Blood Transfusion for Clinicians

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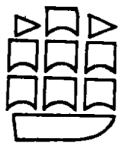

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liable to destroy donor granulocytes before clinical benefit has been produced, and this immune cytolysis often causes severe febrile reactions (p. 253). In addition pre-formed HLA antibodies may cause rapid rejection of allografts (p. 234). Granulocyte therapy using normal healthy donors is practical, provided a cell separator is available, but it is too early to be certain of the benefits and of the hazards.

Whole plasma

The supernatant fluid which is separated, by centrifuging or by standing, from a donation of whole human blood is whole plasma. However, depending on the age of the donation of whole blood from which the plasma has been separated, the composition of the plasma may vary from normal. The most important storage changes in the plasma of whole blood at 4° to 6°C are the loss of the labile coagulation factors V and VIII, and an increase in the potassium content (p. 11). Plasma separated from ACD or CPD blood has an elevated citrate level and an absence of ionized calcium (p. 260). The term whole plasma is usually shortened to plasma, but various forms or modifications of plasma are available as blood products. These variations depend on factors such as the duration of storage of the whole blood prior to the separation of plasma, and the methods of processing and storing the separated plasma. As far as the use of plasma is concerned, one form may produce greater therapeutic benefit than another in a particular clinical situation. Conversely, in certain clinical states one preparation may have more disadvantages or dangers than another form of plasma.

Large scale production of citrated plasma was introduced early in World War II and two practical problems soon became apparent. The first difficulty was the danger of accidental bacterial contamination of liquid plasma, while the second problem was the instability of plasma stored in the liquid state. Normal uncontaminated plasma often showed a cloudiness, and it was therefore impossible to decide from simple inspection whether the cloudiness was associated with bacterial contamination or with the natural instability of liquid plasma. An attempt was made to overcome the problem by a clarifying and then a sterilizing filtration of liquid plasma. This double filtration appeared to solve the problem by producing initially a clear sterile liquid plasma, but a new problem, post-filtration clotting, arose, the coagulation mechanism being activated by the filter pads. The problem of post-filtration clotting was largely overcome by adsorbing most of the clotting factors from citrated plasma with kaolin prior to filtration, and the end product was a clear and relatively stable liquid plasma which was remarkably free from febrile transfusion reactions. It seemed likely that the kaolin process adsorbed not only coagulation factors, but also any toxins or pyrogens which were present in the plasma. However, the final product contained virtually no coagulation factors.

During World War II the lyophilization of liquid plasma was introduced in the UK in the hope that the dried product would be stable. Some of the early batches of dried plasma were sent to tropical areas, and a few months later it was found that the dried powder would not reconstitute properly on

the addition of sterile distilled water, a gel being formed. Investigation showed that the presence of small amounts of water in dried plasma allowed denaturation of proteins to occur in high environmental temperatures. It was essential to lyophilize the plasma thoroughly so that the moisture content of the dried plasma was not more than 0·5 per cent of the weight of the final dried product. The sealing of the bottle containing the final product was also important in that the entry of moisture should be prevented as far as possible. With these additional safeguards and precautions it was possible to store dried plasma satisfactorily for several years.

Wartime experience with plasma highlighted three other important practical issues, namely its potassium content, the presence of anti-A and anti-B alloagglutinins, and the transmission of viral hepatitis. Although it was recognized that diffusion of potassium from the red cells into plasma occurred during the storage of whole blood (p. 11), an elevated potassium content was not a serious problem because the bulk of wartime plasma was prepared from relatively fresh blood. This situation arose from the fact that in the UK during the greater part of World War II the amount of donor blood collected was well in excess of the clinical requirements for whole blood transfusion, and it was possible to separate the plasma from many donations soon after collection. The problem of elevated potassium levels in plasma became much more obvious in peacetime practice when virtually all the blood collected was stored as whole blood, and plasma was prepared almost entirely from time-expired blood. Today, even in the era of component therapy there are clinical situations in which whole plasma therapy may be beneficial, and it may be essential to use plasma separated from donations of fresh whole blood (p. 66, Table 3.1). The freshly separated plasma may be transfused immediately as liquid plasma, but is usually preserved as fresh frozen plasma (FFP) or as fresh dried plasma (FDP). One clinical situation in which FFP or FDP may be required in relatively large amounts is replacement therapy in intensive plasmapheresis (p. 221). The number of continuous-flow cell separators is increasing, and consequently there will be greater facilities for the intensive plasmapheresis of patients and of donors. When large volumes of whole plasma are removed and replacement therapy is required in respect of volume and constituents, a therapeutic dilemma arises. Is it preferable to use whole plasma or an appropriate mixture of blood components and fractions? The answer to this question is not at present clear. For the present there would seem to be a clinical need for FFP and FDP, but experience will show their exact place in the therapeutic armamentarium of the future.

The anti-A and anti-B content of blood products is not a new problem (p. 121), and in particular there is a danger of anti-A and anti-B in donor plasma causing immune haemolysis of recipients' A and B cells respestively. It has already been mentioned in relation to the cryopreservation of red cells (p. 21) that the ultimate aim in clinical blood transfusion is preservation and purification of components of donor blood, and that the process of cryopreservation of red cells removes the unwanted and potentially injurious alloagglutinins anti-A and anti-B. These alloagglutinins are present in plasma

and may therefore be present in blood components derived from the donations of whole blood (p. 113). There is also the possibility of protein fractions containing alloagglutinins which may become concentrated in the process. This important practical problem was recognized during World War II when large volumes of donor plasma were pooled to provide individual bottles or units of liquid plasma and later of dried plasma (p. 31). However, the difficulty of avoiding a final plasma product with a high titre of anti-A or anti-B was overcome relatively easily because of the peculiar wartime situation. It was a not uncommon practice to store as whole blood at 4°C group O donations only; that was the so-called universal donations for the treatment of casualties with massive blood loss (p. 141). Fresh donations of groups A, B and AB were used to provide the plasma pools from which liquid or dried plasma was prepared (p. 31). These plasma pools were usually composed of 500 donations, the proportions of A to B to AB donations being of the order in which these groups occurred in the population. Plasma of group A donations provided soluble A substance and anti-B, group B donations contributed B substance and anti-A to the pool, whereas group AB donations contained soluble A and B substances (p. 115). The result of pooling was specific neutralization of anti-A by soluble A substance and of anti-B by soluble B substance. Any unneutralized anti-A or anti-B was greatly diluted in the large volume pool, and the final plasma product contained little or no alloagglutinins anti-A and anti-B.

This apparently satisfactory situation in respect of the absence of anti-A and anti-B from plasma was upset by two civilian practices which became operative soon after the end of World War II. These were the establishment in large hospitals of banks at 4°C containing whole blood of groups O, A, B and AB, and the use of small pools of not more than 10 donations for the preparation of plasma. The availability of donations of each of the four ABO groups made possible the transfusion of donor blood of the same (homologous) ABO group as the recipient, thus permitting a more economical and a safer use of blood (p. 142). Small pool plasma reduced the danger of transmitting communicable diseases such as viral hepatitis (p. 269). This is an excellent illustration of progress in transfusion practice creating new problems or of converting what was largely a theoretical difficulty or risk into a practical problem or hazard. Such events occur throughout the development of an applied clinical discipline, and the situations become more complex as the discipline introduces advanced scientific methods and sophisticated therapeutic products into modern medical practice (Ch. 11). Although the practical problem of the presence of anti-A and anti-B in plasma was recognized 30 years ago, it can be a recurring problem with modern sophisticated blood products (p. 48). A detailed account of the method used to try to overcome this past, yet recurring, difficulty will illustrate the practical implications and the fact that a completely satisfactory solution may be impractical or impossible to find.

Any donations of ACD whole blood which by the end of the normal shelf life of 21 days had not been transfused were used for the preparation of

plasma. These time-expired donations included plasma from group O donors in addition to groups A, B and AB from which the wartime plasma had been prepared. This group O plasma contained anti-A and anti-B, and almost 50 per cent of the donations available for pooling were from group O persons. The anti-A and the anti-B in group O plasma are usually of higher titre respectively than the anti-A in group B plasma and the anti-B in group A plasma, and are therefore more difficult to neutralize with soluble group substances (p. 118). Furthermore the need to use small pool plasma of 10 donations means that little dilution of a high titre of anti-A or anti-B in a single donation occurs in the pool, and great care has to be taken in balancing the proportion of the ABO groups in the small pool to allow adequate neutralization to occur. In the monograph on Dried Human Plasma in the British Pharmacopoeia (1973) it is stated that in order to ensure cross-neutralization of haemagglutinins by soluble blood group substances, the donations of plasma should be pooled so that contributions from donors of A, O, and either B or AB groups are represented in approximately the ratio 9:9:2. Since the monograph also states that not more than 10 separate donations are pooled, the difficulty in practice of achieving the recommended ratio even only approximately is obvious. This practical difficulty is also greater in the North of Scotland where more than 55 per cent of the population are group O than in the South-West of England where less than 45 per cent are group O. It is also clear that one single donation containing a high titre of either anti-A or anti-B will result in substantial amounts of agglutinin in the final product, hence it is inadvisable to include in a small pool any single donation of plasma containing a high titre alloagglutinin. Such a donation may well be used to provide an excellent laboratory reagent. Although the monograph refers to ensuring specific neutralization, the result of the recommendation is in practice partially, but not completely, successful. Pooled plasma from 10 donations almost inevitably contains some detectable anti-A and anti-B, but if high titre individual donations have been excluded and the proportions of groups are approximately as recommended, the final product will not contain dangerous amounts of anti-A and anti-B. Immune haemolysis of recipient's red cells by alloagglutinins in donor plasma is unlikely to occur from the use of properly prepared small poll plasma, except perhaps when very large volumes have to be transfused as in extensive burning injuries (p. 78).

The third lesson of major importance learned from vast wartime experience in the laboratory processing and clinical use of plasma was the danger of transmitting disease, particularly viral hepatitis, by large pool plasma. A single donation containing an infective agent may contaminate an entire plasma pool. When this occurs with a 500 donation pool, approximately 200 bottles of dried plasma prepared from the pool will contain the infective agent, whereas with the 10 donation pool only four bottles will harbour the infective agent. Theoretically therefore large pool plasma puts at risk many more recipients than does the small pool product. Wartime plasma had acquired a bad reputation for causing type B viral hepatitis—homologous serum jaundice or serum

hepatitis, as it was termed at that time—and it was considered that the introduction of the small pool product would lower the incidence of transfusion-transmitted hepatitis (p. 273). The disease certainly became less prevalent following the introduction of small pool plasma, but other factors may have been responsible for, or may have contributed to, the reduced incidence. For example the prevalence of virus B infections in the general population in the UK may have fallen after the end of World War II, and certainly a higher standard in donor selection was adopted in that volunteers who had had overt hepatitis during the war were not accepted as donors (p. 273). It is however difficult in this complex field to measure the effect of a single modification: even the single step of changing from large pool to small pool plasma, and the explanation of the way in which this reduces the number of recipients exposed to an infective agent may be an over-simplification of the situation. An infective agent from a single donation may become so highly diluted in large pool plasma as to be only mildly infective, and the larger the pool of plasma the greater is the probability of the pool containing specific neutralizing antibody to an infective agent. The chances of a plasma pool containing an infective blood-borne agent depend on the relative incidences and amounts of the infective agent and of specific neutralizing antibody in the population of donors. In the uncertainty which exists about some infective agents, it is not surprising that transfusion practices vary from the use of single donor plasma to large pool plasma. With whole plasma for clinical use however the common practice is to use single donor plasma or small pools of not more than 10 donations, whereas in plasma fractionation it is common to use large pools of 500 or more donations (p. 44).

In addition to recommendations about restricting the number of donations in a plasma pool to 10, and about ensuring the correct ratio of ABO groups in the donations contributing to the pool, the monograph in the British Pharmacopoeia (1973) makes other important recommendations on dried human plasma. To avoid untoward effects due to the products of bacterial growth in the transfused product (p. 253), no individual donation is used if there is any evidence of bacterial contamination, and no pool is used unless it complies with the official test for sterility which is advocated. The danger of denaturation of plasma proteins is recognized by recommending that the liquid plasma is dried by freeze-drying or by any other method which will avoid denaturation of proteins and will yield a product readily soluble in a quantity of water equal to the volume of the liquid from which the dried product was prepared. It is also stipulated that when dissolved in a volume of water equal to the volume of Water for Injections stated on the label, the solution should contain not less than 4·5 per cent w/v of protein. The label on the container of dried human plasma according to the British Pharmacopoeia (1973), should state (i) the name and percentage of anticoagulant and of any other material introduced, (ii) that the contents are derived from not more than 10 donations of blood, (iii) the quantity of Water for Injections necessary to reconstitute the solution, (iv) the protein content of the reconstituted solution, (v) that the reconstituted solution must be used immediately, (vi) the date after which the

preparation is not intended to be used for transfusion and (vii) the conditions under which it should be stored.

As far as storage conditions are concerned the official monograph recommends that dried human plasma should be kept in an atmosphere of nitrogen in a sterile container sealed so as to exclude micro-organisms and as far as possible moisture; should be protected from light; and stored at a temperature below 25°C. The absence and exclusion of oxygen and of moisture diminish the risk of denaturation of proteins during storage as does the maintenance of a storage temperature of less than 25°C. At the end of the drying process the vacuum is broken by admitting an atmosphere of filtered oxygen-free nitrogen to the container, and this container is thoroughly sealed in an attempt to prevent the entry of air and of moisture to the dried powder. Quality control of any product for administration to the human subject is essential, but a special problem with blood products is the relative scarcity of human donations, and consequently a desire to avoid wastage of therapeutically valuable material. The amount of dried plasma used for quality control of the finished product will depend upon the size of the batch, but at least two bottles of dried powder must be sacrificed for essential quality control. One bottle must be reconstituted with the appropriate volume of water to demonstrate that the substance dissolves completely within 10 minutes at 15° to 20°C. In practice this solution of properly lyophilized plasma should be complete well within five minutes. Provided sterile water is used for reconstitution, and care is taken to avoid accidental bacterial contamination, this sample of reconstituted plasma may be used for the necessary sterility tests. A second bottle of dried plasma is used for moisture determination. The loss of weight in a sample of dried powder is measured when the powder is further dried over phosphorus pentoxide at a pressure not exceeding 0·02 mm of mercury for 24 hours. To demonstrate that the lyophilization process has been satisfactory the loss of weight on drying the sample under controlled conditions should be not more than 0·5 per cent of its weight. It should be noted that storage of dried plasma does not normally require refrigeration, although storage at 4°C may well be desirable or even necessary in warm climates in which it is difficult or impossible to maintain room temperatures below 25°C. In practice it is usually satisfactory to store dried plasma in a clean, dry room which should preferably be dark. If the room does admit daylight the bottles of dried plasma must be protected from direct sunlight, otherwise denaturation of proteins may occur. Under good storage conditions dried plasma should remain suitable and safe for clinical use for periods up to eight years. Thereafter it may be difficult to reconstitute the material and gel formation may occur: this difficulty is a sign of denaturation of proteins. Reconstitution difficulties arising within the normal eight-year storage period suggest that either the storage conditions have been faulty or that a leaking container has allowed air and moisture to enter. Clinical users experiencing reconstitution difficulties should return the offending bottle to the hospital haematology laboratory with a report of the observation. In turn the hospital haematologist should report the matter to the regional transfusion director who will wish to examine other bottles of material from the same

drying process. It is therefore essential to record the individual and batch number of any bottle of dried plasma or indeed of any blood product used.

Another important point to note in respect of information on the container label is that the reconstituted solution must be used immediately. The need for sterile blood products has been emphasized (p. 32) and the serious consequences of bacterial contamination are discussed later (p. 254). Every reasonable care is taken in preparing dried plasma to avoid accidental contamination, and a sample is taken for sterility testing at various stages of the process including the finished product. The container is properly sealed to prevent the entry of bacteria. It is therefore most unlikely that the dried plasma will be contaminated, but a common practice is to add sterile pyrogen-free distilled water to the dried powder by an open process when reconstituting the plasma, and minimal bacterial contamination of the solution may occur accidentally, hence the need to use the solution immediately. In practice this means that dried plasma should not be reconstituted until it is certain that the product is required for therapy, and the solution should be administered within three hours of reconstitution.

The solution which results from the reconstitution of freeze-dried plasma is cloudy in appearance. This cloudiness is associated with the breakdown of lipo-protein complexes during the process of freezing the plasma to low temperatures. The presence of fat globules in reconstituted dried plasma leads to consideration of the clinical significance of this and other particulate matter in the solution. Respiratory failure may be the cause of death in patients who have been severely traumatized, and this condition is now termed adult respiratory distress syndrome or shock lung (p. 228). Microemboli may be responsible for the pulmonary effects, and one source of the embolic material may be reconstituted dried plasma. The use of microfiltration of blood products in the prevention of respiratory failure is discussed later (p. 228). Liquid plasma separated from time-expired whole blood will contain some aggregates (p. 11), and these will be present in dried plasma. Thus reconstituted dried plasma contains aggregates and globules which can pass through the standard filter of a giving set, and these embolic materials may contribute to the development of adult respiratory distress syndrome in severely traumatized patients given large volumes of plasma. This disadvantage of dried plasma can be overcome by the use of fresh frozen plasma (FFP) or fresh dried plasma (FDP), because the microaggregates form during storage of blood at 4°C, and in preparing FFP and FDP the plasma is separated within four hours of collecting the whole blood. The detailed clinical management of massive haemorrhage is discussed later (p. 73) and will be governed by the availability of blood products including FFP and FDP which have a place in the transfusion therapy of massive haemorrhage. Ultimately more sophisticated products such as plasma protein fraction or PPF (p. 55) may be used as the main blood volume expander, and red cells recovered from frozen banks (p. 18) may supply the essential carriers of oxygen to the tissues. PPF and cryopreserved red cells contain little embolic material. Having met the essential clinical requirements of volume replacement and oxygen-carrying capacity in this way

in grossly traumatized patients who are likely to develop shock lung, other needs can be provided by the use of appropriate products such as platelets (p. 24), coagulation fractions (p. 45) and immunoglobulins (p. 52).

