period after plasma exchange, the overall response of patients in the three groups was basically similar and thus was not influenced by the choice of the replacement fluid used in the study. Clinical examination confirmed that the response of patients from all three groups to plasma exchange under these conditions was essentially similar. No differences were observed in the incidence of side effects as a consequence of the plasma exchange procedures and which could be attributed to the use of Polygeline I, plasma protein solution, or Polygeline II.

The findings of the present more comprehensive study involving much greater numbers of patients, are in general accord with the results of more limited studies on relatively few patients and in which polygeline (Haemaccel) and plasma protein fraction were used as replacement fluids.[1,2,3,264]

4.2 Study of selected disorders.

4.2.1 Cold haemagglutinin syndrome.

Cold haemagglutinin syndrome[265-268] accounts for 18% of all cases of autoimmune haemolytic anaemia. It is classified as:-

1. Idiopathic - which occurs in the elderly and is

characterised by a gradual onset with a peak incidence in persons over 60 years. The antibody is due to a proliferation of a clone of IgM cells. It is usually a chronic problem and not severe.

2. Secondary - which commonly occurs in
   (a) certain infections, such as mycoplasma pneumonia, infectious mononucleosis and viral infections, particularly cytomegalovirus infection. Only rarely will the titre of cold agglutinins rise to a particularly high level and bring about haemolysis in-vivo. The problem is rarely severe and the titres return to normal in 3-4 months.
   (b) Lymphoreticular disease - certain patients with lymphoreticular disorders develop cold agglutinin disease. It appears to be the result of a malignant proliferation of a clone of B cells, the lesions being classed as histiocytic lymphoma. There is also an association with adenocarcinomas occasionally with squamous cell carcinomas.

Clinical features are characterised by acrocyanosis with purplish discolouration of the skin on exposed parts of the body of many patients. When patients are severely affected, they may be unable to endure contact with any cold materials including cold food, such as ice cream: this will cause both pain in the fauces and oesophagus. The agglutination may occasionally be seen in conjunctival vessels or in the nail bed on microscopic examination. The spleen is frequently enlarged, but rarely massively so.

All patients exhibit the characteristics summarised in Table 48 below.

TABLE 48

Serological Characteristics of Patients

DAGT	ELUATE	IMMUNOGLOBULIN TYPE	SPECIFICITY
POSITIVE	NO ACTIVITY	IgM	ANTI-I

Plasma exchange is known to bring about a decrease in the cold antibody titre with a variable increase in haemoglobin level. In general only transient responses to plasma exchange have been obtained, the antibody titres returning to their pre-exchange levels in only seven days. The results of the present limited study confirm this view.

It should be noted that technical issues can arise with the removal of plasma because of the expectant agglutination of blood as it approaches room temperature.[265] In one case,[266] presumed to be post-infectious, the cold agglutinin titre actually rose during a period of intensive plasma exchange undergone in conjunction with steroids and cyclophosphamide. It was suggested, that this conformed with the observation that, during a presumed primary response, plasma exchange resulted in the loss of feed back control of B cell function with subsequent amplification of antibody production. This suggests caution in the use of plasma exchange in cases of low titre polyclonal IgM cold agglutinins that occur in an acute presumably post-infectious condition.

A study[267] of the effect of cytotoxic treatment on auto-antibody rebound that follows auto-antibody withdrawal showed that after each of two control plasma exchanges by the filtration method the titre in cold agglutinins declined markedly. Thereafter the gamma globulin endowed with auto-antibody activity increased steeply in parallel with the agglutinin titre to values exceeding the pre-treatment levels. There was only a slow rise of polyclonal IgM to pretreatment levels. Two further plasma exchanges were administered 15 days later, this time in combination with ARA-C and cyclophosphamide. No rebound occurred and the serum agglutinin titre remained stable at about the same level for the ensuing three months. The polyclonal IgM level was not greatly affected by the treatment.

The results of my own limited study confirm that plasma exchange is most successful at temporarily reducing the cytotoxic antibody levels and in allowing a prolongation of survival of the transfused cells.[268] It would be an appropriate treatment in acute haemolysis resistant to chemotherapy.

4.2.2 Waldenstrom's Macroglobulinaemia

Waldenstrom's Macroglobulinaemia[181-183,269] is a relatively uncommon, slowly progressive B-lymphoproliferative disorder in which the cells are intermediate in immunological maturation between those of the small cell lymphomas and the plasma cell dyscrasias. The disorder is rarely found in patients below the age of 40 years but occurs more frequently in patients of advancing age.

Small lymphocytes, plasmacytoid lymphocytes and plasma cells accumulate in the bone marrow, in lymph nodes and in the spleen. Many cells synthesise a monoclonal immunoglobulin, cytoplasmic or on the surface membrane and the same immunoglobulin is transferred in the serum. If the concentration of immunoglobulin becomes sufficiently high then Waldenstrom's macroglobulinaemia will result.

The clinical manifestations of Waldenstrom's Macroglobulinaemia are dominated by the large amounts of IgM secreted by tumour of small bulk which may not be detectable clinically. High serum concentrations may occur in the absence of physical signs and little or no bone marrow infiltration. Even when numerous lymph nodes are enlarged and the bone marrow is heavily infiltrated, bone marrow function may be well preserved and the lymphomatous element of the condition may remain static for several years.

IgM paraprotein, of molecular weight, IM Daltons, increases the viscosity of the blood at lower concentrations than IgG or IgA proteins. The value of viscosity increases very rapidly with increasing concentration in serum, so that above a concentration of about 30g/l small increases lead to large increases in viscosity.[270] Conversely a reduction in concentration over the same range rapidly lowers the viscosity. Thus the removal of the macroglobulin contained in 1 litre of blood usually lowers the viscosity sufficiently to bring about a rapid and dramatic relief of symptoms.

Because of its high molecular weight most of the macroglobulin produced is intravascular, so that any lowering of the

concentration achieved by its removal, is not rapidly reversed by the entry into circulation of macroglobulin from the extravascular compartment. Unfortunately the rate of production of macroglobulin is often so high that the serum concentration rises to its former level in a few weeks. The symptoms of hyperviscosity[271,272] include muscular weakness, lethargy, mental confusion proceeding to coma, purpura, haemorrhages from the gums, nose, gastrointestinal and genitourinary tracts, neuropathies, visual disturbances (e.g. bilateral retinal exudates, papilloedema) and deafness. Other neurological complications include ataxia, diplopia and dysarthria.

Symptomless patients with static Waldenstrom's disease are kept under observation without treatment and they may remain well for a decade or more. Symptoms arising from hyperviscosity are rapidly relieved by plasma exchange and may be managed for several years by periodic procedures.[272] Patients with progressive lymphomatous disease and declining bone marrow function require chemotheraphy to reduce the total mass of lymphocytes, e.g. chlorambucil, cyclophosphamide or melphalan are used alone or in combination with prednisolone. This reduces the bone marrow infiltration leading to an improvement in bone marrow function and it reduces the serum concentration of paraprotein. The hyperviscosity syndrome in patients who are refractory to chemotherapy may be controllable by plasmapheresis but rarely for more than a year. Similar characteristic responses to plasma exchange were observed in the present study of 18 patients all of whom exhibited hyperviscosity in association with Waldenstrom's Macroglobulinaemia.

Hyperviscosity due to IgA and IgG myeloma.

Hyperviscosity due to IgA and IgG M components are the second and third most common causes of hyperviscosity syndrome after IgM-M components.[185,186,187] IgA-M components tend to aggregate forming true polymers linked by disulphide bonds and containing J chains. With IgG-M components there is usually a very high concentration of the components present, or the presence of molecules with asymmetric configurations, or the presence of circulating aggregates of IgG-M components. Although hyperviscosity has been reported in association with the other IgG subclasses, most cases are due to IgG-3 myeloma proteins. The clinical manifestations are similar to those in Waldenstrom's Macroglobulinaemia, and the treatment is similar. Generally at least two to four litres of plasma have to be exchanged, every week or two.[273,274] Following plasmapheresis appropriate chemotherapy namely Melphalan and Prednisolone is instigated.

Similar characteristic responses to plasma exchange were noted in the present study of 12 patients all of whom exhibited hyperviscosity in association with multiple myeloma.

The observed response of patients to plasma exchange in the present study is similar to that reported by others. On one investigation[275] 9 patients with myeloma of the IgA type and 2 patients with myeloma of the IgG type, complicated by the laboratory and clinical features of hyperviscosity, were treated by plasma exchange. In those patients with the IgA

myeloma the hyper viscosity syndrome was related to the presence of high molecular weight complexes in the serum. Clinical improvements after plasma exchange coincided with a reduction of whole blood viscosity and in those patients with IgA myeloma, with a parallel reduction of the high molecular weight complexes. IgA and IgG are evenly distributed between the intravascular and extravascular spaces and following plasma exchange re-equilibrium would result in a secondary rise in plasma immunoglobulin concentration and in whole blood viscosity. The more delayed phase of the secondary rise of whole blood viscosity did occur but in the majority of patients this was of no clinical significance and only one patient required a second plasma exchange.

As a part of a more extensive study,[276] 14 patients with monoclonal and polyclonal gammopathies were treated by partial plasma exchange. The patients with varying clinical conditions (2 with light-chain disease, 6 with multiple myeloma and 4 with Waldenstrom's macroglobulinaemia),were subjected to repeated manual plasma exchange procedure (0.3 to 0.61 plasma/session). The removed plasma was replaced by 5% albumin solution or FFP according to the clinical condition of the patients. The two patients with light chain disease were treated in 3 sessions and plasmapheresis was effective. The remaining 10 patients were subjected to a total of 22 sessions of partial plasma exchange. In all but 5 sessions where the amount of plasma exchanged was inadequate, blood viscosity decreased and the clinical signs of hyperviscosity improved in these patients. In monoclonal gammopathies the improvement could not be

attributed to partial plasma exchange alone, because the patients were concurrently treated by cytotoxics. In polyclonal gammopathies the partial plasma exchange without any other specific treatment was sufficient to stop severe clinical symptoms.

4.2.3 Warm Immune Haemolytic Anaemia

It is not known why a person should suddenly produce antibodies apparently directed against his own inherited antigens. The incidence of the disease is difficult to assess, but it appears to be relatively high, perhaps, in the order of 1 in 80,000 population.

Warm autoimmune haemolytic anaemia[192-195] occurs in both sexes, but the incidence is higher in females than males. It can occur at any age but is more frequent in females over 40 years and males over 50 years of age. The mode of presentation is extremely variable. In some cases the haemolysis can be present insidiously and it may be months before the doctor is made aware of the patient's symptoms. In other cases there may be very rapid development of anaemia with extreme prostration and jaundice occurring over the period of a few days.

Autoimmune haemolytic anaemia may occur in association with various well defined disorders, in which case there will also be the signs and symptoms of the other condition. Fifty-six percent of cases are thought to be due to the idiopathic form of the disease where the autoimmune haemolytic anaemia with, or without, thrombocytopenia was unaccompanied by definite signs of another disease. Some 15% of cases were associated

with lymphoma and certain other malignant diseases, for example, carcinoma of the breast, carcinoid tumour of the small intestine and also teratoma of the ovary. The association of auto-immune haemolytic anaemia with SLE and other autoimmune disorders, namely thyrotoxicosis, Hashimoto's thyroiditis, myasthenia gavis, autoimmune hepatitis, ulcerative colitis, rheumatoid arthritis and pernicious anaemia is well known.

The commonest form of autoimmune haemolytic anaemia is defined as "Coomb's positive", i.e., positive direct antiglobulin test. All patients exhibit the characteristics summarised in the Table 49 below.

TABLE 49

Serological Characteristics of A I H A Patients

DAGT	COATING	ANTIBODIES	IMMUNOGLOBULINS
Positive	Non-complement	Rh antigens anti-LW	1 IgG IgG 1 2 IgA IgG 3 3 IgM rarely

The premature destruction of red cells is triggered by an immunoglobulin IgG or by complement components which are attached to antigens on the surface of the cell. Since the production of immunoglobulins in all immune anaemias is not dependent on a specific antigen, the presence of tumour-associated antigens and/or antigens of infectious organisms may play a role in the development of this immune disorder. The antibodies produced will have cross-reactivity with the

red cells and therefore initiate the haemolysis. The repetitiveness of destruction is closely related to the amount of antibodies or components of the complement system and the avidity of antibody binding to the red cells. In the human body there are not only single immune products but also immune complexes and the application of plasma exchange would be justified, although the removal of endogenous antigens such as those associated with the onset of autoimmune haemolytic anaemia would be very difficult. The removal of the rest of the humoral components, responsible for the severity of the haemolytic process, may be helpful in controlling rapidly progressing cases.

The standard treatment of acute haemolysis is usually corticosteroid therapy, splenectomy, and immunosuppressive therapy. These aim to decrease the destruction of antibody-coated red cells, thus producing antibody protection. Generally, however, these modalities take some time to act. Similarly, plasmapheresis offers scope for the removal of antibody, for the removal of antigen, for the removal of antigen and antibody complexes and for the removal of a combination of the first three. The response to plasmapheresis is much faster than that to the standard modalities and in the case of fulminant haemolysis plasma exchange could be beneficial in the interim period.

The reported evidence on the efficiacy of plasma exchange in the management of haemolytic anaemia is conflicting and the present study is too limited to offer clarification. The trends in the three cases are however similar to those noted

in other studies.

