RBC Production

a. pleuripotent stem cell
- capable of self-renewal & differentiation
- produces rbc's, granulocytes, monocytes & platelets

b. proerythroblast
- first committed stage
- undergoes 3-4 cell divisions
- receptors for ***erythropoeitin***

c. normoblast
- last nucleated stage

d. reticulocyte
- formed with expulsion of the nucleus
- remains in the marrow for 2-3 days
- retains mitochondria & ribosomes for 24-48 hrs

e. ***erythropoetin***
- glycoprotein (MW 30-36,000) produced by the kidney in response to ***hypoxia***
- ~ 10-15% produced in the liver
- interacts with cell surface receptors on proerythroblasts → pronormoblasts
- also acts on later cell lines → ↑ Hb synthesis

f. mature rbc
~ 7.5 µm diameter by 2 µm thick
~ 3×10^{13}
~ 900 g of Hb (15 g/dl x 6 l)
~ 7.5 g Hb/day turnover (< 1%)
~ 120 days survival time

Haemoglobin Synthesis

- anhydrous MW ~ 65,000
- tetramer composed of 2 pairs of 4 possible polypeptide chains → a b g d
- each of these is linked to a ***haem*** group → protophoryrin IX + Fe^{++}
- each haem group may reversibly bind 1 molecule of O_2 → ***oxygenation***
- O_2 affinity increases with binding → ***sigmoid*** shape of curve
- in normal adults,

a. HbA ~ 97% 2 alpha / 2 beta

b. HbA_2 ~ 3% 2 alpha / 2 delta

c. HbF < 1% 2 alpha / 2 gamma

Disorders of Haemoglobin Synthesis

1. decreased production of a ***normal*** chain
   - these have recessive inheritance, ∴ occur as ***homozygous*** & ***heterozygous***
   i. alpha thalassaemia
   ii. beta thalassemia
      - results in elevated HbF and HbA_2 levels
      - heterozygous form may be asymptomatic, or present with mild anaemia
2. production of an ***abnormal*** chain
   - eg. sickle cell anaemia
3. persistence of a developmental chain - HbF

Haem Biosynthesis

- in hepatocytes & rbc precursor mitochondria

$$\text{glycine + succinyl-CoA} \xrightarrow[\text{δ-ALA synthase}]{} \text{δALA}$$

- δALA-synthase is,
   a. under negative feedback from haem
   b. induced by increased requirements for haem
   c. induced by many drugs which are cytochrome P_{450} inducers

- δALA is the converted to ***porphobilinogen***, under the influence of δALA-dehydratase, which is a Zn^{++} containing enzyme inhibited by lead
- this is then converted to ***hydroxymethylbilane***, which is the precursor of the ***porphyrins***
- porphyrins are ***tetrapyrrole*** pigments which serve as intermediates in haem biosynthesis
- haem is required for,
   1. haemoglobin
   2. myoglobin
   3. some respiratory enzymes

Haemoglobin Function

- 1g of Hb_A fully saturated combines with 1.39 ml O_2 (STPD)
- iron remains in the ferrous state, thus the reaction is ***oxygenation***
- competitive binding of the ***beta chains*** with 2,3-DPG results in ***decreased*** O_2 affinity
- as haem takes-up O_2 the 2,3-DPG is displaced, further increasing O_2 affinity
- in the absence of 2,3-DPG the curve would shift to the extreme ***left*** → P_{50} ~ 1 mmHg
- Hb_F (α_2/γ_2) has lower affinity for 2,3-DPG → P_{50} ~ 19 mmHg

- factors affecting O_2 affinity are rbc,
  a. $[H^+]$ → *Bohr effect*
  b. P_{CO2}
  c. temperature
  d. 2,3-DPG
  e. $[Cl^-]$

  NB: ↑ in any of these → shift to the ***right*** and ↑ P_{50}

  originally, the *Bohr effect* was in reference to P_{aCO2} , however H^+ is more important

- ***2,3-Diphosphoglycerate 2,3-DPG***

- an intermediary in the Embden-Meyerhof glycolytic pathway, the ***Rapoport-Luebering shunt***
- synthesised from 1,3-DPG by ***2,3-DPG mutase***
- re-enters the glycolytic pathway → 3-phosphoglycerate, catalysed by ***2,3-DPG phosphatase***
- the plasma elimination half-life, $t_{½}$ ~ 6 hrs
- exerts a permissive role for the effects of CO_2 and pH
- thus, in stored blood deficient in 2,3-DPG, the Bohr effect is less
- ↓ pH → ↓ ***mutase*** activity & ↑ phosphatase activity →
  1. ICF pH has the strongest control over synthesis
  2. ***acidosis*** → ↓ rbc glycolysis & ↓ 2,3-DPG formation
     → shifting the curve to the ***left*** in chronic states
     - opposite to the direct effects of pH, and with chronic acidosis the P_{50} is ***reduced***
  3. ***alkalosis*** may be associated with a shift of the curve to the ***right***

- thyroid hormones, GH, and androgens increase 2,3-DPG
- ***exercise*** increases 2,3-DPG within 60 mins, but this effect may not be seen in athletes
- ***high altitude*** triggers a substantial rise in 2,3-DPG secondary to the respiratory alkalosis
- an increase in 2,3-DPG has been described in disorders of ↓ CO
- however, in congenital heart disease, anaemia, cirrhosis, CAL and thyrotoxicosis, both increases and decreases in 2,3-DPG have been described
- AMI results in an increase in 2,3-DPG

