BLOOD AND BLOOD PRODUCTS

Presented By: Dr. Abha Doshi
(Principal of MET’s Institute of Pharmacy, Bandra(W), Mumbai)
Assisted by: Akul Mehta

BLOOD (Whole Human Blood)

Plasma
(- Dried Human Plasma)
(- Human Plasma Protein Fraction)
(- Dried Human Plasma Protein Fraction)

Red Blood Corpuscles
(Concentrated human RBCs)

Clotting Factors

Serum
(Dried Human Serum)

Immunoglobulin - Gamma Globulin
(Human Normal Immunoglobulin Injection)

Fibrinogen
(Human Fibrinogen)

Thrombin
(Human Thrombin)

Fibrin Clot
(Human Fibrin Foam)
WHOLE HUMAN BLOOD

• Definition: It is the human blood mixed with a suitable anticoagulant

That is:

Human Blood + Anticoagulant = Whole Human Blood

Conditions for Being a Donor

Any person in good health is accepted as a donor provided that he or she:

1) Is not suffering from any disease that can be transmitted by transfusion. This includes syphilis, malaria, and serum jaundice.

2) Is not anemic. The haemoglobin content of the blood should not be less than
   - 12.5% for females
   - 13.3% for males
   (checked by allowing a drop of blood to fall into a copper sulphate solution of specific gravity 1.053 for females, and 1.055 for males. If the drop sinks, the sample is satisfactory)

3) Has been taking medication which might prove toxic or have allergic reactions in a patient e.g. antibiotics
Collection of the blood

• Blood is collected aseptically from the median cubital vein, in the front elbow.

• This blood is put into a sterile container containing an anticoagulant solution and the bottle is gently shaken to ensure that blood and anticoagulant are well mixed, thus preventing the formation of small fibrin clots.

• A maximum of 420ml of blood is taken in one attendance.

• Immediately afterwards the container is sealed and cooled to 4-6 degrees centigrade for storage.

Equipment Used for the Collection

Equipment used for taking the blood is made from plastics, and is disposable.

The container earlier consisted of bottles, but Plastic bags have started being used and are the containers of the future.
Blood Clotting

Two important steps in the clotting of blood are:

In presence of THROMBOPLASTIN + PROTHROMBIN (Soluble)

THROMBIN (Soluble)

In presence of THROMBIN

FIBRIN (Insoluble Clot)

In response to injury, the tissues and blood platelets free substances that activate the clot promoting enzyme THROMBOPLASTIN. Thromboplastin, with the assistance of ionized calcium and other factors, converts PROTHROMBIN to active clotting enzyme THROMBIN. Thrombin then acts on FIBRINOGEN, converting it into insoluble FIBRIN, the matrix of the clot.

ANTICOAGULANTS

HEPARIN

CITRATES

DISODIUM EDETATE

ANTICOAGULANTS USED
CITRATES

- The solution most often used as a blood anticoagulant is known as Acid-citrate-dextrose (ACD), composed of:
  - Sodium Citrate (2.0 to 2.5 g)
  - Dextrose (3.0 g)
  - Water for Injection (q.s. to 120 ml)
- The citrate prevents clotting by binding the calcium ions as unionized calcium citrate, thus preventing a vital step of clotting.

Why Acid Citrate and not Normal Citrate??

- Earlier the normal, trisodium citrate was used but it has a very high alkaline pH in solution which causes considerable caramelisation of the dextrose (darkening) during sterilization and the two solutions have to be autoclaved separately.
- The Acid Citrate produces a pH of about 5 and causes little or no caramelisation.
- In addition, it is less likely to induce flaking of the glass of the container.
- The higher concentration (2.5g / 120ml) is often preferred because it more effectively reduces the formation of small clots.
Why add Dextrose?

• The dextrose delays haemolysis of the erythrocytes in vitro and prolongs their life after transfusion.
• Its function is hypothesized to be connected with the synthesis of compounds, such as ATP, that are important in making energy available to living cells.

HEPARIN

• Naturally occurring anticoagulant.
• Made by the mast cells of the connective tissue surrounding blood vessels.
• It inhibits clotting in the circulatory system.
• Occasionally, it is used in blood for transfusion when large volumes must be given to one patient and the corresponding amounts of citrate would be harmful, e.g. in cardiac surgery.
• It quickly loses activity in blood in vitro and normal quantities are effective for about a day.
• ACD on the other hand, prolongs the storage life to three weeks.
• Heparin is expensive and may continue its action even after transfusion, thus needing administration of neutralizing substances such as protamine sulphate.

DISODIUM EDETATE

• This is also a chelating agent like ACD.
• It has a strong affinity for divalent metals, and thus will bind to calcium firmly.
• It is sometimes preferred when preservation of blood platelets is essential, although the stability of these seems to depend much more on preventing contact with the glass surface: if plastic bags or silicone-treated glass is used, ACD is almost as effective as Disodium Edetate.
• The survival of red blood cells in dextrose-edetate solutions is as good as in ACD.
TESTING OF WHOLE BLOOD

• At the time that blood is collected, two small additional amounts are collected:
  – One, which is often obtained by draining the collecting tube, is put into a small 5 ml bottle and is firmly attached to the main container. This is for testing compatibility with the blood of the recipient. This separate specimen avoids the dangerous procedure of attempting to remove a sample from the main bottle without causing bacterial contamination. If a plastic bag is used, it is possible to leave the blood-filled collecting tube attached to the bag and to seal it at several points with a special tool; then a section can be separated for testing without contamination.
  – The second, somewhat larger sample is used as soon as possible for:
    • Serological test to confirm the absence of syphilis and other diseases
    • To determine the ABO blood group of the cells and plasma and the Rh grouping of the cells.

BLOOD GROUPS

• Fundamental Aim: is to prevent antigen-antibody reaction.