There are isolated case reports[265,277,278] suggesting that plasma exchange was effective in the treatment of Evans' syndrome in the rapid stabilization of an acute, possibly post-infectious haemolytic process[195,265] and in the treatment of non-Hodgkin's lymphoma.[265]

It should be noted that plasma exchange can be dangerous in patients with haemoglobin concentrations of less than 3g/dl who cannot tolerate shifts in their plasma volume. However, plasma exchanges of even as little as 2 l. of plasma can often result in a dramatic decrease in the intensity of the direct anti-globulin test, thus allowing a patient's blood to be matched, permitting blood transfusion and ameliorating symptoms. Where other forms of treatment have failed plasma exchange offers alternative therapy.[194,279,280]

4.2.4 Factor VIII Inhibitors

Acquired pathological inhibitors of coagulation are directed against a specific coagulation factor. Factor VIII inhibitors are the most frequent and occur as a complication of treatment in patients with classical haemophilia A. They can also occur in patients regarded previously as haemostatically normal. The reported incidence in patients with haemophilia of inhibitors of Factor VIII varies between 5-20%, and presents some of the most serious and challenging problems that can be associated with the treatment of haemorrhagic disorders. Acquired inhibitors are associated with a variety of chronic

disorders of auto-allergic type. The latter include rheumatoid arthritis, rheumatic heart disease, systemic lupus erythematosus, pemphigus, dermatitis, herpetiformis, penicillin hypersensitivity, Crohn's Disease, and ulcerative colitis. They also occur in women after childbirth and spontaneously in apparently healthy people.

The clinical course of inhibitors is unpredictable, but they usually disappear within months or years provided that the patient does not succumb to haemorrhage in the acute stages of the disease. There is not much evidence that treatment with steroids or immunosuppressive therapy alters the natural course of the disease.

The majority of the antibodies to Factor VIIIC are of the IgG class although IgA and IgM inhibitors have been described. They show remarkable homogeneity with only a single light chain type present, usually kappa and they have been found to be of IgG subtype. The antibodies can be detected by incubating test plasma at 37°C with a source of Factor VIIIC and performing serial coagulation tests. In our laboratory the limits of inhibitor activity are measured in Bethesda units.

The antibodies are known to be present in plasma and serum and are stable at 56°C for 30 minutes. They slowly inhibit FVIII during incubation at 37°C.

The treatment of bleeding in haemophiliacs with antibiotics is difficult since the inhibitor titre rises after replacement

therapy. Where the bleeding is moderate, FVIIIC replacement should be avoided if possible. However if life threatening haemorrhage occurs, large amounts of FVIII are required to neutralise the antibody and increase the level of FVIII coagulant activity. In some cases porcine or bovine FVIII have been infused.[281] Activated FIX preparation, and daily infusions of activated concentrates (FEIBA) have also been used successfully. Immunosuppressive therapy has also been used.

The clinical course in non-haemophiliac patients with inhibitors is much more variable than in haemophiliac patients. Bleeding episodes can be exceedingly difficult to treat and there is significant mortality.[204,282] Following FVIII infusions an anamestic response is not invariable but may still be quite marked.

Plasma exchange has long been recognised as an option in the management of Factor VIII inhibitors and the results of the present limited study of patients with a variety of conditions confirms this view. The observed trends in the values of coagulation screening Factor VIIIC, Factor VIII Rag and inhibitor screen are similar to those reported in other studies.

In one such study[283] 2 plasma exchanges were performed in a three week period in the surgical management of a subdural haematoma. An elderly patient with a sub-lingual haematoma was given 6 exchanges of between 1.5 to 6.0 l each over a period of 13 days whilst a patient being prepared for elective surgery was exchanged 8 times over a 6 week period.

Further,[282] a patient who had developed a very large abdominal wall haematoma after appendicectomy was given 14 plasma exchanges each of 4 l over 15 days. Bleeding stopped after the second exchange with factor concentrate and complete healing followed without other treatment. Haemostasis was also achieved in a patient with benign prostatic hypertrophy after a 4 l plasma exchange followed by Factor VIII prior to transurethral section. The patient made a complete recovery and his plasma showed only minimal residual inhibitory activity.

A further study[284] reported the successful use of plasma exchange in the treatment of a patient with a massive anterior abdominal wall haematoma following an uneventful caesarian section for foetal distress, a patient with large ileopsoas haematoma and femoral nerve palsy and a patient with prolonged post-dialysis bleeding from fistula arterial and venous puncture sites.

To be effective large volumes of plasma (3-5 l)have to be exchanged and the flow of antibody from the extra vascular space requires the procedure to be repeated on several occasions, although immediately following each exchange full Factor VIII coagulant activity may be achieved with Factor VIII infusions.

Plasma exchange is much less expensive than the use of highly purified porcine Factor VIII, superdose Factor VIII and activated prothrombin complex concentrates.

4.2.5 Relapsing Inflammatory Polyneuropathy and Chronic Inflammatory Polyneuropathy

The natural history of acute inflammatory polyneuropathy[175,285-287] is progressive over one to two weeks with a subsequent delay of one to two weeks prior to an improvement which is then followed by further improvement which may be rapid or gradual. Less than 10% of patients experience relapse. Thus chronic inflammatory polyneuropathy occurs in people who have had a previous episode of acute polyneuropathy with a recurrence in rapid or slow fashion. The relapsing form may show excellent recovery if the episodes are acute at onset, but with repeated episodes accumulating disability may occur. Chronic inflammatory polyneuropathy occurs when there is progression in a monophasic or stepwise manner over more than 30 days, usually over more than three months.[285]

In most instances the aetiology is uncertain and the following associated diseases are known to be connected with disorders in the immune system: monoclonal gammopathy, paraproteinaemia, multiple myeloma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, systemic lupus erythematosus, macroglobulinaemia, cryoglobulinaemia, amyloidosis and polyclonalgammopathy. This association is probably not by chance.[285]

Conventional treatment has been based on corticosteroids and immunosuppressive drugs, but their efficiacy is often incomplete and in some cases patients do not respond.

Plasmapheresis was first used in 1978 for the treatment of acute polyneuropathy. Subsequent reports[285,286] have suggested improvement in some or all of the patients treated. The limited response to treatment by plasma exchange by patients in the present study is similar to that found in other investigations. Whilst there have been many reports[288] of successful use of plasma exchange in the treatment of chronic inflammatory polyneuropathy even in cases where Prednisolone and Azathioprine had failed,[286-289] there are also reports where treatment has been unsuccessful[288,289] and it does not seem possible to predict which forms will respond to plasma exchange therapy. In one study,[289] patients who did not respond had electrophysiological signs, suggesting severe axonal lesions. The beneficial effect was also observed in patients whose liver biopsy showed segmental demyelination with little axonal destruction.[290] Since patients with prominent axonal degeneration failed to respond to plasma exchange, the magnitude of the axonal lesions could thus be important in determining the efficacy of plasma exchange.[290]

In a controlled double blind trial[175]29 patients with a disability score of greater than 50 points were randomly assigned into twice-weekly whole plasma volume exchanges or sham plasma exchanges for a period of 3 weeks. All patients in the sham procedures were subsequently treated by plasma exchange. The neurological deficit was monitored periodically; nerve conduction, computer assisted sensory examination and various quantitative measurements of muscle strength were noted. At entry the groups were comparable. Five of 15 patients treated

by plasma exchange showed a greater improvement in the disability score than was observed in the control group. Of the 14 patients receiving plasma exchange after the sham procedure, 4 showed a greater improvement than had been found after the sham treatment only.

If plasma exchange is beneficial its mechanism is yet to be clarified. The clinical improvement is much too rapid to be explained by a remyelinating process. It has been suggested improvement may stem from the clearance of a substance responsible for a reversal of conduction block.[290] Several findings are consistent with the role of a humoral factor in the pathophysiology of the disease. Many reports have shown evidence of IgG and IgM deposits at the surface of nerves as well as the existence of antimyelin antibodies. In one responding patient antibodies of the IgG class have been found directly against the human sciatic nerve. A conduction block has been observed after injection of the serum in one patient. The block although markedly present before plasma exchange persisted but decreased as the patient's condition improved. No conduction block could be induced with the serum of another patient. No evidence of myelination was found after injection of serum of patients who were successfully treated with plasma exchange. The existence of a circulating factor responsible for a conduction block has yet to be confirmed.[290] At the present time it is not possible to determine whether plasma exchange should be used in:

1. Chronic inflammatory demyelinating polyneuropathy resistant to steroids and or cytotoxic drugs.
2. Severe forms with respiratory impairment for which prompt amelioration is desirable.
3. Any case of chronic inflammatory demyelinating polyneuropathy in the hope that should the patient improve it will be possible to reduce the dosage of steroids and cytotoxic drugs required.

4.2.6 Myasthenia Gravis

Myasthenia gravis[209-212,291-293] is a disorder of the neuromuscular junction characterised by weakness and easy fatigue which have a marked diurnal variation with noticable deterioration as the day progresses. Symptoms are generally improved by rest. The symptoms occur in women, generally, between the ages of 20 and 30 years and in men between the ages of 50 and 70 years. In the younger age group the onset is gradual with spontaneous improvement over the first one to two years. In the older age group the disease usually shows consistent progression.[291]

The disorder is characterised by asymetrical ptosis, diplopia, nystagmus of the extra ocular muscles, difficulty with chewing and swallowing and proximal weakness predominantly in the deltoid and iliopsoas muscles.[291] Myasthenia gravis is an auto-immune disorder in which the pathogenic role of the specific auto-antibodies for the neuromuscular symptoms is well documented. These antibodies are directed against the acetylcholine receptor in the post-synaptic end-plate of the

skeletal muscle. The receptor antibodies disturb the neuro-muscular function by blockade of transmitter binding, by increased rate of internalisation of receptors and by complement-mediated destruction of the post-synaptic end-plate. All these mechanisms lead to a relative defect in functioning receptors.[294]

There is now evidence for a spontaneous occurrence of anti-bodies complimentary to the receptor antibodies. These anti-bodies bind idiotypic determinants on the receptor antibody and should be defined as auto-anti-idiotypic antibodies.

Auto-anti-idiotypic antibodies have also been reported to occur in other auto-immune disorders. Auto-anti-idiotypic anti-bodies are, under certain conditions, able to drive the differentiation of B cell pre-cursors to the state of anti-body production.[294]

The prevalence in concentration of acetylcholine receptor auto-anti-idiotypic antibodies is especially high during the early stages of myasthenia. Receptor antibody of the IgM type, although on very low concentrations, is also found more often in early and in late disease. Both receptor antibodies of IgM class and acetylcholine receptor auto-anti-idiotypic anti-bodies are decreased in concentration as the IgG receptor anti-bodies rise. The disease may be treated by administering

1. Anticholinesterase drugs to enhance remaining functions.
2. Immunosuppressive drugs for auto-antibody production.

> Acetylcholine esterase antibody synthesis can be reduced by steroid, anti-metaboliser alkalating agents, but they are not rapidly effective in controlling symptoms. (At least 3-12 months are required to achieve maximum benefit).[295]

Thus the combination of plasma exchange (which rapidly produced onset of benefits) with steroids and antimetabolites (which give long term control) should offer scope in the treatment of this disorder.

In the present study plasma exchange failed to secure improvement in only three patients whilst in 15 patients there was an improvement usually of 2 or 3 grades. This trend is similar to that observed in other studies.

A reported protocol[295] for the treatment of myasthenic patients, consisting of 3 exchanges each of 2 plasma volumes or 4 exchanges of 1.5 plasma volumes over a 7 day period sufficed to clear 65% of IgG and 84% of anticholinesterase antibodies. In the present study, the protocol used removed from 60 to 88% of Anti-Ach R.

In one study[245] 27 patients were submitted to immune-modulatory protocol treatment because their condition was deteriorating rapidly and was not responsive to immunosuppression alone or they exhibited chronic disabling symptoms incompletely responsive to immunosuppression alone or were steroid dependent. Twenty-two percent of patients achieved complete remission at

the end of the plasma exchange cycle, 56% showed marked improvement, 15% mild improvement and 7% were unchanged. After 12 months only 22% of the patients worsened and received a new cycle of plasma exchange therapy. Patients with short duration disease (less than 6 years) responded promptly to treatment while patients with long duration disease were low responders suggesting a very low number of reworkable acetyl-cholinesterase receptor. No correlation could be found in the non-responder group. Side effects included 12 cases of citrate toxicity, 6 allergic reactions to plasma components, one case of mild myasthenic crisis in a non-responder patient and 5 cases of non-A and non-B hepatitis.

In another study[296] 60 patients were treated with plasma exchange and varying immunosuppressive therapy, namely corticosteroids, and azathioprine, azathioprine alone and cyclophosphamide alone. Thirty nine patients had prior thymectomy, 42 had prior corticosteroid therapy. In patients with a mean illness of about 3 years duration the disease disappeared. In patients with a mean illness of greater than 10 years duration the response to treatment was less marked. The incidence of thymoma was much higher in the best responding patients and non-thymoma patients appear to be less amenable to plasma exchange although this could be related to the duration of the illness.

The anti-acetylcholinesterase receptor titre did not predict the response to plasma exchange but the percentage reduction of autoantibody titre correlated with the clinical response to the exchange.