Microaggregates in donor plasma are of potential importance in relation to the development of pulmonary insufficiency, but have another potential clinical danger in respect of alloimmunization of recipients to red cell antigens (p. 140) and to leucocyte and platelet antigens, including those of the HLA system (p. 235). Any solution of plasma obviously contains soluble antigens such as Gm and Am (p. 140) and adverse reactions associated with these blood group systems are discussed later (p. 267). Plasma also contains soluble antigens of the HLA system of transplantation or histocompatibility antigens (p. 236). Furthermore donor plasma may contain alloantibodies in addition to anti-A and anti-B (p. 145) which are capable of causing immune reactions in recipients (p. 175). It is however less well appreciated that potential immunogens may be present in cells or cellular debris in plasma. Not only does plasma separated from stored blood contain microaggregates of leucocytes and platelets, but all separated plasma may contain a small number of red cells. Freezing of plasma will cause destruction of these contaminating cellular elements, but the cell remnants and debris may well retain their immunogenicity. One reason for the apparent lack of appreciation of the potential immunogenicity of plasma is that the main use of plasma is as a volume expander after haemorrhage or burning injuries (p. 78), and although large volumes may be transfused, it is usually a single episode in an acute clinical situation. The clinical problem of alloimmunization to antigenic constituents of plasma and of subsequent adverse reactions is only likely to arise in the small number of patients given repeated transfusions of whole plasma as replacement therapy for a deficiency in one fraction of plasma. In future such patients are more likely to be treated by the appropriate concentrated fraction rather than whole plasma. Another potential hazard of plasma transfusion is the transmission of malaria (p. 288), because of the possible presence of a few red cells containing malarial parasites. It may be that freezing such plasma damages the red cells and renders the plasma non-infective, but it is safer to use plasma from donors who have resided in an endemic malarious area for the preparation of protein fractions only, and not for FFP or FDP.

The monograph in the British Pharmacopoeia (1973) makes no mention of red cells or of haemoglobin in the product, but plasma drying centres pay attention to this in quality control. Plasma which is carefully separated from the red cells of a whole blood donation should contain only a few red cells, and although the cell stromata will be potentially immunogenic, the small amount of free haemoglobin liberated is unlikely to cause adverse reactions in recipients. Nevertheless it is important to ensure in the processing of whole plasma that the amount of contaminating haemoglobin is kept to a minimum. Liberation of haemoglobin into plasma is an inevitable occurrence during storage of whole blood at 4°C, and occurs particularly when the plasma is not separated until several days after the scheduled expiry date for ACD blood of 21 days or for CPD blood of 28 days. Hospital blood banks should return

time-expired whole blood to regional transfusion centres immediately after the expiry date, otherwise potentially valuable plasma may become unsuitable for processing. The liberation of haemoglobin may be aggravated by faulty storage temperatures. Haemolysis will follow thermal damage to red cells on freezing (p. 18) and accidental freezing may occur during storage of whole blood if the temperature of the storage cabinet falls below minus 3°C. Because of this danger it is advisable that all blood storage cabinets should be fitted with low temperature as well as high temperature visible and audible alarm systems. Localized freezing may occur in a pack or bottle of stored blood even when the general storage temperature of the cabinet is within the permissible limits: a unit of blood may have been placed in close proximity to the freezer or lack of proper circulation of air within the cabinet may produce 'cold' pockets. Free haemoglobin in plasma may be the result of whole blood having been at temperatures higher than 10°C for a few hours. The need for correct storage of blood is particularly important in the period between donations leaving the main bank at the regional transfusion centre and being placed in the bank of the receiving hospital (p. 302); another danger area for faulty storage is the laboratories of either the regional centre or the hospital during the performance of essential tests. According to the British Pharmacopoeia (1973) a separate sample of blood mixed with anticoagulant solution should be provided for compatibility testing, and this practice of separate specimens for use in laboratory tests avoids having the main donation out of the storage cabinet for more than a few minutes. Although these recommendations refer primarily to stored whole blood, their application is stressed at this stage, because of the subsequent processing of whole blood to provide plasma for clinical use as whole plasma or as protein fractions: it is important to obtain a high yield of good quality plasma.

A list has already been given (p. 32) of the information which should be stated on the label of the container of dried human plasma (British Pharmacopoeia, 1973). Two additional items of clinical importance may with advantage be stated on the label: these are the technique used for the detection of HBsAg, the hepatitis B surface antigen (p. 274) and the electrolyte content of the product (p. 37). Every blood donation is tested for the presence of HBsAg, and no donation should be issued for clinical use until it has been shown that the test is definitely negative. However, clinicians have been apprehensive of blood products prepared from even small pool plasma transmitting viral hepatitis type B. Three years ago the author introduced a new label for dried plasma, FFP and FDP, stating that the product had been prepared from individual donations, tested and found negative for HBsAg by a named technique. This information and knowledge appears to have reassured clinicians and to have given the users confidence, particularly with the introduction of more sensitive methods of testing. The importance of communication between interested parties in the global practice of transfusion therapy is stressed in relation to organization (Ch. 10) and to future developments (Ch. 11). A simple statement on the label of a blood product container is an effective way of conveying important information to laboratory workers and to clinical users.

Conveying accurate information about the electrolyte content of donor plasma is not straightforward because of the changes which take place in the electrolyte content of the plasma of individual donations of whole blood during storage (p. 11). The average level of potassium in the plasma of whole ACD blood stored for 21 days is 23 mmol/litre and for CPD blood stored for 28 days 30 mmol/litre, but there is some variation in these levels, and in the resultant levels in pooled plasma prepared from 10 donations. These differences in the electrolyte content of pooled plasma are also caused by the variations in the stage of shelf-life at which the supernatant plasma is separated from the whole blood donation. To advocate at this stage the inclusion on the label of the plasma container of information about the electrolyte content may seem to be unnecessary since the promotion of component therapy may cause the disappearance of whole plasma from the list of blood products. It is however changing circumstances which have prompted the author to include such information on the label of fresh dried plasma (FDP). Undoubtedly more and more donor plasma, from freshly donated blood and from time-expired whole blood, will have to be sent to protein fractionation centres to provide sophisticated blood products (p. 42) and the product, dried human plasma (British Pharmacopoeia, 1973) may well not be produced in the future, but for the immediate future there is a place for FDP in the therapeutic armamentarium of clinically valuable blood products (p. 66). The electrolyte levels of FDP will depend on factors such as the sodium content of the anti-coagulant into which the donation is collected, the relative volumes of anti-coagulant solution and of donor blood, and the time after collection at which the fresh plasma is separated. Large volumes of FDP may be administered to patients who are badly traumatized and have suffered massive haemorrhage (p. 73) and it seems clinically desirable, apart from exhibiting good quality control of a blood product, to state the electrolyte content. A typical example of this information for a unit of FDP prepared from freshly donated CPD whole blood is Sodium 160 mmol/litre, Potassium 3·5 mmol/litre and Chloride 70 mmol/litre.

Plasma may be separated from whole blood donations by differential centrifugation or may be obtained from plasmapheresis donors (p. 215). ACD and CPD are suitable anticoagulants for the storage of either whole blood or red cells (p. 12). Tribasic sodium citrate is an excellent anticoagulant, but is not satisfactory for the preservation of red cells; however, it is a suitable anti-coagulant for use when the red cells are returned to the donor immediately, as in plasmapheresis. Donor plasma for the production of fresh frozen plasma (FFP), of fresh dried plasma (FDP), of cryoprecipitate (p. 40) and of other labile coagulation concentrates (p. 45) should if possible be separated within four hours of the collection of the fresh donation of whole blood. Freezing of the separated plasma to preserve the labile coagulation factors should take place at minus 30°C or lower temperatures. This rapid freezing of the fresh plasma is particularly important for the preservation of factor VIII, the component which is deficient in classical haemophilia A and in von Willebrand's disease (p. 90). Low temperatures for freezing may be provided by the use of

dry ice-ethanol baths or mechanical refrigerators giving temperatures as low as minus 70°C. Plastic packs may be subjected to chemical alteration when placed in a liquid freezing bath, and may become rigid and brittle at freezing temperatures. The plastic container in which the liquid plasma is frozen in a liquid chemical bath must be placed in an outer protective plastic pack, and subsequently must be protected from breakage by storing in a cardboard container.

FFP should be kept constantly at minus 30°C or lower, and at such temperatures the product should have a shelf life of 12 months. Until a few years ago it was considered that a temperature of minus 20°C was adequate for the preservation of labile coagulation factors in FFP, and some hospitals still rely on storage temperatures no lower than minus 20°C, at which the shelf life of FFP should be limited to six months. FFP is usually presented for use as a single donation in a plastic pack containing approximately 200 ml plasma, although some transfusion services still issue FFP in glass bottles. Many centres now issue paediatric packs of FFP for use in haemorrhagic disorders in neonates (p. 203): these packs contain approximately 50 ml plasma. FFP is prepared from individual donations, and the ABO group of the donor should be stated on the label of the container. Whenever possible patients treated with FFP should be given material of the homologous ABO group (p. 113). It is advisable to exclude from FFP production any donation of group O with a high titre of either anti-A or anti-B alloagglutinins or allohaemolysins which may by mischance be given to a recipient of group A, B or AB (p. 121) and any other donation with a high titre antibody which may cause immune destruction of recipient's cells (p. 145). Rarely it may be necessary to provide FFP of a selected Gm phenotype (p. 267) or of a predetermined Am phenotype (p. 140) for regular recipients of FFP who have been alloimmunized to a soluble plasma antigen, and consequently experience severe adverse reactions to plasma transfusion therapy. FDP is prepared in the same way and with the same precautions as human dried plasma (British Pharmacopoeia, 1973), the important difference being in the starting plasma from which the product is prepared (p. 34). Some of the clinical advantages of FDP over dried plasma prepared from time-expired whole blood have already been outlined (p. 29), and the clinical uses of FDP and of FFP are indicated in Chapter 3. Since FDP, like dried plasma, is a freeze-dried product, it can be stored satisfactorily at room temperature (less than 25°C) for periods up to eight years.

In the UK dried plasma and FDP are usually prepared in glass bottles containing 400 ml liquid plasma. Reconstitution of the dried product is therefore performed with 400 ml sterile, pyrogen-free distilled water. The dried powder is easily soluble in water, and by reducing proportionately the volume of water used for reconstitution it is possible to administer these dried products as double, triple or even quadruple strength plasma. This was a not uncommon practice a few years ago as a means of giving proteins such as fibrinogen (factor I) or albumin in a relatively small infusion volume to patients deficient in one of these proteins. Now that protein fractions are becoming available to treat these specific deficiencies, it is unlikely that concentrated solutions of

whole plasma will be required for this form of specific replacement therapy. However, fibrinogen and albumin fractions are not yet abundantly available, and continued use of concentrated whole plasma may be practised for a few years yet. It is therefore important to state two disadvantages or dangers associated with the use of concentrated plasma, namely circulatory overloading and electrolyte imbalance. When liquid plasma is lyophilized, the only constituent removed in the process is water, the proteins and particularly the salts remaining in the freeze-dried powder. This means that the solution resulting from reconstitution with only a half, a third or a quarter of the volume of water normally used, is hypertonic and hyperosmotic. Transfusion of this concentrated plasma results in an increase in circulating blood volume significantly greater than the volume infused, and may cause circulatory overloading and cardiac failure. Pre-existing electrolyte imbalance may be a feature of some clinical conditions associated with hypofibrinogenaemia and hypoalbuminaemia. For example, hypofibrinogenaemia occurs in DIC (p. 81) which may complicate conditions such as massive haemorrhage, oligaemic shock and obstetric trauma in each of which upsets of electrolyte balance occur. Similarly hypoalbuminaemia is a feature of liver and kidney failure in which there is gross oedema, fluid retention and electrolyte imbalance. Preparations of human fibrinogen and of human albumin indicate on the label the electrolyte content, and indeed in the case of albumin there is a salt-poor preparation available (p. 55), because of the clinical desirability of giving large quantities of albumin and only small amounts of electrolytes to hypoalbuminaemic patients. The importance of knowing the amount of electrolytes given in some clinical situations is another reason for stating on the container of FDP the electrolyte content (p. 37) since in the non-availability of fractions such as fibrinogen and albumin, FDP may have to be used as an alternative blood product.

FDP or FFP may require to be used as an alternative to the immunoglobulin fraction. Although human normal immunoglobulin (HNI) is now a readily available fraction (p. 52) it has the disadvantage of containing only the IgG fraction and of having to be administered by the intramuscular route. FDP and FFP contain the other classes of immunoglobulins and are given intravenously. Human specific immunoglobulins (HSI) are IgG preparations for intramuscular injection, but again there may be clinical advantages with a desperately ill patient in giving humoral antibody intravenously in the form of FDP or FFP prepared from donors with a high titre of specific antibody. Indeed donations of some specific antibodies such as anti-varicella/zoster may be scarce, and it may be difficult to obtain large volumes of plasma for the preparation of HSI. Hyperimmune plasma may be used instead of HSI anti-varicella/zoster in such circumstances (p.105). Specific examples of hyperimmune plasma may be made available for clinical use as a 200 ml donation of FFP, or the single donation may have been divided into aliquots of smaller volumes. The volume of the presentation will depend on the titre of the specific antibody in the individual donation, and on whether the patient is an adult or a child (p. 105). An objection in the past to the use of plasma rather than IgG fractions has been the great risk of transmitting disease, particularly since

many of the recipients exhibit immunosuppression (p. 289). However, the main fear was the transmission of viral hepatitis, and with the introduction of more sensitive methods of testing donations for HBsAg, plasma should be relatively safe (p. 277).

Cryoprecipitate

Cryoprecipitate may be defined as the cold insoluble precipitate remaining when fresh frozen plasma (FFP) is allowed to thaw at 4°C. This precipitate forming in the cold is a concentrate of the labile coagulation factor VIII plus some fibrinogen. The cryoprecipitate from a single donation is recovered by refrigerated centrifugation, and this single donor cryoprecipitate is preserved at minus 30°C or lower. Supernatant plasma from cryoprecipitate production still contains most of the constituents of plasma with the exception of factor VIII and some fibrinogen. This supernate may be returned to the red cells of the original donation to reconstitute whole blood, or it may be sent to the protein fractionation centre to provide a factor IX concentrate and the IgG and albuminoid fractions of plasma. The preparation of factor VIII products for clinical use in the treatment of classical haemophilia A and von Willebrand's disease has been summarized (Wallace, 1973). Materials currently available in the UK are fresh frozen plasma (FFP), fresh dried plasma (FDP), cryoglobulin precipitate, intermediate concentrates or fractions containing factor VIII and commercial concentrates of factor VIII. The Cohn fraction I mentioned by Wallace (1973) is no longer prepared in the UK, having been replaced by a more potent concentrate, the intermediate factor VIII concentrate available from protein fractionation centres through the regional transfusion centres. Currently available commercial concentrates are more potent than intermediate concentrate, but more expensive to produce. Factor VIII products of animal origin are now rarely used, because of the severe thrombocytopenia and sensitization to foreign protein associated with their use. The relative merits of the different factor VIII protein fractions are discussed in relation to fractionation policy (p. 46) and the management of factor VIII deficiencies is described later (p. 90). Some consideration will now be given to the preparation, properties and use of cryoprecipitate.

The production of cryoprecipitate consists of collecting a donation of blood into ACD or CPD, and then separating and freezing the plasma within four hours of blood collection. Rapid freezing is usually achieved in a dry ice-ethanol bath, the effective temperature being approximately minus 60°C. The frozen plasma is allowed to thaw for 18 to 24 hours at 4°C, and the cold-precipitated proteins are then separated by refrigerated centrifugation. Cryoprecipitate can be satisfactorily stored at minus 30°C for at least six months and perhaps for as long as 12 months, but in practice with the high clinical demand for the product, it is unusual to store the material for more than three months. An indication of the extent of cryoprecipitate therapy is the current annual use in the West of Scotland of material from 25 000 donations for 270 haemophiliacs. Almost 90 per cent of this cryoprecipitate is given to the 60 severe and moderately severe haemophiliacs, and these statistics have to be seen against

a total population of 2 900 000 and an annual intake of donations slightly in excess of 120 000.

The one great advantage which cryoprecipitate has over the other concentrates of factor VIII is that it can be prepared quickly. With an average daily intake of 500 donations it is possible to maintain a daily production of 100 single donations of cryoprecipitate, and the processing of donations to the finished product takes only 24 hours. On the contrary, the complete processing of large batches of fresh frozen plasma to provide intermediate concentrate of factor VIII may occupy two to three months from start to finish. The total yield of the respective products may be virtually the same over the long period of months, but limited amounts of cryoprecipitate may be produced literally overnight. A disadvantage of cryoprecipitate is the individual variation in factor VIII levels from one donation to another. This disadvantage is partially overcome by the pooling of several single donations of cryoprecipitate in the treatment of adults, but remains a disadvantage when only a few donations are pooled as in treating children (p. 91). An advantage of concentrates prepared by the fractionation of large pools (in excess of 100 litres or 500 donations) of fresh frozen plasma is that the final product can be standardized in respect of factor VIII content. Thus an exact calculation of the dose of factor VIII to be given in the form of standardized concentrate is possible (p. 90). A common presentation of intermediate concentrate of factor VIII is a vial containing 250 iu factor VIII, but whatever the presentation the factor VIII content in iu is stated on the label. The factor VIII units are defined in terms of an international standard, one international unit being the factor VIII activity of one ml of an average normal plasma collected for testing in the laboratory. One single donation may be expected to yield a cryoprecipitate containing on average 70 iu, but individual donations will vary in practice from 10 to 160 iu. The factor VIII content of an individual cryoprecipitate donation will depend on (i) the actual level of factor VIII in the donor, the range being from 50 to 300 iu, (ii) the care with which blood is collected and processed to ensure the minimal loss of factor VIII activity and (iii) the actual volume of donor plasma used.

