

Historical review

SUPPORTIVE CARE FOR PATIENTS WITH BLOOD DISORDERS

The first efforts to replace blood in man were made in 1667, three and a half centuries ago. The major impediment to replacement of whole blood for patients with severe anaemia or major blood loss was the tendency of the blood to coagulate. The strategy used to avert this was defibrination of the blood, which was introduced more than a century and a half ago in 1835. Blood replacement was still conducted infrequently because the defibrination process was extremely tedious. It was not until 1914 that citrate anticoagulant was introduced, making allogeneic whole blood replacement transfusion a significant modality for supportive therapy (Freireich and Lemak, 1991). In this review, I will cover the developments in supportive therapy over only the last half century, which is the period of my professional life span, and review this topic from a personal perspective. I must apologize in advance to other scientists and physicians who made enormous contributions to this field for omitting or minimizing their contributions, but I felt that it would be helpful to the reader to appreciate a personal perspective rather than an impersonal literature review.

When I completed my medical training and began my internship in 1950, I was required to participate in phlebotomies of normal volunteer donors at the blood bank of the Cook County Hospital in Chicago, IL, which had a plaque indicating that it was the first operating blood bank, i.e. it was a place where blood was collected and stored. Using the acid–citrate–dextrose (ACD) solutions, the blood could be stored for 14–21 d. I recall those experiences vividly because the equipment that we used consisted of glass bottles with rubber tubing of a wide bore and large cutting edge needles, which were not sharp. All these were reutilized (after cleaning). In contrast to today's sophisticated collection centres, it was a primitive operation. In the early 1950s, my career moved in the direction of haematology, and I cared for patients with bone marrow failure of any type, i.e. anaemia, thrombocytopenia or leucopenia. The major modality of support that we had available was whole blood collected in ACD and stored in a blood bank for 7–21 d. The indication for replacement of blood was anaemia. The blood banks of the day had come to appreciate the importance of the plasma component of the blood as opposed to the red cells because of the elegant work of Dr E. J. Cohn during the Second World War. Dr Cohn developed an instrument for collecting plasma and for preparing the fractions of the plasma which were useful for

the treatment of haemorrhage and shock, particularly under battle conditions. Therefore, we had plasma products and red cell products available for transfusion support.

REPLACEMENT OF PLATELETS

When I was recruited to work at the new Research Clinical Center at the National Institute of Health in 1955, I was assigned the responsibility of developing a programme for the treatment of acute leukaemia. By 1955, there were three agents capable of inducing clinical and haematological remissions of acute lymphoblastic leukaemia of children. The first was methotrexate, reported by Dr S. Farber in 1948; second was 6-mercaptopurine, reported by Dr J. Burchenal; and third was adrenocorticoid steroids, reported both by Dr O. Pierson and Dr Farber. Dr Farber recognized that one of the major reasons for the failure of these treatments was not their effectiveness against the leukaemia but rather the occurrence of haemorrhage. He had initiated a programme at the Children's Hospital in Boston which utilized frozen disrupted platelets to repair the coagulation mechanism and had reported some preliminary success. This product was developmental and controversial, and many investigators who attempted to repeat these experiences were unsuccessful. Thus, this treatment was not available to us at the Clinical Center. In addition, infection was a major problem. The antibiotics and sulpha drugs had first become widely available during the Second World War. I recall that when I was an intern in 1950 penicillin was dispensed only for special cases and we had to recover the penicillin from the patient's urine and recycle it because of the scarcity of this product. By 1955, we had penicillin, the sulpha drugs and streptomycin available to treat infections. As my clinical experience with treating these children with leukaemia developed, it was clear that the major cause of failure was haemorrhage, which in many instances was fatal. I can recall making rounds on a leukaemia service of 10–12 children undergoing chemotherapy and the most striking impact of those rounds was the presence of blood on sheets, furniture, drapes and patients in virtually every room. One day on morning rounds with my immediate superiors, Dr C. G. Zubrod and Dr E. Frei III, I recall visiting a semiconscious child with severe bleeding around the nose and mouth and breathing in an interrupted way, continuously spattering blood all over the room. When we emerged from the patient's room, Dr Zubrod turned to me and said, 'You really should do something about this bleeding problem. Since you are a haematologist, it should command your attention.' So, I retreated to my small laboratory and began to study the blood of these children to