Some patients required to be exchanged every 2, 3 or 4 weeks. All were chronically severe, the majority were respirator dependent, all had thymectomy, none had had thymoma and all had received maximum treatment with Prednisolone and Azathioprine and a large number of exchanges (40-100) over a long period of time. Patients were switched from Azathioprine to Cyclophosphamide during treatment and all attained stable remission without further exchanges.

Thus plasmapheresis has an accepted role in the treatment of patients with disabling or life-threatening symptoms, to hasten the onset of control or to maintain control of symptoms during the latent period of immunosuppressive agents.

Lambert Eaton Myasthenic Syndrome.

This is a rare disorder, sometimes associated with carcinoma (usually of the oat-cell type) or with other auto-immune diseases.[297] It produces proximal weakness, loss of tendon reflexes and autonomic effects (dry mouth, constipation, impotence). It is a pre-synaptic disorder in which the number of packages of acetylcholine released by each nerve impulse is greatly reduced.[298] Recent evidence indicates that this defect is due to an IgG auto_antibody binding to nerve terminal determinants.[299] Freeze fracture studies of Lambert Eaton cases show abnormalities of the active zone particles at the nerve terminal which may be the target for the auto-antibody.

Part of the evidence for the auto immune nature of this disorder is the response to plasma exchange.[299,300] The latency

of the clinical response is about 10-20 days (and not all patients respond). This latency is much greater than that observed in myasthenia gravis and presumably reflects the slower turn over rate of the structures to which the antibody binds. For this reason ten or more exchanges in a single course may be required to observe a clear response. Patients with the non-carcinomatous form of the disease respond favourably to immuno suppressive drug treatment (prednisolone and or azathioprine) but as in myasthenia gravis there is no evidence that plasma exchange enhances the efficacy of such treatment.

The results of the present limited study support this view in that the condition of three patients improved whilst that of two patients deteriorated.

In one study [299] the syndrome associated with carcinoma of the bronchus in one patient and with immunological disorders in two patients improved after plasma exchange. Prednisolone and azathioprine treatment led to almost complete remission in one and improvement in the other of the non-neoplastic cases. It also indicated that an IgG auto antibody binding to nerve terminal determinants may be responsible for the disorder of the neuromuscular transmission.

The passive improvement that PE produces, particularly in the less severely affected patients makes the procedure a useful additional treatment for the disease.

4.2.7 Guillain-Barré Syndrome (Acute Inflammatory Polyneuropathy)

Acute inflammatory polyneuropathy[219-222,301-303] is a segmental demyelinating polyneuropathy with paralysis sensory impairment and autonomic dysfunction. The disease may follow many different types of infection, surgical procedures, or vaccinations and has also been known to be associated with pregnancy.

Ventilatory failure and its associated complications or autonomic dysfunction induced by paralysis are major causes of morbidity and a mortality rate of 10%. 10-20% of all patients require assisted ventilation and a number will require a tracheostomy. Full recovery occurs in 60% of surviving patients within one year and many patients still have a residual neurological deficit after 2 years. The interval between peak weakness and the onset of recovery is of prognostic value.[220]

Acute inflammatory polyneuropathy results from a cell-mediated immunological disturbance with a selective attack against myelin. Circulating lymphocytes are sensitised to peripheral nerve antigens and lymphocytes from acute inflammatory polyneuropathy patients will produce demyelination in tissue culture. Humoral factors are also implicated. Complement fixing antibodies against the neural tissue have been found in 50% of AIP cases. Demyelination has been produced in tissue culture by cell-free serum. Both IgG and IgM have been identified on sections of nerves in AIP patients and IgG

directed against peripheral nerve appears to be specific. These antibodies are best demonstrated during early and progressive disease and are reduced during recovery. Immune complexes have been demonstrated in the circulation or deposited in the kidney. Current methods appear to lack discrimination necessary to identify the precise immunological disorder.[220]

Corticosteroid therapy has not been shown to be effective during the acute stages of the disease and may predispose to relapse. Other forms of treatment namely Cyclophosphamide, Methylprednisolone and 6-Mercaptopurine have been moderately successful. The mechanism by which plasma exchange may benefit patients with AIP is unclear but there is definite evidence to suggest some patients can respond rapidly and beneficially to the treatment.

In the present study all patients showed some improvement in clinical status after plasma exchange, 10%, 65% and 25% of patients exhibited improvements of 3, 2 and 1 grades respectively. The greatest response was seen in those patients whose disorder was most quickly diagnosed and subsequently treated.

A review of the literature[301-303] suggests that the response to treatment by plasma exchange has been achieved in 70% of cases and a negative response in 14% of cases.

Improvement was seen during the first exchange procedure,

the progression of the disease being halted after the first session and in most cases motor deficit which appeared latest began to improve. With subsequent sessions benefit appeared to maximise and following this, improvement was slower and further deterioration was prevented. This decreased the necessity for assisted ventilation. Sensory deficit seemed to respond more slowly and the likelihood of response was increased the younger the patient. If the disease had not yet progressed to plateau level and muscle wasting had not commenced before plasma exchange had started the probability of rapid improvement was increased. If there was evidence of denervation, the prognosis for improvement was poor. Once demyelination had occurred it was unlikely that treatment would be effective because nerve function could only be restored with remyelination which takes place very slowly.[288]

Data has been released in a number of controlled trials of plasma exchange in the treatment of Guillain-Barré Syndrome.[221,301-303] In one American study of 224 patients 2 groups were compared in terms of

1. Clinical outcome at 4 weeks.
2. The time taken to improve one clinical grade.
3. The time on a ventilator.
4. The time to improve to walking capability.

By all these criteria, plasma exchange had beneficial effects on the outcome of patients. The early initiation of exchange was associated with a better outcome than when plasma exchange

was not started until later in the disease.

In one French study over a 3 year period, 183 patients were randomised within 18 days of the onset of muscular symptoms. Preliminary results on 63 patients treated by plasma exchange and 70 patients without plasma exchange showed that distinct benefits were conferred by plasma exchange. The frequency of septicaemia, pulmonary, respiratory or cardiac complications was the same in both groups. Intercurrent complications or adverse effects led to the termination of plasma exchange in 11 patients, 6 of the patients not treated by plasma exchange died.

In patients with severe disease when plasma exchange has been started early in the course of the illness, plasma exchange may shorten duration of motor weakness sufficiently to reduce hospital stay. For patients able to breathe without ventilatory support at the time of the first plasma exchange, the procedure can also reduce the time spent on a respirator, if ventilatory assistance is subsequently needed. Plasma exchange is recommended only for GBS patients with, at a minimum, severe weakness at the time of the first plasma exchange (i.e. patients able to walk only with support). It is not clear whether patients able to walk without support will benefit from plasma exchange.

4.2.8 Glomerulonephritis and Goodpastures Disease

There is evidence[110,304-307] that most, if not all, types of

glomerulopathies are caused by deposition of circulating proteins or protein aggregates in the glomerulus. Glomerulonephritis due to auto-antibodies to the glomerular basement membrane (GBM), or associated with the deposition of immune complexes carries a poor prognosis, with a rapid deterioration in renal function leading to renal failure within a year. The role of anti-GBM antibodies is well defined, their pathogenicity having been demonstrated by transfer of the disease to primates, and by the observation that the disease recurs rapidly in renal allografts if circulating antibody is present at the time of operation. However, the role of immune complex deposition in the kidney remains controversial.

The types of glomerulonephritis were classified as:

Non Antibody Mediated GN

1. Mesangiocapillary GN Type 1 - in which there is a layer of tissue beneath the GBM giving it a double contour. This is a sub-endothelial extension of mesangial matrix and cells containing immune deposits and C3.
2. Mesangiocapillary GN Type 2 - in which C3 associated electron dense material is incorporated into the GBM.
3. Mesangial IgA Disease - perhaps the most common glomerular pathology worldwide, characterised by diffuse mesangial proliferation and associated with recurrent macroscopic haematuria.

Antibody Mediated GN

In Anti-GMB Antibody Nephropathy there is a neutrophilic cell

infiltrate, heavy fibrin deposition and extensive epithelial crescent formation. Anti-GM antibodies have been seen to be responsible for the immunologically mediated renal damage in this condition, and it has been demonstrated that there is a positive correlation between antibody levels and plasma creatinine on presentation. Although the production of anti-GBM antibodies is self-linking this may take years in untreated cases, by which time renal failure is usual.

In view of the grave prognosis in both types of disease, treatment with steroids and cytotoxic drugs was introduced in an attempt to control the underlying immune response and limit the effects of inflammation. The response of the kidney is dependent upon the severity of glomerular damage at the onset of treatment, it is apparent that the earlier treatment is instigated, the higher the chance of recovery.

Plasma exchange can be combined with drug therapy in order to rapidly reduce antibodies (to undetectable levels within 6-8 weeks) pending the effects of cytotoxic agents.

From our small series it can be readily seen that those patients presenting with a creatinine of 550 μmol/1 have an encouraging response to aggressive plasma exchange. Where extensive damage has already occurred dialysis is the end result.

In one study,[304] 44 patients with anti GBM disease, ages ranging from 4-72 years were treated to daily 4 l plasma

exchanges using PPF and an immunosuppressive regimen of prednisolone (60mg reducing to 20mg/day in 4 weeks), cyclophosphamide (3mg/kg daily) and azathioprine (1mg/kg daily). Within 8 weeks, antibody was not detectable in 21 of 34 patients. Of 22 oliguric patients, none recovered renal function, 16 remained dialysis dependent and 6 died. Of 5 patients presenting with creatinine $\geq$600 µmol/1 (6.8mg/dl), one improved and 4 proceeded to dialysis. Of 17 patients with creatinine levels $<$ 600 µmol/1, 15 recovered renal function, one became dialysis dependent and one died. Only 2 of the patients subsequently deteriorated.

In another study,[306] 4 patients with diffuse proliferative GN and 2 patients with focal proliferating GN (one of which had $>$ 50% crescents) were treated with either corticosteroids or corticosteroids and immunosuppressive drugs. This failed to improve kidney function proteinuria and/or the amount of circulating immune complexes. 9.8 $\pm$ 3 1 plasma exchanges were performed on each patient using 5% Human albumin and the immunosuppressive treatment was maintained.

In 5 of the 6 patients serum creatinine, creatinine clearance, proteinaemia and/or the amount of circulating immune complexes declined. Kidney biopsies from 4 patients who were re-biopsied showed a change towards sclerosing GN in all cases and in the patient with focal proliferating GN, a decrease of the number of crescents to $<$50%.

A study[307] has been made of 22 patients with rapidly pro-

gressive glomerulonephritis and severe renal impairment (12 cases were idiopathic, 7 had a positive immunofluorescence, 5 had a negative immunofluorescence, and in 8 cases a vasculitis could be demonstrated). All patients received prednisolone (1mg/kg) and cyclophosphamide (2mg/kg). An intensive plasma exchange regime was applied in 11 cases, 2 positive IF-RPGN, 4 negative IF-RPGN and 5 cases of vasculitis.

Improvement was seen in 5 over 6 plasma exchange treated idiopathic RPGN cases, 4 of them showing negative IF pattern, in 3 a sustained improvement was obtained and 2 went into ESRF after 17 and 33 months. In the group of 6 idiopathic RPGN cases on which plasma exchange was not carried out 3 patients died and 3 did not show any improvement.

Renal function improved in 2 patients with vasculitis-associated RPGN treated by plasma exchange. In one case improvement remained 34 months after treatment but in the other case a progressive loss of renal function was observed, starting haemodialysis five months after. In three other cases, no changes were seen in one and two patients died.

Some beneficial effect of plasma exchange treatment in idiopathic RPGN mainly in its negative IF form seems possible. The effectiveness of plasma exchange in the treatment of RPGN vasculitis-associated was not established.

Goodpasture's Syndrome.

Goodpasture's Syndrome[118,213,214,215,308,309] is characterized

by pulmonary haemorrhage and glomerulo nephritis. The syndrome is most commonly initiated by anti-basement membrane antibodies which can be identified as linear immunoglobulin G (IgG) deposits along the glomerular basement membranes (GBM) and the alveolar basement membranes. Circulating anti-GBM antibody can also be shown by indirect immunofluorescence and more recently by radio-immunoassay. The specificity of the linear deposits detected by immunofluorescence can be confirmed by elution studies.

Steroid therapy is now considered useful in the management of pulmonary haemorrhage but there is less evidence that either steroids and/or immunosuppressive agents are beneficial in the management of the renal manifestations of the syndrome. Nephrectomy has also been considered to be of benefit in some patients with life-threatening pulmonary haemorrhage but the results have not been consistent and fatal pulmonary haemorrhage has occurred in nephrectomized patients.

Intensive plasma exchange permits the immediate removal of antibody and, with the appropriate replacement fluid, can deplete inflammatory mediators such as complement and fibrinogen, whilst the immunosuppressive drugs provide the longer term control of anti GBM.

In the present study plasma exchange was only partially successful in securing improvement in the clinical status of patients treated and this appeared to be dependent on the creatinine concentration on presentation rather than on the depletion of circulating immune complexes. Other studies

have also reported conflicting responses of patients to plasma exchange.

In one study[304] an improvement has been reported in 7 cases of Goodpasture's syndrome treated by combined immunosuppression and plasmapheresis. The contribution of plasmapheresis to the survival of 6 of this group is difficult to assess because of the large doses of immunosuppressive drugs employed at the same time. There is often a striking fall in the level of antibody at the initiation of therapy. Some of the effects of plasma exchange may be due to the removal of inflammatory mediators such as complement C-reactive protein and fibrinogen.

