BP 605 T. Pharmaceutical Biotechnology (Theory)

Blood products and Plasma Substitutes

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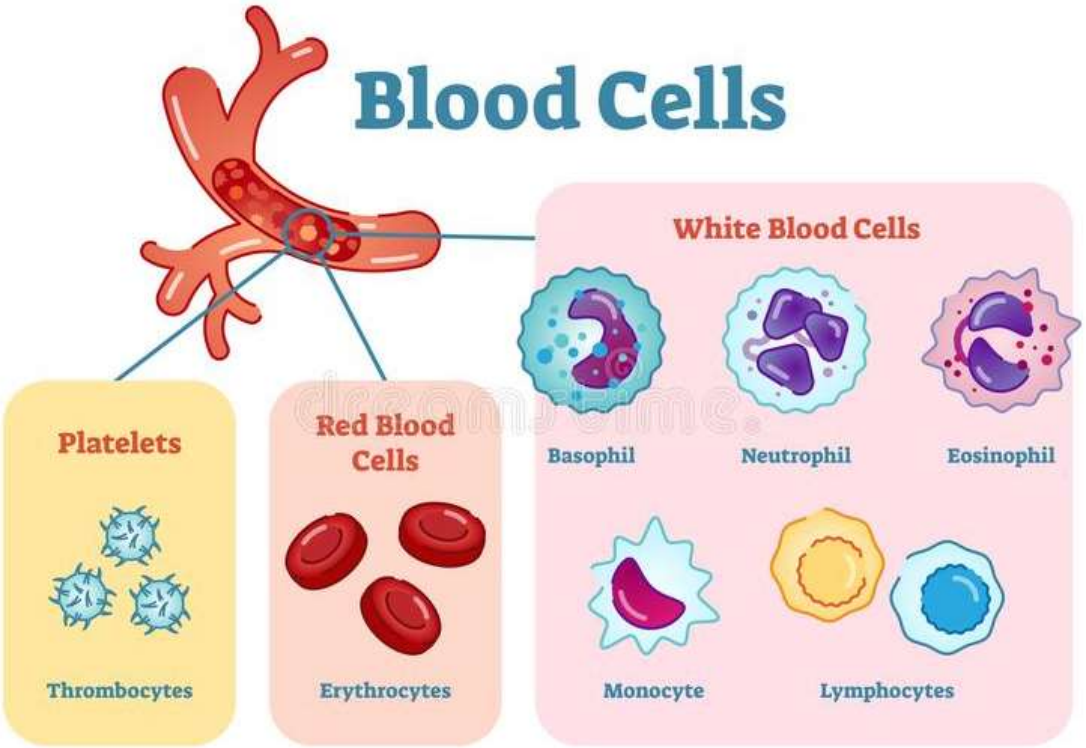
Overview

- Introduction
- Blood Products Components
- Plasma substitutes

BLOOD

- ✓ Main transport system in the body.
- ✓ Blood carries raw materials and finished products from where they originate to where they are used and transports waste products to disposal sites.
- ✓ Accounts for about 7% of the body weight of a normal adult.
- ✓ Blood is composed of plasma and cells suspended in plasma (RBC, WBC, PLATELETS)
- ✓ Plasma is largely made up of water in which many constituents are dissolved. These constituents include;
- ✓ **PROTEINS:**
  - ALBUMIN: The most common protein in blood.
  - Blood clotting proteins made by the liver