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seek the mechanism of the haemorrhagic diathesis and its potential correction. The literature indicated that, although a lack of blood platelets was characteristic, thrombocytopenia itself was not sufficient to result in haemorrhage and other factors, particularly anticoagulants, infection and damaged vessels, were the precipitating events which resulted in haemorrhage. Moreover, in a series of brilliant experiments conducted by Drs G. Brecher and E. Cronkite and Frank Gardner, it had been shown in experimental animals and in man that the transfusion of platelets was effective only transiently and that isosensitization occurred rapidly. Therefore, the replacement by platelet transfusion was not a successful therapeutic strategy, and it had no potential. I turned to the possibility of replacing the procoagulant from platelets. After studying the blood from a series of children with bleeding, I confirmed that the frozen lyophilized platelet product could, *in vitro*, at least, correct the lack of coagulability of the blood. My first studies involved the separation of the lipoprotein from platelets in an ultracentrifuge gradient. We separated what is now known as platelet factor 3 from sonicated platelets, which corrected the coagulation disturbance *in vitro*, and we were able to prepare a sterile suspension of such products and infused them into children. What we observed was what had been observed with the lyophilized product. Although there was dramatic cessation of haemorrhage in children who were actively bleeding, this effect lasted only as long as the infusion continued, and when it was discontinued the haemorrhage promptly recurred. This experience convinced me that the blood platelet was the missing ingredient for the treatment of haemorrhage. Therefore, we turned our attention to studying the effect of whole platelets mixed with the blood of children with leukaemia, and we found in virtually every instance that this corrected the coagulation defect *in vitro*. However, when we studied blood that had been stored for 48 h or more in the blood bank in ACD solutions, we found that this corrective effect was rapidly lost. We therefore initiated a study of administering freshly drawn blood collected in ethylene diamine tetra-acetic acid (EDTA) (a calcium-chelating agent) and collected the blood in the newly developed equipment, i.e. plastic tubing and plastic bags, which allowed blood to be collected in a closed system. After we injected the freshly collected blood into a number of children, we observed correction of the haemorrhagic diathesis, and the effect lasted several days. This encouraged us to confine our replacement of blood for children who were bleeding to freshly collected whole blood. The magnitude of the problem soon overwhelmed our blood bank, and we had a major academic confrontation with our colleagues in the blood bank. Because we were requesting freshly drawn whole blood and had failed to provide conclusive evidence that it was indicated, this put an enormous strain on the mechanics of the operation of the blood bank. As a result, we undertook what to my knowledge was the first prospective randomized comparative study of supportive therapy with platelets (Freireich *et al.*, 1959). We used the technique first introduced in 1948 by A. B. Hill which had proven successful in the study of antibiotics, particularly streptomycin. We applied this

technique to compare the transfusion of one unit of whole fresh blood to one unit of whole banked stored blood which was more than 48 h old. The results of this study were dramatic. The frequency of haemorrhage cessation measured objectively by quantifying blood loss in urine and by physical examination was associated with the fresh products. This difference was highly significant. The amazing thing is that when we studied the change in circulating platelets we found that on average the increase in platelet count was only $5.0 \times 10^9/l$. Today, that would not surprise anyone but, when it was reported in 1959, it created quite a sensation. Because in most haematology laboratories platelets were counted indirectly, i.e. the number of platelets on a stained blood film were compared with the number of red cells, and because platelets have a tendency to clump and adhere to glass, these counts were extremely variable and had reproducibilities of less than 100%. However, Drs Brecher and Cronkite had introduced the method of phase contrast microscopy direct counting in a haemocytometer of platelets in platelet-rich plasma. With that technique, it was objectively possible to detect changes of 100% over the baseline with great accuracy and reproducibility.

When this study was reported, a young physician working in the blood bank, Dr Alan Kliman, approached me and suggested that if one unit of fresh whole blood was useful could we improve our transfusions if we increased the dose by a 100%. Towards this goal, we devised a two-unit closed plastic system which would allow us to collect one unit of blood, centrifuge it, reinject the red cells, save the platelet-rich plasma, bleed a second unit, again return the red blood cells and transfuse two units of platelet-rich plasma all in a closed system (Fig 1). We realized that this was a practical and effective method, and we began to apply this systematically to the treatment of patients who were bleeding (Kliman *et al.*, 1961). We then undertook a study of

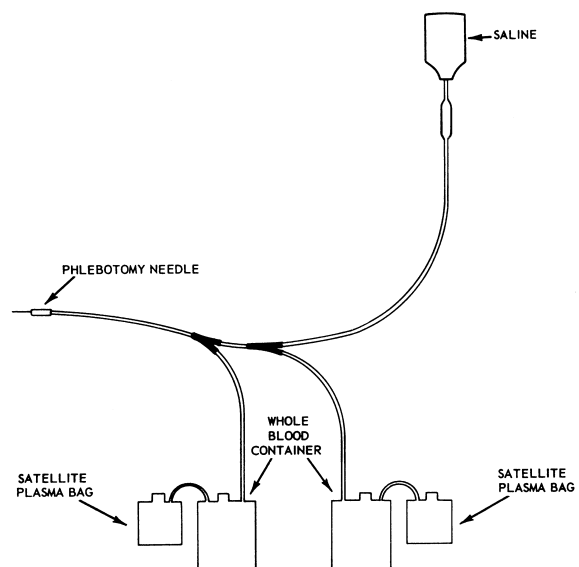


Fig 1. Equipment for obtaining 500 ml of plasma by plasma-pheresis.

