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The Supply of Fractions Containing Blood Clotting Factors VIII and IX

The cryoprecipitate method of making Factor VIII has current popularity, particularly in the United States and Canada. I believe that this popularity reflects the extreme shortage of any concentrated material and the shortage is particularly severe in the United States and Canada. The enthusiasm is understandable because the therapeutic effectiveness of concentrates is great.

As a National policy the values of one method of fractionation compared to another must be assessed according to four main criteria:-

1. Economy in the use of human blood.
2. Economy in time, personnel and money.
3. The quality, safety and reliability of the product.
4. Estimated requirements of plasma fractions.

Economy in the use of blood

In the cryoprecipitate method as originally described, the blood is collected into double plastic packs. The whole blood is centrifuged, the plasma separated into the second pack which is frozen, thawed and again centrifuged and the plasma returned to the red cells. This method ends up with two products:-

1. Whole blood with low Factor VIII concentration.
2. Cryoprecipitate Factor VIII.

In a comprehensive alcohol or ether fractionation procedure the red cells would be used as packed cells and the whole plasma fractionated to provide:-

1. AHG.
2. Gamma Globulin.
3. Albumin.
4. Factor IX.

The comprehensive fractionation procedure thus has advantages if all of these fractions are required. It is true that gamma globulin and albumin may be obtained from out-dated plasma but in our experience Factor IX cannot be obtained from this source. The Factor IX fraction which is essential for the safe treatment of patients with Christmas disease also contains Factors II, VII and X and the fraction is valuable in the treatment of patients with rare congenital deficiencies of these factors and in those with liver disease in whom all four factors are reduced. Thus any National Fractionation scheme must cater for the production of this concentrate.

Economy in time, personnel and money

The cryoprecipitate method as first described uses double packs and these are very expensive. The product (cryoprecipitate) is stored in the second pack and reconstituted for use by adding citrate saline. When administered to an adult patient, the material from as many as 24 packs may occasionally be required for one dose and commonly the material from 10-15 packs is used. The cryoprecipitate from separate units cannot be pooled before use both because such pooling might involve bacterial contamination and because a significant amount of activity may be lost during handling (thawing, reconstitution and refreezing). Thus the preparation of a dose must be time consuming as each unit of concentrate must be reconstituted individually and the total number thereafter pooled.

In blood banks which serve haemophilia centres the material for treating haemophilic patients may be stored as frozen plasma in 400 ml. amounts in blood bottles. When necessary a proportion of this plasma may be made into cryoprecipitate. In this way the number of units of concentrate to be pooled for a single dose is halved. But this method could scarcely be used to store a large stock of cryoprecipitate because

of the deep freeze storage space that the bottles would occupy. The method is convenient to use where dose co-operation exists between a blood bank and haemophilia centre so that specific doses for particular patients can be anticipated and planned.

At some centres the cryoprecipitate is reconstituted for use by leaving a small volume of the supernatant plasma on the precipitate after centrifuging instead of removing all of the plasma and adding citrate-saline. It is our impression that large doses of this plasma-reconstituted material given daily causes a cumulative increase in plasma volume in the recipient.

The cryoprecipitate fraction, however it is reconstituted, contains a significant quantity of blood group antibodies and may cause haemolytic anaemia if administered to patients with incompatible red cells. It is therefore necessary to prepare the material in batches of known blood group and select the appropriate batch for each patient. The amount of blood group antibodies in the material made by alcohol or ether fractionation is much less and these precautions are unnecessary.

The alcohol or ether fractionation process viewed as a large scale fractionation in one or two centres would probably start from frozen plasma; the red cells in this case being used as packed cells. With large scale apparatus there is no doubt that fractionation would be far less laborious and time consuming than the original cryoprecipitate method.

<u>The quality, safety and reliability of the product</u>

The quality of the product may be assessed from many points of view. One of these is the proportion of the original plasma activity that is recovered in the product. Using the cryoprecipitate method about 70% of the activity of the frozen plasma may be recovered whereas with the alcohol or ether fractionation procedure about 50% is recovered.

This comparison suggests a superiority for the cryoprecipitate but in practice many factors will influence the results. For example, normal plasma samples vary very much (50-200%) so that some samples of precipitate will inevitably have a low value and the dose can never be calculated reliably. Moreover, the quality of cryoprecipitate varies greatly according to the attention to detail in handling and processing. At present the products made at different centres are said to vary in activity from 150 to 600%. Only those centres which take great care make the good material and care in this context may be very time-consuming.

As mentioned above, uniformity of the product is important because exact calculation of the dose is often necessary. This uniformity can never be attained with the cryoprecipitate method but should be the rule with alcohol or ether fractionation.

The product of the cryoprecipitate method if reconstituted in plasma has more protein than that obtained by fractionation with alcohol. The amounts of material that may be needed are large and it may be possible to overload the circulation using cryoprecipitate but probably not using the fractionated product. This problem needs further study.

The final product should be bacteriologically safe. In a large scale fractionation procedure this can be ensured. In small scale local manufacture of cryoprecipitate using any but the original method this would be less certain.

The incidence of jaundice and reactions should be taken into account. Until both products have been studied over several years on a large scale it is not possible to forecast which material is likely to be least dangerous.

Estimated requirements of Factors VIII and IX

Modern treatment of haemophilic patients has the objective of prevention of crippling as well as life-saving.

To achieve this objective at least a tenfold increase in supply oB both human AHG and of Factor IX is likely to be needed. Fractionation on this scale could undoubtedly be achieved by a series of small units working at many centres but this would entail an uneconomic replication of expensive apparatus and staff and to be efficient each centre would require a competent coagulation laboratory if a uniform high quality product is to be made. Moreover Factor IX concentrate would not be made in these local centres and material for its manufacture would need to be refrozen and sent for processing to a main fractionation centre.

Although Factor IX deficient patients are rarely encountered they are probably as numerous as patients with agammaglobulinaemia and any national policy must take them into account. We estimate the need for a ten-fold increase in Factor IX and this could only reasonably be supplied by a centralised fractionation service.

Conclusion

The cryoprecipitate method of making AHG is an invaluable interim measure partially to redress a temporary acute shortage of material for treating haemophilic patients. The method is time-consuming and very expensive interms of personnel, both in manufacture and administration to the patient. It is impossible to conceive of the process ever providing a standard product and difficult to be certain that the product would be universally as safe as the alcohol or ether fractionated material. It is difficult to see how the cryoprecipitate method, developed as a series of local projects, could safely be integrated with a fractionation process to supply other essential fractions.

It is therefore our opinion that the ain should be to provide the main bulk of Factor VIII from a central

supply will however be inadequate and the local provision of cryoprecipitate should be encouraged in the meantime. Two lines of research which might do much to improve the present situation may be mentioned:-

1. The cryoprecipitate method provides a simple way of separating the main bulk of the Factor VIII activity from plasma. It is possible that the use of this method as a first step in fractionation might simplify the production of Factor VIII and permit larger volumes to be handled at one time and thus increase the output of a fractionation laboratory.
2. The provision of a reliable standard of Factor VIII activity might be a great help to transfusion services trying to prepare a product of good quality.