A well prepared and potent cryoprecipitate may be rendered therapeutically ineffective by improper handling prior to administration. Certain aspects of administration will be emphasized. There are two alternative methods of completing the process of cryoprecipitate production. In one method as much supernatant plasma as possible is removed from the satellite pack containing the cryoprecipitate before freezing the product. When such material is thawed prior to use, the cryoprecipitate is redissolved in saline. The alternative method is to leave 10 to 15 ml plasma in the cryoprecipitate pack, in which case there is no need to add saline solution to redissolve the material prior to use. Depending on the ABO group of the donor, the residual plasma on the cryoprecipitate may contain anti-A and/or anti-B (p. 95). Group O cryoprecipitate will contain anti-A and anti-B, and as a result large doses of group O cryoprecipitate may cause immune haemolysis and adverse reactions in recipients of groups A, B and AB. Provided supplies of cryoprecipitate are adequate,

it is preferable to give patients cryoprecipitate of the homologous ABO group (p. 95). The reconstitution of cryoprecipitate for infusion is an important task and should not be delegated to an unskilled or an uninformed person. A calculation of the number of packs of cryoprecipitate required for therapy should be made (p. 91), and the appropriate number of packs of the homologous ABO group is removed from the low temperature storage cabinet. The usual practice is to thaw the packs of cryoprecipitate in a water bath at 37°C, but it is advisable to place the pack inside a protective outer plastic bag, because the water in the bath is not sterile and there is a danger of contaminating the blood product. If the cryoprecipitate has been prepared by removing as much supernatant plasma as possible, it will now be necessary to add saline to redissolve the material. This is done by injecting aseptically 10 ml sterile pyrogen-free normal saline with a sterile syringe and needle into the cryoprecipitate pack through the outlet site. Application of gentle pressure to the pack with the fingers will help to dissolve the precipitate. The plastic pack containing the cryoprecipitate should be inspected carefully to see if any leaks have appeared during the thawing process, and any container which leaks must not be used. Cryoprecipitate can be administered from individual packs of redissolved material using a special infusion set with a syringe and filter, but the usual practice is to pool the material from the appropriate number of individual thawed packs into a single transfer pack or a 250 ml sterile bottle. The pooled material is infused through a giving set with a filter, and the infusion should be completed within three hours of thawing.

The final preparation of cryoprecipitate contains on average 40 per cent of the original factor VIII content of the donor plasma in approximately 5 per cent of the original plasma volume. It also contains from 20 to 25 per cent of the fibrinogen present in the original plasma, and cryoprecipitate is sometimes used to supply fibrinogen to a patient, because it is prepared from single donations and is less likely to transmit viral hepatitis. Adverse reactions to cryoprecipitate are less frequent than reactions to FFP, and there is no evidence that cryoprecipitate therapy has increased the number of patients with antibodies (inhibitors) to factor VIII. The advantages of concentrated fractions of factor VIII over the concentrated component, cryoprecipitate are discussed later (p. 46 and p. 90), but cryoprecipitate can be prepared so simply and quickly, and has proved so effective in treating factor VIII deficiencies that it is difficult to imagine cryoprecipitate disappearing from the range of blood products. Much will depend on the production capacity of fractionation centres, and on the incidence of adverse reactions when using concentrated fractions. Satisfactory supplies of fraction, free from adverse reactions, will undoubtedly displace cryoprecipitate in part, but cryoprecipitate may well remain the product of choice for treating minor episodes in hospital and will be the standby in difficulties over the supply of more sophisticated products.

Plasma fractions

Plasma contains a highly complex mixture of proteins. Patients may present with deficiencies in one or more of these proteins. Some protein deficiencies

are inherited, while others are acquired. Replacement therapy in the form of transfusion is often desirable, and is sometimes essential in the management of protein deficiencies. Whole blood or whole plasma may contain the protein in which the patient is deficient, but it is unlikely that these products will contain enough of the particular protein to meet the recipient's clinical requirements. In addition, whole blood or plasma transfusions, in the volumes which would be required, may well cause circulatory overloading even in patients with normal cardiac function. The ideal therapy is a concentrate of purified protein which can be injected into the patient in a small volume, with great therapeutic benefit and without adverse reaction. Donor plasma can be separated into therapeutically valuable proteins, and the various fractions can be further purified and concentrated. This process of plasma or protein fractionation provides, in particular, three physiological classes of blood products, (i) coagulation concentrates, (ii) immunoglobulin preparations and (iii) albuminoid fractions. Before considering the various fractions available within each of these three classes, a brief outline will be given of the methods of protein fractionation.

New developments in large-scale plasma fractionation are described by Watt *et al.* (1972). The classical method of separating plasma protein is the salting out process in which concentrated solutions of salts, such as ammonium sulphate, are added to plasma. This method has been widely used in the laboratory for analytical purposes, but has several disadvantages for large-scale production of protein fractions for clinical use, namely (i) the isolated fractions are crude, (ii) the addition of large amounts of salts causes denaturation of proteins and (iii) the added salts have to be removed by dialysis, and this process is difficult to perform with large volumes under aseptic conditions. The separation of proteins by electrophoresis is a satisfactory method for analytical purposes in the laboratory, but again is difficult to perform with large volumes of plasma. A method of organic solvent precipitation of proteins was introduced by the late Professor Edwin Cohn of Boston at the beginning of World War II, and this method became established throughout the world as the conventional method of fractionating donor plasma for the preparation of products for clinical use. The Cohn method had several advantages over other methods:

(a) the precipitant, ethyl alcohol, was cheap

(b) the process was conducted at low temperatures, which condition along with the use of bacteriostatic alcohol reduced the risk of bacterial growth

(c) vacuum drying of the product could be used to remove the final traces of alcohol from the washed precipitated protein

(d) good separation of proteins was achieved by manipulation of five variables, temperature, pH, ionic strength, protein concentration and alcohol concentration: these variations achieved separation on the basis of solubility differences between proteins.

This Cohn procedure has undergone modifications during the past 30 years. For example in Britain a method of separating protein fractions for clinical use was developed using ether instead of ethanol as the precipitant, but the trans-

fusion services and commercial firms in most countries in which plasma fractionation is practised have adopted the Cohn system or one of its several variants. The conventional Cohn procedure involves static batch processing of donor plasma. Small pilot batches may have a total volume of 5 litres or 25 donations. Large batches may have a volume in excess of 100 litres or 500 donations. Watt *et al.* (1972) have introduced a continuous process of organic solvent precipitation in which time is a sixth variable. Large volumes of plasma can be handled on an industrial scale operation, the system being under automatic control using a process control computer. It is claimed that the essential difference between continuous and static batch fractionation is that the precipitation of plasma protein is a near-instantaneous phenomenon with the continuous method, provided all the variables including time are fixed at correct levels.

More recently, methods of fractionation involving ion exchange resins and precipitation by amino-acids or by non-ionic polymers have been developed for the production of fractions for clinical use. These newer methods are proving particularly valuable in the preparation of concentrates of coagulation factors. For example factor VIII concentrates have been prepared using either glycine or polyethylene-glycol (PEG) as the precipitant. It has already been noted (p. 40) that cryoprecipitate prepared from freshly donated plasma is a moderate concentrate of factor VIII, although contaminated by substantial amounts of fibrinogen. Cryoprecipitated plasma may be used as the starting material from which to prepare a 'superconcentrate' of a purer factor VIII by a sophisticated procedure such as PEG precipitation. Concentrates of factor IX and other factors of the 'prothrombin complex' may be prepared for clinical use by adsorbing these factors from plasma donations onto a suitable medium followed by elution. These adsorbing substances include DEAE-cellulose, calcium phosphate $Ca_3(PO_4)_2$ and aluminium hydroxide $Al(OH)_3$ gel. Ion-exchange separative techniques can be used to provide an immunoglobulin IgG preparation suitable for intravenous therapy. The IgG preparations made by conventional ethanol precipitation methods contain aggregated polymers of protein which have anti-complementary activity, and as a result adverse reactions to intravenous therapy may occur. These conventional IgG preparations are normally given by intramuscular injections, and great caution must be exercised in giving such preparations intravenously (p. 98). There are obvious clinical advantages in being able to administer immunoglobulin preparations intravenously, so that much effort is now being applied to the development of methods of preparing concentrated IgG fractions suitable for intravenous administration. Further developments of fractionation procedures using separation techniques such as molecular sieve chromatography or electrophoresis can be expected to increase yields of the existing fractions and to make available new fractions of greater purity and specific activity.

Mention has already been made (p. 33) of the problem of quality control assessment of blood products arising from the fact that donor blood is a relatively scarce commodity, and the blood products derived from these scarce donations have great therapeutic potential and value. Directors of transfusion

services and protein fractionation centres therefore find themselves in a great dilemma when handling and issuing blood products. There is a desire and an obligation on the one hand to provide products of the highest quality in respect of potency, purity and safety, while on the other hand there is a need to avoid wastage of scarce and valuable therapeutic substances. This special and peculiar problem of the quality control of protein fractions for clinical use is discussed by Watt *et al.* (1972), and one aspect of the problem, namely clinical trials, is considered later (p. 314). In the past in countries like Britain, the difficulty in providing large amounts of plasma fractions has been the inadequacy of the capacity of fractionation plants. Now that greatly increased fractionation capacity is available, the problem is obtaining sufficient starting plasma to provide the desired fractions. The problem of supplying donor plasma to the protein fractionation centre is one of quality as well as quantity. For example the provision of concentrated fractions of labile coagulation factor VIII demands a supply of plasma which has been separated from fresh whole blood preferably within four hours of collection (p. 37), and human specific immunoglobulins (HSI) can only be prepared (p. 53) provided regional transfusion services select plasma donations with a high titre of the appropriate specific antibody. When it comes to the albuminoid fractions the emphasis is on the volume of plasma obtained from fresh and from time-expired donations, because large amounts of starting plasma are required to provide concentrated albumin and plasma protein fraction (PPF). Watt *et al.* (1972) discuss some of the practical problems of providing plasma for processing, and suggest that an abundance of plasma fractions could be provided for clinical use if all hospitals followed the example of one hospital in the USA, in which all but 2 per cent of units of collected blood were transfused as red cells and not as whole blood. Wallace (1972) describes new approaches to the supply of blood and plasma, and emphasizes that the production of large amounts of components and fractions from donations of whole blood is facilitated by the clinical use of red cells rather than whole blood. The use of red cells, and the advantages for recipients of component therapy, rather than whole blood transfusion, are discussed in the appropriate sections (p. 65 and p. 299). Some attention will now be paid to the three main functional classes of plasma fractions, namely coagulation concentrates, immunoglobulin preparations and albuminoid products and to the various preparations for clinical use within each of these three main classes.

Coagulation concentrates

The fractions presently produced in this category are factor VIII for replacement therapy in classical haemophilia A or other clinical conditions in which there is a deficiency of factor VIII (p. 90); factor IX preparations for the treatment of Christmas disease (haemophilia B) and products containing factors II, VII, IX and X (the prothrombin complex) for the acute management of conditions in which the prothrombin complex is deficient (p. 96); and factor I (fibrinogen) which may be required in some states of fibrinogen deficiency (p. 97).

Factor VIII concentrates. It has already been emphasized (p. 40) that cryoprecipitate is a moderate concentrate of factor VIII, but that being prepared from individual donations, cryoprecipitate preparations reflect the variations in factor VIII levels from one healthy donor to another. Although a well prepared single donation cryoprecipitate provides on average 70 iu factor VIII, the great individual variation in potency may result in wastage of the product due to excess therapy or in low *in vivo* levels of factor VIII and continued bleeding due to inadequate therapy. Concentrated fractions of factor VIII are prepared from multiple donations of fresh plasma, and the individual ampoules contain freeze-dried material of known potency which is stated on the label in international units (iu) of factor VIII. It is possible to predict accurately the number of iu factor VIII required to achieve haemostasis in a particular clinical situation, and these fractions have an obvious advantage over cryoprecipitate in providing well balanced therapy. In addition it is sometimes possible with previously assayed fractions to neutralize accurately inhibitors of factor VIII, and thus produce temporary but effective haemostasis in the life-threatening situation of having to perform a surgical operation on a haemophilic patient with an inhibitor (p. 93). Since concentrated fractions are in respect of iu factor VIII more concentrated than cryoprecipitate, the volume to be injected is smaller when using fractions than when administering cryoprecipitate, and this has advantages particularly in home therapy (p. 92). It should be noted however that fractions with varying degrees of concentration of factor VIII can be prepared for clinical use. Modern methods of preparing factor VIII fractions can be used to produce highly concentrated fractions of great purity, but although the final product is very concentrated much of the factor VIII in the starting plasma has been lost in the complex process. An intermediate fraction of factor VIII concentrate which is the type of concentrate currently available through the National Health Service in the UK, is similar in yield or in loss of factor VIII in processing to cryoprecipitate; approximately 40 per cent of the factor VIII in the fresh donor plasma is present in the final product. This intermediate concentrate of factor VIII is however on average twice as concentrated as pools of individual donations of cryoprecipitate, so that a given dose of factor VIII in the form of intermediate concentrate requires only half the injection volume that is needed to give the same dose by the use of cryoprecipitate. Relatively high potency concentrates of human factor VIII are available commercially, but these products contain probably only 20 per cent of the factor VIII present in the starting plasma. Even more potent concentrates can be prepared, but with still greater loss of factor VIII in the process. Superconcentrates of high purity factor VIII prepared by PEG or glycine precipitation may contain only 10 per cent of the factor VIII present in the starting plasma. The price of purification and concentration of factor VIII is its loss in the fractionation process, and the practical question which arises is how far can transfusion services and the donor community go in sacrificing valuable factor VIII to meet an unproven clinical demand for superconcentrates of factor VIII (p. 64 and p. 299). For the present the answer in Britain is to provide only an intermediate concentrate, although there may

be a case for a small and limited production of superconcentrate for the selective treatment of haemophiliacs with inhibitors (p. 93).

Non-human sources of factor VIII are currently out of favour for the control of bleeding. Pig and cow blood are particularly rich in factor VIII, and preparations of porcine and bovine factor VIII have been available commercially as freeze-dried concentrates. The animal protein is immunogenic to man, and adverse immune reactions to foreign animal protein may occur after 10 days of therapy. Indeed although clinical manifestations of an immune response may not become obvious until 10 days after the start of the course of animal factor VIII, a silent immune reaction may occur earlier in the form of immune destruction of the infused factor VIII. It is therefore particularly important to monitor the response to animal factor VIII by assaying the plasma level of factor VIII achieved in the patient. Another disadvantage of, or contraindication to, the use of animal factor VIII fractions is the tendency for thrombocytopenia to occur soon after the injections. This thrombocytopenia is often transient, but may be severe when large doses of animal factor VIII are given at short intervals in an attempt to neutralize a factor VIII inhibitor: the development of severe thrombocytopenia in that situation may aggravate already dangerous bleeding. It should be noted that there is no cross-reactivity between the porcine and bovine proteins. Thus a patient who has become immunized to the proteins of one species may be given factor VIII concentrates from the other species.

The risks attached to the use of factor VIII concentrates of human origin are the transmission of viral hepatitis (p. 269) and immune haemolysis of the recipient's red cells by anti-A or anti-B present in the concentrate (p. 95). Considering the large amount of blood products given to haemophilic patients, it seems surprising that hepatitis occurs relatively infrequently. Probably over the years of treatment the patients have had multiple exposures to the infective agents of viral hepatitis: certainly 30 per cent of haemophilic patients in Britain have demonstrable anti-HBs when their sera are tested by a sensitive method. It is probable that all the older and severe haemophiliacs have an active immunity to type B hepatitis, and the introduction of more sensitive methods of testing individual donations for the presence of HBsAg should diminish the risk of transmitting the infection to younger and non-immune haemophiliacs (p. 277). The availability of clinically effective commercial concentrates of factor VIII has however renewed and increased the fears of transmitting viral hepatitis, because it seems that these high potency concentrates are prepared from very large donor pools of fresh plasma, and that these donors who are paid for their services originate from countries with a high prevalence of HBs antigenaemia (p. 271). The infectivity of concentrates in respect of viral hepatitis type B depends not only on the size of the pool, but on the sensitivity of the method of testing individual donations for the presence of HBsAg and on the amount of anti-HBs in the plasma pool (p. 282). If the donor population from which the fresh plasma is obtained has a high prevalence of HBs antigenaemia, it would be expected that many donors would have anti-HBs which would neutralize partially or completely any HBsAg that escapes

detection in the screening test. Indeed it is not inconceivable that the large plasma pool contains an excess of anti-HBs, and some of this antibody may be present in the factor VIII concentrate, giving the recipient passive immunity to viral hepatitis type B (p. 105 and p. 281). One possible complication of preparing concentrates for clinical use is that not only is the desired fraction concentrated, but other contaminating proteins and components of plasma may also be concentrated. This can happen with the agglutinins anti-A and anti-B, and may happen with other immunoglobulins, and perhaps even with some infective agents. Although active steps can now be taken to minimize the risk of transmitting type B hepatitis, there is growing concern over the possible transmission of the postulated, but not yet proven, long incubation type C hepatitis (p. 283). This infective agent would appear to be prevalent among donors in the USA, but so far type C hepatitis would not seem to be a problem in the UK. Some of the commercial concentrates of factor VIII which are now being used to treat haemophilic patients in the UK are produced in the USA, and may contain the so-called virus C to which British patients may have little or no immunity. It is unwise to cause fear over the possible consequences of using a valuable therapeutic substance, but recipients of these newer products should be observed closely for any evidence of hepatitis developing. An important practical point applicable to the administration of all blood products, but especially to these newer concentrates, is that full details, including batch numbers, should be recorded in the patient's case notes (p. 226).