six healthy adult male donors to determine the limits of platelet donation by plasmapheresis (Kliman *et al*, 1964). Whole blood donation was limited to every 90 d; so, the donor pool for the repeated transfusions of platelets two to three times per week for each patient resulted in the need for an enormous number of donors. However, with platelet pheresis, we demonstrated that an adult donor could donate four units of platelets every week without significant depletion of any haematopoietic formed elements in the blood; the limiting factor being the loss of protein, which was regenerated at the rate of 1 l per week. We now had a supply of platelets which was in excess of 50-fold over single whole blood units, and it was now possible to begin systematically replacing platelets in these leukaemic children. While these studies were ongoing, one of our clinical associates, Dr L. A. Gaydos, and I decided to examine the relationship between the platelets and haemorrhage to determine whether there were factors other than the platelets responsible for the haemorrhagic diathesis. Retrospectively, we examined the records of a large number of children with leukaemia. For every day that they were in hospital, we determined whether there was any form of haemorrhage, and we rated this haemorrhage as superficial, i.e. petechial haemorrhage alone, minor bleeding from mucus membranes which was intermittent or major bleeding which was grossly visible and life threatening. We recorded these from the records of the physicians and nurses attending to these patients, and each day we recorded the patients' platelet counts. The results of these studies were striking, and this publication is now a citation classic being referred to frequently to guide platelet replacement transfusion (Gaydos *et al*, 1962). This publication showed that there was a direct and continuous relationship between the degree of thrombocytopenia and the occurrence of haemorrhage. This relationship was not a linear one, but it was continuous. As a result, we stated in the paper that life-threatening haemorrhage occurred infrequently at platelet counts below $20 \times 10^9/l$ (Fig 2).

That statement led to the use of a platelet level of $20 \times 10^9/l$ as a trigger for platelet replacement for many years after that publication; even though we clearly stated that there was no threshold for bleeding but rather a continuous risk of haemorrhage. Moreover, we demonstrated that when the platelet count was falling the level at which haemorrhage occurred was higher than if the platelets were chronically low, an observation which has been repeatedly confirmed.

Armed with sufficient platelets for replacement transfusion and with the knowledge that the level of platelets to eradicate haemorrhage or to prevent it was known, we undertook a programme of platelet replacement transfusion systematically when the platelet level fell below $20 \times 10^9/l$ (Freireich *et al*, 1963). We rapidly demonstrated that such a programme could substantially reduce the occurrence of thrombocytopenic haemorrhage. In a retrospective review by E. Hersh and G. Bodey, we demonstrated that, with the introduction of systematic replacement platelet transfusion, haemorrhage as a cause of death was reduced to the level of almost being eliminated and the major cause of death was now replaced by infection (Fig 3); a situation which is true to this day (Hersh *et al*, 1965). There was still the problem of isosensitization, and we were amazed to find that in these children isosensitization occurred infrequently (Fig 4). The major causes of platelet resistance were shown to be the occurrence of systemic infection and/or splenomegaly rather than isoantibody formation. With improvements in the equipment used to collect platelets, whether with continuous flow or intermittent blood flow separators or with multiple plastic bags, it is now possible to obtain four units of platelets, i.e. 4×10^{11} platelets from a single donation. With such donations, the initially reported increments of $12.5 \times 10^9/l/10^{11}$ platelets injected provide a systematically reproducible measurement for replacing platelets. Other technical improvements include the observation of the acid environment, the importance of motion to prevent platelets from clumping and the observation that refrigeration induces clumping. Therefore, platelets

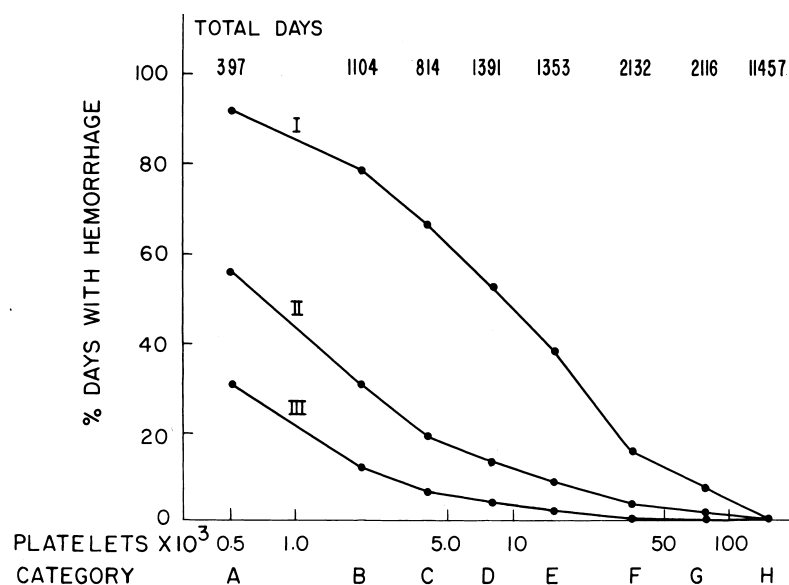


Fig 2. Platelet count and frequency of haemorrhage.