In another study[306] a case was reported of antiglomerular basement membrane antibody induced Goodpasture's syndrome in which the patient required haemodialysis and was treated with immunosuppressive agents and plasmapheresis. A severe (80%) crescentic lesion was reversed and crescentic lesion was stablized at 2.5mg/dl at one year follow-up. Fifteen other reported cases of Goodpasture's syndrome in which the patients were treated with plasmapheresis were reviewed. Short term follow-up showed that nine of these patients were alive without need of dialysis, five were receiving dialysis and 2 had died.

There have been instances of the recurrence of circulating anti-glomerular basement membrane antibody at different times after immunosuppressive treatment and plasma exchange. In one

patient, antibodies reappeared after an absence of 3 years and linear IgG staining of the glomeruli was shown by immunofluorescent studies. Renal function did not change and there was no evidence of pulmonary haemorrhage. Antibody levels then fell spontaneously over the succeeding 18 months. Two cases have been reported in which Goodpasture's syndrome recurred 13.5 and 38 months after kidney transplantation. In one patient there were additional recurrences at 18.5 and 30 months associated with kidney graft dysfunction and proteinuria. Dramatic improvement of graft function occurred after each series of plasma exchange and has persisted for 41 months after the last plasma exchange. The second patient returned to haemodialysis one month after plasma exchange when graft biopsy showed vascular lesions of chronic rejection. Anti GBM antibodies initially detected in both patients disappeared after the first plasma exchange.

4.2.9 Aplastic Anaemia and Red Cell Aplasia.

When there is a deficiency or defective utilization of an essential factor, erythropoiesis may fail and haemopoietic tissue may disappear. Haemopoietic hypoplasia may result from damage to the haemopoietic stem cells by means of a toxin acting against later erythroid cells or from damage to marrow stroma. These result in pancytopenia, i.e., anaemia, leukopenia with neutropenia, and thrombocytopenia and are referred to as aplastic anaemia. When the white cells and platelets are not affected, pure red cell aplasia is the result.

Aplastic Anaemia.

A number of different mechanisms[223,224,225,310] lead to marrow aplasia. In about a third of all patients, this appears to be due to a drug, e.g., Chloramphenicol. The maintenance of an adequate vascular microcirculation is necessary to support growth of haemopoietic tissue. Destruction of the sinusoidal structure of a marrow may result in aplastic anaemia from atrophy of haemopoietic tissue and recovery requires restoration of medullary vascularity. Stem cell defects may be primarily responsible in this case. An autoimmune mechanism may operate in some cases, e.g., selective aplasia of the erythroid cells, where there is frequently an association with other aberrations in the immune system such as thymoma and IgG antibodies to erythroblasts. There is some evidence of lymphocyte-mediated stem cell inhibition in cases of aplastic anaemia.

Haematopoietic stem cells and their progenitors differentiate to mature blood cells under the influence of bone marrow microenvironment and haemopoietins. Stem cells have surface antigens that are different from those of mature cells and antibodies specific for precursor cells do not bind to mature cells. The exact nature of the elements of bone marrow environment responsible for haematopoietic differentiation are unknown. In-vitro granulopoiesis is dependent on colony stimulating factors secreted by the monocyte macrophages and probably regulated by prostaglandins, serum factors and cellular interactions. The role of immune mechanisms in bone

marrow failure associated with aplastic anaemia provides the rationale for treatment in plasma exchange.

The present very small study shows some evidence that plasmapheresis could be beneficial in very few patients in whom antibodies are responsible for the inhibition. In the patients described in this study, the serum factor was IgG-anti DNA antibody in the patient with SLE; IgM in the patient with cryoglobulinaemia and also in the remaining patients.

Similar beneficial results of plasma exchange have been reported in other investigations. One study[224] of in-vitro granulopoises in 21 patients with aplastic anaemia showed that 12 patients had no in-vitro abnormality which accounted for the failure of the marrow to form colonies. It was thus assumed that the pancytopenia was secondary to a still unknown stem cell or micro-environmental abnormality. The three abnormalities identified were (1) bone marrow hyperactive suppressor T cells directed against the precursor cells in three patients (2) circulating immunoglobulins directed against marrow colony forming units in culture (CFU-C) in three patients, and (3) abnormalities in the function of colony-stimulating factor in three patients. Plasmapheresis combined with cytotoxic therapy proved to be effective in removing the serum antibody and in the recovery of bone marrow in the three patients in whom serum factors, that were inhibitory to the bone marrow precursor cells, were depleted.

Plasma exchange[310] has been used in the treatment of a patient,

with aplastic anaemia and serological evidence of systemic lupus erythematosus, whose serum contained complement-dependent immunoglobulin IgG antibody that inhibited both granulocyte and erythroid colony formation by normal human bone marrow in-vitro. Incubation of normal human bone marrow cells with the patient's serum and a source of complement caused a 95% reduction in myeloid colony formation. The addition of the patient's serum plus complement, to normal marrow cultured in methylcellulose, also produced a 98% reduction in erythroid colony numbers. Assay of serum immunoglobulin fraction showed that inhibitory activity was contained in the IgG function. After a series of procedures in which 5 to 6 litres of plasma were exchanged isovolumetrically with type AB fresh frozen plasma at monthly intervals, the titre of the inhibitor fell, the marrow cellularity and peripheral counts returned to normal. Incubation of the patients recovery marrow with stored pre-plasma pheresis serum and complement did not produce reduction in myeloid colony formation.

Red Cell Aplasia.

Some types of aplastic anaemia[225,226,311-313] have their counterparts in cases where only the erythroid cells are affected. They are characterised by the main absence of erythroblasts in an otherwise normal marrow. In some cases tryptophane metabolism may be involved in the genesis of erythroblastic hyperplasia, i.e., it may be associated with riboflavin deficiency. Other acquired cases appear to be due to antibody-directed specificity against erythroblasts and/or

erythropoietic factors. This is often associated with a thymoma, occasionally with myasthenia gravis and in a proportion of cases it may be possible to demonstrate the serum anti-nuclear factor, LE cells, and a positive direct antiglobulin test. IgG serum inhibitor erythropoiesis has been demonstrated by both in-vitro and in-vivo assays.

The effects of autoantibodies include interference with haem synthesis, as demonstrated in suspension cultures of normal bone marrow, complement-dependent lysis of erythroblasts and complement-independent inhibition of erythroid progenitors. Occasionally antibodies are directed against erythropoietin.

The treatment of red cell aplasia of the antibody type is based on thymectomy for thymoma and immunosuppression, e.g. cyclophosphamide or antilymphocyte globulin. Plasma exchange offers potential for the treatment of patients who may not respond to immunosuppressive therapy.

The present study, involving only two patients, was too limited to properly reflect the efficacy of plasma exchange in the treatment of aplastic anaemia. In one reported study,[311] plasma exchange was applied to a patient with pure red cell aplasia who did not respond to immunosuppressive therapy. The patient's plasma contained antibodies that inhibited erythroid formation of autologous and normal bone marrow cells. Extensive plasma pheresis removed the auto antibody and resulted in complete normalisation of erythropoiesis that had persisted for more than 2 years.

In a further study,[313] a patient with fulminant lupus pneumonitis, renal failure and red cell aplasia not responsive to massive corticosteroid therapy showed sustained improvement after plasma pheresis and haemodialysis. The patient's respiratory and renal function improved and gradually returned to normal.

For selected patients with life threatening disease unresponsive to conventional therapy plasmapheresis provides an albeit temporary experimental therapeutic option. Additional experience may show whether patients with less severe disease activity may be responsive to such therapy.

4.2.10 Polymyositis

Polymyositis identifies a group of diseases in which muscular weakness and wasting may be associated with muscle pain and tenderness or with evidence of some form of connective tissue disease or collagen disorder. The term is used to include cases with florid skin change, i.e., dermatomyositis, usually taken to indicate the so-called idiopathic syndrome and excludes disorders such as polymyalgia rheumatica and acute myositis resulting from infections with micro-organisms and viruses.[314-318]

The aetiology suggests an organ-specific autoimmune disease, while in cases showing involvement of skin or joints, it can be accepted as being the muscular manifestation of non-organ specific-auto immune disease.

The commonly demonstrated relationship between polymyositis and dermatomyositis on the one hand and malignant disease on the other suggests that the condition may also on occasion be the result of conditioned auto-immune response in patients suffering from cancer. It is a condition occurring in many races world-wide and in both sexes.

Apart from occasional acute cases in which widespread muscle pain, fever, constitutional upset and rapidly progressive paralysis occurs, the condition usually runs a sub-acute or chronic course. Muscle pain and tenderness occur in 50% of cases, as does dysphagia. Cutaneous manifestations are seen in 66% of all patients. Helioptrope erythema and periorbital oedema are particularly characteristic and so is congestion of the nail beds. In 25% of cases, joint pain and stiffness occur at some stage. Proximal limb muscles are invariably involved and the neck muscles are affected in about 60% of all cases. Involvement of distal limb muscles is less common, but weakness may be generalised in about 33% of all cases. Because of the progressive nature of the disease, it has been suspected that a pathogenic autoantibody might be involved. Although it has not yet been demonstrated, there is some evidence from muscle biopsies carried out, that an autoantibody with specificity for muscle tissue may be present. Diagnosis is usually by demonstrating a markedly elevated serum creatinine phospho kinase, a myopathic pattern of volitional activity electromyographically and by means of a muscle biopsy. The latter demonstrates wide-spread necrosis, and phagocytosis of muscle fibres and interstitial and peri-

vascular infiltrates of inflammatory cells.

The mainstay of treatment is cortisteroid therapy in moderate to high dose in the first instance. In those patients refractory to this therapy, immunosuppressive and cytotoxic therapy is instituted.

If cellular and humoral immune mechanisms contribute to the muscle and skin injury then plasma exchange should provide some relief. In the present study 7 patients exhibited an improvement in clinical status of 2 grades and 3 patients of one grade after plasma exchange following significant reductions in the values of creatinine kinase, asparate transaminase, alanine transminase and lactate dehydrogenase. Similar behaviour has been reported by other workers.

In one study,[314] 27 patients with polymyositis were treated with a weekly series of large volume plasmapheresis (5-6% body weight) until an end point was reached where the CK had normalised or reached a plateau and there was no additional benefit. All patients had been treated previously with moderate to high dose corticosteroids but had either responded inadequately or could not tolerate a dosage adequate to suppress disease activity because of side effects. Cyclophoasphamide was added daily at the time of the first exchange. Patients who developed side effects were switched to chlorambucil. All but 2 patients showed at least 1 grade of improvement on a 7-step scale of muscle testing. The best responses were seen in patients whose disease was quite

active as determined by the rate of clinical progression and the height of the CK activity. CK was found to be a muscle enzyme which seemed to correlate better with disease activity than other commonly measured enzymes such as SGOT adolase or LDH.

Patients with slowly progressive disease had the least and no response to plasma exchange. Serum CK activity decreased in all patients in whom it was initially elevated and normalized at some point in two-thirds of the patients. It took up to 30 treatments over prolonged period of time to completely normalize CK in some patients.

Post-exchange biopsies from patients with highly active disease showed no evidence of fibre necrosis, phagocytosis and interstitial and vascular inflammation. Two patients had mild perifascicular atrophy.

In another study,[315] electro-myography and muscle biopsy were abnormal in each patient. An IgG kappa monoclonal paraprotein was found in the serum of all three patients. Muscle biopsy specimens showed linear deposits of IgG kappa along the sarcolemmic basement membrane of the individual fibres. Stains for lambda light chains, other immunoglobulins and complement components were negative. The patients failed to respond to prednisolone for periods longer than 3 months. The addition of plasma exchange performed 3 times a week for 3 weeks produced a fast clinical improvement in all 3 patients. Muscle biopsies after plasma exchange showed no immune de-

posits. Monoclonal immunoglobulin is demonstrated in the serum and along the sarcolemmic basement membrane in three patients.

Two of the four patients with polymyositis clinically improved after an intensive course of plasma exchange.[316] One patient showed marked, and the other moderate clinical response. No relation was found between the degree of clinical improvement and the duration of illness before plasma exchange. No statistically significant biochemical response could be demonstrated.

4.2.11 Clinical Status of Patients.

The side effects observed in the study are in accord with those recorded in an extensive literature.[236-244].

The most frequently reported side effects of Haemaccel are minor skin reactions, urticaria and burning sensations. An occasional case of facial oedema, a rise in body temperature, hypotension and respiratory difficulties have been observed. Cases of bronchospasm and circulatory collapse have occurred very infrequently. Haemaccel is generally considered to be non-immunogenic, i.e., it does not trigger production of antibodies on repeated challenge.

Whilst not encountered in the present study, it is known that the rapid infusion of plasma substitute can cause the release of detectable small amounts of histamine. The exact mechanism is unclear but it may be due to direct chemical action on

mast cells. It has also been suggested that histamine liberation may be related to individual sensitivity and to the rate of infusion of solution.

Feverish reactions constitute the majority of side effects experienced with plasma protein solution. These tend to be short lived and usually occur in cases of known sensitisation to blood and its derivatives. Other side effects which have arisen were usually due to the procedure. In France there are many reports of fever and shivering associated with the use of 4% albumin as replacement fluid. This was attributed to the purity and variability of the preparation. Albumin of placental origin purified by chromatographies substantially reduced the frequency of the febrile reactions. Where volumes of fresh frozen plasma larger than those employed in the present study have been used significant side effects have been encountered.

