VII.—Plasma Coagulation Factors. By A. S. Douglas, B.Sc., M.D., F.R.C.P. (Lond., Ed., Glasg.), F.R.C.Path., Regius Professor of Medicine, University of Aberdeen

SYNOPSIS

(1) Factor VIII preparations in the treatment of haemophilia and von Willebrand's syndrome. It should be appreciated that despite the recognition of many genetically determined coagulation defects, haemophilia is the only relatively common hereditary coagulation defect. The incidence is of the order of 1 per 20 000 of the population. Although relatively rare, the severe haemophiliac has so many problems that relevant therapeutic issues impinge more frequently on the physician than the incidence rate would indicate. These preparations also have a role in the rarer disorder of von Willebrand's syndrome, which is a genetically determined haemorrhagic state characterised by a prolonged bleeding time and often by a deficiency of factor VIII.

Plasma is used in the treatment of these conditions either as whole plasma or as one of its fractions —a simple prepared fraction called cryoprecipitate or as a lyophil dried preparation made by much more sophisticated and expensive techniques.

(2) Consideration must also be given to factor IX in the treatment of haemophilia B or Christmas disease.

In some ways this is easier to treat while in others it is more difficult. It is easier in the sense that factor IX is much less labile than factor VIII, but cryoprecipitate does not contain therapeutic amounts of factor IX and there is no equivalent simple fractionation procedure for factor IX. The 'purified' fractions are prepared by relatively more sophisticated and expensive techniques.

(3) In the management of vitamin K deficiency, oral anticoagulant therapy or liver disease then purified preparations of factors II, VII, IX and X may be required.

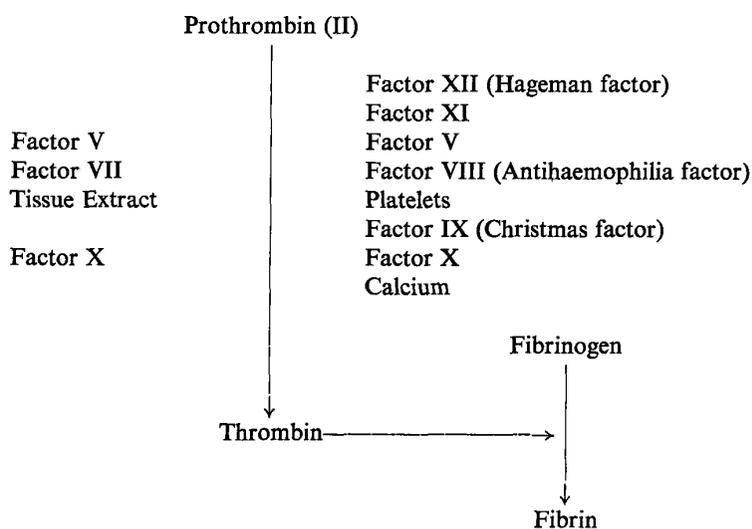(4) In the defibrination syndrome fibrinogen may be needed.

1. INTRODUCTION

In any haemostatic defect where the total blood loss has been massive, then the first step in replacement therapy is to give whole blood. In the bleeding of liver disease or defibrination (the latter usually due to intravascular coagulation), fresh blood is preferable. This is because both of these conditions may be associated with thrombocytopenia. In respect of blood loss it is important to recall that haemophiliacs may have massive blood loss, without blood being lost outside the body. For example, the haemophiliac with a massive retroperitoneal ileopsoas haematoma may have lost three-quarters of his total blood volume into this site of haemorrhage.

As way of introduction, a brief account of the current concept of blood coagulation is outlined.

The theory of blood coagulation at the beginning of the century was that prothrombin was converted to thrombin under the influence of thromboplastin and calcium. This was the Morawitz or classical theory of blood coagulation.

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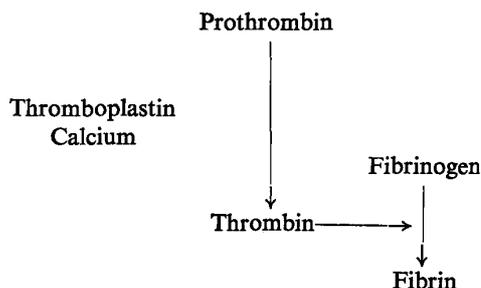
The term thromboplastin was used in the context that this was tissue. In the modern theory the term thromboplastin has been expanded, so that the mechanism for prothrombin conversion is now much more complex with the components shown in this diagram responsible for rapid and effective thrombin formation.