PLASMA

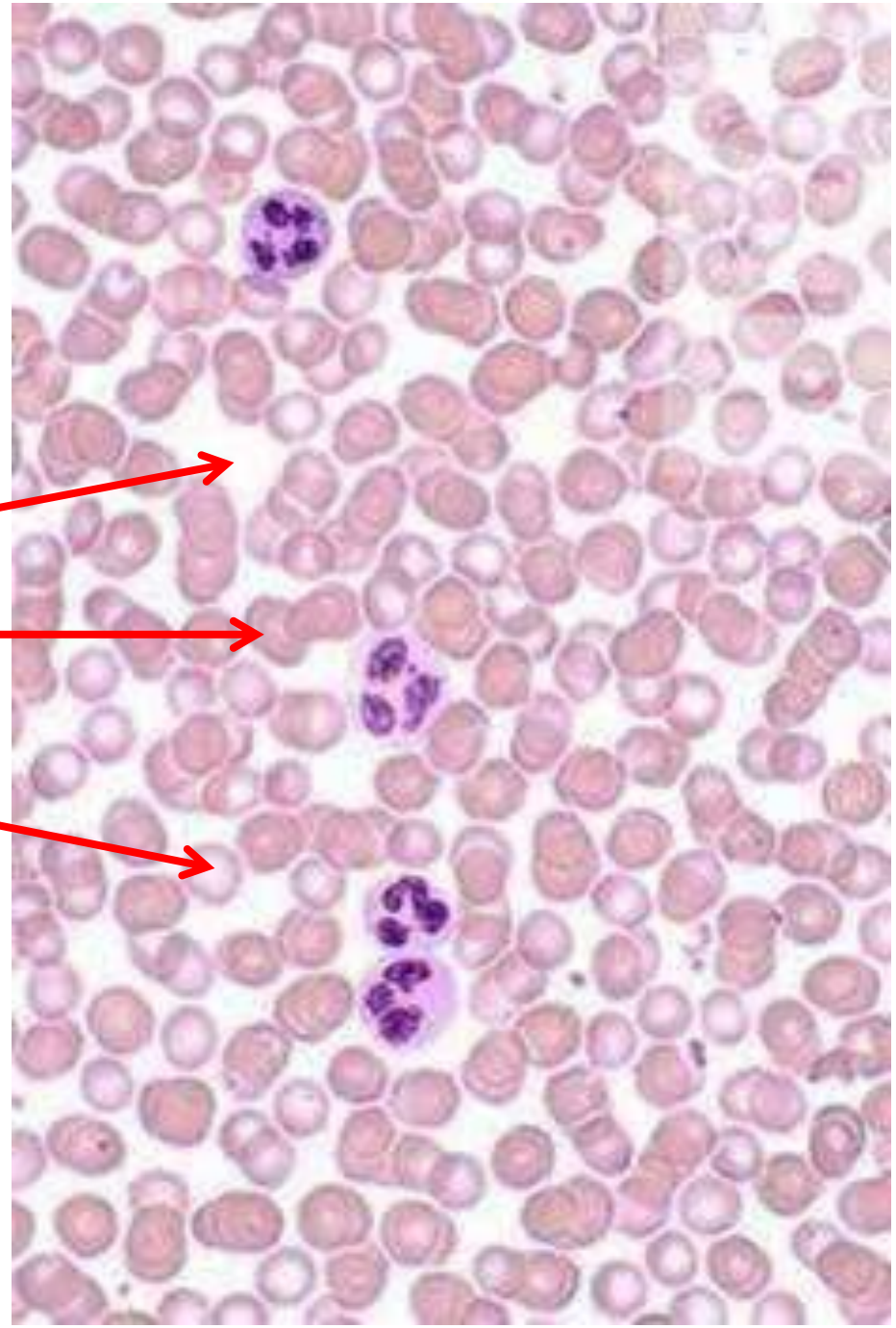
- ✓ 55% of blood.
- ✓ Liquid part of the blood.
- ✓ Plasma transports:
  - Soluble food molecules.
  - Waste products.
  - Hormones.
  - Antibodies.

RED BLOOD CELLS (RBCs)

- ✓ 5-6 million/ml.
- ✓ Transports oxygen.

WHITE BLOOD CELLS (WBCs)

- ✓ The body's defence.
- ✓ Part of the immune system.
- ✓ Larger than RBCs.
- ✓ Have nucleus.
- ✓ 4000-13000 per mm^2.
- ✓ Two types:
  - Phagocytes.
  - Lymphocytes.



BLOOD

- ✓ **ERYTHROPOIETIN:** A protein made by the kidneys that stimulates red cell production.
- ✓ **IMMUNOGLOBULINS:** Antibodies made by plasma proteins in response to infections.
- ✓ **HORMONES**

  Such as thyroid hormone and cortisol.
- ✓ **MINERALS**

  Such as iron and magnesium.
- ✓ **VITAMINS**

  Such as folic acid and B12.
- ✓ **ELECTROLYTES**

  Such as calcium, potassium and sodium.

BLOOD

- ✓ **BLOOD PRODUCTS:** Therapeutic substance prepared from human blood.
- ✓ **WHOLE BLOOD:** Unseparated blood collected into an approved container containing an anticoagulant preservative solution.
- ✓ **BLOOD COMPONENT:** A constituent of blood, separated from whole blood such as
  - Red blood concentrate.
  - Red blood suspension.
  - Plasma.
  - Platelet concentrates.
  - Plasma or platelets collected by apheresis.
  - Cryoprecipitate, prepared from fresh frozen plasma: rich in factor VIII and fibrinogen.

PLASMA DERIVATIVES:

- ✓ Human plasma proteins prepared under pharmaceutical manufacturing conditions such as,
  - Albumin.
  - Coagulation factor concentrates.
  - Immunoglobulins.