4.3 Leukapheresis

Acute leukaemia[18-22] results from the uncontrolled proliferation of poorly differentiated immature cells (blasts) in the blood and bone marrow and their invasion into the rest of the body organs is chiefly responsible for the devastating effects of the disease. The clinical manifestations and the response to therapy may vary with the type of blast cell involved in the leukaemia process. Acute lymphatic leukaemia accounts for over 80% of the cases in children, whilst acute myeloid leukaemia constitutes at least 85% of adult patients.

There are two fundamentally different forms of chronic leukaemia, namely chronic granulocytic and chronic lymphocytic leukaemia. The latter is a disease of the elderly, the former occurs at all ages and is especially associated with middle age.

Cell morphology provides the basis of diagnosis and classification and immunological techniques are used to detect antigens and other molecules on the cell surface. Standardised treatment strategies have now been devised based largely on intensive drug regimes.

However leukapheresis, by continuous flow centrifugation, has been employed in the management of both acute and chronic leukaemia . It is a method by which a large and relatively pure population of white blood cells can be harvested in a short period of time. This controlled depletion of peripheral white cells occurs without a significant loss of red cells or platelets. It has been employed for:-

1. The therapeutic cell depletion for the reduction of the tumour burden in chronic leukaemia and the prevention of leukastasis in acute leukaemia.
2. The collection of peripheral stem cells from patients with chronic myeloid leukaemia.
3. The collection of allogenic leukaemic blasts for immunotherapy as an adjunct to chemotherapy in prolonging remission in acute myeloid leukaemia.

It is known that leukostasis, i.e., the occurrence of intravascular leukocyte aggregates and thrombi, is most frequently

associated with acute non-lymphatic leukaemia with peripheral leukocyte counts in excess of 100 x 10^9/1. Any increment added to the total cytocrit by transfusion may aggravate morbidity and mortality, as may the institution of conventional chemotherapy. Clinically the morbidity and mortality is predominantly of pulmonary and central nervous system origin and onset symptoms may occur in the last few hours of life. Despite few histological confirmations of leukostasis in patients subjected to leukapheresis, dramatic improvement in the clinical status of patients has been noted.

The urgent removal of 0.5 to 2.4 x 10^{12}/1 cells can rapidly reduce the WBC to less than 50,000 x 10^9/1.

Leukapheresis, as sole therapy for chronic myeloid leukaemia, requires repetitive procedures at high cost relative to medical therapy and does not achieve marrow remission. The transient lowering of leukocyte counts to relieve symptoms attributed to extreme leukocytosis, while cytotoxic therapy is being intiated, would seem justified.

The occasional leukapheresis procedure can be useful in selected patients with chronic lymphatic leukaemia in whom the WBC is 100,000 x 10^9/1. In some patients there is a reduction in magnitude of the hepatomegaly and/or splenomegaly over the duration of the procedure. Leukapheresis should never be employed as sole therapy in cases of chronic lymphatic leukaemia as it is unsuccessful in alievating anaemia or thrombocytopenia.
Occasional patients (less than 10% with stage IV disease) appear

to respond to leukapheresis at one to four week intervals with increases in haematocrit and/or platelet count.

The present study has confirmed that leukapheresis provides a relatively rapid means of safely reducing the circulating white cell count. It will certainly bring clinical improvement, by reducing the circulating WBC by 40% within three hours of the first procedure. Leukapheresis relieved respiratory symptoms in 2 out of 10 patients, and neurological symptoms in 3 out of 10 patients. The optimal treatment would therefore appear to be leukapheresis followed by chemotherapy because the response to chemotherapy is improved if the white cell count has been reduced by serial leukapheresis.

In the treatment of chronic myeloid leukaemia leukapheresis has not proved to be a superior form of treatment and does not appear to delay blastic transformation. In the treatment of chronic lymphatic leukaemia leukapheresis will result in reductions in lymphocyte count, spleen size, lymphnode size, bone marrow infiltration and liver size, but it should be used for those patients failing other forms of treatment.

There is considerable literature [319-324] on the application of leukapheresis in the treatment of leukaemias. In one study leukaemia patients with extremely high peripheral white blood counts and who had experienced a cellular hyperviscosity syndrome were leukapheresed to reverse the associated neurological dysfunctions.[319] Leukapheresis had effectively been used to rapidly reduce high blast counts in acute leukaemia for the management of

leukostasis.[320] Temporary control of leukocytosis has also been managed by cytopheresis techniques.[321,322] Regression of the disease has been reported with intensive leukapheresis in chronic lymphocytic leukaemia. Leukapheresis has been safely used as an alternative to chemotherapy in the treatment of leukaemia during pregnancy.[323]

A single urgent leukapheresis has been recommended[19] prior to conventional chemotherapy to reduce the leukocyte count to the 50 x 10^9/l range. Considerable variation was found in the separation conditions for leukaemia cells but there were immediate clinical benefits, namely improved cerebral function, reduction in spleen size and decreased dyspnoea. There was considerable CNS and pulmonary morbidity and late mortality in the treated group. A serum LDH rising to greater than 4 x 10^3 units was associated with a poor prognosis.

Nineteen patients with acute leukaemia (6 lymphoblastic, 8 myeloblastic and 5 myelomoncytic) and 13 patients with chronic leukaemia (9 myelocytic, 3 lymphocytic and 1 hairy cell) have been subjected to leukapheresis procedures.[21] The patients, 16 male and 16 female, ranged from 5 to 80 years, and were subjected to 17 and 38 procedures respectively. The lymphoblastic patients, myeloblastic patients, myelomoncytic patients, myelocytic patients, lymphocytic patients and hairy cell leukaemia patient were subjected to 6, 8, 5, 9, 6, and 21 procedures respectively.

In most of the acute cases the leukapheresis was performed as an emergency procedure and was lifesaving in some instances. The

chronic patients were leukapheresed either because of blastic transformation (CML) or because they were refractory to conventional therapy. In some cases there were minor improvements in respiratory function, WBC, neurological dysfunction and anaemia after leukapheresis, but no improvement was noted in splenomagaly, hepatomegaly, nodular involvement and fever. Only 44% of the acute leukaemia patients survived 21 days past the first leukapheresis. Those not surviving died shortly after the procedure because of leukostasis or haemorrhage. All of the chronic leukaemia patients survived beyond 21 days.

In one study,[324] 20 leukapheresed patients were compared with 78 non-leukapheresed patients (control group), 12 male and 8 female (aged 11-59 years), 9 newly diagnosed patients with acute lymphoblastic leukaemia and 11 with acute non-lymphoid leukaemia. All patients were given 1, 2 or 3 leukapheresis (42 in all) followed by induction chemotherapy. A 30-35% decrease in initial WBC was obtained after each leukapheresis.

In ALL, 7 of the 9 leukapheresed patients achieved complete remission without early death. In ANLL, 5 of the 11 leukapheresed patients achieved complete remission, while 6 died.

It was concluded that early death rate was lower in the leukapheresed patients, but statistically significant only in ALL patients ($p < 0.01$) and the complete remission rate was higher in the leukapheresed patients than in the control group.

The leukaemic mass removed after second leukapheresis procedure did not increase any more.

5. FUTURE DEVELOPMENTS

5.1 Technical

If, in the course of time, therapeutic apheresis is assessed to be no more than adjunctive or palliative and not curative and of short-term rather than long term benefit, the application of blood cell separators and apheresis techniques will be justified by their contribution to the improvement of the clinical management of several disorders by cellular or plasma depletion or by rapid erythrocyte or plasma replacement. Randomized clinical trials being undertaken at present or in the future will undoubtedly increase the scope of potential applications of the procedures and, hopefully will add to the understanding of the separation characteristics of different cells and the kinetics of cell population and lead to an improvement in the rationale of treatments based on apheresis procedures.

The separation of blood components by centrifugation methods is relatively crude and some measure of inter-constituent contamination is inevitable. Further, because of the diminishing incremental effect of the removal or addition of a substance in a patient by plasma exchange, the complete removal of a harmful substance will be difficult and the removal of even the bulk of a substance will require a number of large volume exchanges with appropriate intervals to allow the diffusion of extra vascular antibody into the intravascular space for subsequent removal. This method of removal of a substance is inefficient and wasteful in that

the number of desirable components are also indiscriminatingly removed and then require to be replaced by appropriate fluids which in themselves can introduce further complications. Clearly there is a need for procedure that will permit the expeditious return of the patient's own plasma from which the offending component or components have been selectively removed in the course of the exchange. Apheresis by filtration rather than by centrifugation offers advantages. The resulting plasma should be completely free of particulate matter above 0.6 μm in size which should render it more resistant to blockage in long procedures or in small bead packed columns. The continuing development of less costly, more efficient, faster operating membranes for the specific removal of plasma constituents may be expected.

It has been generally predicted[325] that the likely overall demands for the future will require an input from every million of the population of between 10,000 and 12,000 litres of plasma annually, the bulk of which will have to be fresh. There is general agreement that the efficient and economical use of blood implies that patients be transfused only with the specific component of blood required. The cellular components of blood can be separated into packed red cells, platelets and white cells. Plasma is a source of albumin or labile clotting factors. The most economical source of plasma is from whole blood drawn for red cell transfusion. There is clearly scope for equipment and procedure development that will maximize the yield of components, minimize the inter-component contamination and component cost.

Improvements can also be expected aimed at reducing the variability of components particularly in cellular quantity and character.

It is likely that by combining apheresis procedures with existing and future column techniques greater progress will be made in achieving the above objectives. The bio-compatibility of column packing can be markedly improved by passing only plasma through the column and returning the by-passed cellular elements directly to the patient.

There are already indications that progress is being made in the selective isolation and collection of plasma constituents.[73] There is increasing application of protein A which not only can remove IgG sub-classes 1, 2 and 4 but can apparently re-activate a suppressed immune system either by removing blocking antibodies or stimulating the cellular system. Cells bearing IgG on their surface may also be adsorbed out provided the size of bead in the column is increased to 300 μm to allow free passage of cells through the interstices.

A and B blood group antibodies have been removed by totally synthetic antigens rendered bio-compatible by cellulose nitrate/albumin coating cholesteral and/or low-density lipo-proteins have been removed by Heparin-agrose. Inhibitors to Factor VIII and Factor IX have been adsorbed by protein A - sepharose. Further studies along these lines may be expected to continue.

Questions remain regarding composition of column or filter

effluents and the modified plasma. The mechanics of specific and non-specific removal of factors such as coagulation, anti coagulation, complement and other plasma constituents requires further clarification. Further evaluation is required of the reactions encountered in the course of plasma-perfusion to determine whether they can be prevented. The premise that a specific sorbent should exist for each of the many plasma constituents likely to require removal provides the impetus for further developments.

The perfusion of leukocytes will become feasible if the problems of cell blockage can be overcome. The use of large microbeads (300 μm diameter) has been advocated but these have very low capacity and a tendency to high non-specific binding. The newer microspheres (0.1-0.2 μm diameter) may offer greater scope, particularly when coated with appropriate antibodies and labelled with fluorescein or magnetic colloids. They can bind to the target cell surface at multiple sites and be removed in a second stage filter column of immobilised anti-fluorescein or magnetic filters. Such a procedure would be useful in the treatment of diseases where changes in the T cell suppressor/helper subsets is known to be beneficial, e.g., multiple sclerosis.[73]

Advantages have been conferred by combining membrane and column techniques in a single integrated film absorber in which carbon is embedded in cellulose nitrate film. This improves the internal diffusion limits set by larger beads and can be expected to form the basis for further development.

5.2 Therapeutic.

Lymphopheresis is not yet of practical use in haematologic - oncologic disorders. It would be applicable if the lymphocytes producing the harmful substances could be identified. The ability to remove only specific lymphocytes would be of value in diseases mediated by lymphocyte immune activity but this awaits the development of labelling techniques. Lymphopheresis is currently used in graft versus host disease following bone marrow transplantation.

The contributions of transfusions of donor granulocytes are now reasonably well defined. It is unlikely that further studies in the forseeable future will significantly alter the indications of their use. If haemopoietic stem cells are present in the human circulation in appreciable numbers, their use in preference to stem cells harvested from bone marrow could have various specific advantages.

5.3 Replacement Fluids.

By the end of March 1986, 340 cases of Acquired Immune Deficiency Syndrome had been recorded in the United Kingdom. Of these 5.5% were the recipients of blood products. Most at risk were haemophiliacs because some 20,000 donors could have contributed to each vial of American Factor VIII concentrate. Since the introduction of heat-treated Factor VIII, the risk of infection has been reduced.

Patients who had had transfusions of blood and blood products, namely, platelet concentrate and fresh frozen plasma, are also at risk, although the risk is much lower. British blood donors, who are voluntary, have always had a lower incidence of hepatitis than those in the U.S.A., who are paid; the same is true of HIV. The screening of blood donors is now routine in the U.K., the U.S.A. and an increasing number of other countries. The potential donor is warned that this is happening and is asked not to donate if he considers himself at risk of infection. Despite this, and because of the increased incidence of Acquired Immune Deficiency Syndrome and the possibility of transmitting the HIV through plasma exchange, great concern has been raised about the use of fresh frozen plasma as a replacement fluid.