• Red cells carry an antigen that reacts with the corresponding antibody in the plasma of individuals of certain other groups. If the cells are transfused into an individual with the equivalent antibody in his plasma, they are rapidly destroyed, with serious consequences.

• Although some 9 blood groups are known, only the ABO and Rh are of major importance as causes of haemolytic transfusion reactions.
1) ABO System

- The first sign of the haemolytic antigen-antibody reaction is agglutination and, therefore, red-cell antigens and plasma antibodies are called agglutinogens or agglutinins respectively.
- The agglutinated cells haemolyse, freeing haemoglobin and other constituents and causing jaundice and kidney damage: if the latter is extreme, the patient may even die.
- Fortunately most transfusion reactions are mild.

Compatibility Chart

<table>
<thead>
<tr>
<th>Group</th>
<th>Red Cell Antigen (Agglutinogen)</th>
<th>Plasma Antibody (Agglutinin)</th>
<th>Can Donate To:</th>
<th>Can Receive From:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>Anti-B</td>
<td>A or AB</td>
<td>A or O</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>Anti-A</td>
<td>B or AB</td>
<td>B or O</td>
</tr>
<tr>
<td>AB</td>
<td>A &amp; B</td>
<td>None</td>
<td>AB only</td>
<td>All groups. (Universal Recipient)</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
<td>Anti-A and Anti-B</td>
<td>All groups. (Universal Donor)</td>
<td>O only</td>
</tr>
</tbody>
</table>

NOTE - Generally, the recipient’s cells are not seriously harmed by the agglutinins in the donor’s plasma, because the latter quickly becomes diluted in the recipient’s blood (thus a donor of blood group O can donate to all even though it has agglutinins Anti-A and Anti-B)
2) Rh System

- Rh factor, so named cause it was found in the rhesus monkey.
- The red cells of some individuals carry an antigen that is known as the Rh factor.
- If Rh+ blood is transfused into an Rh- recipient, production of antibodies to the Rh+ blood may be stimulated.
- If this occurs, subsequent transfusion of Rh+ blood will cause a haemolytic reaction.

- Haemolytic disease in a new born:
  - If a foetus is Rh+ from it’s father, and the mother is Rh- and has the Rh+ antibody in her blood (either from previous transfusion of Rh+ blood or as a result of stimulation by antigens of the foetus), the mother’s antibodies may cross the placenta and destroy the foetal erythrocytes.
  - This haemolytic reaction may kill the foetus or cause the infant to be severely anaemic.
STORAGE

- Blood collected must be kept at a temperature between 4 to 6 degrees centigrade, at all times except during short periods of transport and examination, which must not exceed 30 mins.
- Even at this low temperatures, deleterious changes do take place.
  - The leucocytes disintegrate in a few hours
  - The platelets disintegrate in a few days
  - The red cells show a fall in ATP and other organic phosphates, a reduction in oxygen-carrying capacity and, due partly to loss of lipid from their membranes, increased fragility.
- Storage at room temperature even for a day, seriously reduces post-transfusion survival of the erythrocytes.

- The fitness of blood for transfusion is based on its appearance. On standing, the cells sediment, leaving a layer of yellow supernatant plasma.
- If the blood has been taken shortly after a heavy fatty meal, the plasma may be turbid and show a white layer of fat on it’s surface. On top of the red cells there may be a complete or partial greyish layer of leucocytes.
- The most important feature, however, is the line of demarcation between cells and plasma, which must be sharp: if it is obscured by a diffuse red coloration, indicating haemolysis, the blood is unfit for use.
- Complete haemolysis, especially if it occurs rapidly, is usually a sign of bacterial infection, but its absence is not confirmation of sterility since certain psychrophilic bacteria, predominately pseudomonads and members of the coli-aerogenes group, can grow in blood at refrigerator temperatures without causing haemolysis.
- Many of the organisms isolated from contaminated blood have been capable of using citrate as their sole source of carbon and, as would be expected, this has led to clot formation, as citrate which is the anticoagulant gets assimilated by the bacteria.
USES

- Haemorrhage, shock, burns and uncontrollable diarrhoea and vomiting can all cause significant losses of blood.
  - Haemorrhage and other diseases may result in deficiency or absence of vital blood constituents such as red cells, platelets, or clotting factors.
  - The transfusion of whole blood can be of great value in all these circumstances but often, because of the risk of transfusion reactions, it is not used where the need is solely to make up blood volume but is restricted to haemorrhage and certain diseases where there is deficiency of the vital oxygen-carrying erythrocytes.
- Normally whole blood is not administered unless the ABO and Rh groups of the donor and recipient are known and a sample of the donor’s blood has been tested for compatibility with that of the recipient.
- In an emergency, group O, Rh negative blood may be given while taking necessary precautions.

CONCENTRATED HUMAN RED BLOOD CORPUSCLES

Definition: This is the solution of human RBC’s which have been concentrated using centrifugation.
Preparation

• It is prepared by removing most of the citrated plasma from whole blood that is not more than a fortnight old and has been allowed to stand or has been centrifuged to deposit the cells.

• More than 40% of the supernatant fluid after the settling, is siphoned off using sterile tubes, taking strict aseptic precautions throughout.

• Since there is a risk of bacterial contamination the product must be used within 12 hours.

• The cells are matched with the recipient’s plasma and may then be mixed with matched cells from other bottles.

• The haemoglobin content must not be less than 15.5%.

Uses

• This product is used when administration of whole blood might overtax the circulation, i.e., in treatment of diseases, such as chronic anaemia (where blood volume has not been reduced), rather than haemorrhage (which would require a replenishment of blood volume as well and thus would require whole blood).