Major Functions of Blood

- ✓ **Respiration**—transport of oxygen from the lungs to the tissues and of CO2 from the tissues to the lungs
- ✓ **Nutrition**—transport of absorbed food materials
- ✓ **Excretion**—transport of metabolic waste to the kidneys, lungs, skin, and intestines for removal.
- ✓ Maintenance of the normal **acid–base balance** in the body
- ✓ Regulation of **water balance** through the effects of blood on the exchange of water between the circulating fluid and the tissue fluid
- ✓ Regulation of **body temperature** by the distribution of body heat
- ✓ **Defense** against infection by the white blood cells and circulating antibodies
- ✓ Transport of **hormones** and regulation of metabolism
- ✓ Transport of **metabolites**
- ✓ **Coagulation**

INDICATIONS

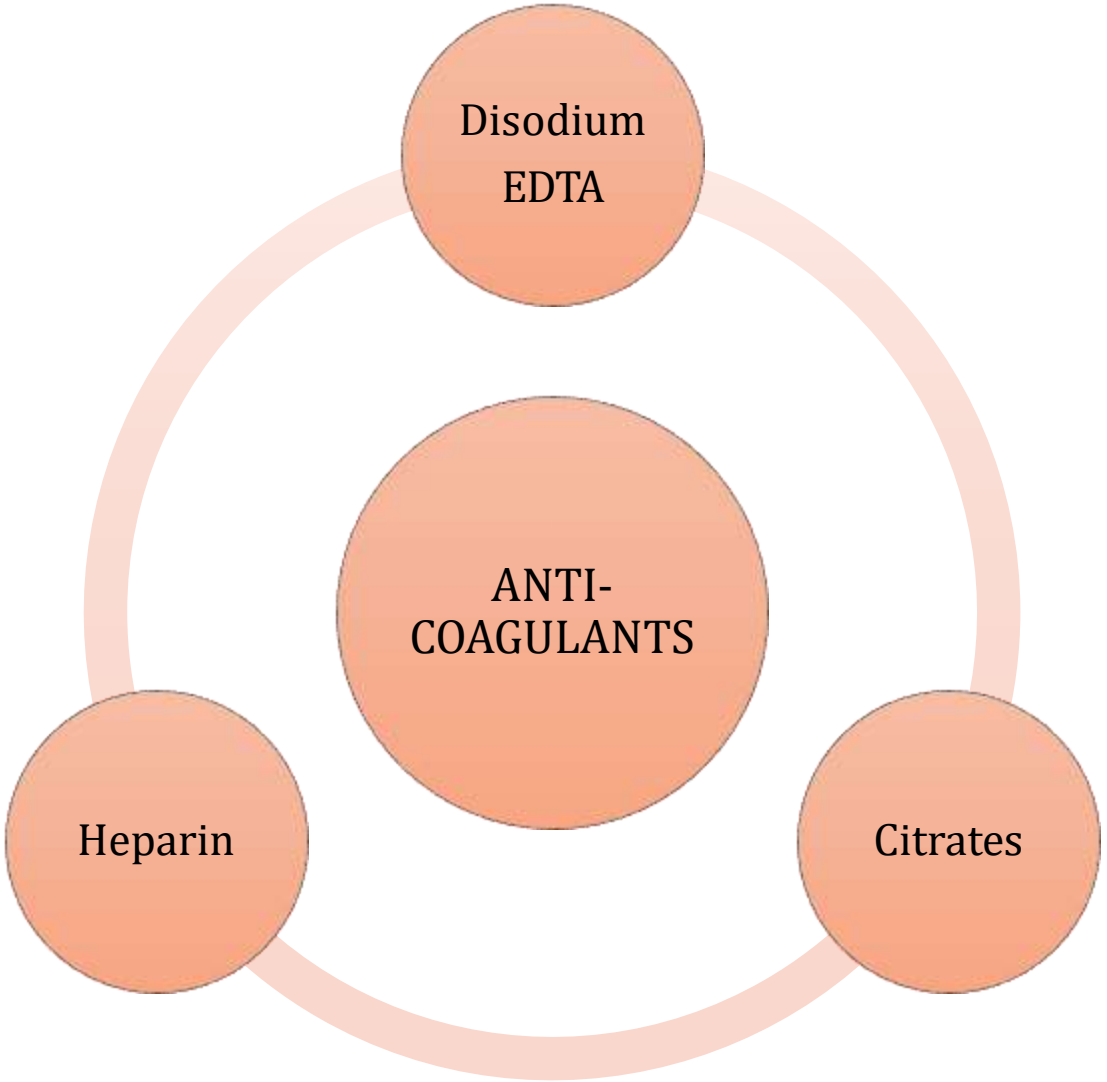
- ✓ Anemia.
- ✓ Major surgical operations.
- ✓ Accidents resulting in considerable blood loss.
- ✓ Cancer patients requiring therapy.
- ✓ Women in childbirth and new born babies in certain cases.
- ✓ Patients of hereditary disorders like Haemphilia and
- ✓ Thalassemia.
- ✓ Severe burn victims.