The danger of immune haemolysis of recipients' red cells by anti-A or anti-B alloagglutinins in blood products has been mentioned already (p. 47), and the practical significance is discussed later (p. 95). Part of the quality control assessment of factor VIII concentrates is the titration of anti-A and anti-B in the final product. The low content of these alloagglutinins in the fractions issued for clinical use is not normally significant in the dosage used for small incidents in recipients of group A or B or AB. However when large amounts of factor VIII concentrate have to be given to recipients other than those of group O, frank immune haemolysis may occur, and the patient should be observed closely for signs of haemolysis. The occurrence of hyperbilirubinaemia in a multi-transfused haemophilic patient immediately raises the possibility of viral hepatitis, but other causes of hyperbilirubinaemia such as extensive bleeding into tissues, immune haemolysis and the taking of hepatotoxic drugs should be considered. Anaphylactic reactions to cryoprecipitate or to concentrated fractions of factor VIII appear to be much less frequent than these reactions were with fresh whole plasma, but a severe anaphylactic reaction does occur rarely with concentrates. Reactions make haemophiliacs apprehensive about future therapy and it is a not uncommon practice to give antihistamine drugs prophylactically (p. 253). All antihistamine drugs have powerful antiplatelet actions which may cause unexpected bleeding. Chlorpheniramine (Piriton) 10 mg iv seems to have less antiplatelet activity than other antihistamines. It is important to report adverse reactions to concentrated factor VIII fractions to the director of the regional transfusion service so that appro-

priate investigations can be undertaken and, if necessary, an early warning can be issued about material from the same batch.

Factor IX concentrates. Factor IX is less labile than factor VIII *in vitro*, and 80 per cent of the original factor IX activity may be present in ACD whole blood stored for 21 days or in CPD blood stored for 28 days at 4°C. However, fresh plasma is a more reliable source of factor IX for the preparation of concentrates to be used in the management of patients with haemophilia B (Christmas disease). The factor VIII deficient supernatant plasma from cryoprecipitate production remains a good source of factor IX as long as regional transfusion centres are preparing cryoprecipitate. When the future management of haemophilia A is undertaken largely by means of concentrated fractions of factor VIII rather than by cryoprecipitate, the fresh frozen plasma which is sent from regional transfusion centres to the protein fractionation centre will become the source of factor IX as well as of factor VIII for the production of the respective concentrates. Dike, Bidwell and Rizza (1972) summarize methods for the preparation of concentrates of factor IX. The main clinical use of these factor IX or 'Christmas' concentrates is the maintenance of haemostasis in patients with haemophilia B, particularly following serious trauma and surgery. These concentrates of factor IX can also be used for 'on-demand' treatment of spontaneous bleeding in patients with haemophilia B either in hospital or at home, in the same way as factor VIII concentrates are used in haemophilia A (p. 92). A therapeutic dose of factor IX concentrate may be injected intravenously by syringe, and this offers a great advantage over other preparations such as fresh frozen plasma (FFP) or fresh dried plasma (FDP) in which the relatively low factor IX concentration necessitates administration by continuous intravenous infusion. As already indicated (p. 44) the preparation of concentrate involves the use of ion-exchange cellulose, the factors II, VII, IX and X of the prothrombin complex being adsorbed from the plasma onto a suitable medium, and then these coagulation factors are recovered by elution from the adsorbing medium. The methods currently used for ACD or CPD plasma provide suitable conditions for adsorption and recovery of factors II, IX and X, but unfavourable conditions for factor VII. As a result the system provides a satisfactory concentrate of factors II, IX and X, but the product contains little or no factor VII. These 'Christmas' concentrates are satisfactory for the management of haemophilia B, but doubts have been raised about the clinical effectiveness of these concentrates in the treatment of patients with deficient levels of the factors of the prothrombin complex which includes factor VII.

There are undoubtedly clinical conditions in which the missing factors of the prothrombin complex are required urgently for substitution therapy (p. 96). The existing 'Christmas' concentrates often appear to be clinically satisfactory in the treatment of haemorrhagic complications of severe liver disease and in the rapid reversal of anticoagulant therapy, and it may be that in many clinical situations of this kind, sufficient amounts of factor VII are present in these concentrates of factors II, IX and X to correct deficiencies in the prothrombin complex. However, sometimes these concentrates of factors II, IX and X do not

control bleeding effectively and factor VII has to be administered. This need for factor VII may be met by the use of fresh frozen plasma (FFP), but the patient may be elderly or may have impaired cardiac function for some reason other than old age, and it is clinically undesirable to transfuse the large volume of FFP which may be required to provide haemostatic levels of factor VII. There would therefore appear to be a limited need for a concentrate of the prothrombin complex containing substantial amounts of factor VII in addition to factors II, IX and X. Using a calcium phosphate adsorption technique it is possible to prepare a clinically acceptable concentrate containing factors II, VII, IX and X. This product is sometimes called PPSB, a term introduced by the French workers who devised the method of production (Wallace, 1973). PPSB is an abbreviation for Prothrombin (factor II), Proconvertin (factor VII), Stuart (factor X) and haemophilia B (factor IX). Some workers in this field have altered the order of the capital letters to PPBS to conform to the serial order of the numbers II, VII, IX and X. In practice, particularly when orders for blood products are given over the telephone in an emergency, the author has found that the use of numbers is less confusing than the use of letters. The product PPSB or PPBS will therefore be termed a concentrate of factors II, VII, IX and X.

A practical disadvantage in the preparation of this concentrate of factors II, VII, IX and X is that the material has to be prepared from EDTA plasma (p. 13) and not from ACD or CPD plasma. Unfortunately EDTA is not a suitable anticoagulant for the preservation of red cells, so that this procedure of collecting blood into EDTA anticoagulant for the provision of plasma for the production of a concentrate of factors II, VII, IX and X is costly in the loss of red cells. It is therefore important to limit the clinical use of the concentrate of factors II, VII, IX and X to situations in which the administration of the concentrate of factors II, IX and X, supplemented if necessary by FFP, is not completely effective because of inadequate amounts of factor VII. Another disadvantage or danger is that concentrates of factors II, VII, IX and X may cause adverse reactions including intravascular coagulation (DIC). The potential thrombogenicity of these concentrates of factors of the prothrombin complex creates reservations or fears about the widespread clinical use of these products which will become more readily available with the expansion of fractionation centres. This thrombogenicity arises from damage to coagulation factors during the fractionation process and the formation of activated clotting factors, particularly activated factor X (Xa). It is thought that this risk of thromboembolism is greatest in patients with liver disease, receiving these prothrombin complex concentrates. The liver normally removes activated procoagulant substances from the circulation, and hepatic dysfunction may result in delayed clearance from the blood. Thrombogenicity is however a potential hazard in whatever clinical situation concentrates of the prothrombin complex are used, and caution should be exercised before these concentrates are widely used, but particularly in patients with liver disease. Extensive studies of the problem are being conducted to determine the relative effectiveness and safety of various prothrombin complex concentrates in

different clinical situations. Some fractionation centres have been so concerned over the risks of thromboembolism in patients receiving these concentrates that heparin has been added to the final product, and this addition of heparin would appear to prevent the complication. An interesting development in this field is the use of non-heparinized concentrates of the prothrombin complex in the management of patients with an inhibitor to factor VIII (p. 94). These concentrates contain activated clotting factors, particularly IXa and Xa, and it is thought that these non-heparinized concentrates cause the bypassing of the factor VIII part of the cascade or waterfall system of coagulation and lead to thrombin formation. The use of material containing activated clotting factors carries the risk of DIC (p. 80), but bleeding in haemophilia A patients with a factor VIII inhibitor may in itself be a life-threatening situation. It may be a case of having to take desperate and dangerous measures to save life, but clearly such therapy should only be undertaken by experienced staff within a haemophilia centre which has the full facilities of a diagnostic and monitoring laboratory service and of a blood transfusion laboratory to provide supportive therapy.

Apart from the thrombogenic complication of non-heparinized concentrates of the prothombin complex, these concentrates may be associated infrequently with allergic or anaphylactic reactions. Although properly prepared derivatives of human plasma rarely cause severe adverse reactions, it cannot be guaranteed that any blood product is free from side effects. As with factor VIII concentrates (p. 48) it is important to record and to report adverse reactions to any factor IX product so that thorough investigations and appropriate action can be taken by the hospital haematologist and by the regional transfusion director.

Fibrinogen fraction. The blood preparations section of the British Pharmacopoeia (1973) contains a monograph on Dried Human Fibrinogen. This is to be prepared from liquid human plasma by precipitating with organic solvents under controlled conditions. The label on the container should state (i) the amount of fibrinogen, sodium ions and citrate ions, (ii) the volume of water required to reconstitute the freeze-dried powder, (iii) the expiry date, (iv) the conditions of storage and (v) that the reconstituted solution should be used immediately. Like other fractions, the methods of preparation have been modified from the original Cohn system in an attempt to provide a purer, safer and more concentrated preparation of fibrinogen. A few years ago human fibrinogen had acquired a bad reputation among blood products as being likely to transmit viral hepatitis, and its clinical use became greatly restricted. Clinicians who wished to administer fibrinogen tended to use cryoprecipitate, because it was prepared from single donations, whereas fibrinogen fraction was produced from multiple donations in a large plasma pool. Now that individual donations are tested to exclude the use of HBsAg positive material, and fibrinogen fraction is more highly purified than previously, it seems unlikely that there is a high risk of transmitting viral hepatitis by fibrinogen fraction. In modern transfusion practice the use of fibrinogen is limited. Hereditary deficiencies of fibrinogen are rare (p. 97) and the need for concentrated fibrinogen therapy in acquired deficiencies of fibrinogen, such as dis-

from bacterial endotoxins (p. 253). A great advantage of TF_D is that the one process separates active transfer factor from all the macromolecular cell constituents, histocompatibility antigens (p. 235) and probably all viruses including hepatitis viruses. These undesirable constituents remain in the dialysis sac. TF_D is not immunogenic in man, and does not appear to cause adverse reactions on repeated injections, which is a great clinical advantage. Properly prepared lyophilized TF_D can be stored at 4°C for five years, without loss of potency, and a reserve of specific preparations of TF_D can be accumulated for future use.

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form of community service. In addition, lay members of the community may seek advice from medical practitioners about fitness to donate blood and what is involved in donating blood.

A recurring theme in this book is the care of the donor, of the donation and of the recipient. The volunteer arriving for the first time to donate blood is almost certainly apprehensive. It is important to allay fear, to make the donor feel welcome, and to generate a friendly, but professional, atmosphere. What happens to a volunteer at the first donation may well determine whether or not the individual becomes a regular donor. This and subsequent advice may be particularly helpful to hospital practitioners who occasionally have to withdraw blood from donors.

Selection of volunteers

Age

Most transfusion services adopt an arbitrary limit of 18 to 65 years.

Medical history

Much valuable information can be elicited by asking a few general questions. Specific questions, particularly about communicable diseases and injections, must be asked. It is important to ask these questions in simple language and in a relaxed atmosphere with privacy. The following are essential questions.

General enquiries. Do you enjoy good health? Have you attended a doctor recently? Have you had any serious illness or operation? Have you been absent from work recently?

The answers given to these questions may lead to further questioning about the details of an illness or operation or absence from work. Volunteers with a recent acute infection such as a sore throat or influenza should not be accepted immediately, unless the illness has been mild, but should be advised to postpone donation for say one month. A female donor who has been pregnant within the previous six months should in general not be accepted, but an exception may be made if the donation is to take the form of plasmapheresis (p. 215) because of a valuable maternal antibody. Donors who have undergone major surgery should not be accepted until at least six months have elapsed since the operation. Minor surgical procedures should not debar a volunteer from donating, provided the general health is good. In this context, the term 'minor operation' may include appendicectomy, tonsillectomy, reduction of simple fractures, hernia repairs, simple gynaecological procedures and dental surgery.

While general guidelines are important in the selection of donors, each volunteer should be regarded as an individual. For example a history of recent active tuberculosis is a cause for rejection, but not to accept a volunteer who had a mild attack of tuberculosis 10 years ago, and has since enjoyed good health, may be psychologically bad. Similarly in general, a history of rheumatic heart disease or of coronary artery disease is a cause for rejection. However, single episodes of rheumatic fever, a history of a cardiac murmur or of the surgical repair of a congenital cardiac defect, should not necessarily condemn

the volunteer to permanent rejection. Indeed it is not unknown for cardiac surgeons to encourage patients who are now enjoying satisfactory cardiac function and good health to donate blood. Certainly it is wise not to accept a volunteer if any doubt exists about the individual's fitness to donate.

Specific enquiries. Detailed questions are now discussed:

i. Have you donated blood before?
ii. Have you ever had hepatitis?
iii. Have you been vaccinated recently?
iv. Have you taken any medicines recently?
v. Have you ever had malaria or been in a malarial area?
vi. Do you suffer from fainting attacks?
vii. Do you have any allergies?
viii. What is your occupation?

i. Have you donated blood before? The usual practice in the UK is to allow six months to elapse between donations, although in some countries the interval is as short as two months. Certainly some male donors maintain their haemoglobin levels and enjoy good health when giving blood regularly every three to four months. When enquiring about previous donations, it may be helpful to ascertain tactfully if the individual has ever been rejected, or has had an adverse reaction to donation.

ii. Have you ever had hepatitis? Have you been in contact with a case of hepatitis in the past six months? (p. 280) Many donors will not understand the term 'hepatitis' but most will be familiar with the expression 'yellow jaundice'. Closer questioning will then reveal whether the icterus was likely to have been associated with hepatitis, or to have had some other cause. Volunteers with a history of viral hepatitis are now accepted as donors, provided it is more than 12 months since recovering from the illness, but the donation will only be used for transfusion if it is shown to be hepatitis B surface antigen (HBsAg) negative by a sensitive method of testing (p. 280). As far as contacts are concerned it is only necessary to exclude volunteers who have had close contact with a patient with viral hepatitis. The type of contact hospital staff encounter in their routine work is not generally regarded as a cause for rejection. Inmates of prisons and other institutions should be treated in the same way as other volunteers, provided the donation is proved to be HBsAg negative (p. 279).

In respect of the transmission of viral hepatitis, particular attention should be paid to volunteers who suspected of being drug addicts or who have a tattoo (p. 271). It is probably wise not to accept a volunteer who has been a drug addict. Donations from volunteers with a tattoo should be tested for HBsAg by a sensitive method, but it is advisable to exclude those who admit to having been tattooed within the previous six months.

iii. Have you been vaccinated recently? Have you had any injections in the past three months? Healthy volunteers who have been vaccinated against smallpox may be accepted two weeks after vaccination. Apart from general fitness, the decision to accept or reject a volunteer who has had prophylactic

injections depends on the nature of the immunogen and the time interval. Some prophylactic injections stimulate the production of immune anti-A and anti-B (p. 119). A volunteer who has received toxoids or killed bacterial, viral or rickettsial vaccines may be accepted after 24 hours. Donors are acceptable two weeks after ingestion of oral poliomyelitis vaccine or after immunization against yellow fever. Three months should elapse after rubella vaccination before accepting a volunteer.

iv. Have you taken any medicines recently? (p. 2) A history of taking analgesics, oral contraceptives, vitamin preparations or minor tranquillizers is not by itself a cause for rejecting a volunteer. It is however inadvisable to prepare platelets from a donor who has taken any salicylate-containing drug within the previous 48 hours, because of depression of platelet function (p. 305). Not uncommonly a volunteer will give a history of having been given a five day course of antibiotics for an upper respiratory infection, and provided all the symptoms have disappeared and the volunteer is feeling fit, it is usually in order to accept the volunteer, but the final decision must be taken by the practitioner at the donor session. If any doubt exists the donation should be postponed for say four weeks (p. 206).

v. Have you ever had malaria or been in a malarial area? (p. 288) All volunteers must be questioned closely. If an otherwise fit donor admits to having had an attack of malaria, it is advisable to collect blood for the preparation of plasma fractions only, and to use the red cells for laboratory purposes. Donations from UK residents who have visited or passed through endemic malarial areas may be used for normal transfusion purposes, provided the donors have been in the UK for at least eight weeks since the return from their visit, have had no febrile illnesses since returning, and have taken antimalarial therapy for one month after return. Particular attention should be paid to immigrants who have resided in the UK for many years and go for a short holiday in an endemic area without taking antimalarial drugs.

vi. Do you suffer from fainting attacks? Volunteers with a history of epilepsy, even when well controlled, should not be accepted, because blood withdrawal may precipitate a fit. Similarly, a donor who is subject to vasovagal syncope or to hyperventilation tetany should be rejected (p. 294).

vii. Do you have any allergies? (p. 292) Provided the general health of the volunteer is good, it is usually safe to accept a donation. If the donor is presently having manifestations of allergic disorders such as hay fever, asthma or urticaria, it is advisable to defer donation until the volunteer is symptomless. Volunteers who have had a desensitizing injection in the past four days should not be accepted.

viii. What is your occupation? Some volunteers may have a hazardous occupation. For example a donor whose occupation involves working at heights on ladders or scaffolding should donate blood at the end of a working day, and not resume work until the day following the donation. A similar rule should apply to bus drivers and engine drivers who are responsible for the safety of others (p. 294).

Physical examination

i. general appearance
ii. weight
iii. haemoglobin level
iv. pulse and blood pressure
v. laboratory tests

General appearance. A volunteer who looks unwell should not be accepted. In cases of temporary indisposition the donation may be postponed until the volunteer is well again. Persons obviously under the influence of drugs or of alcohol should be rejected, at least temporarily.