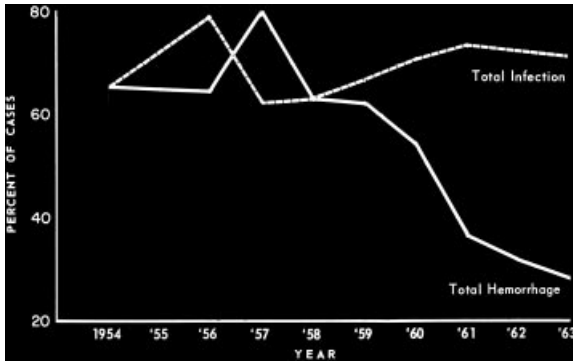


Fig 3. Changing patterns of fatal infection and haemorrhage in acute leukaemia.

currently are collected and continuously agitated in their acidified plasma at room temperature before transfusion for periods of up to 48 h.

Platelet replacement transfusion programmes still require better understanding of the isoantigens and the occurrence of platelet resistance, as well as better methods for *in vitro* storage. Of course, the cycle will be complete when we finally develop a chemical substitute for the platelet which can be used as a pharmaceutical. Many investigators are pursuing this, and many products have appeared on the horizon. I am confident that we will soon replace the use of allogeneic whole blood platelets for the treatment of thrombocytopenic haemorrhage.

REPLACEMENT OF WHITE BLOOD CELLS

Flushed with the success of the use of plasmapheresis technique for increasing the number of platelets available for transfusion, we began to examine the literature to see whether we could use a similar approach to replacing granulocytes for the treatment of infection. Dr Bodey had conducted a retrospective study of the relationship between the white blood count and the occurrence of infection (Bodey *et al.*, 1966). This paper is also a citation classic and

made many important and original observations. The first was that, like platelets, there was a direct and continuous relationship between the degree of leucopenia on any given day and the occurrence of an infectious complication on those days (Fig 5). This was true both for fevers of unknown origin and for proven infections. This relationship held true for both the absolute number of granulocytes and the absolute number of lymphocytes in the patients. Therefore, we wondered whether we could use the same plasmapheresis technique to collect sufficient numbers of granulocytes to increase the number of granulocytes, following the experimental plan that we had used with platelets. By this date, 1962, the physiology of the granulocyte had been well studied in experimental animals and in man using radioisotopes. It was found that the granulocytes had a half-life of 6–8 h in the circulation and that their loss was exponential, i.e. random. Thus, it was evident that to increase the granulocyte concentration to normal in a child would require all of the granulocytes present in the circulating blood of an adult, a task which we thought could not be accomplished easily (Table I). For proof of principle, we had the idea that if we turned to the experiment of nature, i.e. patients with chronic granulocytic leukaemia (CML) whose white blood counts were an order of magnitude higher than in normal donors, we could use the same technique. Thus, we initiated a programme of the collection of peripheral blood granulocytes, i.e. buffy coats, in the form of white cell and platelet-rich plasma supernatants from the blood of patients with CML in benign phase using the same two-bag technique that we had successfully used for platelets. These cells were collected with an ACD anticoagulant and immediately transfused into children who had proven major life-threatening haemorrhage. As with platelet transfusion, these transfusions proved dramatically effective (Freireich *et al.*, 1964). We were able to collect up to 10^{11} granulocytes per donor, and we were able to show that the increase in granulocytes in the recipients was directly correlated with the number of granulocytes collected and injected. Moreover, we were able to show a quantitative dose relationship between the increment in the recipient, or the dose of granulocytes administered and the response of

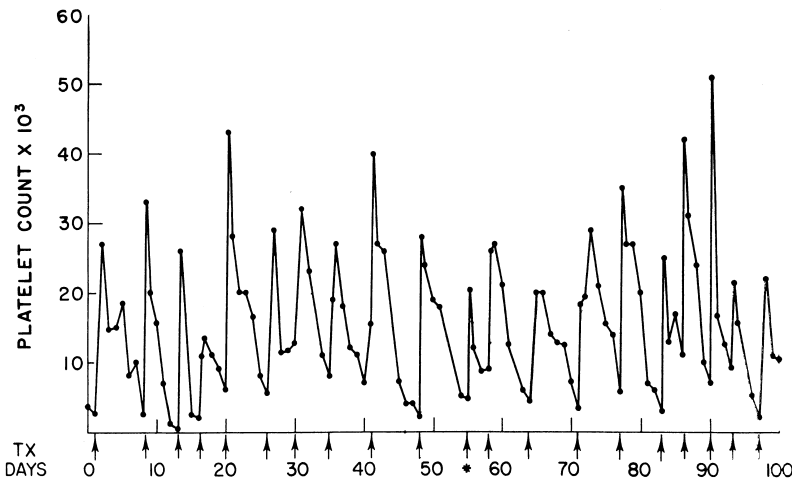


Fig 4. Effect of 19 repeated platelet transfusions (Tx) from a single donor.