CONDITIONS FOR BEING A DONOR

A person in good health is accepted as a donor provided that the individual is;

- ✓ Not suffering from any diseases that can be transmitted by transfusion. This includes syphilis, malaria, and serum jaundice.
- ✓ Not anaemic. The haemoglobin content of blood should not be less than 12.5% for females and 13.3% for males.
- ✓ Not taking medications which might prove toxic or have allergic reactions in a patient e.g. antibiotics.

ANTI-COAGULANTS



CITRATES

The solution most often used as a blood anticoagulant is known as

- **Acid-citrate dextrose (ACD)**
  - Sodium citrate (2.0 – 2.5 g)
  - Dextrose (3.0 g)
  - Water for injection (q.s. upto 120ml)

✓ The citrate prevents clotting by binding the **calcium ions as unionized calcium citrate,** thus preventing a vial step of clotting**.**

CITRATES

Why acid citrate and not normal citrate?

- ✓ The normal trisodium citrate has a very **high alkaline pH in solution** which causes considerable **caramelisation of 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.
- ✓ The higher concentration (2.5g/120ml) is often preferred because it more efficiently reduces the formation of small clots.

Why add dextrose?

- ✓ The **dextrose delays the hemolysis** 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** surrounded 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 less 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**.
- ✓ ACD is almost as effective as disodium edetate.
- ✓ The survival of red blood cells in dextrose edetate is as good as in ACD.

WHOLE HUMAN BLOOD

DEFINITION: One unit whole blood contains 450ml of donor blood plus anticoagulants.

VOLUME: 450ml.

STORAGE: between 2-6°C in approved blood bank refrigerator. Transfusion should be started within 30 minutes of removal from refrigerator.

SHELF LIFE: 21 days.

INDICATIONS:

- Red cell replacement in acute blood loss with hypovolemia.
- Exchange transfusion.

CONTRAINDICATIONS: risk of volume overload in patients with

- Chronic anaemia.
- Incipient cardiac failure.

CHANGES IN COMPOSITION OCCUR DURING STORAGE

- ✓ Significant detoriation of clotting factors.
- ✓ Total loss of functioning granulocyte and platelets.
- ✓ Concentration of hydrogen and potassium ions in plasma increases during storage.
- ✓ **Thus the infusion of large amount of stored whole blood results in infusion of undesirable constituent.**

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 5ml bottle and is firmly attached to the main container. This is for **testing compatibility** with the blood of the recipient.
- ✓ 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.

RED BLOOD CELLS

Red blood cells contain **haemoglobin** and serve as the **primary agent for transport of oxyge**n to tissues.

PREPARATION : It is prepared by removing **most of the citrated plasma** from whole blood by **centrifugation or sedimentation**.

DESCRIPTION: 150-200ml red cells from which most of the plasma has been removed.

INFECTION RISK: Same as whole blood.

INDICATIONS: Replacement of red cells in anaemic patients(chronic anaemia).

DESCRIPTION- Generally available in two types:

- RCC without additive solution.
- RCC with additive solution.

ADVANTAGE OF TRANSFUSING RBC OVER WHOLE BLOOD:

- ✓ RBC increases oxygen carrying capacity without increasing blood volume. This is useful in chronic anemia and CHF.
- ✓ Removal of plasma decreases plasma protein thus decreases the chances of allergic or anaphylactic reactions.

ADVANTAGES OF USING RBC WITH ADDITIVE SOLUTIONS:

- ✓ Reduces the viscosity of blood, hence making the transfusion easy.
- ✓ Shelf life of RCC increases from 35 days to 42 days.
- ✓ Post transfusion viability of red cells increases.

PLATELET COMPONENTS

Platelet therapy may be achieved by infusion of either

- **APHERESIS PLATELETS**
- **PLATELETS** (whole blood- derived platelet concentrates).

✓ In either component, platelets are suspended in an appropriate volume of original plasma, which contains near normal levels of stable coagulation factors that are stored at room temperature.

- **STORAGE:** Between 20-24°C. **SHELF LIFE:** 5 days.
- **INDICATIONS:** The therapeutic goal of platelet transfusion is to provide adequate numbers of normally functioning platelets for the prevention or cessation of bleeding.

Treatment of bleeding due to :

- Thrombocytopenia.
- Platelet function defects.