Weight. An acceptable weight level, for example 8 stones or 112 lbs (51 kg) is an arbitrary decision. A volunteer who appears to be underweight should be questioned closely, because weight loss may be indicative of serious underlying illness.

Haemoglobin level. The British Pharmacopoeia (1973) states that the acceptable haemoglobin value should be not less than 12·5 per cent w/v for female donors or 13·3 per cent w/v for male donors. The copper sulphate specific gravity method is a simple, rapid and reasonably reliable screening test for use at donor sessions. A solution with a specific gravity of 1·052, equivalent to a haemoglobin level of 12·5 g/dl is used for female donors. Male donors are tested using a solution with a specific gravity of 1·055 which has a haemoglobin equivalent of 13·3 g/dl. One drop of blood is allowed to fall from a height of 10 mm into the appropriate copper sulphate solution. This drop becomes encased in black copper proteinate. If the haemoglobin level is not satisfactory, the drop will start to descend, will then halt and rise up the solution before finally falling to the bottom.

This screening test for haemoglobin is not quantitative, and simply shows whether or not the donor's haemoglobin level is acceptable. When the level is not acceptable, it is desirable to establish the exact level of haemoglobin, and to institute an investigation of the anaemia through the donor's general medical practitioner.

Pulse and blood pressure. It is undesirable to collect a donation from a volunteer who is known to be hypertensive or to have received treatment for hypertension. The development of vasovagal syncope associated with venesection is more prolonged and potentially more serious in hypertensive than in normotensive donors. Routine measurement of blood pressure in prospective blood donors is of doubtful value, because many donors, particularly those volunteering for the first time, are excited and have a temporarily raised blood pressure. Similarly routine examination of the pulse is of limited value since there is often tachycardia. Any gross irregularity of the pulse should be investigated before accepting the volunteer for donation. The sudden development of bradycardia during blood collection may mark the onset of vasovagal syncope, and to discontinue the collection of blood at that stage may prevent a severe and prolonged faint (p. 294).

Laboratory tests. Apart from the haemoglobin screening test, it is not essential

to perform laboratory tests at the donor session. Some transfusion services do perform a simple rapid ABO grouping test on new volunteers at the blood collecting session. After collection the following tests are performed routinely:

i. ABO and Rh(D) group (p. 113)
ii. antibodies to red cell antigens (p. 145)
iii. hepatitis B surface antigen, HBsAg (p. 275)
iv. syphilis serology (p. 286)

Additional tests such as determination of red cell antigens other than A, B and Rh(D) may be needed to provide compatible blood for recipients with unusual antibodies (p. 144). Tests for leucocyte antibodies may be performed on parous female donors to provide reagents for HLA typing (p. 236). Screening tests for anti-HBs, anti-tetanus and other antibodies may be used to find plasma donations suitable for the production of specific immunoglobulins (p. 218). Laboratory tests are performed on specimens other than the main donation in the primary plastic pack, because to sample the actual donation may contaminate the blood which is to be transfused (p. 10).

Detailed records of the medical history, physical examination, and laboratory tests must be kept for each donor. A numerical system should be used to identify each donation and to relate the plastic pack or glass bottle containing the donation to the blood samples for laboratory tests and to the records of the individual donor. Great care must be taken to ensure correct identification of donation, specimens and records. Clerical errors pose a great danger in the practice of blood transfusion, and mistakes can occur at any point in the long succession of steps from the acceptance of the voluntary donor, right through to the administration of a blood product. At the time of blood collection, it is good practice to (i) identify the donor records with the donor by name: the donor should be asked to identify himself by stating his name, (ii) fix identical numbered labels to the records of the donor, the container in which the blood donation is being collected and the specimen container for laboratory tests, (iii) use a system such as the integral donor line on plastic packs or a sterile pilot tube firmly attached to glass bottles to provide donor red cells for a matching test prior to transfusion (p. 113): this ensures that the donor cells used in the matching test are identical with the red cells to be transfused, (iv) recheck all the numbers on labels, on records and on containers.

Salient medical aspects of blood collection

Full details of systems and methods of blood collection are given by James (1958). The technique of blood withdrawal is described by Mollison (1972), and manufacturers of plastic packs provide step by step guidance on the use of their packs. Important practical aspects are now discussed.

i. choice of vein
ii. tourniquet
iii. preparation of skin and use of local anaesthesia
iv. inspection of blood container
v. rate of flow of donor blood

It is generally agreed that respiratory failure is a not uncommon cause of death in a major trauma and in other clinical situations in which massive amounts of stored blood are liable to be transfused, such as open heart surgery. There is also general agreement that particulate matter in stored blood which is not prevented by the filter on the standard blood giving set from entering the venous circulation, is one of several factors which may precipitate respiratory failure. Disagreement exists however over the value of fine screen filtration of stored blood in the prevention of adult respiratory distress syndrome or shock lung. What is needed is a proper and thorough evaluation of micropore filters and the following five aspects should be considered: (i) the properties of the filter, (ii) the effect of the filter on individual constituents of donor blood, (iii) the amount and nature of the particles retained, (iv) the composition of the filtrate and (v) a controlled clinical trial.

Basically there are three varieties of microaggregate blood filters (a) the depth filter using Dacron wool or polyurethane foam, (b) the screen filter using polyester mesh and (c) a combined depth and screen filter. These fine filters are designed to have a pore-size ranging from 40 μm, whereas the filter on a standard blood giving set has a pore size of approximately 170 μm. The efficiency of filtration is determined not only by the pore-size, but by the adhesiveness of the filtering material. A disadvantage and potential danger of passing blood through these fine filters is that the formed elements in the blood may be trapped as well as the undesirable particulate matter. For example when filtering fresh blood, valuable platelets may be filtered off, and the platelets trapped on the surface of the filter may initiate coagulation. Similarly the trapping of red cells by the filter, and the subsequent haemolysis may also be thrombogenic. Damage to the formed elements is particularly liable to happen if the material of which the microfilter is composed has irregularities. The introduction of thromboplastic material into the circulation of a badly traumatized recipient, or of a patient undergoing open heart surgery, may aggravate a clinical situation, in which there is already a deficiency of platelets and of coagulation components, such as factor V and factor VIII (p. 82). These are the very situations in which DIC may be precipitated (p. 80).

Thus while there are advantages in using microfilters when giving massive transfusions of stored blood, there are also disadvantages and even dangers. The need for a thorough clinical evaluation, preferably in the form of a controlled clinical trial, is clear, but in practice this is difficult to perform, because a substantial number of patients would be required in view of the almost certain multifactorial cause of adult respiratory distress syndrome (shock lung), and several possible causes of death in badly injured and exsanguinated patients. However, the high cost of a multicentre clinical trial may be justifiable, because fine microfilters are themselves costly articles, and reliable information on the cost benefit may be revealing.

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9. Hazards of Transfusion Therapy

Definition of adverse reactions

Any unfavourable event occurring in a patient during or following the transfusion of whole blood or of a blood product may be regarded as an adverse reaction to transfusion. The untoward symptoms or signs may however be manifestations of the primary disease from which the patient is suffering, or a combined effect of transfusion and disease. For example, a rise in the recipient's temperature, during or immediately following transfusion, may be a feature of the disease rather than a true febrile transfusion reaction. Similarly, the development of hyperbilirubinaemia within 48 hours of transfusion immediately suggests the possibility of a haemolytic transfusion reaction due to red cell incompatibility, but it is possible, particularly following massive transfusion, that the hyperbilirubinaemia is a manifestation of impaired liver function. Any blood donation will contain some effete red cells, and the proportion will increase the longer the blood is stored at 4°C. These effete red cells will be rapidly destroyed *in vivo*, and consequently bilirubin will be produced. The level of the patient's plasma bilirubin will depend on the volume and age of the blood transfused, and on the ability of the recipient's liver to conjugate the bilirubin produced from the destruction of effete red cells. If liver function is impaired as the result of disease or severe hypovolaemic shock, hyperbilirubinaemia and clinical icterus may develop, especially following the transfusion of large volumes of blood nearing its expiry date. Investigations should be instituted whenever the recipient of any blood product develops unfavourable clinical manifestations, but the untoward symptoms and signs should be regarded as an adverse transfusion reaction until proved otherwise.

It must not be assumed however, that all adverse reactions are overt and occur within 48 hours of the termination of transfusion therapy. There are delayed or even silent reactions. The transmission of viral hepatitis type B by transfusion may have an incubation period as long as 180 days, and the hepatitis may be anicteric or even asymptomatic (p. 272). Another important adverse effect of transfusion which may be delayed in its manifestations, is primary alloimmunization to antigens in donor red cells, leucocytes, platelets or plasma (p. 140).

Thus adverse reactions may be immediate or delayed, overt or silent. Even a silent reaction may have important clinical significance. If the therapeutic benefit of transfusing a blood product is of shorter duration than expected, then the possibility of a silent reaction should be considered. Ideally, the length of survival of any transfused blood component should be similar to the biological survival in the healthy donor. A significant reduction in survival

in vivo may result from damage to or deterioration of the blood product during collection, processing or storage. Alternatively reduced survival may be caused by an immune reaction in the recipient. If immune destruction of a transfused blood component arises from an antibody already present in the recipient, the reaction is likely to be acute and overt. On the other hand a primary immunogenic response to transfusion may result in the slow destruction of a component, in which case the reaction may be silent. For example, the transfusion of red cells may result in alloimmunization, and within 14 days of transfusion a recipient may be producing antibodies to a red cell antigen in donor blood. The circulating donor red cells, apparently compatible at the time of the pre-transfusion matching test, are now incompatible and will be destroyed gradually without acute clinical symptoms arising. Such recipients may become anaemic if unable to make an adequate haemopoietic response to the elimination of incompatible donor red cells. Alloimmunization to any one of the many immunogens in either the formed elements or the plasma proteins of donor blood may not cause an overt reaction at the initial transfusion, but may result in severe reactions at subsequent transfusions (p. 140).

An important aspect of transfusion practice is the prevention of adverse reactions. Reasonable care must be taken in the collection of donor blood, in the subsequent processing of donations, and in the storage of blood products, to prevent unfavourable clinical manifestations in recipients (Ch. 2). Similarly, appropriate laboratory tests must be performed prior to transfusion to reduce the hazards of transfusion therapy.

General hazards of intravenous therapy

IgG immunoglobulin preparations are normally given by intramuscular injection, although some specially prepared IgG products (p. 54) can be administered intravenously which is the normal route for blood products. On rare occasions the arterial or peritoneal route (p. 186) is used. Thus adverse reactions to transfusion will include hazards which are common to all forms of intravenous medication. These include pyrogenic reactions, bacterial contamination, thrombophlebitis, circulatory overloading, air embolism and electrolyte imbalance.

Special hazards of transfusion therapy

There are special hazards which are inherently associated with transfusion therapy. These are immune or allergic reactions, the transmission of specific diseases from donor to recipient, and transfusion haemosiderosis.

The occurrence of immune reactions is not surprising: human blood contains a multiplicity of immunogens, and a great variety of already formed antibodies. Thus there is a possibility of an immediate antigen-antibody reaction when donor blood is transfused. Alternatively, primary alloimmunization of the recipient by an immunogen in donor blood, may result in a delayed transfusion reaction, or in complications at a subsequent transfusion. Particularly dangerous recipients in respect of immune reactions are those who have been given previous transfusions, or multiparous women already alloimmunized by fetal blood.

In theory, many diseases may be transmitted by transfusion provided (a) the infective agent is present in the donation, (b) the processing and storage of the donation does not remove or destroy the pathogen and (c) the recipient is susceptible to the infection. A single donation may be processed to provide as many as six different products (p. 2). Thus an infective agent in one donation may be disseminated into multiple blood products. Similarly protein fractions are prepared from large volume pools of plasma to which many donors have contributed (p. 44). One donation containing a microbiological agent may contaminate an entire plasma pool, and render a large batch of blood products potentially infective to many recipients. The transmission of disease may have serious consequences for the patient, who may be in a debilitated state from the primary disease which necessitated transfusion therapy. In turn staff may be placed at risk by being exposed to the infective agent firstly in donor blood and secondly in the recipient. Many patients given supportive blood therapy exhibit immunosuppression, associated either with disease or therapy. Such recipients may become chronic carriers of infective agents, thus exposing staff and relatives to risk and increasing the circulation of pathogens in the population. The essence of transfusion practice is the care of the donor, of the donation and of the recipient. Application of this in the context of the transmission of disease involves careful selection of donors, testing of donor blood for evidence of infection, and prophylactic measures in recipients.

Transfusion haemosiderosis is a rare complication of red cell therapy, but is an excellent illustration of a delayed type of adverse reaction. Patients with a refractory anaemia, not associated with iron deficiency, may be kept alive over a period of many years by frequently repeated transfusions of red cells. Iron, from the breakdown of haemoglobin in donor red cells, will be deposited in large amounts in the organs and tissues of such recipients. Haemosiderosis tends to be a late complication in patients requiring repeated red cell transfusions.

Classification of hazards

The adverse effect of transfusion therapy should be avoided, if possible. This is mainly a matter for the transfusion service and the hospital laboratory, but the clinician has an important part to play. Indeed the most important preventive measure is the avoidance of unnecessary transfusions. In spite of precautions and preventive measures, unfavourable reactions can occur, and some of these may be serious and life threatening. Clinicians should be aware of the diverse nature of these hazards, of the clinical management of adverse effects, and of the relevant information and appropriate specimens required for a thorough investigation. A classification of hazards is helpful in providing a logical approach to investigation and therapy.

Because of the diversity of adverse reactions, different forms of classification have been adopted. Since reactions fall mainly into two categories, immediate reactions observed within 48 hours of transfusion and delayed reactions, this

time sequel may form the basis of a classification. In practice it is important to have a comprehensive and workable classification. Since transfusion includes the selection and care of donors (p. 206) it is desirable to be aware of unfavourable reactions in donors as well as recipients. Whatever system is adopted, adverse reactions do not always fit neatly into a classification table, and there are individual variations in the manifestations of a particular type of reaction, such as differences in time of onset, presenting signs and symptoms, duration and severity. More than one type of reaction may occur from a single transfusion, and there may be considerable overlap in the manifestations of different types of reactions. The author has found this general classification of hazards of practical value:

A. *Recipient* (p. 252)
   i. febrile (p. 252)
   ii. bacterial contamination (p. 254)
   iii. mechanical (p. 255)
   iv. metabolic (p. 259)
   v. haemorrhagic (p. 261)
   vi. immune (p. 262)
   vii. transmission of disease (p. 268)
   viii. haemosiderosis (p. 290)
B. *Donor* (p. 291)
   i. local injury (p. 292)
   ii. infection (p. 293)
   iii. cardiovascular (p. 293)
   iv. miscellaneous (p. 295)

Each category of these hazards in respect of recipient and donor will now be described and discussed. The aim is firstly to avoid hazards and secondly to recognize reactions. Suspected reactions should be investigated and appropriate steps taken in the clinical management of patients and of donors who exhibit unfavourable manifestations.

A. Recipient

i. *Febrile reactions*

These reactions are often termed pyrexial or pyrogenic, and older textbooks considered that a rise in temperature was an almost inevitable accompaniment of a blood transfusion. The main cause was almost certainly the presence of pyrogenic material in either (a) the distilled water, chemicals or containers used to prepare the anticoagulant solution or (b) the giving set used to administer the transfusion. Prevalence of febrile reactions was substantially reduced when preventive measures were introduced to eliminate pyrogenic material from these sources. It is now the practice when preparing anticoagulant solutions to use freshly distilled water, thoroughly cleaned containers and purified chemicals, and to sterilize the solutions without delay. Some bacteria will multiply even in distilled water which is left standing at room

sensitivity and to penicillin antibodies. In the former case the recipient was known to be sensitive to nickel, and it was thought that the nickel-steel shafts of the needles used for transfusion caused the reaction. Antibodies to drugs such as penicillin may be present in the plasma of a healthy donor, and may cause an allergic reaction (or even a haemolytic reaction) in a recipient being treated with the drug (p. 156). Some allergic reactions cannot be explained in spite of careful investigations, and it may be that unsuspected and unrecognized antigen-antibody reactions are involved. For example no explanation had been found for an occasional allergic reaction in a recipient of repeated blood transfusions over many years. Then by chance it was discovered that the patient was a chronic carrier of HBsAg (p. 278). In the absence of other findings to explain the occasional mild allergic reaction, it is possible that the reactions followed the transfusion of blood containing anti-HBs in the plasma, because approximately 1 per cent of donations contain this specific antibody.

Febrile responses may be manifestations of immune reactions (p. 253). In addition to possessing HLA surface antigens, granulocytes, lymphocytes and platelets, each have specific antigens, which are peculiar to the type of cells (p. 243). Alloimmunization to any one of these several antigens can result from transfusion or from pregnancy, and a common manifestation of an antigen-antibody reaction involving white cells or platelets is a rise in the recipient's temperature. Cough and dyspnoea may also be features of severe febrile reactions and it seems that agglutination of leucocytes or of platelets, may cause microaggregates which are trapped in pulmonary capillaries. Radiological examination may show perihilar nodules and infiltration of the lower lung fields. The development of these febrile reactions causes great apprehension in recipients of repeated transfusions, and it becomes essential to prevent these reactions. If the recipient's serum contains antibodies to leucocytes or platelets, and the patient requires the transfusion of red cells, it is important to give leucocyte-poor blood (p. 17).

vii. *Transmission of disease*

The combined efforts of regional transfusion services, hospital laboratories and clinical users of donor blood have promoted and achieved safer transfusion practices. Care in blood collection and storage, the introduction of sensitive grouping and matching tests and clinical attention to the volume and rate of transfusion have reduced the incidence of adverse reactions. As a result, attention is now focused on the prevention of transmissible disease, a subject reviewed by Wallace (1976).