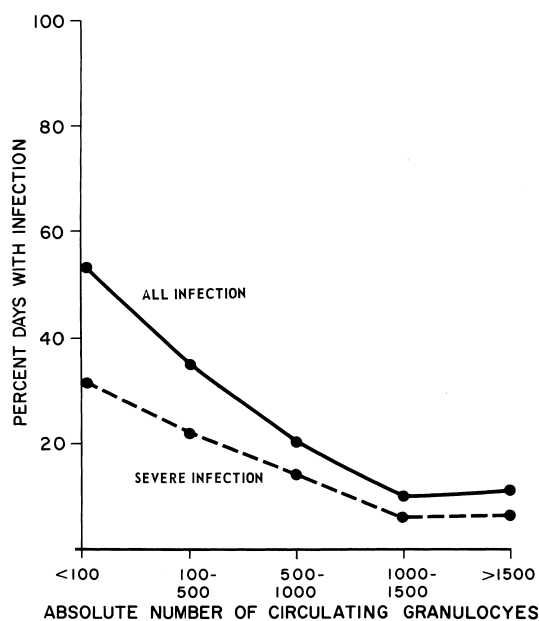


Fig 5. Relationship between granulocyte count and infection.

the patient's infection and/or fever. Another important observation that would prove subsequently to be quite important was that the half-life of these transfused granulocytes was not 6–8 h but was 24 h. We reasoned that the prolonged presence of these granulocytes in the circulation was a result of the injection of immature myeloid cells, which continued to divide and produce mature granulocytes which we counted in the blood of these children. We observed that these increases in circulating granulocytes lasted for a week or more, and we were able to demonstrate there was persistence of Philadelphia chromosome-positive mitoses in the bone marrow of some of these children for up to 30 d. Therefore, we demonstrated temporary allografts from the peripheral blood of CML donors in recipients with acute leukaemia (Levin *et al.*, 1963).

As with the platelet transfusion, we realized that although proof of principle had been demonstrated there was not a supply of CML donors for providing granulocytes for supportive therapy for patients. Rather, we had to develop a method for processing large volumes of peripheral blood if this strategy was to be successful for the treatment of infection. So, we initiated work on biomechanical equipment to process blood continuously, in the manner

of an artificial kidney, and skim off only granulocytes, leaving the other formed elements in plasma to be returned to the donor. At this point, the father of one of our CML patients was an engineer with the IBM Corporation; Dr J. Block referred him to me. Thus, Mr George Judson came to my laboratory and asked if he could help to develop such a piece of equipment with the help of many talented engineers. This began a collaboration which eventuated in what became known as the IBM 2990 Blood Cell Separator (Freireich *et al.*, 1965). The instrument was developed in my laboratory with component parts that had been discarded by engineers at IBM, plus materials that we could purchase at hardware stores and plastic equipment which we could manufacture in our own laboratory. When Mr Judson asked what the goals of the project should be, we set out the following objectives.

1. Leucocytes should be separated from whole blood at a reasonable efficiency by sedimentation in a centrifuge.
2. Operation should be conducted on a continuous flow basis to allow processing of large quantities of blood at optimal speed and efficiency.
3. A vein-to-vein procedure should be used to avoid arterial puncture.
4. An anticoagulant that does not require anticoagulation of the donor, with its associated risks, should be used.
5. The loss of platelets, red cells and plasma should be minimal to allow processing of large volumes of blood in a single donor.
6. The system should be completely closed, needle-to-needle, without any air–blood interface to obviate the danger of air injection or bacterial contamination.
7. The entire system should contain a volume of blood under 500 ml at all times.
8. The system should be easily cleaned, mostly disposable and sufficiently automated to be operated by a single non-professional operator.

When we began this project, we were aware of the Cohn–Latham blood cell separator, which was a falling film instrument that processed blood intermittently at approximately one 500 cc unit at a time. We felt that to be effective our instrument would need to process whole blood volumes (i.e. 5–10 l). To accomplish this, we felt it would need to be able to collect, simultaneously supernatant and precipitate, while collecting the buffy coat from the centre of the centrifugal field. This was a new concept and it was patented.

The first contact that I had with Mr Judson was on 14

Table I. Transfusion dose requirement for the formed elements of the blood.

	% Recovery in circulation	Dose factor, donations to replace 25% (child)	Survival, 1/2 time (d)	Factor dose × time
Red cells	> 90	1	60	1
Platelets	30	3	1	180
Granulocytes	5	18	1	1080

Table II. Effectiveness of etiocholanolone (ETIO) and hydroxyethyl starch (HES) in increasing granulocyte collection.

	Number of leucaphereses	Total WBC* ($\times 10^9/l$)	Total polys* ($\times 10^9/l$)
Controls	29	1.35	0.38
With ETIO	13	1.95	0.77
With HES	24	2.42	1.58
With ETIO and HES	35	3.52	2.73

*Mean values. polys, polymorphonuclear leucocytes, i.e. granulocytes.