Since March 1986, no fresh frozen plasma has been used as replacement fluid at the Royal Infirmary. Since November 1985, the Canadian Apheresis Group decided to dispense with fresh frozen plasma because it considered that the product posed a serious threat to continued patient enrolment.

Thus, in the future only Albumin and Gelatins will be used as replacement fluids and this should decrease concern over the possibility of infectious complications.

6. CONCLUSIONS.

1. The fluids normally used to replace large volume plasma removed during plasma exchange procedures were plasma protein fraction, albumin 5% and very occasionally fresh frozen plasma. It was found that the consumption of albumin 5% could be substantially reduced by substituting wholly or partly with polygeline. The response of patients treated for myasthenia gravis, Guillain-Barré syndrome, polyneuropathy and polymyositis by plasma exchange was essentially similar when plasma protein solution, plasma protein with Haemaccel or Haemaccel were used as replacement fluids.

2. Therapeutic plasma exchange has not proved to be the panacea for all disorders. It is, however, finding its level of application; specific for some treatments, useful for others and to be used in certain cases when all conventional therapy has failed.

Although crude in its selectivity, plasma exchange is capable of removing any toxin located mainly within the blood stream. The problem is more complicated when the toxin is distributed both within and outside the vasculature, with the two compartments in dynamic equilibrum. If the source of supply of fresh toxin can be interrupted (and provided that no irreversible harm has been done to the target organ) then again the removal of the toxin from the plasma in sufficient quantities will benefit the patient. The toxins in Goodpasture's syndrome and myasthenia gravis are respectively antibodies against the glomerular basement membrane and acetylcholine receptors and their production may be diminished by

immunosuppressive drugs. Survival and recovery of renal function in Goodpasture's syndrome is improved by plasma exchange, whilst its use to circumvent the known delay in the action of immunosuppressive drugs is clearly indicated in severe and life-threatening myasthenia gravis.

The success of plasma exchange in specific antibody mediated diseases does not justify its use in others. In most cases treatment by immunosuppressive drugs will prove effective. If immunosuppressive drugs cannot be used then the antibody will rebound to the initial or even higher concentrations. In diseases mediated by immune complexes, plasma exchange can lift the blockade imposed by circulating complexes on their clearance by the reticuloendothelial system. It is also possible that plasma exchange may modulate the immune response by influencing the composition of the complexes and their distribution between body components. However, the effectiveness of plasma exchange in the treatment of immune complex disease has yet to be established.

There is a need for more satisfactory controlled studies which are not easy to organise because the diseases are rare, action is often urgent and protocols may often prove inappropriate. Further, without sufficient knowledge of the underlying pathogenic mechanisms interpretation of the results must be difficult.

3. Leukapheresis may be used for acute myeloid leukaemia patients with high white cell counts and associated symptoms; it is not recommended as routine therapy or as a form of tumour debulking. It can be useful in the treatment of some patients to whom chemo-

therapeutic drugs cannot be administered immediately or in whom initial chemotherapy has not produced the required rapid fall in white cell count.

Intensive repeated leukapheresis for chronic myeloid leukaemia patients produces falls in peripheral leukocyte count, improvement in systemic symptoms and reduction in hepatosplenomegaly, but only rarely are these sustained. The overall course of the disease appears to be unchanged. In the treatment of patients with advanced chronic lymphocytic leukaemia intensive leukapheresis may offer some benefit from falls in circulating lymphocyte counts, variable regression in the degree of bone marrow infiltration, adenopathy and splenomegaly. However, it is unlikely that the proliferation of leukaemia cells could be influenced by bulk removal of cells, so as to increase sensitivity to specific chemotherapy.

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APPENDIX A.

RESULTS OF STATISTICAL ANALYSIS.

TABLE 17S

Full Blood Counts.

	t-Test	Pre-Plasma Exchange			5 Days Post-Plasma Exchange		
		t	n	p	t	n	p
Haemoglobin	Group 1 Polygeline 1 v Group 2 Plasma Protein Solution	1.48	60 60	N.S.	0.69	60 60	N.S.
	Group 1 Polygeline 1 v Group 3 Polygeline 11	0.43	60 20	N.S.	0.64	60 20	N.S.
	Group 2 Plasma Protein Solution v Group 3 Polygeline 11	1.74	60 20	0.10 > p > 0.05 N.S.	1.94	60 20	0.10 > p > 0.05 N.S.
White Blood Cell Count	Group 1 Polygeline 1 v Group 2 Plasma Protein Solution	1.72	60 60	0.10 > p > 0.05 N.S.	0.63	60 60	N.S.
	Group 1 Polygeline 1 v Group 3 Polygeline 11	0.22	60 20	N.S.	0.45	60 20	N.S.
	Group 2 Plasma Protein Solution v Group 3 Polygeline 11	1.90	60 20	0.10 > p > 0.05 N.S.	0.94	60 20	N.S.
Haematocrit	Group 1 Polygeline 1 v Group 2 Plasma Protein Solution	1.19	60 60	N.S.	1.24	60 60	N.S.
	Group 1 Polygeline 1 v Group 3 Polygeline 11	0.77	60 20	N.S.	0.77	60 20	N.S.
	Group 2 Plasma Protein Solution v Group 3 Polygeline 11	2.29	60 20	0.05 > p > 0.02	2.58	60 20	0.02 > p > 0.01

n = Number of Patients per Group. N.S. = Not significant. i.e., p > 0.05.
Probabilities between 10% and 5% (0.1 > p > 0.05) are noted.

TABLE 17S

Full Blood Counts (Continued)

	t-Test	Group 1 Patients Polygeline l			Group 2 Patients Plasma Protein Solution			Group 3 Patients Polygeline ll		
		t	n	p	t	n	p	t	n	p
Haemoglobin	Pre-Plasma Exchange v Post-Plasma Exchange	0.55	60	N.S.	1.96	60	0.10 > p > 0.05 N.S.	0.95	20	N.S.
White Blood Cell Count	Pre-Plasma Exchange v Post-Plasma Exchange	0.89	60	N.S.	2.23	60	0.05 > p > 0.02	0.9	20	N.S.
Haematocrit	Pre-Plasma Exchange v Post-Plasma Exchange	1.0	60	N.S.	1.68	60	0.10 > p > 0.05 N.S.	1.05	60	N.S.

n = Number of Patients in Group. N.S. = Not significant, i.e., $p > 0.05$

Probabilities between 10% and 5% ($0.1 > p > 0.05$) are noted.

TABLE 19S

Platelet Counts.

t-Test	30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange			24 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline I v Group 2 Plasma Protein Soln.	4.4	60 60	$p < 0.001$	10.22	60 60	$p < 0.001$	2.06	60 60	$0.05 > p > 0.02$
Group 1 Polygeline I v Group 3 Polygeline II	3.1	60 20	$0.01 > p > 0.002$	7.67	60 20	$p < 0.001$	4.19	60 20	$p < 0.001$
Group 2 Plasma Protein Soln. v Group 3 Polygeline II	0.93	60 20	N.S.	1.09	60 20	N.S.	6.08	60 20	$p < 0.001$
	3 Days Post-Plasma Exchange			5 Days Post-Plasma Exchange					
	t	n	p	t	n	p			
Group 1 Polygeline I v Group 2 Plasma Protein Soln.	6.79	60 60	$p < 0.001$	0.81	60 60	N.S.			
Group 1 Polygeline I v Group 3 Polygeline II	8.68	60 20	$p < 0.001$	1.25	60 20	N.S.			
Group 2 Plasma Protein Soln. v Group 3 Polygeline II	5.2	60 20	$p < 0.001$	2.12	60 20	$0.05 > p > 0.02$			

n = Number of Patients in Group. N.S. = Not significant, i.e., $p > 0.05$.

Probabilities between 10% and 5% ($0.1 > p > 0.05$) are noted.

TABLE 20S

Coagulation Screening.

	t-Test	Pre-Plasma Exchange			30 Minutes Post-Plasma Exchange		
		t	n	p	t	n	p
Pro-Thrombin Time	Group 1 Polygeline I v Group 2 Plasma Protein Soln.	3.46	60 / 60	p<0.001	3.46	60 / 60	p<0.001
	Group 1 Polygeline I v Group 3 Polygeline II	0	60 / 20	N.S.	2.94	60 / 20	0.01>p>0.002
	Group 2 Plasma Protein Soln. v Group 3 Polygeline II	3.87	60 / 20	p<0.001	3.87	60 / 20	p<0.001
		6 Hours Post-Plasma Exchange			24 Hours Post-Plasma Exchange		
		t	n	p	t	n	p
	Group 1 Polygeline I v Group 2 Plasma Protein Soln.	0	60 / 60	N.S.	0	60 / 60	N.S.
	Group 1 Polygeline I v Group 3 Polygeline II	0	60 / 20	N.S.	0	60 / 20	N.S.
	Group 2 Plasma Protein Soln. v Group 3 Polygeline II	0	60 / 20	N.S.	0	60 / 20	N.S.

n = Number of Patients in Group. N.S. = Not significant, i.e., p>0.05.

Probabilities between 10% and 5% (0.1>p>0.05) are noted.

TABLE 20S

Coagulation Screening (Continued).

	t-Test	Pre-Plasma Exchange t	n	p	30 Minutes Post-Plasma Exchange t	n	p
Kaolin Cephalin Clotting Time	Group 1 Polygeline] v Group 2 Plasma Protein Soln.	3.10	60 / 60	0.01 > p > 0.002	2.4	60 / 60	0.02 > p > 0.01
	Group 1 Polygeline] v Group 3 Polygeline]]	2.36	60 / 20	0.05 > p > 0.02	2.72	60 / 20	0.01 > p > 0.002
	Group 2 Plasma Protein Soln. v Group 3 Polygeline]]	0	60 / 20	N.S.	3.87	60 / 20	p < 0.001
		6 Hours Post-Plasma Exchange t	n	p	24 Hours Post-Plasma Exchange t	n	p
	Group 1 Polygeline] v Group 2 Plasma Protein Soln.	0	60 / 60	N.S.	0	60 / 60	N.S.
	Group 1 Polygeline] v Group 3 Polygeline]]	1.02	60 / 20	N.S.	0	60 / 20	N.S.
	Group 2 Plasma Protein Soln. v Group 3 Polygeline]]	1.29	60 / 20	N.S.	0	60 / 20	N.S.

n = Number of Patients in Group. N.S. = Not significant, i.e., $p > 0.05$.

Probabilities between 10% and 5% ($0.1 > p > 0.05$) are noted.

TABLE 20S

Coagulation Screening (Continued).

	t-Test	Pre-Plasma Exchange			30 Minutes Post-Plasma Exchange		
		t	n	p	t	n	p
Thrombin Clotting Time	Group 1 Polygeline 1 v Group 2 Plasma Protein Soln.	0	60 / 60	N.S.	11.1	60 / 60	$p<0.001$
	Group 1 Polygeline 1 v Group 3 Polygeline 11	0	60 / 20	N.S.	3.88	60 / 20	$p<0.001$
	Group 2 Plasma Protein Soln. v Group 3 Polygeline 11	0	60 / 20	N.S.	3.88	60 / 20	$p<0.001$
		6 Hours Post-Plasma Exchange			24 Hours Post-Plasma Exchange		
		t	n	p	t	n	p
	Group 1 Polygeline 1 v Group 2 Plasma Protein Soln.	5.56	60 / 60	$p<0.001$	0	60 / 60	N.S.
	Group 1 Polygeline 1 v Group 3 Polygeline 11	3.88	60 / 20	$p<0.001$	0	60 / 20	N.S.
	Group 2 Plasma Protein Soln. v Group 3 Polygeline 11	0	60 / 20	N.S.	0	60 / 20	N.S.

n = Number of Patients in Group. N.S. = Not significant, i.e., $p>0.05$.

Probabilities between 10% and 5% ($0.1 > p > 0.05$) are noted.

TABLE 20S

Coagulation Screening (Continued).

		Group 1 Patients Polygeline 1			Group 2 Patients Plasma Protein Solution			Group 3 Patients Polygeline 11		
		t	n	p	t	n	p	t	n	p
Pro-Thrombin Time	Pre-Plasma Exchange v Post-Plasma Exchange 24 Hours	2.74	60	$0.01 > p > 0.002$	0	60	N.S.	3.16	20	$0.01 > p > 0.002$
Kaolin Cephalin Clotting Time	Pre-Plasma Exchange v Post-Plasma Exchange 24 Hours	1.83	60	$0.10 > p > 0.05$ N.S.	1.73	60	$0.10 > p > 0.05$ N.S.	8.94	20	$p < 0.001$
Thrombin Clotting Time	Pre-Plasma Exchange v Post-Plasma Exchange 24 Hours	0	60	N.S.	0	60	N.S.	0	20	N.S.

n = Number of Patients in Group. N.S. = Not significant, i.e., $p > 0.05$

Probabilities between 10% and 5% ($0.1 > p > 0.05$) are noted.