DOSAGE AND ADMINISTRATION: One unit of platelets would be expected to increase the platelet count of a 70kg adult by 5,000 to 10,000/µl. The therapeutic adult dose is 1 unit of apheresis platelets or 4 to 6 units of whole blood derived platelets.

PLASMA COMPONENTS

FRESH FROZEN PLASMA (FFP) DEFINITION:

FFP is plasma separated by normal whole blood donation by single donor and rapidly frozen within 6 hours of being collected. It contains all coagulation factors.

- **VOLUME:** 200ml – 220ml (1 unit).
- **STORAGE:** at -30°C or colder.
- **SHELF LIFE:** 1 year.

NOTE: before use, FFP should be thawed in blood bank in thawing bath between 30°C to 37°C. FFP should be administered as soon as possible after thawing.

- **INDICATIONS:** Used in patients with multiple coagulation factor deficiencies
  - Liver diseases.
  - Warfarin (anticoagulant) overdose.
- **DOSAGE:** Initial dose of 10 to 15 ml/kg.
- **ADMINISTRATION:**
  - Must be ABO compatible.
  - No compatibility testing required.
  - Infuse using standard blood transfusion set as soon as possible after thawing.

PLASMA COMPONENTS

CRYOPRECIPITATE DEFINITION:

Cryoprecipitate are precipitated proteins if plasma rich in factor VIII and fibrinogen obtained from a single unit of fresh frozen plasma.

- **VOLUME:** 10ml to 20ml. **STORAGE:** at -30°C or colder.
- **SHELF LIFE:** 1 year.

NOTE: Before use, should be thawed in blood bank thawing bath between 30 to 37°C. once thawed, cryoprecipitate should be transfused immediately but in any case not later than 6 hours.

- **INDICATIONS:** As an alternative to factorVIII concentrate in the treatment of inherited deficiencies of FACTOR VIII (haemophilia A).
- **DOSAGE:** 2 units/10 kg wt. one bag contains more than 80 units of factor VIII and more than 150mg of fibrinogen. Each unit of factor VIII per kg raises plasma factor VIII by 2%.
- **ADMINISTRATION:**
  - No compatibility testing is required.
  - After thawing, infuse as soon as possible through a standard blood transfusion set.
  - Must be transfused immediately or within 4 hours of thawing.

NEUTRALIZATION OF PLASMA AGGLUTININS

- ✓ Agglutinins in the donors plasma usually do not damage the recipients red cells.
- ✓ Occasionally, however, the plasma agglutinins are very powerful and can cause serious hemolysis 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 cells agglutinogen 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 agglutinogen, producing a preparation that is safe to transfuse to all groups.
- ✓ Most satisfactory ratio for mixing is 9 of A : 9 of O : 2 B or AB.

NEUTRALIZATION OF PLASMA AGGLUTININS

STORAGE:

- ✓ Dried plasma, kept below 20°C and protected from light, moisture, and oxygen, remains usable almost indefinitely.
- ✓ 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.
- ✓ It must dissolve completely within ten minutes at room temperature.
- ✓ Gel formation or incomplete solution indicates deteoriation.
- ✓ After reconstitution it must be used immediately.