Prothrombin (II)

Factor XII (Hageman factor)
Factor XI
Factor V — Factor V
Factor VII — Factor VIII (Antihaemophilia factor)
Tissue Extract — Platelets
Factor IX (Christmas factor)
Factor X — Factor X
Calcium

Fibrinogen

Thrombin——————→

Fibrin

Haemophilia A, as already indicated the commonest type of genetically determined haemorrhagic disease, is due to deficiency of factor VIII and haemophilia B (Christmas disease) to lack of factor IX; replacement therapy in both of these disorders requires to be considered. It is probable that patients with haemophilia may either lack the appropriate clotting factor or have an abnormal protein, functionally inactive, representing factor VIII. Haemophilia A and Haemophilia B (Christmas disease) are both sex-linked recessive disorders affecting males; von Willebrand's syndrome is an autosomal defect. *In vitro* comparisons of the plasmas from patients with haemophilia A and those with von Willebrand's syndrome show that they are not mutually corrective. However, there are differences in the plasmas on *in vivo* comparisons. When plasma from a patient with haemophilia A is infused into a patient with von Willebrand's syndrome, after an interval there is a relatively prolonged rise in the factor VIII level in the recipients plasma (see below).

2. MANAGEMENT OF HAEMOPHILIA

The problem in management of haemophilia is that the half-life of factor VIII infused into a haemophiliac is approximately 9 hours, and the rise in level from a pint of plasma may be from zero to a level of 10 per cent. One might therefore require

5 pints of plasma (say 10 donations) to bring the level up to 50 per cent. These are only rough approximations, but give some idea of the magnitude of the issue of replacement therapy.

Plasma. This requires to be fresh for the best levels to be obtained in the patient. There are obvious administrative difficulties in making available fresh plasma for every haemorrhagic emergency in the haemophilic patient. To overcome this difficulty fresh plasma is collected, separated from the red cells and maintained at −20°C. When thawed for use there is reasonable recovery of factor VIII, but a gradual loss down to approximately 50 per cent. over 3 months. The surviving level of factor VIII is more related to the initial level of factor VIII in the donor plasma than to the effects of storage. Donors with high levels can be detected by screening donated blood; male donors of Group A have a higher level than average and blood given for transfusion may be selected for processing on this basis. The rise in factor VIII which accompanies exercise is seldom exploited, although brief exertion in normal subjects can readily double the factor VIII level in circulating blood, and this increased level has the usual storage survival time.

Cryoprecipitate. This is the sheet anchor of current replacement therapy in haemophilia; a paper published by Pool and Robinson in 1959 described how 'it was by chance observed that the AHG content of the last few drops of plasma left in a bottle after the transfusion was greater than that of the freshly transfused unit'. (AHG is antihaemophilic globulin-factor VIII.) From this fortuitous finding sprang the development of one of the most widely used and cheapest sources of factor VIII called 'cryoprecipitate'.

Blood is usually collected for the production of 'cryoprecipitate' into a double plastic pack consisting of two connecting bags. The usual ACD anticoagulant is employed. Plasma is separated by centrifugation at 4°C as soon as possible after collection; this plasma is decanted into the satellite bag, a temporary clip applied to the connecting tubing and the plasma container immersed in a freezing bath (dry ice-ethanol mixture). When plasma frozen as described above is thawed at only a few degrees above zero (i.e. 4°C) then the fibrinogen precipitates as a 'sludge', with which much of the factor VIII is associated. This sludge forms the 'cryoprecipitate'. About 5–15 ml of supernatant plasma is retained and the remainder decanted back on top of the red cells. The remixed red cells and plasma can be used for most normal transfusion requirements, i.e. the factor VIII has been harvested, without the sacrifice of blood for routine use.

When the cryoprecipitate requires to be used the selected number of packs are thawed in a 37°C waterbath with occasional kneading or mixing to ensure even melting. At this temperature the fibrinogen redissolves and a clear solution should be obtained. Pooling of cryoprecipitate from a small number of donations in a single container immediately before refreezing for storage greatly simplifies the task of subsequent administration. A device for pooling the units under sterile conditions has been described by Dr Davidson from the Royal Infirmary in Glasgow (Davidson and Muir 1968). A mobile van unit has been constructed by Dr Wallace and his colleagues at the West of Scotland Blood Transfusion Service so that plasma can be processed for cryoprecipitate production immediately after donation.

'Purified' Fractions. By a number of different fractionation procedures, dried fractions of plasma have been made as sources of factor VIII.