• Another application is in exchange transfusion in infants: a toxic amount of citrate might be given if whole blood was used.
DRIED HUMAN PLASMA

- It is the portion of the blood which has been separated from the cell content, and is dried, and can be used after reconstitution with water.

THUS, UNDER SUITABLE CIRCUMSTANCES, DRIED PLASMA CAN BE USED AS A SUBSTITUTE FOR WHOLE BLOOD.
Problems to be Overcome During Preparation

• Two major problems have to be overcome
  – Transmission of Viral Jaundice
  – Neutralization of Plasma Agglutinins

1] Transmission of Viral Jaundice

• There are two types:
  – Infective hepatitis (incubation time = 5 weeks, mortality rate = 0.3%)
  – Homologous serum jaundice (incubation time = 20 weeks, mortality rate = 12%)

• Most infections following transfusion are mild

• Control is partly effected by refusing to accept donors with a history of jaundice, but not all cases are recognized and since at present there is no reliable test by which carriers can be detected, an occasional infected bottle is inevitable.

• Attempts have been made to kill the causative viruses by treatment with UV light, but the method is technically difficult.
• Note – if the preparation of a blood product involves pooling material from a larger number of donors, infection in one or two bottles will be distributed throughout the pool and appear in each of the units made from it.

• Nowadays, the pools used for making dried plasma and serum are limited to not more than ten donations, and the incidence of jaundice is only slightly greater than when whole blood is transfused.

• However, in the past, when pools of 300 or more bottles were made, the incidence was 7 to 12%.

2] Neutralization of Plasma Agglutinins

• Agglutinins in the donor’s plasma usually do not damage the recipient’s red cells.

• Occasionally, however, the plasma agglutinins are very powerful and can cause serious haemolysis of the cells of the recipient.

• This means that incompatibility problems are not entirely eliminated by using products such as plasma and serum, that contain no cells.

• The problem has been overcome since the discovery that red cell agglutinogens also occur as water soluble forms in plasma, saliva and other body fluids.

• Consequently, by mixing plasma from different groups in suitable proportions the powerful agglutinins can be cross-neutralized by soluble agglutinogens, producing a preparation that is safe to transfuse to all groups.

• The most satisfactory ratio for mixing is 9 of A: 9 of O: 2 of B or AB.
Preparation of Dried Plasma

- Dried plasma is usually prepared from time-expired citrated blood
- The blood is centrifuged as in the case of concentrated red blood cells and the supernatant fluid is siphoned off.

- This siphoned fluid is then combined and batches of less than 10 bottles are pooled, choosing the correct ratio of blood groups to neutralize the powerful agglutinins.

- The pools are kept at 4 to 6 degrees centigrade while samples are tested for sterility and no pool is used unless it passes.

- Then 400ml quantities are dispensed into bottles and subjected to freeze drying.
- General aspects of freeze drying are followed with special features of the plasma process:
  - Preliminary Freezing
  - Primary Drying
  - Secondary drying

Preliminary Freezing

- The bottles are sealed with bacteriologically efficient fabric pads covered by ring-type closures and then centrifuged at -18 degrees centigrade.
- The liquid snap-freezes and becomes distributed around the inside of the bottle.
Primary Drying

• The bottles of frozen material are mounted horizontally in the drying chamber and a high vacuum is applied.
• The ice sublimes on to a condensing coil kept at -50 degrees centigrade and a small heater provides the latent heat required for evaporation.
• This stage takes about 2 days, after which the residual moisture content is about 2%.

Secondary Drying

• This is done in another chamber by vacuum desiccation over phosphorous pentoxide.
• It takes about a day, and the product is left with about 0.5% of moisture.
• Each fabric seal is then replaced by an MRC type closure perforated by a plugged hypodermic needle. The bottles are returned to the secondary drying chamber, re-evacuated, and then the vacuum is broken with dry sterile nitrogen.
• Finally, the needles are removed and the closure is protected with a sterile viscose cap.
Storage

• Dried plasma, kept below 20 degrees centigrade and protected from light, moisture, and oxygen, remains usable almost indefinitely, although it is customary to impose an arbitrary expiry date of about 5 years.
• Its fitness for use is shown by its solubility when reconstituted in a volume of water for injection (WFI), Sodium Chloride Injection or a solution containing 2.5% dextrose and 0.45% sodium chloride, equivalent to the original volume of plasma.
• It must dissolve completely within ten minutes at room temperature.
• Gel formation or incomplete solution indicates deterioration.
• After reconstitution it must be used immediately.

Uses

• Reconstituted plasma is satisfactory alternative to whole blood in conditions where there is no loss of red cells.
• It is of particular value in the treatment of severe burns and scalds where, because of extensive fluid and protein loss, there is considerable haemo-concentration.
• It may also be given when blood is more appropriate; either because whole blood is unavailable or, in emergency, until the results of matching tests are known.
• Because of its long storage life at a convenient temperature, dried plasma is more suitable than blood as a reserve stock in a small hospital or a remote community.
DRIED HUMAN SERUM

• Prepared in the same way as dried plasma except that the blood is collected into dry bottles and allowed to clot.

• The supernatant serum being separated after the clot has retracted.

• Plasma is usually obtained from blood that is out-of-date, i.e. has been available as whole blood for 21 days.
• By converting blood into serum this period in reserve is lost and, therefore, much less blood is used for serum production

Its, storage and use are the same as for dried plasma.

LIQUID PLASMA AND SERUM

• The only official liquid blood product is human plasma protein fraction.
Why unmodified liquid plasma and serum are no longer recognized?

- Plasma and serum, like blood, are excellent media for bacterial growth and, therefore, the user must be able to detect contamination.
- Unfortunately, these products are often opalescent due to suspended fat.
- Further, turbidity and deposits develop during storage and as a result of movement during transport, thus making infection very difficult to identify.
- Attempts to remove fat by filtration were not very successful because it blocked the filter, but centrifugation proved more satisfactory.
- With serum it was then possible, by passing the product through a sterilizing pad, to obtain a clear preparation that would store reasonably well. It has passed out of use because blood is more economically used for dried plasma production.