TABLE 22S Factor 1 (Fibrinogen)

t-test	30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange			24 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group I Polygeline 1 v Group 2 Plasma Protein Soln	11.08	60 / 60	$p < 0.001$	3.5	59 / 60	$p < 0.001$	6.3	59 / 60	$p < 0.001$
Group 1 Polygeline 1 v Group 3 Polygeline II	8.76	60 / 20	$p < 0.001$	1.57	59 / 20	N.S.	2.73	59 / 20	$0.01 > p > 0.002$
Group 2 Plasma Protein Soln v Group 3 Polygeline II	0.75	60 / 20	N.S.	0.69	60 / 20	N.S.	2.09	60 / 20	$0.05 > p > 0.02$

t-test	3 Days Post-Plasma Exchange			5 Days Post-Plasma Exchange		
	t	n	p	t	n	p
Group 1 Polygeline 1 v Group 2 Plasma Protein Soln.	3.07	60 / 60	$0.01 > p > 0.002$	11.2	60 / 60	$p < 0.001$
Group 1 Polygeline 1 v Group 3 Polygeline II	2.84	60 / 19	$0.01 > p > 0.002$	8.4	60 / 20	$p < 0.001$
Group 2 Plasma Protein Soln v Group 3 Polygeline II	4.9	60 / 19	$p < 0.001$	1.8	60 / 20	$0.10 > p > 0.05$ N.S.

n = Number of patients in Group. N.S. = Not significant, i.e., $p > 0.05$.

Probabilities between 10% and 5% ($0.1 > p > 0.05$) are noted.

TABLE 23S Factor II

t-test	30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange			24 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline 1 v Group 2 Plasma Protein Soln	7.05	60 60	$p < 0.001$	7.8	60 60	$p < 0.001$	4.05	60 60	$p < 0.001$
Group 1 Polygeline 1 v Group 3 Polygeline 11	3.90	60 20	$p < 0.001$	4.9	60 20	$p < 0.001$	4.4	60 20	$p < 0.001$
Group 2 Plasma Protein Soln v Group 3 Polygeline 11	5.0	60 20	$p < 0.001$	0.16	60 20	N.S.	0.86	60 20	N.S.

t-test	3 Days Post-Plasma Exchange			5 Days Post-Plasma Exchange		
	t	n	p	t	n	p
Group 1 Polygeline 1 v Group 2 Plasma Protein Soln	8.2	60 60	$p < 0.001$	3.3	60 60	$0.002 > p > 0.001$
Group 1 Polygeline 1 v Group 3 Polygeline 11	2.78	60 20	$0.01 > p > 0.002$	4.0	60 20	$p < 0.001$
Group 2 Plasma Protein Soln v Group 3 Polygeline 11	2.5	60 20	$0.02 > p > 0.01$	1.96	60 20	$0.10 > p > 0.05$ N.S.

n = Number of patients in Group. N.S. = Not significant, i.e., $p > 0.05$.

Probabilities between 10% and 5% ($0.1 > p > 0.05$) are noted.

Table 24S Factor VIII C

t - TEST	30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange			24 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline 1		60			60			60	$0.05 > p > 0.02$
v									
Group 2 Plasma Protein Soln.	8.8	60	$p < 0.001$	4.0	60	$p < 0.001$	2.0	59	
Group 1 Polygeline 1		60			60			60	
v									
Group 3 Polygeline 11	3.8	20	$p < 0.001$	1.39	19	N.S.	0.17	20	N.S.
Group 2 Plasma Protein Soln.		60			60			59	
v									
Group 3 Polygeline 11	13.3	20	$p < 0.001$	1.55	19	N.S.	1.36	20	N.S.

t - TEST	3 Days Post-Plasma Exchange			5 Days Post-Plasma Exchange		
	t	n	p	t	n	p
Group 1 Polygeline 1		60			60	
v						
Group 2 Plasma Protein Soln	3.2	60	$0.002 > p > 0.001$	2.0	60	$0.05 > p > 0.02$
Group 1 Polygeline 1		60			60	
v	3.86		$p < 0.001$	1.3		N.S.
Group 3 Polygeline 11		20			20	
Group 2 Plasma Protein Soln		60			60	
v	9.7		$p < 0.001$	2.86		$0.01 > p > 0.002$
Group 3 Polygeline 11		20			20	

n = number of patients in group N.S. = not significant i.e., $p > 0.05$

Probabilities between 10% and 5% ($0.1 > p > 0.05$) are noted

Table 25S Factor IX

t - TEST	30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange			24 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline I		60			60			60	
v									
Group 2	9.4		$p < 0.001$	7.5		$p < 0.001$	9.0		$p < 0.001$
Plasma Protein Soln		60			60			60	
Group 1 Polygeline I		60			60			60	
v	11.5		$p < 0.001$	1.0		N.S.	1.24		N.S.
Group 3 Polygeline II		20			20			20	
Group 2									
Plasma Protein Soln		60	$0.10 > p > 0.05$		60	$p < 0.001$		60	$p < 0.001$
v	1.96			5.23			7.6		
Group 3 Polygeline II		20	N.S.		20			20	

t - TEST	3 Days Post-Plasma Exchange			5 Days Post-Plasma Exchange		
	t	n	p	t	n	p
Group 1 Polygeline I		60			59	
v						
Group 2	17.8		$p < 0.001$	9.5		$p < 0.001$
Plasma Protein Soln		59			59	
Group 1 Polygeline I		60			59	$0.01 > p > 0.002$
v	4.3		$p < 0.001$	3.0		
Group 3 Polygeline II		20			20	
Group 2						
Plasma Protein Soln		59			59	
v	9.8		$p < 0.001$	5.48		$p < 0.001$
Group 3 Polygeline II		20			20	

n = number of patients in group. N.S. = not significant, i.e. $p > 0.05$
Probabilities between 10% and 5% ($0.1 > p > 0.05$) are noted.

Table 26S - Factor X

t - TEST	30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange			24 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline I v Group 2 Plasma Protein Soln	6.85	60 60	$p < 0.001$	1.93	60 60	$0.10 > p > 0.05$ N.S.	3.88	60 60	$p < 0.001$
Group 1 Polygeline I v Group 3 Polygeline II	4.55	60 20	$p < 0.001$	1.64	60 20	N.S.	5.33	60 20	$p < 0.001$
Group 2 Plasma Protein Soln v Group 3 Polygeline II	0	60 20	N.S.	3.65	60 20	$p < 0.001$	2.61	60 20	$0.02 > p > 0.01$

t - TEST	3 Days Post-Plasma Exchange			5 Days Post-Plasma Exchange		
	t	n	p	t	n	p
Group 1 Polygeline I v Group 2 Plasma Protein Soln.	3.86	60 60	$p < 0.001$	4.0	60 60	$p < 0.001$
Group 1 Polygeline I v Group 3 Polygeline II	2.38	60 20	$0.05 > p > 0.02$	0.72	60 20	N.S.
Group 2 Plasma Protein Soln v Group 3 Polygeline II	4.71	60 20	$p < 0.001$	1.68	60 20	$0.10 > p > 0.05$ N.S.

n = number of patients in group, N.S. = not significant, i.e., $p > 0.05$
Probabilities between 10% and 5% ($0.1 > p > 0.05$) are noted.

TABLE 27S

Electrolytes

Element	t-Test	Pre-Plasma Exchange			5 Days Post-Plasma Exchange		
		t	n	p	t	n	p
Sodium	Group 1 Polygeline v Group 2 Plasma Protein Soln.	1.73	60 60	0.10 > p > 0.05 N.S.	1.37	60 60	N.S.
	Group 1 Polygeline v Group 3 Polygeline II	1.05	60 20	N.S.	0.9	60 20	N.S.
	Group 2 Plasma Protein Soln. v Group 3 Polygeline II	3.6	60 20	p < 0.001	1.8	60 20	0.10 > p > 0.05 N.S.
Potassium	Group 1 Polygeline v Group 2 Plasma Protein Soln.	2.93	60 60	0.01 > p > 0.002	1.21	60 60	N.S.
	Group 1 Polygeline v Group 3 Polygeline II	1.08	60 20	N.S.	2.05	60 20	0.05 > p > 0.02
	Group 2 Plasma Protein Soln. v Group 3 Polygeline II	2.9	60 20	0.01 > p > 0.002	0.84	60 20	N.S.
Calcium	Group 1 Polygeline v Group 2 Plasma Protein Soln.	1.81	60 60	0.10 > p > 0.05 N.S.	5.46	60 60	p < 0.001
	Group 1 Polygeline v Group 3 Polygeline II	1.67	60 20	N.S.	1.93	60 20	0.10 > p > 0.05 N.S.
	Group 2 Plasma Protein Soln. v Group 3 Polygeline II	2.20	60 20	0.05 > p > 0.02	1.93	60 20	0.10 > p > 0.05 N.S.
Magnesium	Group 1 Polygeline v Group 2 Plasma Protein Soln.	0	60 60	N.S.	1.0	60 60	N.S.
	Group 1 Polygeline v Group 3 Polygeline II	1.82	60 20	0.10 > p > 0.05 N.S.	0.59	60 20	N.S.
	Group 2 Plasma Protein Soln. v Group 3 Polygeline II	1.97	60 20	0.10 > p > 0.05 N.S.	0	60 20	N.S.

n = Number of Patients in Group. N.S. = Not significant, i.e., $p > 0.05$
Probabilities between 10% and 5% ($0.10 > p > 0.05$) are noted.

TABLE 27S

Electrolytes (Continued).

Element	t-Test	Pre-Plasma Exchange			5 Days Post-Plasma Exchange		
		t	n	p	t	n	p
Chloride	Group 1 Polygeline v Group 2 Plasma Protein Soln.	3.10	60 60	0.01> p > 0.002	0.86	60 60	N.S.
	Group 1 Polygeline v Group 3 Polygeline 11	0.52	60 20	N.S.	0.56	60 20	N.S.
	Group 2 Plasma Protein Soln. v Group 3 Polygeline 11	2.01	60 20	0.05 > p > 0.02	0.77	60 20	N.S.
Phosphate	Group 1 Polygeline v Group 2 Plasma Protein Soln.	7.68	60 60	p < 0.001	7.25	60 60	p < 0.001
	Group 1 Polygeline v Group 3 Polygeline 11	3.9	60 20	p < 0.001	5.20	60 20	p < 0.001
	Group 2 Plasma Protein Soln. v Group 3 Polygeline 11	0.9	60 20	N.S.	0	60 20	N.S.
Urea	Group 1 Polygeline v Group 2 Plasma Protein Soln.	2.65	60 60	0.01> p > 0.002	3.05	60 60	0.01> p > 0.002
	Group 1 Polygeline v Group 3 Polygeline 11	0.8	60 20	N.S.	3.14	60 20	0.01> p > 0.002
	Group 2 Plasma Protein Soln. v Group 3 Polygeline 11	3.55	60 20	p < 0.001	1.8	60 20	0.10 > p > 0.05 N.S.
Creatinine	Group 1 Polygeline v Group 2 Plasma Protein Soln.	1.73	60 60	0.10 > p > 0.05 N.S.	0	60 60	N.S.
	Group 1 Polygeline v Group 3 Polygeline 11	0.35	60 20	N.S.	4.84	60 20	p < 0.001
	Group 2 Plasma Protein Soln. v Group 3 Polygeline 11	3.1	60 20	0.01> p > 0.002	9.4	60 20	p < 0.001

n = Number of Patients in Group N.S. = Not significant, i.e., p > 0.05.
Probabilities between 10% and 5% (0.10> p > 0.05) are noted.

TABLE 27S

Electrolytes (Continued).

Element	t-Test	Group 1 Patients Polygeline 1			Group 2 Patients Plasma Protein Solution			Group 3 Patients Polygeline 11		
		t	n	p	t	n	p	t	n	p
Sodium	Pre-plasma v Post-plasma Exchange	2.74	60	$0.01 > p > 0.002$	3.47	60	$p < 0.001$	1.61	20	N.S.
Potassium	Pre-plasma v Post-plasma Exchange	5.86	60	$p < 0.001$	3.63	60	$p < 0.001$	6.25	20	$p < 0.001$
Calcium	Pre-plasma v Post-plasma Exchange	6.2	60	$p < 0.001$	5.2	60	$p < 0.001$	8.0	20	$p < 0.001$
Magnesium	Pre-plasma v Post-plasma Exchange	1.0	60	N.S.	5.48	60	$p < 0.001$	1.39	20	N.S.
Chloride	Pre-plasma v Post-plasma Exchange	2.12	60	$0.05 > p > 0.02$	5.95	60	$p < 0.001$	1.27	20	N.S.
Phosphate	Pre-plasma v Post-plasma Exchange	3.6	60	$p < 0.001$	2.74	60	$0.01 > p > 0.002$	0	20	N.S.
Urea	Pre-plasma v Post-plasma Exchange	1.43	60	N.S.	2.33	60	$0.05 > p > 0.02$	0.34	20	N.S.
Creatinine	Pre-plasma v Post-plasma Exchange	2.1	60	$0.05 > p > 0.02$	1.89	60	$0.10 > p > 0.05$ N.S.	5.69	20	$p < 0.001$

n = Number of Patients in Group. N.S. = Not significant, i.e., $p > 0.05$

Probabilities between 10% and 5% ($0.10 > p > 0.05$) are noted.