THE FRACTIONATION OF PLASMA

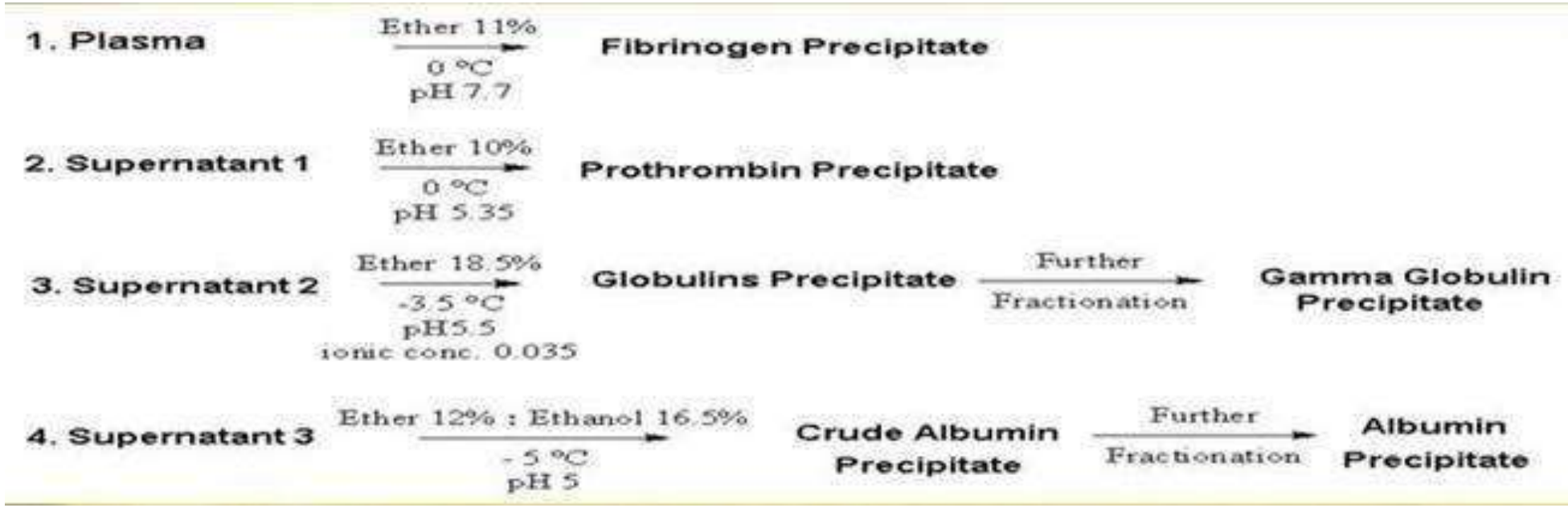
- ✓ About 60% of plasma protein in albumin and therfore, it plays a major part in maintaining the high osmotic pressure necessary to retain fluid in the blood vessels.
- ✓ A very successful solvent precipitation technique was developed by which other proteins, as well as albumin, were separated.
- ✓ 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 fraction nor affect the solubility.
- ✓ 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

- ✓ COHN'S TECHNIQUE – Developed by E.J.Cohn
- ✓ Based on the use of an organic solvent (ethyl alcohol) to reduced the solubility of the proteins, and was given flexibility by alterations of pH, ionic strength (i.e., salt concentrations) and protein.
- ✓ On the other hand, it is necessary to keep the temperature very low (0-5°C) to prevent solvent denaturation of proteins.``



HUMAN PLASMA PROTEIN FRACTION

- ✓ A solution of some of the proteins from liquid plasma.
- ✓ 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°C and protected from light
- ✓ Its use remains the same as dried plasma
- ✓ A stabilizer such as sodium **caprylate or acetylteyptophan** 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°C for 10hrs to destroy the viruses of infective hepatitis and homologous serum jaundice.

DRIED HUMAN PLASMA PROTEIN FRACTION

- ✓ Dried human plasma protein fraction is prepared by freeze drying human plasma protein fraction.
- ✓ Its 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 fractionation, the precipitation is collected by centrifugation, dissolved in citrate-saline, and freeze dried.
- ✓ The air in the containers displaced by nitrogen
- ✓ The citrate prevents spontaneous clotting when material is reconstituted.
- ✓ The solution should be used as soon as possible and not later than 3 hours after preparation.

USE:

- ✓ Occasionally fibrinogen is administered alone to treat fibrinogen deficiency.
- ✓ But it is more often used in conjunction with thrombin.

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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 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 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 clot also acts as a haemostat.

HUMAN FIBRIN FOAM

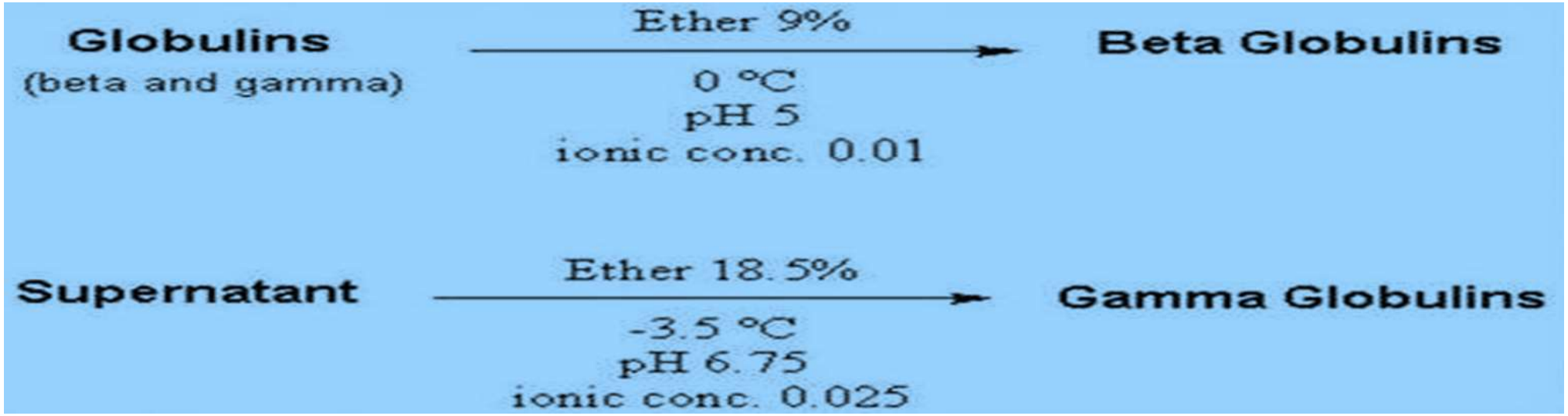
- ✓ This is a sponge like mass of human fibrin.
- ✓ It is prepared by whipping a solution of fibrinogen into a froth by mechanical means and then adding thrombin.
- ✓ The product is poured into trays and freeze dried, then cut into pieces of
- ✓ convenient size and sterlized by dry heat at 130°C for 3hrs.
- ✓ The foam must be stored under dry conditions, protected from light and at a temperature below 20°C. The other storage conditions are similar to that of dried serum expect that fibrin foam not to be kept under fibrinogen.