- With plasma, there are additional difficulties because, unlike serum, it contains the clotting factors, which can be activated fairly easily. For example, if plasma is filtered through fibrous pads, magnesium ions from the asbestos can, like calcium ions in vivo, activate prothrombin and cause clots in the filter and, later, the filtrate.
- Methods were devised for removing some of the clotting factors and an unstable lipoid-globulin complex by adsorption on to kaolin or fractionation with an organic solvent at low temperatures.
- Complex aseptic manipulations were involved and, to confirm freedom from contamination, it was necessary, before issue, to store the products for several weeks at a temperature conducive to bacterial growth (that way, if there was contamination, it would show up).

- Further progress was made less urgent by the success of dried plasma but investigations continued and have contributed to the development of the product known as human plasma protein fraction.
THE FRACTIONATION OF PLASMA

• About 60% of plasma protein is albumin and, therefore, it plays a major part in maintaining the high osmotic pressure necessary to retain fluid in the blood vessels.
• A very successful solvent precipitation technique was developed by which other proteins, as well as albumin, were separated.
• Some of these, i.e. fibrinogen, prothrombin, and gamma globulin, proved so valuable that protein fractionation of plasma quickly became an established procedure.

Conditions for the process of fractionation are:

• The process selected must not alter the biological properties of the fractions nor affect the solubilities.
• It must be possible to carry it out aseptically and, ideally, the conditions should discourage bacterial growth.
• Any additive must be harmless or easily removed after use
Techniques of Protein Separation

• One of the oldest methods of protein separation is salting-out, but this is unsuitable for plasma fractionation because high concentrations of salt are needed and these are not selective enough. Also, dialysis, a technique that is difficult to perform aseptically, is necessary to remove the salt after the precipitation.

• E. J. Cohn and his colleagues (due to the need for transfusion material with a long life and stability, unlike whole blood, during the early part of the Second World War), had developed a technique to separate albumin and other proteins from plasma.

Cohn’s technique was based on the use of an organic solvent (ethyl alcohol) to reduce the solubilities of the proteins, and was given flexibility by alterations of pH, ionic strength (i.e., salt concentrations) and protein.

• The use of an organic solvent, instead of salt, as a major precipitant confers a number of incidental advantages:
  – Because of its volatility it can be removed easily during the freeze drying of the final product.
  – Salt can be used in low concentrations to improve resolution.
  – It helps to control contaminants because of its bacteriostatic activity.
  – Being a liquid, it is easy to add aseptically

• On the other hand, it is necessary to keep the temperature very low (0 to -5 degrees centigrade) to prevent solvent denaturation of proteins.
Ether Fractionation of Plasma

1. **Plasma**
   - Ether 11%
   - 0 °C
   - pH 7.7
   - Fibrinogen Precipitate

2. **Supernatant 1**
   - Ether 10%
   - 0 °C
   - pH 5.35
   - Prothrombin Precipitate

3. **Supernatant 2**
   - Ether 18.5%
   - -3.5 °C
   - pH 5.5
   - Ionic conc. 0.035
   - Globulins Precipitate
   - Further Fractionation
   - Gamma Globulin Precipitate

4. **Supernatant 3**
   - Ether 12% : Ethanol 16.5%
   - -5 °C
   - pH 5
   - Crude Albumin Precipitate
   - Further Fractionation
   - Albumin Precipitate

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Human Plasma Protein Fraction

- This is a solution of some of the proteins from liquid plasma.
- It contains albumin and certain globulins that retain their solubility on heating.
- It is prepared by fractionating pooled citrated plasma and is similar to the fraction shown as crude albumin in the chart shown on the previous slide.
A stabilizer such as sodium caprylate or acetyltryptophan is added. This allows the preparation to be heated for several hours at a low temperature without significant denaturation of proteins.

Sodium chloride is added to make the preparation approximately isotonic.

The solution is sterilized by filtration, aseptically distributed into blood bottles and then heated at 60 +/- 0.5 degrees centigrade for ten hours to destroy the viruses of infective hepatitis and homologous serum jaundice.

Although the use of a bactericide or antibiotic would help to control contamination, neither is allowed.

- A bactericide would be undesirable on toxicity grounds in a preparation given intravenously in large volumes.
- With an antibiotic, there would be a risk of sensitization.

The fractionation process involves concentration of albumin fraction. To ensure that, as a result, the amount of sodium citrate is not raised to a harmful level, the concentration in the final preparation is limited to 0.4%.

The protein content is not less than 4.3% w/v and the product exerts a colloidal osmotic pressure approximately equivalent to that of pooled liquid plasma containing 5.2% w/v of protein.

It must be stored between 5 to 20 degrees centigrade and protected from light.

Since fibrinogen has been removed, the preparation remains clear.

Its use remains the same as dried plasma.

Dried Human Plasma Protein Fraction

- Dried human plasma protein fraction is prepared by freeze-drying human plasma protein fraction
- It’s use is also the same as that of dried plasma
Human Fibrinogen

- Fibrinogen is the soluble constituent of plasma which on addition of thrombin is converted to fibrin (which is insoluble).
- After separation from plasma by fractionation, the precipitate is collected by centrifugation, dissolved in citrate-saline, and freeze-dried.
- The air in the containers is displaced by nitrogen.
- The citrate prevents spontaneous clotting when the material is reconstituted.
- Fibrinogen dissolves slowly. However, like many other protein solutions, it froths a lot if shaken and the solid-stabilized foam is very slow to disperse, thus agitation should be limited to rocking.
- The solution should be used as soon as possible and not later than three hours after preparation.
- The fibrinogen must be stored under dry conditions, protected from light and at a temperature below 20 degrees centigrade. The other storage conditions are similar to that of dried serum.