Blood products are prepared from whole blood, and the monograph entitled 'Whole Human Blood' in the British Pharmacopoeia (1973) refers to disease transmissible by transfusion. In particular, donor blood should not be obtained from a human subject (a) who is known to be suffering from or has suffered from syphilis, (b) whose blood has not been tested with negative results for evidence of syphilitic infection, or (c) whose blood has not been tested with negative results for the presence of Australia (hepatitis-associated) antigen

and its antibody—now known as the hepatitis B surface antigen (HBsAg) and its antibody anti-HBs. In addition every donor should, as far as can be ascertained by a registered medical practitioner after inspection or simple clinical examination and consideration of his medical history, be free from disease transmissible by blood transfusion. The key words in that recommendation are 'as far as can be ascertained'. Blood donors are volunteers and human. Each volunteer is asked standard questions (p. 206) and the vast majority of donors answer truthfully, but a few hide the complete facts of their medical history. Full physical examination of every volunteer would be unrealistic, and indeed unacceptable to many volunteers. Donors volunteer to give blood, not to submit to a medical interrogation and examination. Even the use of diagnostic laboratory tests has limitations. It is enormously expensive and time consuming to perform a battery of laboratory tests on each donation prior to use, and the dividend may be infinitesimally small. Donor blood stored at 4°C has a limited shelf life, usually not greater than 28 days, and there is therefore an urgent desire to make the best use of each donation, by releasing blood for clinical use as soon as possible after collection. In addition, there is a need to use fresh blood or products prepared from freshly donated blood, such as platelet concentrates, so that the patient may derive the maximum therapeutic benefit from transfusion therapy. The need for simple and rapid methods of testing donations is therefore clear. There is a danger of creating shortages of valuable donor blood by rejecting volunteers on the flimsiest of evidence, but any serious doubt about the safety of accepting an individual should result in the rejection of the donor. A transfusion service must pursue a path between the ultracasual and the ultracautious extremes. There is however an important general point to be made about systemic viral diseases, which illustrates and emphasizes the practical difficulties involved in preventing the transmission of disease by transfusion.

A person with a significant bacteraemia is unlikely to attend a donor session. Similarly a donor with symptoms caused by viraemia will either not volunteer, or will be excluded by the medical practitioner at the donor session. The 'dangerous' donors are those with an asymptomatic viraemia which occurs in the following situations:

a. during the incubation period of a specific viral infection

b. in association with an inapparent or asymptomatic viral infection

c. in the persistent carrier state which may follow a viral disease: the development of the apparently healthy carrier state is more frequently a sequel to a mild or covert illness than to an acute viral infection. Thus consideration of the medical history and a simple medical examination may not detect the 'dangerous' donor with a viraemia. Reservations about the value and benefits of laboratory screening tests have already been expressed, and will be discussed in specific detail in relation to individual transmissible diseases.

Viral hepatitis. Maycock (1972) states that the transmission of viral hepatitis is the most serious complication of the use of blood and blood products. Great concern has arisen in the past 15 years from the many serious outbreaks

of viral hepatitis in chronic renal dialysis and transplant surgery units. Blood transfusion appears to have been the probable portal of entry for the infective agent. The disease has not been confined to patients, and there has been a high morbidity and a significant mortality among staff. These serious outbreaks prompted the Central Health Departments in Britain to establish two expert advisory groups to recommend preventive measures. One group (Department of Health and Social Security, 1972a) considered the prevention of hepatitis in patients with chronic renal failure. It was recognized that patients with progressive renal failure exhibit immunosuppression, and that recipients of an organ graft are receiving immunosuppressive therapy. Thus these patients are liable to become chronic carriers of infective agents which gain entry to the blood stream. The most important preventive measure against the transfusion transmissible diseases is the avoidance of unnecessary transfusions, and this measure has undoubtedly helped to reduce the number of outbreaks of hepatitis in renal units in recent years. Some transfusions are however clinically essential, and the second advisory group (Department of Health and Social Security, 1972b) made important practical recommendations about preventing the transmission of hepatitis by transfusion. In particular, every donation prior to use should be tested for the presence of the Australia antigen, now termed the hepatitis B surface antigen (HBsAg) which is a marker of the infective agent of type B viral hepatitis. All persons with HBsAg are considered to be infective, and their blood should not be used for transfusion purposes. Doubts have been expressed about the infectivity of the blood of persons whose serum contains anti-HBs, and about the necessity to exclude from the donor panel healthy volunteers with detectable anti-HBs. Although these doubts have not been resolved completely, a recent recommendation (World Health Organization, 1975) states that donations may be accepted from volunteers with anti-HBs provided a sensitive test has shown that the individual has no detectable HBsAg. A similar recommendation has been made in Britain (Department of Health and Social Security, 1975).

Yellow fever, hepatitis type A and hepatitis type B are the only viral infections of man which consistently have hepatitis as the main clinical manifestation. Other viruses that cause hepatitis do so as a complication of their more usual clinical picture. The most important of these other viruses are cytomegalovirus (CMV) and Epstein Barr virus (EBV). When the term 'viral hepatitis' is used without qualification in respect of transfusion transmitted infection, it usually means viral hepatitis type B, formerly called serum or long incubation hepatitis. Other forms of viral hepatitis which are known to be transmissible by transfusion are viral hepatitis type A, formerly called infective, epidemic or short incubation hepatitis, and hepatitis associated with either CMV or EBV. While HBsAg is a marker for the infective agent of type B hepatitis, and serological tests are available for the recognition of CMV and EBV infections, there is so far no serum antigen marker specific for the infective agent of type A hepatitis. Virus-like particles have been demonstrated in the faeces of patients suffering from viral hepatitis type A, and an antibody which reacts specifically with these faecal particles has been found in human sera but

no serum antigen specific for the infective agent of type A hepatitis has yet been convincingly described.

Recent observations, particularly in USA, suggest that another form of viral hepatitis, type C exists, and the infective agent may be transmitted by transfusion (Prince *et al.*, 1974). Diagnosis of type A hepatitis transmitted by transfusion is, in the absence of a specific serological marker, based on circumstantial evidence such as history of contact and short period of incubation. Type A infections have an incubation period of 15 to 40 days, compared with 40 to 180 days for type B infections. Hepatitis developing within six weeks of a transfusion is considered to be a probable type A infection, provided there is no serological evidence of other infective agents. Sometimes the diagnosis of type A hepatitis in a recipient is supported by the history of a donor having become ill with suspected type A hepatitis soon after donation. Several prospective studies of recipients in the USA have shown that many cases of post-transfusion hepatitis cannot be explained by known types of hepatitis. Diagnosis of type B, CMV and EBV infections has been excluded, because of an absence of serological evidence to support the diagnosis. Type A hepatitis has been excluded, because the incubation period has been too long. For the present, this unexplained and markerless form of long incubation post-transfusion hepatitis is termed viral hepatitis type C. It must be emphasized that while the development of hepatitis in a patient with a history of transfusion in the previous six months must be regarded initially as transfusion transmitted, other portals of entry for the infective agent should not be excluded or ignored completely. Although the introduction of disposable syringes and needles has helped to prevent transmission of viral infective agents from one patient to another, there are still portals of entry such as haemodialysis, cardiac catheterization, tuberculin testing (Heaf gun), paracentesis and electromyography. Furthermore it is now widely accepted that type B viral hepatitis which formerly was considered to be transmitted only parenterally, can be acquired by close contact.

A recent detailed study of post-transfusion hepatitis in Britain has been reported (Medical Research Council, 1974). Among 768 recipients of blood in the London area there were eight cases (1·0 per cent) of hepatitis, of whom three had the anicteric form of the disease. Two of the five recipients with icteric hepatitis died from acute hepatic necrosis. These observations agree with previous reports from other parts of Britain of a low incidence of icteric and anicteric hepatitis, and contrast sharply with reported high incidences from Japan (65 per cent), United States (18 per cent) and Germany (14 per cent). All these studies were made prior to the introduction of testing donations for HBsAg. If the difference is real, explanations must be sought. One possible explanation is differences in the carrier rates of infective agents in donor populations; for example it has been shown that HBs antigenaemia has a high prevalence in tropical areas. Even in temperate zones HBsAg is found more commonly in certain groups, such as immigrants or returned travellers from tropical areas, drug addicts, male homosexuals, prisoners, the tattooed and the sexually promiscuous. Evidence from the USA has indicated

that the incidence of HBs antigenaemia is much higher in paid than in unpaid donors. Some or all of these factors may affect a regional or national donor population, and may account for the variations in the incidence of post-transfusion hepatitis reported from different countries.

An important reason for the reported differences in incidences of post-transfusion hepatitis may however be variations in the criteria used to establish a diagnosis of hepatitis, particularly of the anicteric form of the disease. In prospective studies of post-transfusion hepatitis, recipients of blood products should be observed carefully for six months following transfusion therapy. These observations should include a full medical examination, and the performance of screening tests for liver function. Patients should be seen at least every two weeks for the first two months, and at four weekly intervals thereafter, until the completion of the six months survey. Observed abnormalities of liver function make the performance of several tests of liver function necessary and of liver biopsy desirable. Ideally similar studies should be made on a control series of untransfused hospital patients, carefully matched in respect of factors such as age, sex, duration of stay in hospital and area of residence.

Surveys of this kind must be planned carefully, and their successful completion is only possible, provided staff and money are available, and above all the patients are willing to co-operate. Clearly if the incidence of hepatitis is low, as would appear to be the case in Britain, a large number of patients must be surveyed. Several practical difficulties are encountered in the conduct of such surveys. As many as 25 per cent of the transfused patients may die within six months of the transfusion, from the primary disease or its complications. Surviving patients may become so ill as to be unable or unwilling to attend hospital as out-patients, and to have to visit such patients at home is extremely time consuming and expensive. The patients in the control series may have little motivation for visiting hospital as frequently as the study requires.

Added to these practical difficulties is the problem of deciding the diagnostic criteria for asymptomatic or anicteric hepatitis. For example in the British study (Medical Research Council, 1974) alanine transaminase (ALT) was estimated routinely, and values greater than 30 iu/l were regarded as abnormal. However factors such as underlying hepatobiliary disease, and the administration of potentially hepatotoxic drugs, may have caused the enzyme rise, and when these other factors existed, it was considered that post-transfusion hepatitis was not the cause of the elevated levels of serum enzymes. This rigid exclusion of all patients having other possible causes of their liver damage, may have contributed to the low incidence (1·0 per cent) of post-transfusion hepatitis in the British study. All donors in Britain are unpaid, and this fact may have contributed to the low observed incidence of post-transfusion hepatitis (icteric plus anicteric) at 1·0 per cent prior to the introduction of total screening of donations for the presence of HBsAg. It should however be noted even with a low incidence of 1·0 per cent, that the British survey still indicates a morbidity and mortality equivalent to 27 cases of hepatitis and eight deaths per 10 000 units of blood transfused (p. 271).

Prior to the availability of possible screening tests for the presence of HBsAg in donations, several measures had been taken by transfusion services to try and prevent the transmission of hepatitis by transfusion. In Britain, soon after the end of World War II, small pool plasma was introduced, not more than 10 donors contributing to the pool (p. 32). Some countries felt that even small pool plasma was too dangerous, and insisted on the use of single donor plasma. Other measures introduced to render plasma less icterogenic were incubation of liquid plasma at 37°C, the irradiation of liquid plasma by UVL, and the addition of viricidal chemical agents such as beta propriolactone. Most authorities have serious reservations about the value of these various methods of rendering plasma free from the risk of transmitting viral hepatitis. It was these fears of plasma-transmitted hepatitis that precipitated the introduction of synthetic macromolecular products as volume expanders. The main alternatives to plasma were polyvinylpyrrolidone (PVP), dextrans, and later hydroxyethylstarch (p. 107).

Another preventive measure was the exclusion as donors of persons who were considered likely to transmit hepatitis. In particular, any volunteer who admitted on questioning to having had an illness resembling viral hepatitis, was permanently excluded from the donor panel. It was also considered dangerous to accept a donation from a volunteer who had been in contact with a case of hepatitis in the previous six months. This type of embargo was sometimes extended to volunteers who had received a transfusion in the past, such as war wounded, and to all donors who had been involved in the suspected transmission of hepatitis by earlier donations. The result was the loss of many existing donors and the rejection of a large number of healthy new volunteers. Many members of the public who had earlier indicated their willingness to donate, did not actually volunteer because of the knowledge that they would not be accepted as donors on account of a history of jaundice, often many years ago. This illustrates the consequences of the implementation of a general embargo or blanket exclusion of a large number of apparently healthy persons as donors. There is growing evidence that many of those excluded were safe donors. Policy is now being changed, and the position of volunteers with a history of hepatitis is discussed in the light of present knowledge and methods of testing donations (p. 280).

Preventive measures against transfusion transmitted hepatitis will now be considered from the aspect of improved selection of donors (p. 273), use of safe blood products (p. 280) and the prophylactic treatment of recipients (p. 281).

Improved selection of donors. The recognition of HBsAg as a marker for the infective agent of type B hepatitis introduced the possibility of testing every blood donation for the presence of HBsAg. Considerable discussion ensued as to the benefits resulting from the costly, and time-consuming procedure of total screening. Some authorities stated that the exclusion of HBsAg positive donors would at best reduce the incidence of post-transfusion hepatitis by only 25 per cent. The inability to prevent 75 per cent of cases of transfusion-transmitted hepatitis was considered to be multifactorial:

i. the methods of detecting HBsAg by large scale screening were the relatively insensitive immunodiffusion (ID) and counterimmunoelectrophoresis (CIEP) techniques (p. 276).

ii. it was suspected that HBsAg was not homogeneous, and that different subtypes would make detection difficult (p. 275)

iii. other infective agents might transmit hepatitis: these included virus A, CMV, EBV and viruses not yet identified, such as the predicted virus C (p. 283).

iv. the use of absolutely fresh untested donations: some clinicians considered that fresh blood had clinical merit and insisted on its use

v. a route of transmission other than transfusion: it was recognized that various hepatitis viruses could be transmitted by parenteral routes other than transfusion and by close contact (p. 271).

The prevention of even 25 per cent of cases of post-transfusion hepatitis was however regarded as statistically impressive. It was estimated that in the USA the actual incidence of transfusion-transmitted hepatitis exceeded 150 000 cases annually, and total screening of donations for the presence of HBsAg, even by a relatively insensitive test, would prevent approximately 40 000 cases of hepatitis in a year. A recent British survey (Medical Research Council, 1974) suggested that before the introduction of total screening of donations for HBsAg, there was a morbidity of 27 cases of hepatitis per 10 000 units of blood transfused, and this would mean approximately 4000 cases of hepatitis per annum in the UK using untested donations. Even insensitive methods of total screening might be expected to prevent 1000 of these cases, and in addition the screening of donations reduces the risk of infection being transmitted to staff and to relatives (p. 279). The introduction of more sensitive methods of testing should reduce the risk of transmitting type B hepatitis even more (p. 277).

Detection of HBsAg is discussed in a special scientific report (World Health Organization, 1975). Morphologically the hepatitis B antigen is sufficiently characterized for electronmicroscopy to be used for direct detection. Three types of particle may be visualized. The most numerous are small round forms about 20 nm in diameter. Long forms about 20 nm diameter, but of variable length, are usually present. A third type, called the Dane particle, may be present. The Dane particle is a double-shelled or 'doughnut' form about 45 nm diameter, the inner core being approximately 20 nm diameter. Many authorities regard the Dane particle as the intact hepatitis B virus, while the small round particles and the long forms are derived from the surface coating of the virus. The surface coating of the Dane particles, and the particles derived from this surface coating have characteristic antigenic determinants, which can be detected by serological techniques such as immunodiffusion, counter-immunoelectrophoresis, complement fixation, haemagglutination methods and radioimmunoassay. It is these surface antigenic determinants which are now called the hepatitis B surface antigen (HBsAg). The dense inner core of the Dane particle also has a distinctive antigenic determinant, called the hepatitis B core antigen (HBcAg). There are corresponding and specific antibodies termed anti-HBs and anti-HBc respectively.

Although this is a relatively simple serological concept, there is no doubt about the complexity of the antigenic structure of HBsAg (*Nature,* 1974). All examples of HBsAg share a common antigen *a*. Two other antigens, *d* and *y* are mutually exclusive. Thus there are two main antigenic subtypes *ad* and *ay*. The subtype *ad* predominates among symptomless carriers of HBsAg in northern Europe and in North America, whereas there is a preponderance of subtype *ay* in chronic carriers around the Mediterranean, the Middle East and Pakistan. In general both *ad* and *ay* subtypes are encountered in patients with acute hepatitis type B, but subtype *ay* has been frequently detected among drug addicts and in hepatitis victims in renal dialysis units, even in countries in which the subtype *ad* predominates among asymptomatic carriers of HBsAg. Two new and mutually exclusive antigens *w* and *r* have been observed. Thus four phenotypes of HBsAg have now been recognized, namely *adw, adr, ayw* and *ayr*.