May 1962. The next month was spent planning the project, and on 17 June 1962 the actual assembling of the hardware began in my laboratory. The instrument was an assemblage of components including blood pumps that had been salvaged from the early cardiac surgery days, a centrifuge bowl which was designed and fabricated for the instrument, some discarded motors and solenoids and plastic tubing. Amazingly, by 8 April 1963, 10 months after the laboratory studies began, we proposed, and were approved, to go directly to man. The reason that this was possible is that the *in vitro* work was carried out entirely with human blood from donors who proved to have either syphilis or hepatitis, and therefore could only be used in the laboratory. It was clear from the literature and from other studies that if we were to work with the blood of other primates, dogs or other experimental animals we would have to develop entirely different parameters for centrifugal speeds and flows through the instrument. After extensive safety testing *in vitro* for microbiological contamination, haemolysis and other hazards, we were able to convince the appropriate management individuals to go directly to patients. The first patient studied was a patient with benign phase CML. We specifically elected to initiate these studies in patients with CML because we had extensive experience with collecting leucocytes from these patients in the two-plastic bag closed

system that we used for platelet collection. We understood the sedimentation characteristics, and because the buffy coats were large we realized the physical ability to collect these cells was realistic. Second, we proposed that removal of a large number of leucocytes from patients with CML could have a therapeutic benefit, particularly in patients with high platelet counts and white counts; we felt that the rapid lowering of these counts might provide a benefit risk ratio which was favourable to initiate a new procedure. These early studies were interrupted by two events. The first was the connection between the centrifuge and the stationary phase of the instrument. We were using a machined 'face' seal which had three channels and, despite the elegant engineering, there was clearly a passage of blood cells across the seal faces. Second, the pumps that we had adapted from other uses proved to be unsatisfactory for carefully regulating the flow between the precipitate and the supernatant so that the buffy coat could be positioned over the collecting port. By 2 January 1964, we found that there was a need for significant engineering work before a practical instrument could be tested. Therefore, a contract between the National Cancer Institute and IBM was initiated for a period of 18 months. Unfortunately, a year later, Dr Frei elected to move to The University of Texas M. D. Anderson Cancer Center in Houston, TX, USA. In July, he recruited me to join him in Texas, and the work on the blood cell separator was continued under the supervision of Dr S. Perry and Dr D. Buchner. After arriving in Texas, we began negotiations with IBM to continue the investigations at M. D. Anderson. This culminated in IBM's decision to manufacture three instruments at the current stage of engineering development, and they offered to allow us to carry out the early studies in patients with this instrument, which was called the IBM 2990. We received our instrument on 2 December 1966 and began to work in our laboratory with hand-assembled tubing and locally manufactured anticoagulants (Fig 6). By September 1969, we had accumulated sufficient experience and we expanded our operations to two instruments (Fig 7). Our initial studies began on patients with chronic lymphocytic

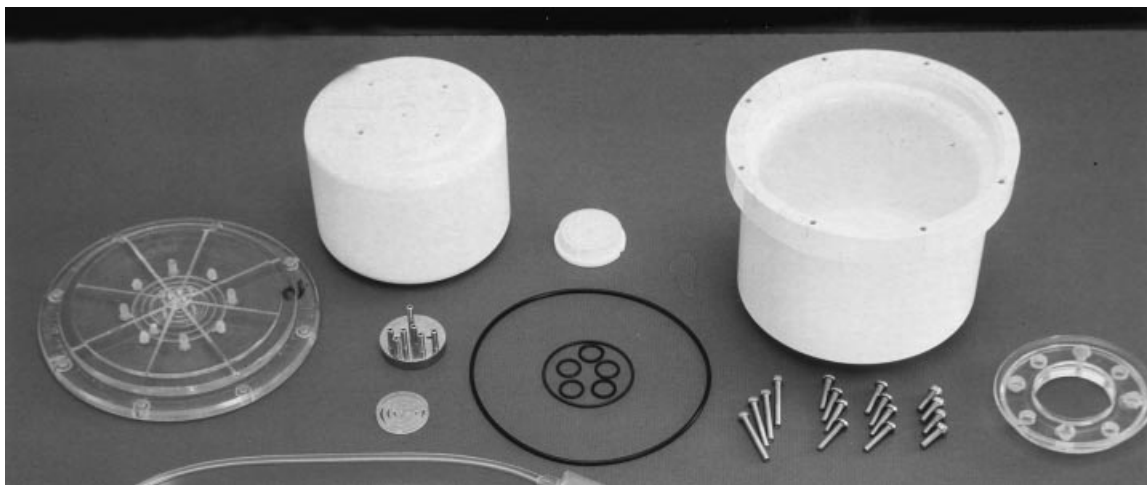
**Fig 6.** Components of the IBM 2990 centrifuge bowl.



Fig 7. The IBM 2990 in early clinical trials.

leukaemia (CLL). The reason for this decision was twofold; first, we knew that under the operating conditions of the instrument with unmodified ACD anticoagulant the lymphocytes accumulated in the buffy coat at a high

concentration. In contrast, the granulocytes were distributed throughout the packed red cell layer and were difficult to separate from the red cells *in vitro* and in the instrument (Fig 8). Second, we chose these patients because of our