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 solution 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 human origin.

HUMAN NORMAL IMMUNOGLOBULIN INJECTION

- ✓ Immuno or gamma globulin 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:



- ✓ 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°C with protection from light.

USES OF IMMUNOGLOBULINS:

- ✓ Used to prevent or attenuate diseases such as

  Measles; Rubella; Infectious hepatitis; Hepatitis B; Chickenpox; and Hypogammaglobulinaemia
- ✓ It is used to prepare specific immunglobulins such as:
  - Human Anti Vaccinia Immunoglobulin – for small pox.
  - Human Anti Tetanus Immunoglobulin.
  - Human Anti D Immunoglobulins – used to suppress sensitization of Rh-ve mothers to the Rh D antigen (Rh +ve infant).
  - Anti HBs Immunoglobulin – this is still under investigation. It is an immunoglobulin for Hepatitis B surface antigen.

QUALITY CONTROL OF BLOOD PRODUCTS

- **STANDARDS**
  - STERILITY AND PYROGENS
  - SOLUBILITY
  - IDENTIFICATION
- **LABELLING**
  - WHOLE BLOOD LABELLING
  - DRIED PLASMA PROTEIN FRACTION LABELLING

IDENTIFICATION

- ✓ **Precipitation tests with specific antisera** are used to show that only human serum proteins are present
- ✓ **The characteristic mobilities of blood proteins in an electrophoretic field.** For example, in the plasma protein fraction there must not be less than 58% 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.
- ✓ **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.

IDENTIFICATION

STERILITY AND PYROGENS

- ✓ All blood products must comply with the official tests for sterility.
- ✓ 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.
- ✓ 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 1 ml of 0.1% fibrinogen in saline buffered at**
- ✓ **7.2 to 7.3 in 15 seconds at 37°C.**
- ✓ Determination 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.**

PLASMA SUBSTITUTES REPLACEMENT FLUID

- ✓ Replacement fluids are used to replace abnormal losses of blood, plasma or other extracellular fluids by increasing the volume of the vascular compartment, principally in:
- ✓ Treatment of patients with established hypovolaemia: e.g. haemorrhagic shock.
- ✓ Maintenance of normovolaemia in patients with ongoing fluid losses: e.g. surgical blood loss.

NEED FOR PLASMA SUBSTITUTES

- ✓ The limited supplies of plasma.
- ✓ The cost of producing the dried form.
- ✓ The risk of transmitting serum hepatitis

LABELLING FOR WHOLE BLOOD

Name of Preparation
• ABO Group • Rh group and nature of antisera used for testing • Total Volume; proportion of blood; nature and percentage of anticoagulant and any other material introduced • Date of Donation • Expiry date • Storage Conditions • A statement that the contents must not be used if there is any sign of deterioration • An indication by which the history of the preparation can be traced

LABELLING OF DRIED PLASMA PROTEIN FRACTION

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

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PROPERTIES OF IDEAL PLASMA SUBSTITUTE

- ✓ The same colloidal osmotic pressure as whole blood.
- ✓ A viscosity similar to that of plasma.
- ✓ A molecular weight such that the molecules do not easily diffuse through the capillary walls.
- ✓ A fairly low rate of excretion or destruction by the body
- ✓ Eventual and complete elimination from the body.
- ✓ Freedom from toxicity. E.g. no impairment of renal function.
- ✓ Freedom from antigenicity, pyrogenicity, and confusing effects on important tests such as blood grouping and the erythrocyte sedimentation rate.
- ✓ Isotonicity, in solution, equal to that of blood plasma.
- ✓ High stability in liquid form at normal and sterilizing temperatures and during transport and storage.
- ✓ Ease of preparation, ready availability and low cost.