TABLE 28S

Liver Function Tests

		Pre-Plasma Exchange			5 Days Post-Plasma Exchange		
		t	n	p	t	n	p
Bilirubin	Group 1 Polygeline v Group 2 Plasma Protein Soln.	1.02	60 60	N.S.	3.2	60 60	0.002 > p > 0.001
	Group 1 Polygeline v Group 3 Polygeline 11	0.96	60 20	N.S.	2.08	60 20	0.05 > p > 0.02
	Group 2 Plasma Protein Soln. v Group 3 Polygeline 11	1.24	60 20	N.S.	0	60 20	N.S.
Asparate	Group 1 Polygeline v Group 2 Plasma Protein Soln.	2.46	60 60	0.02 > p > 0.01	4.0	60 60	p < 0.001
	Group 1 Polygeline v Group 3 Polygeline 11	1.56	60 20	N.S.	2.94	60 20	0.01 > p > 0.002
	Group 2 Plasma Protein Soln. v Group 3 Polygeline 11	0	60 20	N.S.	0	60 20	N.S.
Alanine Trans-aminase	Group 1 Polygeline v Group 2 Plasma Protein Soln.	0.9	60 60	N.S.	1.64	60 60	N.S.
	Group 1 Polygeline v Group 3 Polygeline 11	0.6	60 20	N.S.	1.05	60 20	N.S.
	Group 2 Plasma Protein Soln. v Group 3 Polygeline 11	0	60 20	N.S.	0	60 20	N.S.
Alkaline Phos-phatase	Group 1 Polygeline v Group 2 Plasma Protein Soln.	5.15	60 60	p < 0.001	11.5	60 60	p < 0.001
	Group 1 Polygeline v Group 3 Polygeline 11	11.07	60 20	p < 0.001	7.95	60 20	p < 0.001
	Group 2 Plasma Protein Soln. v Group 3 Polygeline 11	6.29	60 20	p < 0.001	0	60 20	N.S.

n = Number of Patients in Group. N.S. = Not significant, i.e., p > 0.05
Probabilities between 10% and 5% (0.10 > p > 0.05) are noted.

TABLE 28S

Liver Function Tests (Continued).

	t-Test	Group 1 Patients Polygeline 1			Group 2 Patients Plasma Protein Solution			Group 3 Patients Polygeline 11		
		t	n	p	t	n	p	t	n	p
Bilirubin	Pre-plasma Exchange v Post-plasma Exchange	2.15	60	$0.05 > p > 0.02$	0	60	N.S.	1.58	20	N.S.
Asparate	Pre-Plasma Exchange v Post-Plasma Exchange	1.64	60	N.S.	1	60	N.S.	0.55	20	N.S.
Alanine Transaminase	Pre-Plasma Exchange v Post-Plasma Exchange	2.19	60	$0.05 > p > 0.02$	4.38	60	$p < 0.001$	3.07	20	$0.01 > p > 0.002$
Alkaline Phosphatase	Pre-Plasma Exchange v Post-Plasma Exchange	4.7	60	$p < 0.001$	12.6	60	$p < 0.001$	1.07	20	N.S.

n = Number of Patients in Group. N.S. = Not significant, i.e., $p > 0.05$.

Probabilities between 10% and 5% ($0.10 > p > 0.05$) are noted.

TABLE 29S SERUM PLASMA PROTEIN CONCENTRATIONS TOTAL PROTEIN

t - Test	Pre-Plasma Exchange			30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline I		60	0.10 > p >		60			60	
v	1.90		0.05	4.4		p < 0.001	4.4		p < 0.001
Group 2 Plasma Protein Solution		60	NS		60			60	
Group 1 Polygeline I		60	0.02 > p >		60			60	
v	2.64		0.01	1.59		NS	17.0		p < 0.001
Group 3 Polygeline II		20			20			20	
Group 2 Plasma Protein Solution		60			60	p < 0.001		60	
v	1.41		NS	4.76			13.9		p < 0.001
Group 3 Polygeline II		20			20			20	
t - Test	24 Hours Post-Plasma Exchange			3 Days Post-Plasma Exchange			5 days Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline I		60			60			60	
v	7.94		p < 0.001	1.98		p = 0.05	3.97		p < 0.001
Group 2 Plasma Protein Solution		60			60			60	
Group 1 Polygeline I		60			60			60	
v	19.4		p < 0.001	12.1		p < 0.001	5.36		p < 0.001
Group 3 Polygeline II		20			20			20	
Group 2 Plasma Protein Solution		60			60			60	0.01 > p >
v	16.2		p < 0.001	12.4		p < 0.001	3.09		0.002
Group 3 Polygeline II		20			20			20	

n = Number of patients in Group NS - Not Significant i.e. $p > 0.05$
Probabilities between 10% and 5% ($0.10 > p > 0.05$) are noted

TABLE 29S SERUM PLASMA PROTEIN CONCENTRATIONS ALBUMIN

t - Test	Pre-Plasma Exchange			30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline I		60			60			60	
v	5.77		$p < 0.001$	13.4		$p < 0.001$	9.9		$p < 0.001$
Group 2 Plasma Protein Solution		60			60			60	
Group 1 Polygeline I		60			60			60	
v	1.08		NS	6.2		$p < 0.001$	11.1		$p < 0.001$
Group 3 Polygeline II		20			20			20	
Group 2 Plasma Protein Solution		60	$0.01 > p > 0.002$		60			60	
v	2.68			12.3		$p < 0.001$	16.4		$p < 0.001$
Group 3 Polygeline II		20			20			20	

t - Test	24 Hours Post-Plasma Exchange			3 Days Post-Plasma Exchange			5 days Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline I		60			60			60	
v	6.7		$p < 0.001$	6.7		$p < 0.001$	6.7		$p < 0.001$
Group 2 Plasma Protein Solution		60			60			60	
Group 1 Polygeline I		60			60			60	
v	15.8		$p < 0.001$	9.9		$p < 0.001$	7.9		$p < 0.001$
Group 3 Polygeline II		20			20			20	
Group 2 Plasma Protein Solution		60			60			60	
v	14.8		$p < 0.001$	10.7		$p < 0.001$	9.4		$p < 0.001$
Group 3 Polygeline II		20			20			20	

n = Number of patients in Groups, NS = not significant, i.e. $p > 0.05$
Probabilities between 10% and 5% ($0.10 > p > 0.05$) are noted.

TABLE 29S SERUM PLASMA PROTEIN CONCENTRATIONS GLOBULIN

t - Test	Pre-Plasma Exchange			30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline I v Group 2 Plasma Protein Solution	7.97	60 60	$p<0.001$	12.4	60 60	$p<0.001$	25.5	60 60	$p<0.001$
Group 1 Polygeline I v Group 3 Polygeline II	3.74	60 20	$p<0.001$	3.88	60 20	$p<0.001$	10.3	60 20	$p<0.001$
Group 2 Plasma Protein Solution v Group 3 Polygeline II	1.93	60 20	$0.10>p>0.05$ N.S.	14.2	60 20	$p<0.001$	7.75	60 20	$p<0.001$
t - Test	24 Hours Post-Plasma Exchange			3 Days Post-Plasma Exchange			5 Days Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline I v Group 2 Plasma Protein Solution	23.5	60 60	$p<0.001$	14.4	60 60	$p<0.001$	16.8	60 60	$p<0.001$
Group 1 Polygeline I v Group 3 Polygeline II	13.7	60 20	$p<0.001$	10.1	60 20	$p<0.001$	0	60 20	N.S.
Group 2 Plasma Protein Solution v Group 3 Polygeline II	2.58	60 20	$0.02>p>0.01$	0	60 20	N.S.	12.9	60 20	$p<0.001$

n = Number of Patients in Group. N.S. = Not significant, i.e., $p>0.05$.

Probabilities between 10% and 5% ($0.10>p>0.05$) are noted.

TABLE 30S SERUM IRON AND TOTAL IRON BINDING CAPACITY

	t - Test	Pre-Plasma Exchange t	n	p	Post Plasma Exchange t	n	p
Serum Iron	Group 1 Polygeline I v Group 2 Plasma Protein Solution	1.02	60 60	NS	3.8	60 60	$p < 0.001$
	Group 1 Polygeline I v Group 3 Polygeline II	0.46	60 20	NS	0.35	60 20	NS
	Group 2 Plasma Protein Solution Group 3 Polygeline II v	0.34	60 20	NS	2.14	60 20	$0.05 > p > 0.02$
Total Iron Binding Capacity	Group 1 Polygeline I v Group 2 Plasma Protein Solution	2.9	60 60	$0.01 > p > 0.002$	3.00	60 60	$0.01 > p > 0.002$
	Group 1 Polygeline I v Group 3 Polygeline II	1.52	60 20	NS	0	60 20	NS
	Group 2 Plasma Protein Solution Group 3 Polygeline II v	0.35	60 20	NS	1.74	60 20	$0.10 > p > 0.05$ NS

	t - Test	Group 1 Patients Polygeline I t	n	p	Group 2 Patients Plasma Protein Solution t	n	p	Group 3 Patients Polygeline II t	n	p
Serum Iron	Pre-Plasma Exchange v Post-Plasma Exchange	2.3	60	$0.05 > p > 0.02$	2.16	60	$0.05 > p > 0.02$	0.6	20	NS
Total Iron Binding Capacity	Pre-Plasma Exchange v Post-Plasma Exchange	3.4	60	$0.002 > p > 0.001$	2.35	60	$0.05 > p > 0.02$	0.5	20	NS

n = Number of patients in Group, NS = not significant, i.e. $p > 0.05$
Probabilities between 10% and 5% ($0.10 > p > 0.05$) are noted

TABLE 31S SERUM IMMUNOGLOBULIN IgG

t - Test	30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange			24 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline I		60			60			60	
v	1.82		0.10 > p > 0.05	0		NS	1.82		0.10 > p > 0.05
Group 2 Plasma Protein Solution		60	NS		60			60	NS
Group 1 Polygeline I		60			60			60	
v	2.58		0.02 > p > 0.01	2.14		0.05 > p >	0		NS
Group 3 Polygeline II		20			20	0.02		20	
Group 2 Plasma Protein Solution		60			60			60	
v	1.29		NS	2.78		0.01 > p >	1.29		NS
Group 3 Polygeline II		20			20	0.002		20	
t - Test	3 Days Post-Plasma Exchange			5 Days Post-Plasma Exchange					
Group 1 Polygeline I		60			60				
v	3.64		p < 0.001	5.48		p < 0.001			
Group 2 Plasma Protein Solution		60			60				
Group 1 Polygeline I		60			60				
v	6.94		p < 0.001	3.88		p < 0.001			
Group 3 Polygeline II		20			20				
Group 2 Plasma Protein Solution		60			60				
v	4.17		p < 0.001	0		NS			
Group 3 Polygeline II		20			20				

n = Number of patients in Group, NS = Not significant, i.e. $p \geq 0.05$
Probabilities between 10% and 5% ($0.10 > p > 0.05$) are noted

TABLE 31S SERUM IMMUNOGLOBULIN IgA (continued)

t - Test	30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange			24 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline I v Group 2 Plasma Protein Solution	1.82	60 60	0.10> p >0.05 NS	0	60 60	NS	3.65	60 60	p < 0.001
Group 1 Polygeline I v Group 3 Polygeline II	2.58	60 20	0.02 > p > 0.01	2.58	60 20	0.02> p> 0.01	0	60 20	NS
Group 2 Plasma Protein Solution v Group 3 Polygeline II	1.29	60 20	NS	3.34	60 20	0.002> p > 0.001	2.78	60 20	0.01> p > 0.002
	3 Days Post-Plasma Exchange			5 Days Post-Plasma Exchange					
	t	n	p	t	n	p			
Group 1 Polygeline I v Group 2 Plasma Protein Solution	2.74	60 60	0.01> p >0.002	8.2	60 60	p < 0.001			
Group 1 Polygeline I v Group 3 Polygeline II	3.88	60 20	p < 0.001	1.94	60 20	0.10> p > 0.05 NS			
Group 2 Plasma Protein Solution v Group 3 Polygeline II	5.81	60 20	p < 0.001	7.75	60 20	p < 0.001			

n = Number of patients in Group, NS = Not significant, i.e. $p > 0.05$
Probabilities between 10% and 5% ($0.10 > p > 0.05$) are noted

TABLE 31S SERUM IMMUNOGLOBULIN IgM (continued)

t - Test	30 Minutes Post-Plasma Exchange			12 Hours Post-Plasma Exchange			24 Hours Post-Plasma Exchange		
	t	n	p	t	n	p	t	n	p
Group 1 Polygeline 1 v Group 2 Plasma Protein Solution	3.6	60 60	$p < 0.001$	3.09	60 60	$0.01 > p > 0.002$	4.3	60 60	$p < 0.001$
Group 1 Polygeline 1 v Group 3 Polygeline 11	0	60 20	NS	1.29	60 20	NS	5.52	60 20	$p < 0.001$
Group 2 Plasma Protein Solution v Group 3 Polygeline 11	2.58	60 20	$0.02 > p > 0.01$	3.07	60 20	$0.01 > p > 0.002$	6.76	60 20	$p < 0.001$
	3 Days Post-Plasma Exchange			5 Days Post-Plasma Exchange					
	t	n	p	t	n	p			
Group 1 Polygeline 1 v Group 2 Plasma Protein Solution	8.2	60 60	$p < 0.001$	0	60 60	NS			
Group 1 Polygeline 1 v Group 3 Polygeline 11	5.8	60 20	$p < 0.001$	5.16	60 20	$p < 0.001$			
Group 2 Plasma Protein Solution v Group 3 Polygeline 11	0	60 20	NS	5.16	60 20	$p < 0.001$			

n = Number of patients in Group, N.S. = Not significant, i.e. $p > 0.05$
Probabilities between 10% and 5% ($0.10 > p > 0.05$) are noted.

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