Use of Human Fibrinogen

- Occasionally, fibrinogen is administered alone to treat fibrinogen deficiency.
- But it is more often used in conjunction with thrombin as will be seen ahead.
Human Thrombin

- Thrombin is an enzyme that converts fibrinogen to fibrin.
- The prothrombin obtained from the fractionation of plasma is washed with distilled water and dissolved in citrate saline.
- It is converted to thrombin by adjustment of pH to 7 and adding thromboplastin and calcium ions.
- The solution is filtered and freeze dried, and the air in the containers is replaced by nitrogen.
- It is reconstituted with saline when required.
- The thrombin must be stored under dry conditions, protected from light and at a temperature below 20 degrees centigrade. The other storage conditions are similar to that of dried serum.

Uses of Human Thrombin

- The fibrin clot produced when thrombin is mixed with fibrinogen is used in surgery to suture severed nerves and to assist adhesion of skin grafts.
- The mixture clots at a rate that depends on the amount of thrombin present and, therefore, if necessary, it can be kept fluid long enough for adjustments, e.g. of skin grafts to be made.
- The clot also acts as a haemostat (which will be seen ahead in Human Fibrin Foam).
- Since the fibrin is human, it is well-tolerated by the body, and new cells penetrate it rapidly allowing a quicker and better healing to occur.
Human Fibrin Foam

- This is a sponge like mass of human fibrin.
- It is prepared by whipping a solution of fibrinogen into a froth by mechanical means and then adding thrombin.
- The product is poured into trays and freeze-dried, then cut into pieces of convenient size and sterilized by dry heat at 130 degrees centigrade for three hours.
- The foam must be stored under dry conditions, protected from light and at a temperature below 20 degrees centigrade. The other storage conditions are similar to that of dried serum except that fibrin foam need not be kept under nitrogen.

Uses of Human Fibrin Foam

- It is used with thrombin as a haemostat in surgery, when other methods used to arrest bleeding have been unsuccessful.
  - A piece is dipped in thrombin solution and applied to the bleeding area.
  - The combination of thrombin and the large rough surface provided by the sponge causes the blood to clot.
  - The foam can be left in situ, where it will be absorbed because it is entirely of human origin.
Human Normal Immunoglobulin Injection

- Immuno- or gamma globulin is obtained from the globulins fraction separated in stage 3 of the fractionation of plasma, as had been shown earlier.
- The ionic strengths are critical and further fractionation is done as follows:

  ![Diagram of globulins fractionation](http://www.pharmaxchange.info)

  - **Globulins (beta and gamma)**
    - Ether 9%
    - 0 °C
    - pH 5
    - ionic conc. 0.01
  - **Supernatant**
    - Ether 18.5%
    - -3.5 °C
    - pH 6.75
    - ionic conc. 0.025

- The immunoglobulins are dissolved in a suitable solvent, usually 0.8% sodium chloride solution, and a preservative, e.g. 0.01% thiomersal, is added.
- The solution is sterilized by filtration, packed in single-dose containers and stored at 4 to 6 degrees centigrade, with protection from light.
- Normally pools of not less than 1500 donations are used to ensure a satisfactory representation of the various types of adult antibodies.
- However, as in the preparation of antivaccinia and antitetanus immunoglobulins, which is obtained from the blood of recently immunized donors, the pools of blood can be smaller.
Uses of Immunoglobulins

• Used to prevent or attenuate diseases such as
  – Measles
  – Rubella
  – infectious hepatitis
  – hepatitis B
  – Chickenpox
  – Hypogammaglobulinaemia (deficiency in gamma globulins)

• It is used to prepare specific immunoglobulins such as:
  – Human Anti-Vaccinia Immunoglobulin – for smallpox
  – Human Anti-Tetanus Immunoglobulin
  – Human Anti-D Immunoglobulin – used to suppress sensitization of Rh –ve mothers to the Rh(D) antigen (Rh +ve infant)
  – Anti-HB$_s$ Immunoglobulin – this is still under investigation. It is an immunoglobulin for Hepatitis B surface antigen.
Why do quality control on blood products?

- Although all the blood products can save life, many are dangerous.
- If, for example, blood, plasma, or serum become heavily contaminated with micro-organisms, or the container or solvent is not pyrogen free, or a bactericide is added to a preparation given in large volumes intravenously – the risk to the patient is considerable.
- The official standards and labeling are designed to reduce the hazards to a minimum, and quality control of blood products should be done in accordance with them.
Identification

• As all blood products contain proteins, the standard method used in protein identification are often applicable.
• Precipitation tests with specific antisera are used to show that only human serum proteins are present in dried serum, dried plasma, the plasma protein fractions, fibrinogen, thrombin and immunoglobulin.
• The characteristic mobilities of blood proteins in an electrophoretic field are a sensitive means of identifying fibrinogen, immunoglobulin, and the plasma protein fraction. For example, in the plasma protein fraction- there must not be less than 85% of the protein having the mobility of albumin and not more than 1% of gamma globulin.

• Proteins can be identified by their sedimentation rate in an ultra-centrifuge, which is a suitable method for identifying and quantifying the different types of gamma globulins.
• Differences in clotting behaviour are simpler but useful characteristics. Plasma clots when calcium chloride is added, but serum does not. Fibrinogen is identified by the clotting that occurs when thrombin is added, and thrombin by the same result when it is mixed with plasma.
• The determination of blood groups – ABO of plasma and cells and Rh of cells, is an identification test for whole blood, while the descriptions of the latter and of concentrated red blood corpuscles are aids to identify and safeguards against the use of an unsafe product.
Sterility and Pyrogens

• All blood products must comply with the official tests for sterility, and those preparations (i.e. immunoglobulins and the plasma protein fractions) that are exposed to special risk of contamination with pyrogens due to lengthy processing must also pass the test for pyrogens.