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Cohn Fraction I
Ether Fraction
Blombäck Fraction I-O
Glycine Fraction
High potency glycine fraction
Polythene glycol-precipitated Fraction

When studied per mg of protein the last two are very powerful materials. The plant required to produce these fractions is sophisticated and the product is expensive in comparison with cryoprecipitate. Clear advantages for this type of purification have not yet emerged.

Purified fractions (rich in factor VIII) of bovine and porcine plasma are available commercially and are extremely potent.

Principles of Intravenous replacement Therapy in Haemophilia. The first considera-tion is potency for this determines the total volume of salt and protein solution needed to give the patient the required dose of factor VIII. Using plasma, the volume should not be so great as to overload the circulation; in routine clinical practice in the management of haemophilia large volumes of plasma are tolerated surprisingly well. Side-effects to factor VIII include febrile reactions, the transmission of serum hepatitis and the induction of antibodies to factor VIII; thrombocytopenia is particularly a problem with the animal preparations of factor VIII. Using human factor VIII preparations the risk of hepatitis is proportional to the number of donations involved in the material given. In the production, for example, of cryoprecipitate the blood should be screened for Australia antigen.

Dose. Different clinical situations demand different levels of circulating factor VIII if bleeding is to be reliably arrested or prevented. It is convenient to classify these situations into three grades of severity (see Mason and Ingram 1971).

(1) The minor episodes of spontaneous haemorrhage which are a recurring feature of a severe haemophiliac's life. They usually respond to a plasma level of factor VIII of 5–10 per cent. and treatment of these episodes is usually aimed at maintaining this level for 48 hours.

(2) The intermediate episodes where it is very important to achieve prompt haemostasis. Bleeding involving the central or peripheral nervous system or the major airways fall into this category. Levels of about 20 per cent. of factor VIII are recom-mended. Levels are maintained until healing has occurred or complete clinical resolution is apparent.

(3) Major surgical procedures and trauma. Factor VIII levels of at least 30 per cent. should be maintained for at least 48 hours and thereafter maintained at 20 per cent. until healing is complete.

Resistance to Factor VIII. This may be of two sorts. Species specific antibodies develop to animal preparations from bovine and porcine sources. It is usually accept-able that these preparations should be given for 10 days, once in a haemophiliac's lifetime, being reserved for use in a life-threatening situation when human factor VIII is not available. The material has in fact been reused, perhaps surprisingly without the anticipated serious anaphylactic reaction. With the advent of cryoprecipitate therapy the occasions when needed have become less in number. A small proportion of severe haemophiliacs develop antibodies specifically against factor VIII. These

latter are usually potent against human factor VIII, and render therapy in this group particularly difficult.

3. Treatment of Christmas Disease
(Haemophilia B—Factor IX Deficiency)

The levels of factor IX to be aimed for in treating this disease are the same or slightly less than those already given for factor VIII.

Factor IX is considerably less labile than factor VIII *in vitro* so that, in an emergency, plasma may be prepared from ACD blood stored for as long as 3–4 weeks at 4°C. This means that in routine bank blood potency is well maintained; at least 80 per cent. of the original activity may be present at the end of the period of routine shelf life. A few reports however have suggested that more rapid losses occur on storage; for this reason it is more reliable to use, whenever possible, plasma prepared from freshly donated blood. It should be kept until use at between −20° and −40°C. The supernatant plasma from which 'cryoprecipitate' has been prepared is an equally good source of factor IX. It is surprising that freeze-dried whole plasma, on the other hand, is not a reliable therapeutic material.

Christmas disease is rarer than haemophilia; for every patient with Christmas disease there are five with haemophilia and consequently there are fewer opportunities to gather information on the recovery and decay of factor IX after infusion. Twenty-four hours is a convenient estimate of the half-life of factor IX on which to base therapy, and will probably safely underestimate the true rate of decay in most patients. For reasons which are not clear, the expected rise in factor IX is not as great as expected; i.e. the recovery of factor IX *in vivo* is poor compared to that of factor VIII. In calculating the dose to be given it is assumed that about one-fifth of the infused activity appears in the circulation.

There is available to us a variety of preparations; all involve absorbing the 'prothrombin complex' of factors II, VII, IX and X on to a suitable medium, followed by elution. In Scotland two preparations are available.

> (i) Factors II, IX and X, or
> (ii) Factors II, VII, IX and X.

It depends on the method of preparation whether the VII is lost in the procedure. This is linked to whether the blood is collected in ACD (acid citrate dextrose) or EDTA (ethylene diamine tetra-acetic acid). The ACD plasma is available from blood collected for routine transfusion purposes or from the cryoprecipitate supernatant. Blood collected into EDTA is not used for transfusion purposes.