The recognition of hepatitis B core antigen (HBcAg) in the inner component of the Dane particle has increased the serological complexity of the hepatitis B antigen system. Considerable discussion has ensued over the relative significance of anti-HBs and of anti-HBc in respect of the infectivity of the person with the specific antibody. Anti-HBs tends to appear only transiently after acute hepatitis type B, but is found more frequently in those repeatedly exposed to antigenic stimuli from HBsAg, namely nurses, hospital laboratory staff, drug addicts, haemophiliacs and those who live in conditions in which exposure to HBsAg is common. The presence of anti-HBs is serological evidence of exposure to HBsAg, and presumably of contact with the infective agent of type B hepatitis. It is also well established that in overt type B hepatitis, both HBsAg and anti-HBs may co-exist, particularly in the form of antigen-antibody complexes. However although the British Pharmacopoeia (1973) considers that volunteers with detectable anti-HBs are potentially infective, and should not be utilized as normal blood donors, the World Health Organization (1975) considers that healthy volunteers with anti-HBs are safe blood donors, provided the presence of HBsAg has been excluded by the use of a sensitive method of detection (p. 277). The presence of anti-HBc is however an indication of persisting infectivity. All patients with acute type B hepatitis develop anti-HBc, and all chronic carriers of HBsAg have detectable anti-HBc. It has also been observed that 1 per cent of unpaid donors and 5 per cent of paid donors in USA have detectable anti-HBc, even in the absence of HBsAg and anti-HBs, a finding which may reflect insensitivity in the methods of detecting of HBsAg. Persistent anti-HBc may represent a sensitive marker of continuing viral replication, and the development of tests for the presence of anti-HBc in donations may help to prevent the transmission of hepatitis type B.

Ideally, the method used for screening all donations for the presence of HBsAg should be technically simple, rapid, sensitive and specific, as well as being relatively inexpensive. A regional transfusion centre may require to test from 500 to 1000 donations daily, hence the need for a test which can be performed simply. Rapidity is necessary for two reasons. Firstly there is a small,

but essential, clinical demand for fresh blood products, particularly platelet concentrates. Secondly, donor blood stored at 4°C has a relatively short shelf life limited to 28 days, and it is desirable and economical to make all donations available as quickly as possible. The need for a sensitive test is obvious, but one disadvantage of more sensitive methods of testing is loss of specificity. Too many false positive reactions are disadvantageous for two reasons, (i) all presumptive positive reactions have to be verified and confirmed; a large number of repeat tests creates additional work, and causes a delay in issuing blood products to hospitals, (ii) donors may be falsely labelled HBsAg positive, and as a result be stigmatized. Techniques requiring simple equipment and relatively few staff are preferable to methods which require expensive apparatus and a large staff. Each day there is a heavy workload in testing a large number of donations, so that even a temporary breakdown in apparatus can have serious consequences in the release of donations for clinical use.

Confusion has arisen over the meaning of the term sensitivity in relation to testing donations for the presence of HBsAg. One meaning of sensitivity is the strength or titre of HBsAg in an individual serum. For example HBsAg may have a titre of 1 by immunodiffusion (ID) in that a positive reaction by ID is elicited with neat serum only, whereas the same example of HBsAg may have a titre of 10 000 by radioimmunoassay (RIA). It may therefore be claimed that RIA is 10 000 times more sensitive than ID, but this does not mean that in total screening of donations, RIA will detect 10 000 times as many examples of HBsAg as ID. Indeed RIA may not detect even twice as many positive samples as ID. The most sensitive method for total screening of donations is that which is likely to detect the largest number of true positive examples of HBsAg. However detection rate depends not only on the relative sensitivity of the method employed, but on the accuracy of performances. A highly sensitive method badly performed, may detect fewer examples of HBsAg than a less sensitive method performed skillfully, and read accurately.

The advantages, disadvantages and relative merits of the different methods available for the detection of HBsAg are discussed in special reports (World Health Organization, 1973 and 1975). Most transfusion centres started initially by testing with a simple immunodiffusion (ID) technique. ID methods are simple, but relatively insensitive, and too slow for routine screening of a large number of donations. During the past few years, transfusion centres have relied mainly on the technique of counterimmunoelectrophoresis (CIEP) which is simple and rapid, and has a slightly greater sensitivity than ID. A recommendation (World Health Organization, 1973) was made that CIEP should be the method adopted for total screening and it was considered that the HBsAg in most healthy carriers was of a strength likely to be detectable by CIEP. However doubts have been expressed on the detectability of all examples of HBsAg in persistent carriers by CIEP, and the value of other methods of testing has been investigated (Wallace, Barr and Milne, 1975).

Inert particles such as latex have been coated with anti-HBs and these particles are agglutinated by sera containing HBsAg. The method is simple and rapid, but unfortunately has produced too many false positive reactions

for routine use as a screening test. An alternative particle to latex is red cells which have been coated with anti-HBs, and these antibody coated red cells are agglutinated by sera containing HBsAg. This technique is called reversed passive haemagglutination (RPHA). There is no doubt that RPHA is more sensitive than CIEP for the detection of HBsAg in donor sera, the increase in the detection rate being from 30 to 50 per cent. Most transfusion centres in Britain have adopted the simple and rapid RPHA technique as the screening procedure instead of CIEP. However the method which gives the highest titres of HBsAg is radioimmunoassay (RIA), and RIA is the most sensitive method of screening donations for the presence of HBsAg. For example, Wallace (1976) indicated an increased detection rate of 44 per cent by RPHA and of 52 per cent by RIA, compared with the CIEP method of screening donor sera for the presence of HBsAg. The RIA method is expensive in terms of capital cost of sophisticated equipment such as gamma counters, and since a break down would result in delays in completing the tests, expensive reserve equipment must be available. Running costs are also high, since the reagents required for RIA testing are expensive. The RIA test is less rapid than RPHA and CIEP, but the test can be automated, and given an adequacy of apparatus, it is a practical way of screening donations. One great merit which RIA has over the other methods of testing is that it is an objective test. Technical staff often experience difficulty in the subjective reading of precipitin lines and of agglutination of particles, but appreciate the objectivity of a gamma counter.

It is important to ensure that HBsAg positive donations are not used clinically. The action to be taken in respect of donors confirmed to be HBsAg positive is discussed (Department of Health and Social Security, 1975). Throughout Britain there is in each region a reference laboratory which will perform a battery of confirmatory tests on any serum thought to contain HBsAg. It is only after confirmation has been received of the donor being HBsAg positive, that definitive action is taken in respect of the donor. A letter of explanation should be sent to any blood donor confirmed to be HBsAg positive. The most important message to convey is that the individual must not donate blood again, because of the possibility of his donation causing hepatitis in a recipient. It is equally important not to cause unnecessary worry for the donor and his family, so that the communication should stress that a fit person may carry an infective agent for hepatitis in his blood and still enjoy good health. At the same time it is advisable to seek permission from the donor to inform his general medical practitioner of the finding, because the donor may be incubating viral hepatitis type B. However whether the donor is incubating the disease, or is suffering from an asymptomatic attack of type B hepatitis, or is a chronic carrier of HBsAg, tests of liver function should be performed. In the light of the results of these tests, and of his knowledge of the donor, the general practitioner will decide whether or not to refer his patient to a consultant gastroenterologist. Many of the apparently healthy carriers of HBsAg who exhibit impairment of liver function, are heavy drinkers, and an attempt should be made to persuade such individuals to reduce or eliminate their consumption of alcohol.

There is no doubt that the transfusion of a blood product containing HBsAg may cause type B hepatitis in the recipient. Not all recipients of HBsAg positive products however develop serologically proven type B hepatitis, because some recipients may already have an immunity to virus B infections, and all HBsAg positive donations do not necessarily contain the actual infective agent. Studies among household contacts of HBsAg positive persons in Britain suggest that these persistent carriers are not particularly dangerous, in the sense that icteric hepatitis is uncommon among the contacts. There is however serological evidence of the transmission of HBsAg to contacts, although this transmission may be selective, occurring particularly when the contact is close, as between sexual partners. Early work on Australia antigen, now termed HBsAg, indicated that the carrier state had a genetic basis, and this may account in part for some household contacts and blood relatives becoming persistent carriers of HBsAg and others not. In general, the presence of a chronic carrier of HBsAg does not appear to be especially hazardous to other members of a household. It may well be that close contacts are repeatedly exposed to small amounts of the infective agent and develop an active immunity.

However medical and dental practitioners may be apprehensive when investigating and treating a patient who is known to be HBsAg positive, because staff, particularly in renal units, have developed severe or even fatal type B hepatitis. Some reassurance is given by a Public Health Laboratory Services Report (1974) which suggests that the various preventive measures taken in chronic renal dialysis and transplant surgery units, have indeed succeeded in stemming the tide of repeated outbreaks of hepatitis among patients and staff. It may be that strict adherence to codes of practice in these units has made renal units relatively safe places in which to work. What is perhaps needed now is an extension of these codes of practice to other parts of the hospital service. In the pre-antibiotic era, staff working in infectious diseases hospitals were aware of the dangers of cross infection, but overcame their fears by constant vigilance, and strict adherence to codes of practice. Perhaps the growing realization of the need to avoid transmitting infective agents to any patient who exhibits immunosuppression, will lead to a much wider application of preventive measures.

Total screening of donations in the West of Scotland for the presence of HBsAg has shown that approximately 1 in 800 (0·13 per cent) are positive by CIEP, and about 1 in 500 (0·2 per cent) by the more sensitive RIA method. There are geographical variations in the prevalence within Britain, and areas which have a substantial proportion of persons from tropical areas will have a higher incidence of HBs antigenaemia. Evidence suggests that the incidence of antigenaemia is similar in hospital patients and in donors within an area. Thus a hospital practitioner must over the years, treat many patients who are chronic carriers of HBsAg, and relatively few practitioners are aware of having had an attack of acute viral hepatitis. The probable explanation is similar to that suggested for the infrequency of hepatitis in household contacts of carriers, namely that practitioners are repeatedly exposed to small doses of infective agent, and develop an immunity. Some general practitioners are in a dilemma

whether or not to inform hospital practitioners that a patient is a carrier of HBsAg. There is a suspicion that a patient known to be HBsAg positive may not be thoroughly investigated, or may be denied some form of treatment, because of the fear of staff becoming infected. The author has experienced this apprehensive reaction and, admittedly rarely, a reuluctance to operate on a patient with HBs antigenaemia. An understanding explanation, with particular emphasis on the fact that the colleague has unknowingly treated many HBsAg positive patients in the past without developing hepatitis, resolves the problem and ensures that the patient receives the treatment which the diagnosis has indicated should be given. It is also helpful to explain to hospital colleagues that the information about an individual being a carrier of HBsAg has come to light as a result of a preventive measure. The total screening of donations for the presence of HBsAg is undertaken primarily to make transfusion therapy safer for recipients. A dividend of this procedure is the exclusion from the hospital blood bank, ward and operating threatre of HBsAg positive blood products which in turn renders transfusion therapy safer for all hospital staff who are involved in transfusion work. A realization of the purpose of the exercise helps to reassure apprehensive colleagues. Indeed after due and proper explanation and the overcoming of initial fear, most practitioners prefer to be informed that a particular patient is HBsAg positive, rather than be kept in ignorance of the situation. It is generally agreed that we must not create lepers in the 20th century by stigmatizing persons who are carriers of HBsAg.

This is the appropriate time to consider certain controversial features of donor selection in respect of the transmission of hepatitis by transfusion. It has been established that within any potential donor population, certain groups have a higher than average incidence of HBs antigenaemia. In particular, HBs antigenaemia is more prevalent in male prisoners, and in volunteers from tropical areas. Some transfusion services have declined to accept volunteers in prisons and among immigrant populations. This ultracautious approach may be doubly undesirable. Few transfusion services have so much donor blood available that offers of substantial help can be refused in blanket fashion. Indeed visits to prisons to collect blood can often be arranged when the general intake of blood is low because of the holiday season. The incidence of HBs antigenaemia among male prisoners in Scotland is less than 1 per cent using the most sensitive techniques of testing, thus generous offers of useable donations would be lost by placing a total embargo on prison donors. Furthermore it is socially and psychologically undesirable to exclude prisoners and volunteers from tropical areas from the donor population. Acceptance of prisoners as donors helps to rehabilitate, and some of these volunteers become regular donors after their release. Similarly, acceptance of donors from tropical areas helps to integrate immigrants into the community, and provides donations of red cell groups which may be relatively uncommon in the native British population. Immigrants require transfusion therapy in hospital, and this clinical need for compatible whole blood or red cells may be difficult to meet from a native British population which has a different proportion of blood groups (p. 173). Recruitment of donors from immigrant populations should not be

discouraged and the constructive approach is not to exclude social or racial groups of volunteers, but to test all donations for the presence of HBsAg by sensitive methods such as RPHA or RIA.

Another general embargo which has been operated by transfusion services for many years is the exclusion from the donor panel of volunteers with a clinical history of hepatitis (p. 273). Clinical manifestations of hepatitis include inapparent infection, anicteric illness, acute icterus and chronic liver disease, so that the volunteers excluded are only likely to be those who have had overt icteric hepatitis. The most likely causal agent for this past hepatitis is virus A, and it is thought that the viraemic phase of acute hepatitis A infection is brief. Indeed it is doubtful if a chronic carrier state exists following hepatitis A infection, and exclusion of donors with a clinical history of hepatitis A, may not materially diminish the frequency of hepatitis among recipients. A greater proportion of individuals who have had a mild or inapparent type B infection become chronic carriers of HBsAg, than those who have had a more severe illness (World Health Organization, 1973), and the exclusion of donors who, several years ago, had overt type B hepatitis, and are now HBsAg negative may not reduce the incidence of post-transfusion hepatitis. It is now recommended (World Health Organization, 1975) that volunteers with a history of clinical hepatitis can be accepted as donors, provided the clinical illness occurred more than 12 months ago, and the serum of the donor is shown not to contain HBsAg when tested by a sensitive method such as RPHA or RIA.

Reservations have also been expressed about the temporary exclusion of volunteers with a history of contact with a case of hepatitis during the previous six months, reducing the incidence of post-transfusion hepatitis. Epidemics of type A hepatitis tend to occur among children in families, schools and residential institutions, the spread being by the faecal-oral route. The contacts at greatest risk of acquiring the infection are close contacts in the household or institution, but adult contacts tend to escape an icteric illness, probably because of already having an active immunity to virus A. As far as non-parenteral transmission of type B hepatitis is concerned the contact needs to be close, and transmission is most likely to occur between sexual partners. Donors should be questioned about contacts with patients with hepatitis and temporary exclusion of volunteers can be limited to those who have had close contact during the past six months.

The use of safe blood products. The question is often asked as to whether or not any blood product is free from the risk of transmitting hepatitis. A simple answer is that it is never possible to guarantee that a particular blood product is absolutely safe, although it is often claimed that albuminoid fractions, IgG immunoglobulin preparations and red cells recovered from a frozen bank do not transmit viral hepatitis (Ch. 2). There is certainly good experimental evidence that plasma protein fraction (PPF) and human albumin solutions which have been pasteurized at 60°C for 10 hours do not transmit hepatitis, but the practical difficulty is that no absolute guarantee can be given that a particular batch of an albuminoid preparation will not transmit hepatitis. In fact isolated incidents have been reported of a batch of PPF having caused

several cases of hepatitis in recipients, and the explanation has been failure to maintain the bottles in the batch at 60°C for 10 hours. The effectiveness of pasteurization of infective albumin solutions was demonstrated in volunteers many years ago, but similar work today is regarded as unethical. There is no animal suitable or available in large numbers to demonstrate the effectiveness of pasteurization. In the absence of human volunteers and laboratory animals, there is a gap in the viral sterility testing of the product. Strict attention should be given to the control of the temperature and duration of pasteurization. With the increasing use of sensitive RPHA and RIA methods of testing individual donations for HBsAg, and strict control of the processing of plasma, it is most unlikely that albuminoid fractions will transmit hepatitis. There is good circumstantial evidence that IgG fractions prepared by chemical precipitation, and red cells recovered from frozen banks, are unlikely to transmit hepatitis, but again an absolute guarantee cannot be given. The use of these relatively safe blood products is particularly valuable in selected clinical situations. For example in progressive renal failure, anaemia and hypoalbuminaemia may occur, and there is the fear of transfusion therapy introducing viral hepatitis into renal units. If it is considered necessary to treat the anaemia and hypoalbuminaemia by transfusion, then red cells recovered from the frozen bank and albuminoid preparations should be used. Another advantage of using that form of red cell transfusion is that the cells recovered from the frozen bank provide a leucocyte-poor and platelet-poor product. The chance of alloimmunization of potential recipients of a kidney graft, particularly to HLA antigens, is therefore reduced (p. 71).

The prophylactic treatment of recipients. Spread of viral hepatitis type A in the community cannot be prevented, but when special protection is required, human normal immunoglobulin (HNI) is of proven value (Public Health Laboratory Service Report, 1968). HNI suppresses the overt features of type A hepatitis, although infection may still occur, in which event the subject is temporarily infective to his close contacts, but subsequently develops an active immunity to virus A (p. 100). It is doubtful if, in general, HNI attenuates viral hepatitis type B, although claims that HNI is of prophylactic value in type B infections have been made. The explanation of some apparent benefit from the use of HNI in the prevention of type B infections is almost certainly that the particular batches of HNI have had a substantial content of anti-HBs. Just as the prevalence of HBs antigenaemia varies in different populations, so the incidence of anti-HBs varies. Another operative factor in the variable anti-HBs content of HNI is the extent to which plasma donations known to contain anti-HBs are removed from the global plasma pool used for HNI production in order to prepare HSI anti-HBs (p. 100). Certainly in Britain in recent years all donations have been screened for anti-HBs, and plasma shown to contain anti-HBs, has been used to produce HSI anti-HBs and not HNI. However the HNI currently produced does contain a low titre of anti-HBs derived from donations containing weak examples of anti-HBs, which have not been detected in the antibody screening test (p. 282).