Solubility

• Complete solubility in an appropriate volume of the usual solvent, sometimes in a specified time, is required for all solid preparations except fibrin foam.

• It indicates that the protein constituents have not deteriorated.
Assays

- For whole blood and concentrated RBCs the assay is a determination of the haemoglobin value.
- For the remaining products, except fibrin foam (which has no assay) and thrombin, the protein constituent is determined chemically.
- In thrombin there must be a minimum number of clotting doses per mg, a clotting dose being the amount of thrombin required to clot 1ml of 0.1% fibrinogen in saline buffered at 7.2 to 7.3 in 15 seconds at 37 degrees centigrade.
- Determinations of Na and K ions in plasma protein fraction ensures that the level are not high enough to disturb the electrolyte balance of the recipient.
- An assay for sodium citrate in the same product prevents toxic effects from excess of this salt.

Labelling for Whole Blood

<table>
<thead>
<tr>
<th>Name of Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>• ABO Group</td>
</tr>
<tr>
<td>• Rh group and nature of antisera used for testing</td>
</tr>
<tr>
<td>• Total Volume; proportion of blood; nature and percentage of anticoagulant and any other material introduced</td>
</tr>
<tr>
<td>• Date of Donation</td>
</tr>
<tr>
<td>• Expiry date</td>
</tr>
<tr>
<td>• Storage Conditions</td>
</tr>
<tr>
<td>• A statement that the contents must not be used if there is any sign of deterioration</td>
</tr>
<tr>
<td>• An indication by which the history of the preparation can be traced</td>
</tr>
</tbody>
</table>
Labelling of Dried Plasma Protein Fraction

<table>
<thead>
<tr>
<th>Name of Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Volume of water of injection necessary for reconstitution</td>
</tr>
<tr>
<td>• Total amount of protein in reconstituted solution</td>
</tr>
<tr>
<td>• Concentrations of potassium, sodium and citrate ions</td>
</tr>
<tr>
<td>• Names and concentrations of stabilizing agents or other added substances</td>
</tr>
<tr>
<td>• Expiry Date</td>
</tr>
<tr>
<td>• Storage Conditions</td>
</tr>
<tr>
<td>• A statement that the contents must not be used if, after adding water, a gel forms or solution is incomplete</td>
</tr>
<tr>
<td>• An indication by which the history of the preparation can be traced</td>
</tr>
<tr>
<td>• An instruction to discard the reconstituted solution if not used within three hours</td>
</tr>
</tbody>
</table>

PLASMA SUBSTITUTES
The need for plasma substitutes?

• The limited supplies of plasma, the cost of producing the dried form and the risk of transmitting serum hepatitis stimulated attempts to find substitutes of non-human origin that could be used to restore the blood volume temporarily while the recipient replaced the lost protein.

Properties of an Ideal Plasma Substitute

1. The same colloidal osmotic pressure as whole blood.
2. A viscosity similar to that of plasma.
3. A molecular weight such that the molecules do not easily diffuse through the capillary walls.
4. A fairly low rate of excretion or destruction by the body.
5. Eventual and complete elimination from the body.
6. Freedom from toxicity, e.g. no impairment of renal function.
7. Freedom from antigenicity, pyrogenicity, and confusing effects on important tests such as blood grouping and the erythrocyte sedimentation rate.
8. Isotonicity, in solution, equal to that of blood plasma.
9. High stability in liquid form at normal and sterilizing temperatures and during transport and storage.
Gum Saline

- This is a synonym for Injection of Sodium Chloride and Acacia, which was official in the 1932 British Pharmacopoeia.
- In the First World War Bayliss experimented with soluble starch, dextrin, and gelatin as plasma substitutes and finally used 6% acacia in 0.9% Sodium Chloride solution.
- It was transfused extensively until signs of liver dysfunction disclosed that the gum was not metabolized but stored in various organs.
Polyvinylpyrrolidone

• In the Second World War, the Germans introduced a synthetic colloid, polyvinylpyrrolidone, for the treatment of shock.
• It was marketed in the 1950s but was later withdrawn because of suspected carcinogenicity.

Dextran

• To date this is the most satisfactory plasma substitute.
• It is a polysaccharide produced when the bacterium Leuconostoc mesenteroides is grown in a sucrose-containing medium.
• In the sugar industry it occurs as a slime that clogs pipes and filters and interferes with crystallization.
• The organism secretes an enzyme that converts sucrose to dextran according to the following equation:

\[
\text{n sucrose} \xrightarrow{\text{dextran-sucrase}} \text{n(glucose - H}_2\text{O)} + \text{n fructose (Dextran)}^2
\]

• Different strains produce dextrans of two main groups-
  – Long, practically unbranched chains of glucose units joined by 1-6 glucosidic linkages.
  – Highly branched polymers consisting of short chains of 1-6 units joined by 1-4 and 1-3 linkages to branches.

• Branched chains are more likely to give rise to allergic reactions when injected, and in dextrans used for plasma substitutes the linkages should be almost entirely of the 1-6 type. This is achieved by choosing a suitable specially developed strain of the organism that produces dextran in which about 95% of the linkages are 1-6.
Production

- Production involves laboratory culture followed by growth in seed tanks in the factory and then in 4500 cubic dm fermenters. (similar process to antibiotic production).
- Because synthesis of the enzyme and its action on the sucrose are rapid, the high degree of asepsis maintained in antibiotic fermentation is not necessary here.
- Also, as the process is inhibited by aeration, there is no need for a costly supply of sterile air.
- Another special feature is the need to prevent the hydrolysis of sucrose to glucose and fructose during sterilization of the culture media. If this occurs, dextran will not be produced because in nature the conversion does not involve inversion but is a straight transglycosidation. Preventive measures include adjustment of the media to neutral pH before sterilization, and the avoidance of overheating.