PROPERTIES OF IDEAL PLASMA SUBSTITUTE

GUM SALINE

- ✓ Synonym for Injection of Sodium Chloride and Acacia having 6% acacia in 0.9% Sodium Chloride solution.

DISADVANTAGES:

- ✓ Signs of liver dysfunction as gum was not metabolized but stored in various organs.
- ✓ Polyvinylpyrrolidone – a synthetic colloid. Disadvantage – suspected carcinogenicity.

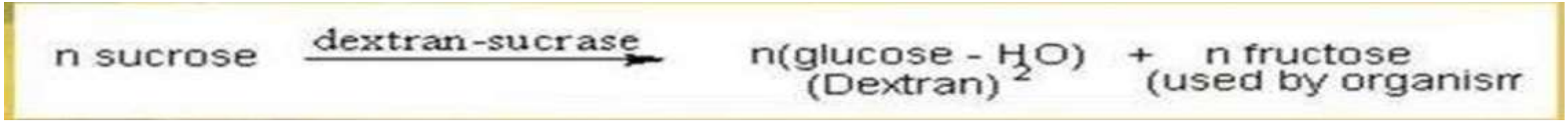
DEXTRAN

- ✓ 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.

PROPERTIES OF IDEAL PLASMA SUBSTITUTE

GUM SALINE

- ✓ The organism secretes an enzyme that converts sucrose to dextran according to the following equation-



- ✓ Different strains produce dextrans of two main groups-
  - o Long practically unbranched chains of glucose units joined by 1-6 glucosidic linkages.
  - o Highly branched polymers containing 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-
  - o Choose a suitable specially developed strain of the orgainsm that produces dextran in which about 95% of the linkages are 1-6 production.

PROPERTIES OF IDEAL PLASMA SUBSTITUTE

- ✓ Production involves laboratory culture followed by growth in seed tanks in the factory and then in 4500 cubic dm fermenters.
- ✓ PRECAUTIONS – need to prevent the hydrolysis of sucrose to glucose and fructose during sterilization of the culture media. Prevention measures include the 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. Production involves laboratory culture followed by growth in seed tanks in the factory and then in 4500 cubic dm fermenters.

PROPERTIES OF IDEAL PLASMA SUBSTITUTE

- ✓ 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.

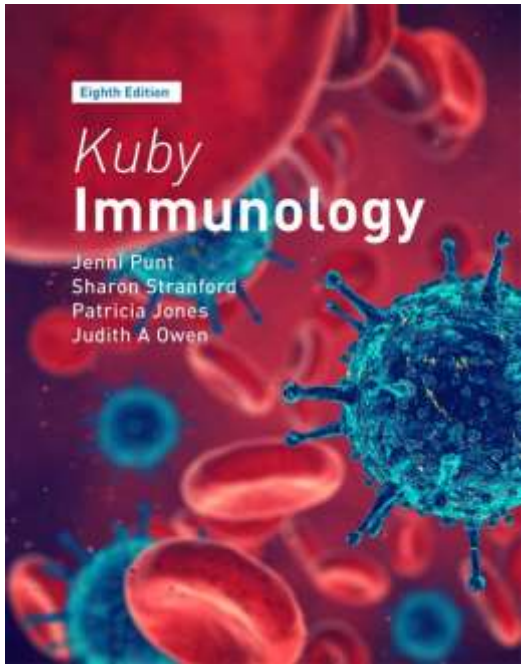
PROPERTIES OF IDEAL PLASMA SUBSTITUTE

- ✓ 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 (most widely used).
  - Thermal degradation.
  - Ultrasonic disintegration.
  - Seeding the fermenter.
- ✓ The very small molecules, i.e. those of below a molecular weight of about 60,000 also have disadvantages:
  - They are rapidly excreted in urine.
  - They pass into the tissue fluids causing an adverse osmotic pressure

Additive Solution (mg/100 mL.)	Dextrose	Adenine	Monobasic Sodium Phosphate	Mannitol	Sodium Chloride	Sodium Citrate	Citric Acid	Shelf Life
AS-1 (Adsol)	2200	27	0	750	900	0	0	42 days
AS-3 (Nutricel)	1100	30	276	0	410	588	42	42 days
AS-5 (Optisol)	900	30	0	525	877	0	0	42 days

- ✓ 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.
  - Colour – by adsorption on to activated charcoal.
  - Pyrogrens – by adsorption on to asbestos, or cellulose derivatives.
  - Micro organisms – by filtration.
- ✓ 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.

Acknowledgement



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