In the management of Christmas disease the preparation used is usually the one containing factor II, IX and X—a plasma equivalence of 1½ litres being administered.

Patients with Christmas disease or haemophilia may develop non-specific reactions to plasma but have no problem when given the concentrated materials.

4. Treatment of von Willebrand's Disease

Three haemostatic components are found to be abnormal in this disorder on testing these patients. A prolonged bleeding time was reported in von Willebrand's original

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description and since then the response of this to a variety of therapeutic materials has been investigated. Nilsson *et al.* (1957) showed that the bleeding time was shortened by infusion of subfraction I-O of Cohn fraction I prepared from normal or haemophilic plasma. The 'bleeding time factor' is not present in Fraction I-O from patients with von Willebrand's disease. Cryoprecipitate, but not the depleted supernatant plasma, contains the factor. Mason and Ingram (1971) found that factor VIII concentrate prepared by Kekwick and Wolf's method did not shorten the bleeding time in a case of von Willebrand's disease responding adequately to plasma. This issue of a 'bleeding time factor' as a distinct entity is not finally resolved, but the overall evidence suggests that simply raising the factor VIII level does not necessarily correct the bleeding time to normal.

The second haemostatic defect which may be present in von Willebrand's disease is a reduced level of factor VIII. Although this is very variable, it is only occasionally as marked as in severe haemophilia. The unique factor VIII response to transfusion therapy is frequently commented upon. Not only normal plasma but preparations devoid of measurable factor VIII activity such as serum or haemophilic plasma will stimulate factor VIII synthesis for a period of 12–24 hours after infusion. Whatever the explanation of this phenomenon, it can be exploited by treating patients with plasma or most of the available human concentrates such as cryoprecipitate. Many of these materials also contain the 'bleeding time factor'; hence, both haemostatic defects are usually treated simultaneously by current clinical materials.

There is no widely accepted dosage schedule. For surgery, levels of factor VIII in the normal range should be maintained, and are usually achieved by infusions on consecutive or alternate days, depending on the patient's synthetic ability. Alternate-day infusions of cryoprecipitate will usually keep the factor VIII level above 30 per cent. For elective surgery, the first infusion may be given the day before to give time for the factor VIII response to build up. In urgent situations the first infusion should itself contain sufficient factor VIII activity to raise the patient's level immediately to the required haemostatic value.

In von Willebrand's syndrome, under certain rigidly standardised conditions the adhesion of platelets to glass beads can be shown to be defective. So far, no well-defined therapeutic issue has followed from this.

5. Preparations of Factors II, VII, IX and X

These are used in vitamin K deficiency or on patients on oral anticoagulant therapy when the urgency of the situation demands reversal of the defect more rapidly than can be attained after administration of vitamin K_1. In such circumstances the preparation containing factors II, VII, IX and X should be administered in an adult in a plasma equivalence of $1\frac{1}{2}$ litres, at the same time as the vitamin K_1.

One of the most frequent recent indications has been the patient on chronic dialysis who is on oral anticoagulant therapy who as an emergency procedure is to receive a kidney in a transplant procedure.

In liver disease any of the coagulation factors may be deficient; surprisingly factor VIII levels are often well maintained. In addition to platelets, there may be a need for use of the preparation containing factors II, VII, IX and X as well as fibrinogen.

6. FIBRINOGEN DEFICIENCY

Disseminated intravascular coagulation is becoming recognised more and more frequently. In many of the circumstances it is a relatively slow process; for example, widespread metastatic disease or pre-eclampsia and specific replacement therapy may not be needed. It may be that heparin therapy is the proper management if the vascular system is intact. In obstetrical defibrination or occasionally after major surgery, e.g. heart-lung bypass operations, fibrinogen and fresh blood (for platelets) are needed. In the obstetrical situation this is a short-lived storm where the object of therapy should be to 'ride out' the situation until the haemostatic system returns to normal. Under these circumstances all possible steps should be taken to expedite delivery, when the triggering mechanism will cease. Together with fresh blood for replacement as required, 4–8 grams of clottable fibrinogen should be given intravenously. A suitable freeze-dried preparation is available from the transfusion services

Iatrogenic defibrination with the purified fraction from the Malayan Pit Viper may need antivenin followed by fibrinogen. When streptokinase has been given therapeutically for thrombolysis and the effect demands rapid reversal then epsilon aminocaproic acid should be given followed by fibrinogen.

7. CONCLUSION

From the above account it should be appreciated that blood component therapy has now a major role in the management of haemostatic defects.

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