- When maximum conversion to dextran has been obtained it is precipitated by adding a suitable organic solvent.
- Natural dextran consists of chains of approximately 200,000 glucose units with molecular weights up to about 50 million.
- Very large molecules i.e. those with a molecular weight above about 250,000 have serious drawbacks:
  - They yield very viscous solutions that are difficult to administer.
  - They may cause renal damage and allergic reactions.
  - They interfere with blood matching and sedimentation tests by causing rouleaux formation. Rouleaux are aggregates of red cells that resemble piles of plates.
  - They produce colloidal osmotic pressures that are lower than those of small molecules.
Therefore to produce a material suitable for medical use it is necessary to reduce the size of the natural molecules. This can be accomplished in several ways:

- **Acid Hydrolysis (the method most widely used)**
- **Thermal Degradation**
- **Ultrasonic Disintegration**
- **Seeding the Fermenter**

### Acid Hydrolysis –
- The dextran is adjusted to a pH of 2 and heated at 90 degrees centigrade.
- As hydrolysis proceeds, the preparation becomes less viscous and the reaction is stopped at the required viscosity.
- Acid hydrolysis is the method most often used. The hydrolyzed product contains molecules ranging from 10,000 to 1 million in molecular weight.

### Thermal Degradation –
- A solution of dextran is heated under pressure at 160 degrees centigrade in the presence of sodium sulphite, to prevent oxidative deterioration, and calcium carbonate, to neutralize acidity.
- The method is slower than acid hydrolysis but the yield of the preferred molecules is better and fewer reducing groups are produced.
Ultrasonic Disintegration –

• Bombardment with ultrasonic waves splits the molecules into fragments of approximately the same size and the product is clinically acceptable, unlike the material from the previously mentioned methods, which requires considerable fractionation.

• Unfortunately, this technique is much more expensive to use.

Seeding the Fermenter –

• If a low molecular weight dextran is added in the culture medium before fermentation, the organism will use it as a template on which to build more glucose molecules.

• The average molecular weight of the product is much lower than if no template dextran is provided.

• The very small molecules, i.e. those of below a molecular weight of about 60,000 also have disadvantages:
  – They are rapidly excreted in the urine.
  – They pass into the tissue fluids causing an adverse osmotic pressure.

• Therefore, the product should contain the minimum of molecules of molecular weight less than 60,000 (i.e. the number of molecules with a molecular weight of less than 60,000 should be kept to a minimum).
To summarize

- This suggests that the ideal fraction should have a majority of molecules within the range of 100,000 to 250,000 with a bias towards the lower end.

- The American type of dextran has an average molecular weight of about 75,000.
- The aims are to restore colloidal osmotic pressure quickly and to ensure fairly rapid elimination of the foreign colloid from the body.
- The latter advantage, combined with a reduction in rouleaux formation is considered to outweigh the disadvantage of the high dosage necessary to compensate for excretion losses.
• The British dextran of the 1963 BP (Dextran 150 injection) has an average molecular weight of about 150,000 and produced a more prolonged effect due to the preponderance of larger molecules that are not lost from the blood vessels. This preparation caused rouleaux formation, a condition known as sludging because it slows the flow of blood in the capillaries and post-capillary veins.

• To overcome the problem, which is less evident with lower molecular weight fractions, the 1968 BP has replaced Dextran 150 with Dextran 110, which has an average molecular weight of 110,000.

• To obtain the clinical ranges of molecular weight, the neutralized hydrolysate is subjected to a long process of fractional precipitation.

• A water-miscible organic solvent, in which the polysaccharide is insoluble (e.g. acetone or alcohol) is added under very carefully controlled conditions and the required fraction is gradually separated by repeated retreatment of either the precipitate or supernatant fluid.

• Cost decides the narrowness of the fraction finally accepted.

• In all samples, because of entrainment of one fraction with another, there are small proportions of very large and very small molecules.
The selected fraction still requires considerable purification to remove –

- Reducing Sugars – by further solvent precipitation. The main contaminant is fructose, the by-product of fermentation.
- Fractionation solvents – by evaporation under reduced pressure.
- Inorganic salts – by demineralization in a mixed bed ion exchanger. It is particularly important to remove phosphates because they cause precipitation during sterilization and storage.
- Colour – by adsorption on to activated charcoal.
- Pyrogens – by adsorption on to asbestos, or cellulose derivatives.
- Micro-organisms – by filtration. Between each treatment the preparation is passed through a fibrous pad and just before bottling a membrane filter is used.

- The solution is diluted to a concentration of 5% in either 5% Dextrose Injection or Sodium Chloride Injection, packed in sulphur treated soda-lime bottles, and closed with lacquered rubber plugs.
- Finally, it is sterilized, usually by heating in an autoclave.
Control for Dextran

- The following tests from the official specification for Dextran 110 Injection illustrate the precautions taken to confirm that the product is suitable as a plasma substitute.
- Chemical techniques limit the amount of lead, acetone and alcohol, reducing sugars, nitrogen (from culture medium) and acid and alkali.
- Biological methods show that the preparation is not pyrogenic, is sterile, and is free from proteins that could cause anaphylaxis.

- The dextran content is determined by polarimetry and there are limit tests for small and large molecules.
  - The former involves the injection into rabbits; the urine collected throughout the succeeding 48 hours must not contain more than 30% of the injected dose. (as small molecules are excreted in the urine).
  - The latter necessitates precipitation of the top 10% of the fraction with alcohol and determining its intrinsic viscosity; this must not be greater than 0.4% which is equivalent to an average molecular weight of about 240,000.
  - The intrinsic viscosity of the fraction as a whole is also found and must indicate an average molecular weight of about 110,000.
Dextran 40 Injection

• A number of conditions, including severe burns, crush injuries and acute peritonitis, are accompanied by a severe degree of sludging in the blood.