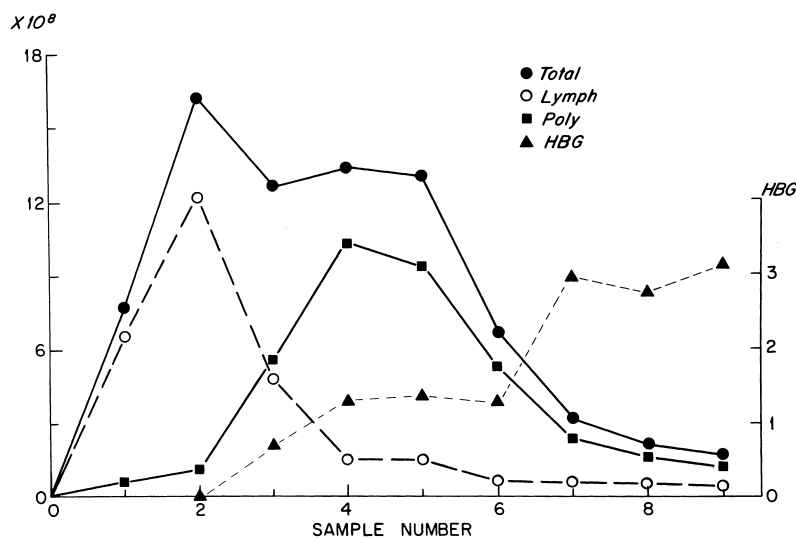


Fig 8. Collection of buffy coat samples 0 (plasma) to 9 (packed red cells).

interest in the potential of immunotherapy of malignant diseases, and we thought that the lymphocytes collected with the instrument could be studied for adoptive transfer of immunity from donor to recipient. As with the CML studies, we hypothesized that because of the known long life span of lymphocytes in CLL such a procedure could provide therapeutic benefit. These early studies in patients with CLL demonstrated transient benefit for the patients (Curtis *et al.*, 1972), and we were able to demonstrate the adoptive transfer of immunity (Curtis *et al.*, 1970). These studies have proven to be quite important for the subsequent use of the instrument for such sophisticated studies as the LAK cell transfusion treatment experiments reported by Rosenberg for patients with melanoma and renal cell cancer, which is a proven therapeutic modality.

However, our primary goal of collecting granulocytes to treat infections was frustrated by the lack of ability to collect sufficient granulocytes with the instrument. From studies *in vitro*, it was clear that it was necessary to improve the separation efficiency using a red cell rouleuxing agent. We began to explore the available macromolecules that could be used for this purpose. We were concerned about proteins, such as fibrinogen and gamma globulin, because of the potential contamination with hepatitis virus and other blood-borne pathogens, so we turned to synthetic agents that had been used for volume expansion. We chose to investigate hydroxyethyl starch which proved both *in vitro* and subsequently *in vivo* to be highly effective in this regard. In addition, we reasoned that we could further increase the efficiency of the separation technique if we utilized agents which could increase or mobilize granulocytes, both from the marginating pool and from the bone marrow pool. Therefore, from the literature, we identified etiocholanalone as an agent which was available and previously used as a therapeutic agent. It did have the property of regularly increasing the number of granulocytes in the blood. We subsequently demonstrated that with use of etiocholanalone injections into the donors and with the use of hydroxyethyl starch added *in vitro* we could increase the number of granulocytes collected by an order of magnitude (McCredie *et al.*, 1974; Table II). The era of replacement transfusion of normal granulocytes into recipients began (McCredie *et al.*, 1973).

Between 1970 and 1973, we systematically delivered granulocytes, collected at a dose of approximately $2 \times 10^{10}/\text{m}^2$ surface area of recipient, from siblings, parents and identical twins. We recognized the great importance of histocompatibility for effective granulocyte replacements. In identical twins, on average, the per cent recovery in the circulation was approximately 40%, which agreed well with studies using labelled granulocytes. For sibling donors, on average, this figure was only 15%, and for parental donors it was below 10%. From random donors, the average recovery was approximately 5% in the circulation. Despite these limitations, we systematically treated patients with proven infections refractory to antibiotics, and we found a success rate of 64% in 73 consecutive patients treated, which far exceeded the response rate expected from continuing antibiotics alone.

Two important observations were made during these studies. The first being that patients who had prolonged periods of neutropenia showed minimal benefit, and that to carry people through episodes of severe neutropenia and infection required a minimum of four transfusions, i.e. 2×10^{10} given daily for 4 consecutive days. Of the 28 patients who received four to eight transfusions, 78% responded (McCredie *et al.*, 1973). However, to accomplish this required dedicated family members and friends because one had to have at least two donors who were willing to donate on alternate days.

Following these reports, a number of groups began to study the usefulness of granulocyte replacement transfusion. There were several large randomized prospective comparative trials and in virtually all these studies the results were negative and the positive results we initially reported were not confirmed. However, it should be emphasized that in all of these studies the major limitation was an inadequate dose of granulocytes delivered for each transfusion. From our work with CML cells, it was clear that at doses below 10^{10} , certainly at the level of 10^9 , there was no expectation for response. In the majority of these studies, doses of $0-5 \times 10^9$ were the administered doses. Second, we reported that one had to continue treatment on a daily basis for a minimum of 4-8 d. In most of these studies, single or two transfusions were studied and, again in our own experience, successful outcomes were rarely observed. As a consequence of these studies, granulocyte transfusion study therapy fell into disrepute in most institutions. In fact, the matter became quite controversial and many investigators reported on the observed unfavourable site effects, which included anaphylactic-like reactions with dyspnoea, shortness of breath and pulmonary infiltrates that were recorded in a subset of patients who received repeated granulocyte transfusion. There was also concern about the transmission of pathogens such as cytomegalovirus from donor to recipient. This was particularly important in the bone marrow transplant setting. In our institution, we continued to use granulocyte transfusion in circumstances where patients were demonstrated refractory to available antibiotics and had persistent established infection. In general, our experience continued at the level that it was initially reported, but for many patients donors were not available and this practice even at our own institution was not highly successful nor was it a routine method of supportive therapy.