There are encouraging reports that HSI anti-HBs is of prophylactic value

in selected clinical situations (p. 105). These include exposure to HBsAg in genuine accident situations in laboratory, ward or operating theatre, in the accidental transfusion of a blood product containing HBsAg, in chronic renal dialysis units, and in infants born to mothers who are known to be persistent carriers of HBsAg. However, only limited amounts of prophylactic HSI anti-HBs are available, and its use should be carefully controlled (Kerr, 1973). There are three basic problems in the prophylactic use of HSI anti-HBs (i) the prophylactic value in various clinical situations is not yet proven, (ii) the amount available is limited and (iii) the dosage is empirical. These three factors are inter-related, and pose practical problems for transfusion services in respect of the production and presentation of the fraction HSI anti-HBs. Transfusion services are no longer obliged to perform total screening of donations for the presence of anti-HBs, since it is considered that donors with detectable anti-HBs are unlikely to be harbouring virus B (p. 275). There is still a need to find donations containing anti-HBs for the production of HSI anti-HBs, but the performance of total screening of donations for the presence of anti-HBs is expensive, and occupies valuable technician time. This need may be met for the present by the intensive plasmapheresis of donors who are already known to have anti-HBs. It is unethical to boost the titre of anti-HBs in these donors by injecting inactivated HBsAg, but supplies of plasma for HSI anti-HBs production will have to be supplemented in the future by screening new donors for anti-HBs.

Another practical problem in the future provision of plasma containing anti-HBs is the method of screening to be used for the detection of anti-HBs. Approximately 0·2 per cent of blood donors in the West of Scotland have detectable anti-HBs when screened by CIEP, but the incidence rises to approximately 1·0 per cent when passive haemagglutination (PHA) is used as the screening method. The corresponding increase in the amount and yield of HSI anti-HBs is not however fivefold, because the PHA method is detecting many low titre antibodies which are not discovered by CIEP. This problem of the method of screening donations and the subsequent yield is relevant to the therapeutic dose of HSI anti-HBs, and to the form in which the product is presented for clinical use. At present in Britain the dosage is empirical at 0·5 g IgG, given by deep intramuscular injection as soon as possible after, and within five days of exposure to HBsAg. Until international units for anti-HBs are established, the dose is expressed as g IgG, the fraction having so far been prepared from plasma donations in which the anti-HBs has been detected by a CIEP screening technique. If in future the lower titre donations, in which the specific antibody is detectable by PHA, but not by CIEP, are to be used for the production of HSI anti-HBs, then the dosage and presentation of the product may have to be greater than the current 0·5 g IgG. This is an excellent example of the practical problems which face the blood transfusion service in particular, and the National Health Service in general, in forward planning and expenditure. It illustrates the need for co-operation between the transfusion service, the clinician, and the administrator, in reaching decisions and in formulating policy (Ch. 11).

The difficulties in establishing the diagnosis and incidence of post-transfusion hepatitis have already been discussed (p. 272). For similar reasons it is difficult to assess the benefit of total screening of donations for the presence of HBsAg, but careful studies from USA have provided valuable information. Prince *et al.* (1974) in a detailed prospective study of 204 cardiovascular-surgery patients found that an agent other than virus B, seemed to be the cause of 36 (71 per cent) of 51 cases of overt post-transfusion hepatitis. The sera of these 36 cases of hepatitis showed no evidence of the presence of HBsAg or of anti-HBs. Incubation periods and clinical and epidemiological features were inconsistent with a diagnosis of type A hepatitis, while the serological evidence indicated that it was unlikely that CMV or EBV infections had caused the post-transfusion hepatitis in these 36 cases. It was concluded that another infective agent, virus C, was involved and that complete control of post-transfusion hepatitis will require identification of this postulated hepatitis virus. There was serological evidence that the remaining 15 (29 per cent) of the 51 cases of overt post-transfusion hepatitis were type B infections. In addition, in this series of 204 patients receiving large volume transfusions, another 25 patients had serological evidence of exposure to HBsAg without developing overt hepatitis. Thus a total of 40 patients, that is almost 20 per cent of the entire series of recipients, developed evidence of the transmission of type B hepatitis by transfusion, and the potential value of screening all donations for the presence of HBsAg by sensitive methods was indicated. The degree of benefit will depend on the prevalence of HBs antigenaemia in the donor population, and on the extent of transfusion transmitted type B hepatitis prior to the introduction of screening tests.

The prevalence of HBs antigenaemia is high in tropical areas and among professional donors. In Britain, which is a temperature zone with unpaid blood donors, there is a low incidence of HBsAg positive donations, and the available evidence (Medical Research Council, 1974) indicates that post-transfusion hepatitis is statistically much lower in Britain than in other countries such as the USA, Japan and Germany (p. 271). The benefit of total screening for the presence of HBsAg in donations in Britain is therefore expected to be less dramatic than in countries with a high incidence of post-transfusion hepatitis. Wallace, Barr and Milne (1975) report their experience in Scotland of total screening of donations by CIEP during a period of four years. A sample of frozen serum from each donor was preserved for at least six months with the following three objectives (i) when a previous donor is found to be HBsAg positive, it is possible to retest the serum from the earlier donation which had given a negative reaction; (ii) comparative trials with newer methods of screening such as RPHA and RIA can be undertaken on preserved donor sera already screened by CIEP; (iii) alleged cases of post-transfusion hepatitis can be thoroughly investigated by retesting by CIEP and by testing for the first time by RPHA and RIA, the preserved serum from the actual donations which may have transmitted hepatitis. In this way false negative reactions for HBsAg by the CIEP method may be revealed by the more sensitive RPHA and RIA tests. The CIEP method detects examples of HBsAg within a limited

range of strength of HBsAg; very strong and very weak examples will not be detected by CIEP. Donations containing these very weak or very strong forms of HBsAg may transmit type B hepatitis, and it is recommended that the CIEP method of screening donations should be replaced by RPHA or RIA. Of these two sensitive methods, RPHA is simpler and more rapid, but RIA is more sensitive, and has the great merit of being an objective test (p. 277). Sensitive techniques tend to produce more false positive reactions as well as revealing more true positive donations. It is extremely important not to report voluntary blood donors as being HBsAg positive until there is reasonable certainty that the observed serological reactions are truly positive (p. 277). In general confirmatory tests should include specific neutralization of the suspected HBsAg by anti-HBs.

There is evidence that a donation containing a very weak form of HBsAg not detectable by RPHA, but just detectable by RIA may transmit type B hepatitis, and some transfusion services have introduced RIA rather than RPHA, as the routine method of testing donations. Whatever method of screening donations is used, it is important to follow up every suspected case of post-transfusion hepatitis. The diagnosis of hepatitis should be established clinically and by appropriate laboratory tests, and similarly the type of hepatitis should be determined. Hospital practitioners must include in the case notes of any recipient of a blood product, detailed records of each unit transfused, so that in the event of a complication, the transfusion service can identify all the donations involved (p. 226). If, in turn, the transfusion service preserves serum from each donation for at least six months, it is possible to use serological tests in an attempt to find evidence of a specific infective agent in the donations transfused. Careful studies and investigations of this kind will help to evaluate the place of total screening of donations for HBsAg in the prevention of post-transfusion hepatitis. Even at this stage however, some reservations should be expressed.

Evidence from the USA (p. 283) indicates that a long incubation form of hepatitis other than type B exists, and this has been named type C or 'non-A, non-B' (*Lancet*, 1975). Present evidence on this infection in Britain is scanty, but most cases of post-transfusion hepatitis seem to be type B, although some cases with relatively short incubation periods are associated with type A or CMV or EBV infections. It may be that at present type C infection is rare in Britain. More evidence on this subject will emerge as RPHA and RIA are introduced as the method of testing donations for HBsAg. There is however evidence that some donations which give negative reactions for HBsAg by the most sensitive methods of testing transmit type B hepatitis. In such circumstances it is possible for patients to have acquired the infective agent by a portal of entry other than transfusion, but controlled studies suggest that transmission by transfusion has occurred in some patients. There is good evidence that the spheroidal particles (p. 274) which are present in the largest numbers in HBsAg positive donors are immunogenic, but non-infective. The inner core particles of the large Dane particles contain DNA, and are thought to be infective. There is also evidence of free viral DNA which may be infective.

It is therefore possible that a few donors who give negative reactions for HBsAg have, in their blood, viral DNA, either in core particles or free in the plasma. Techniques are being evolved for the detection of DNA, and these may detect the potentially dangerous donors, who seem to have no detectable surface antigen HBsAg. One serological test which is being evaluated is the detection of anti-HBc in donor sera (p. 275). It is known that the sera of chronic carriers of HBsAg contains anti-HBc, and it is thought that this antibody appears in an individual in whom replication of the viral agent is occurring. The presence of anti-HBc in a donor serum which contains no detectable HBsAg may indicate the presence of hepatitis B virus. Further studies are required to evaluate the full significance of free DNA or of anti-HBc in donor sera.

Complete understanding of the transmission of type B hepatitis by transfusion will not be achieved until the actual infective agent has been isolated and cultured. Even then, however, it will still be necessary to employ a rapid screening test of donations for the presence of a marker of the infective agent. Viral sterility tests take time, and some blood products must not only be prepared from freshly donated blood, but have to be used soon after preparation, in order to be therapeutically effective. While type B hepatitis seems to be the form of post-transfusion hepatitis most commonly encountered in Britain, it would be advantageous to recognize markers for the infective agents of 'non-B' hepatitis such as type A and type C, if the latter really exists. Another desirable development is effective protection against viral hepatitis. The present state of passive immunization has already been discussed (p. 281), but uncertainty over active immunization will exist, until the infective agent has been isolated. The 20 nm spheroidal particles of HBsAg (p. 274) are immunogenic and probably non-infective. These particles are present in large numbers in the plasma of chronic carriers of HBsAg, so that a source of starting material for a vaccine is available. It will be essential to ensure that these particles are free from infective agent, before being used as a vaccine to induce active immunization. The core of the Dane particles contains DNA, and it is possible to produce a preparation of 20 nm spheroidal particles which is free from Dane particles, but there is uncertainty about the absence of free DNA. In spite of the great advances which have been made in recent years, type B hepatitis remains a mystery.

Hepatitis transmitted by cytomegalovirus (CMV) and Epstein-Barr virus (EBV). CMV and EBV infections of the liver transmitted by transfusion have been reviewed by Stern (1972). These infective agents are normally associated with infectious mononucleosis, CMV being an important cause of Paul-Bunnell-negative infections, and EBV being the cause of Paul-Bunnell-positive illnesses. Hepatitis is probably always present as a complication of infectious mononucleosis, although the manifestations are usually anicteric. An illness resembling infectious mononucleosis occurs as a complication of open heart surgery, and has been called the post-pump or post-perfusion syndrome. It is a systemic disorder associated with fever, hepatosplenomegaly and atypical lymphocytosis. Liver function tests are abnormal, and some patients develop

severe icteric hepatitis. Virological studies in these patients clearly established CMV as the aetiological agent in the infective illness. This type of disorder is not confined to cardiac surgery patients, but is found in other patients who have received large transfusions, usually of fresh blood from multiple donors. A similar disorder occurs in association with EBV infections.

Most adult blood donors in Britain have detectable EBV antibodies, and more than 60 per cent have detectable CMV antibodies. However some of the donors with antibodies may carry the infective agent in a latent form in the circulating leucocytes, and transmission of EBV and CMV may occur. In turn, most adult recipients already have EBV antibodies, but almost 40 per cent lack CMV antibodies. It would therefore be expected that the incidence of post-transfusion EBV infections would be low compared with CMV infections. A recent study (Medical Research Council, 1974) revealed only five cases of CMV-associated anicteric hepatitis in 712 recipients of blood. Four were examples of primary CMV infections, and the remaining case was an example of reinfection. In the same series there was no clinical illness or disturbed liver function associated with EBV infections. Both CMV and EBV infections transmitted by transfusion are essentially benign conditions, but blood products containing these infective agents can be hazardous for patients on immunosuppressive therapy and for pregnant women. The infective agents are carried in the circulating leucocytes of donors, and the risk of transmitting CMV and EBV by transfusion is greatest with large volumes of fresh blood, or with granulocyte concentrates. Infection can, however, be transmitted by blood which has been stored at 4°C for several days. There is currently no established method of preventing the transmission of these viruses by transfusion. Screening of donors for the absence of EBV antibodies does not help because relatively few adults lack these antibodies. A similar screening procedure may help to prevent the transmission of CMV infections, but it will be difficult to detect the occasional donor undergoing primary infection at the time of giving blood. Other preventive measures include the utilization of stored rather than fresh blood although this is not completely protective, and the addition of antiviral agents to potentially infected blood products.

Syphilis. In Britain it is not customary to question donors directly about syphilitic infections, but there is an obligation to perform a serological test for syphilis on each donation (British Pharmacopoeia, 1973). The evidence of confirmed positive serological reactions for syphilis among donors in the West of Scotland is less than 1 in 10 000, and statistically transfusion-transmitted syphilis is not a serious hazard with voluntary unpaid donors. Routine serological testing is not however a complete safeguard, because a healthy person may harbour the infective agent in his blood in the early stages of a systemic infection, and be sero-negative. Sometimes a clinician insists on transfusing absolutely fresh blood, or a product derived from the fresh donation, before there is time to perform serological tests for infective agents, and must be advised of the risk. In such circumstances, the donation should, if possible, be collected from a volunteer who has donated blood on previous occasions, and has been consistently sero-negative. Any such donation must

collection, air is already present in the flask and it is essential to use an airway during blood collection to allow the expanding air to escape. Air embolism can still occur even when an airway is used, if the airway is defective. Blockage of an airway can arise from the use of a wool filter which is packed too tightly or from the use of a glass connecting tube which, instead of being open at each end, is closed at one end. When the flow of donor blood into a non-vacuumized bottle suddenly slows there are several possible explanations of which one is a blocked airway. Replacement of a faulty airway by a functioning airway may well cause the flow of blood to accelerate again. If an operator is unaware of a build-up of positive air pressure as the real cause of this sudden slowing of blood flow, and releases the pressure in the cuff on the upper arm, the result could well be fatal. The air trapped in the bottle can escape rapidly into the vein of the donor and a catastrophe may result. Another potential hazard with non-vacuumized bottles for blood collection is the use of a vacuum pump to assist the flow of donor blood. Some operators prefer to use a relatively fine needle, so that the donor is less likely to complain of pain, and try to ensure a steady flow of blood by connecting the airway to a vacuum pump. Because it is simple, a reversed 'Higginson's syringe' may be used for this purpose, but there is an obvious danger of accidentally blowing air into the bottle. Most transfusion services now use plastic packs for blood collection, but some centres still prefer glass bottles. Air embolism is fortunately a rare complication in blood donors, but it is potentially dangerous and is avoidable. The fact that a healthy volunteer may die from a simple operation as a result of defective equipment or an improper use of functionally sound apparatus emphasizes the need for thorough training of personnel and for constant vigilance on the part of the blood collecting team. Prevention is better than cure, but the treatment of air embolism has already been discussed (p. 258).

A generalized reaction to the use of local anaesthetic in blood donors is fortunately an extremely rare event. The author has observed only one example of this alarming complication in 35 years of transfusion practice and this was associated with the use of procaine. Anaphylaxis in man is rare, but may occur from one of a wide variety of injected substances to which the individual has been sensitized by a previous injection. Injection of a local anaesthetic such as procaine has been incriminated in acute anaphylaxis, and it must be emphasized that although the small amount of local anaesthetic injection prior to blood donation is given intradermally and subcutaneously, some of the injected material may accidentally enter a small blood vessel. It is advisable to have adrenaline or one of its analogues available at donor sessions. The adult dose of adrenaline (1:1000 solution) is 0·5 ml intramuscularly immediately an acute anaphylaxis occurs, and 0·5 ml every 20 minutes if the systolic blood pressure remains below 100 mm Hg.

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products have an obligation to make the best possible use of each donation, and of the derived blood components and fractions.

Blood transfusion services which have relied on unpaid voluntary donors wish to maintain the system. Some services which have hitherto paid donors now wish to change to an unpaid system. Indeed the World Health Organization is encouraging the expansion of the unpaid voluntary donor system through the world. Payment for blood donations does attract a potentially dangerous type of donor; there is an increased danger of transmitting communicable diseases, particularly viral hepatitis, when paid rather than unpaid volunteers are used (p. 272). Inevitably the offer of financial reward attracts drug addicts, alcoholics and the sexually promiscuous, who are more likely to be harbouring infective agents which may be transmitted by transfusion therapy. The routine performance of sensitive tests for evidence of the infective agents of viral hepatitis type B and of syphilis undoubtedly detects some potentially dangerous donations, but others are missed because of the relative insensitivity of the tests, or by the fact that the donor is in a sero-negative phase of the disease. Dissemination of infective agents is aggravated by the development of component therapy, because an infective agent in a single donation may contaminate several derivatives prepared from that donation, or one infected donation may contaminate a pool of plasma made from many donations (p. 31).

It has often been assumed that the payment of individual donors for their services attracts so many volunteers that there is never a shortage of donors, but this is a wrong assumption. All systems, with the exception of one in which blood donation is legally compulsory, are voluntary in that the individual is at liberty to decide whether or not to give blood, and to decide the time and place of his donation. There may also be an element of commercial competition between two local transfusion services, each of which pay donors, and it is not unknown for paid donors to refuse to donate blood until the payment is increased. Payment of donors does not therefore guarantee a regular and adequate intake of donor blood. A modern transfusion service also requires selected donors for the supply of a product of a particular blood group, or for the provision of a special product at an early, convenient or appropriate time, and therefore needs the full cooperation of donors. Experience has shown that these requirements are more likely to be met with unpaid rather than paid volunteers.

While there are good reasons for wishing to have a system of unpaid voluntary donors, consideration will have to be given locally, if not nationally, to the payment of reasonable expenses to some volunteers, particularly those donors required for special blood products. Most donors in Britain will be invited approximately every six months to donate blood (p. 214). For many of these volunteers the arrangements will be convenient and inexpensive in that a mobile blood collecting unit will visit a local hall or the place of work. Some donors work near a static donor centre, and find it convenient to make regular donations there. Other volunteers make themselves available by telephone at work or at home, and are very pleased to be given the opportunity