• This can be reduced by the administration of Dextran 40 injection which, because it contains polymers of low molecular weight, lowers plasma viscosity and improves capillary flow.

• Both changes reduce cell aggregation and this in turn, further improves the flow.

• A crude dextran of low molecular weight is manufactured by including very small template molecules in the fermentation medium.

• Then fractionation is used to produce the clinical material which has an average molecular weight of 40,000.

Absorbable Haemostats
Absorbable Haemostats

• These materials are used to control bleeding when it cannot be checked by more conventional means.
• They are gradually absorbed by the tissue and, therefore, if used during surgery can be left in the body when the incision is closed, and if applied to a surface wound need not be removed when the dressing is changed.

• There are four important types:
  – Human Fibrin Foam (which has been covered earlier)
  – Gelatin Sponge
  – Oxidized cellulose
  – Calcium Alginate
Absorbable Gelatin Sponge

- This is prepared by adding a small percentage of formaldehyde to a warm solution of good quality gelatin.

- Which is then whisked into a foam and freeze-dried.

- The porous product is cut into pieces of suitable size and sterilized by dry heat at 140 degrees centigrade.

- It is marketed as while or near white, rectangular, very porous pieces that are extremely light and have a papery feel.

- It absorbs many times its own weight of blood and the official standard for absorbency requires absorption of not less than thirty times its weight of water.

- When pressed tightly on to a bleeding area, blood is taken up and clotting is encouraged by the large rough surface which causes platelet disintegration.

- The sponge also acts as a plug by sticking to the underlying tissues and mechanically supporting the clot over the oozing vessels.

- Some times it is previously soaked in saline, antibiotic or thrombin solution, when it must be pressed to remove air and excess liquid before application.
• Since some organisms liquefy gelatin it should not be used in septic wounds; nor is it suitable for arresting haemorrhage from large vessels.
• Absorption occurs in four to six weeks.
• The sponge is non-antigenic and tissue reactions have been mild.
• The standards include a test for digestibility in acid pepsin solution.
• Unless aseptic handling has been perfect it is advisable to discard any unused part of the contents of a container. Resterilization should not be attempted.

Oxidized Cellulose

• Cellulose can be converted into polyanhydro-glucuronic acid, an absorbable haemostatic material, by oxidation with nitrogen dioxide.
• Early products were made from cotton in the form of wool, lint, and particularly, gauze.
• More recently a knitted material, in which cotton is replaced by regenerated cellulose (viscose rayon) has been introduced. Fibres of the latter are of uniform diameter and the size distribution of the polymers is less wide and more constant than in cotton. As a result the oxidation can be carried out in a more reproducible manner and the product is claimed to show less variation in the absorption time, haemostatic efficiency, and tissue reactivity.
• Oxidation is carried out on the fabricated dressing involves conversion of about 20% of the primary alcohol groups to carboxyls.
• Gaseous sterilization, often by formaldehyde, is used because heat causes serious deterioration.
• The material has the appearance of the original dressing except that it may be less white in colour.
• It has a faint odour and acid taste.
• In contact with blood it turns dark brown and swells to a gelatinous coagulum.

• It’s haemostatic activity may be partly due to chemical reaction between the polyuronic acid and haemoglobin or other blood proteins.
• But the fabric also acts as a scaffolding for clot formation and a plug at cut ends of the vessels.
• It is more effect if used dry.
• Small pieces are absorbed in 2 to 7 days but very large amounts may take several weeks.
• It inactivates thrombin, unless previously neutralized with sodium bicarbonate injection, and is incompatible with penicillin.
• It should not be used in bone surgery because callus formation is inhibited
• Unused pieces from an opened container should be discarded.
Calcium Alginate

- This is derived from alginic acid, a colloidal substance obtained from seaweeds Laminaria digitata and Laminaria cloustoni which grow off the scottish and Irish coasts.
- Alginic acid is a polyuronide built up from d-mannuronic acid units.
- Its carboxyl groups react with the metallic ions to form alginates and, since the parent acid is unstable, the water-soluble sodium salt is used as the source of other alginates.

If ionized calcium salt is added to sodium alginate solution instantaneous precipitation of calcium alginate occurs, a sensitive reaction that can be used for preparing foams, fabrics and other physical forms.
- These can be sterilized by autoclaving or dry heat.

\[2 \text{Na-alginate} + \text{Ca}^{++} \xrightarrow{\text{reaction}} \text{Ca-alginate} + 2 \text{Na}^+\]
• Calcium alginate dressings have a marked haemostatic effect that is probably due mainly to mechanical pressure.

• As long as twelve weeks may be necessary for complete absorption and, although it is possible to make products that are absorbed in about 10 days, by including a small proportion of sodium alginate, the tendency is to restrict the use of alginates to the arrest of external bleeding, e.g. from surgical incisions, tooth sockets, and sites from which grafts have been taken.

• A transparent protective film can be made in situ over a burn, wound or incision by applying a solution of sodium alginate and then spraying with calcium chloride solution. This film is impervious to water but permeable to water vapour.

• Alginate dressings can be removed, if necessary, by washing with a solution of sodium salt, e.g. 5% sodium citrate, which reverses the reaction shown in the equation above.

• They are compatible with penicillin and can be resterilized if necessary.
REFERENCES

Main reference
• Cooper and Gunn’s – Tutorial Pharmacy

Please add points from
• Bentley’s Text Book of Pharmaceutics by E. A. Rawlin