There was a dramatic change in the early 1990s, when the myeloid cytokines granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) were approved for clinical use. The concept of administering cytokines, specifically G-CSF, to volunteer donors was immediately attractive and was soon reported to increase donor circulating granulocyte levels by an order of magnitude, mimicking the circumstance of the CML donor (Bensinger *et al.*, 1993). With the assistance of G-CSF mobilization, it was now regularly possible to collect between 5 and 10×10^{10} granulocytes from a single donor, which made granulocyte replacement transfusion a reality (Freireich, 1993). Many investigators have been

studying the optimal conditions for donor treatment and for collection parameters to optimize granulocyte transfusion (Jendiroba *et al.*, 1998). Currently, the major limitation to the utilization of granulocyte replacement transfusion is the availability of appropriate donors. As it is established that for the treatment of infection the earlier the treatment begins the more effective the replacement and if long periods of replacement are needed, i.e. more than 1 week, the strain on the donor pool becomes a limiting factor. The second important limitation is the compatibility between donor and recipient; as already outlined from a transfusion effectiveness point of view, the closer the histocompatibility between donor and recipient the better the results in the recipient. For granulocyte transfusion, isosensitization is a major problem and in the presence of isoantibodies major reactions can occur after granulocyte transfusion. Finally, there is no proven method of *in vitro* preservation. At the present time, the only product that seems useful is the freshly collected granulocyte preparations. The final problem with granulocyte transfusion is a concern with most replacement transfusions, i.e. the transmission of leucocyte-carrying pathogens, such as cytomegalovirus, etc., which requires a good deal of thought in matching donors and recipients.

Can granulocyte replacement be practised on a prophylactic basis in the same fashion as platelet replacement? The answer to this is undoubtedly negative because of the limitations described above. However, it needs to be emphasized that granulocyte transfusion effectiveness depends on two important parameters of the host. The first limitation is the early institution of granulocyte replacement in patients who have documented infections and are refractory for antibiotics. In our institution, if a patient remains febrile 48 h after the institution of the appropriate antibiotics, granulocyte replacement is instituted. The second limitation, the duration of cytopenia, for instance for patients undergoing remission induction where cytopenia periods may last from 14 to 28 d, replacement on a daily basis of sufficient granulocytes to maintain a normal granulocyte level is technically extremely difficult. Thus, much research is needed in the field of granulocyte replacement, but certainly the tools are at hand to improve systematically our ability to support patients with severe granulocytopenia with granulocyte replacement transfusion.

PERSPECTIVES

Two major points emerge in this review.

First, the enormously contemporary nature of our knowledge. This review has covered only a half century or one professional lifetime. The dramatic change in supportive therapy for bone marrow failure is evident from the review.

Second, the important role of patient-orientated research in the development of new concepts of physiology and biology relevant to the treatment of patients. The role of patient-orientated research has declined in academic importance in recent years but, as with granulocyte transfusion, there is a rebirth of enthusiasm for the training

of physician scientists to engage in the scientific objective practice of medicine to make fundamental discoveries caring for patients at the bedside (Hirsch, 1999).

It seems appropriate to close such a review with a feeble attempt to predict the future. Clearly, the collection and expansion of haematopoietic stem cells from the peripheral blood, both autologous and allogeneic varieties, will assume a growing importance in supportive therapy. The blood bank of the future, I believe, will become a laboratory for the preservation and expansion for haematopoietic stem cells. It is clear that this expansion can be lineage specific so that it should be possible to create transfusable doses of each of the formed elements of the blood; specifically platelets, granulocytes, monocytes, antigen-presenting cells, eosinophils, basophils, etc. Such a tissue culture facility will also have the capacity to match closely donor-recipient pairs for allogeneic transfusions to avoid the type of complications that are currently an important part of allogeneic replacement transfusion.

The idea of replacing the function of the formed elements of the blood with pharmaceutical products is an activity which is almost certain to succeed. Red cells may well be replaced by molecules which have the same capacity to exchange oxygen and carbon dioxide. Platelets may soon be replaced with liposomal preparations of procoagulants which function in the same capacity as platelets, and the control of infections from all forms of microbes will increasingly be controlled with chemotherapeutic agents specific for the pathogens involved. Finally, the products of the immune system, such as monoclonal antibodies to replace specific immune deficiencies and monoclonal antibodies combined with cytotoxins, may well replace the host defence features of the T cell. In the next century, the blood bank may be in large part replaced by a pharmacy, dispensing pharmaceuticals which can replace the function of the formed elements of the peripheral blood.

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