NB: the effects of DPG are only seen in the range **P_{50} ~ 15-34 mmHg**

Porphyrias

Def'n: group of metabolic disorders of porphyrin production, 2 types,

1. ***hepatic porphyrias***
   i. acute intermittent porphyria (AIP)
      → ***uroporphyrinogen synthetase I*** deficiency

   ii. porphyria cutanea tarda (PCT) * ***commonest form***
      → uroporphyrinogen decarboxylase deficiency

   iii. variegate porphyria (VP)
      → ? protoporphyrin oxidase deficiency

   iv. hereditary coproporphyria (HC)
      → coproporphyrin synthetase deficiency

2. ***erythropoietic porphyrias***
   i. congenital erythropoietic uroporphyria (CEU)*
      → uroporphyrinogen synthetase II deficiency

   ii. erythropoietic protoporphyria (EP)
      → ferrochetelase deficiency

NB: all are ***autosomal dominant***, except the rare CEU*

LIGW states inherited or acquired ??

- ***Clinical Features***

• usually relate to either ***skin*** or ***neurological*** abnormalities
• the ***hepatic porphyrias*** are characterised by the 4 "P's",
   1. abdominal pain
   2. peripheral neuritis
   3. psychosis
   4. port-wine / purple urine

Clinical Features						
Type	**AIP**	**PCT**	**VP**	**HC**	**CEU**	**EP**
photosensitivity	-	+	+	±	+	+
liver affected	+	+	+	+	-	+
CNS involvement	+	-	+	+	-	+
barbiturate sens^{y}	**+++**	-	++	++	-	-
Abnormal Metabolites						
red cells	-	-	-	-	+	+
urine	+	+	+	+	+	-
faeces	-	-	+	+	+	+
urine colour	black	pink brown			red	

- ***Skin Lesions***

• porphyria cutanea tarda, congenital erythropoeitic porphyria and protoporphyria,

a. sensitivity to sunlight
b. blistering
c. excessive fragility & scarring

NB: may be associated with hirsuitism & hyperpigmentation, especially face & hands

• CEP also associated with haemolytic anaemia, splenomegaly and erythrodontia

- ***Neurological Lesions***

• AIP, variagate porphyria, and the rare hereditary coproporphyria,

a. ***central***
   i. confusion, hysteria, depression, psychosis
   ii. epilepsy
b. ***peripheral*** *neuropathy is often ***reversible***
   i. LMN disorders - generalised weakness, flaccid quadraparesis
      - foot drop, wrist drop, bulbar palsy, absent DTR's
      *differential diagnosis for GBS
   ii. neuritic pain & hyperaesthesia
c. ***autonomic***
   i. abdominal pain, constipation, colic, N&V
      • normally ***no*** abdominal rigidity & minimal abdominal tenderness
      • mild fever & leukocytosis may be present
   ii. hypertension, postural hypotension & angioneurotic oedema

Investigations

- during acute attack, differentiation by,
    1. screening of urine for ***porphobilinogen***
    2. feces & rbc's for excess porphyrins

    NB: ***all*** hepatic porphyrias, except PCT, are associated with ↑ urinary PBG
    only hepatic porphyria with a ***negative*** fecal screen is AIP

Acute Intermittent Porphyria

- ***autosomal dominant*** disorder of porphyrin metabolism
- most serious of the hepatic porphyrias
- ***uroporphyrinogen I synthetase*** deficiency → accumulation of ***porphobilinogen***
- diagnostic features include,
    a. raised urinary δALA and porphobilinogen during an attack
    b. urine turns ***black*** on standing
    c. low rbc uroporphyrinogen synthetase level

- clinical features,
    a. usually young to middle aged female
    b. episodes of acute ***abdominal pain***
    c. variable neurological defects due to ***demyelination***,
        i. motor weakness
        ii. arreflexia
        iii. autonomic dysfunction
        iv. occasional bulbar and cerebellar signs
    d. trigger factors
        - starvation, dehydration
        - sepsis
        - pregnancy
        - drugs
    e. alleged trigger drugs
        * ***barbiturates & benzodiazepines***
        - ketamine, althesin, etomidate
        - ethanol, phenytoin
        - glutethimide
        - pentazocine
        - steroids and sulpha's
    f. alleged "safe" drugs
        - volatiles, N_2O
        - fentanyl, morphine, pethidine
        - ***propofol***, droperidol, propanidid
        - relaxants, anticholinergics & anticholinesterases
        - promethazine, chlorpromazine

- ***Management***

- protection against UV light → clothing, sunscreens, etc
- use of beta-carotene (30 mg/day) & haematin are still experimental
- activated charcoal has been used in CEP to bind excess porphyrins in the GIT
- patient should have a personnal list of "safe" drugs which have been used without consequence

- ***acute attack,***

a. suportive
   i. rehydrate
   ii. correct electrolyte abnormalities

b. ***dextrose*** ~ 20 g/hr
   ~ 100 ml 20% dextrose / hr
   - decreases porphobilinogen production

c. ***haematin*** ~ 3-4 mg/kg x infused over 10 mins q12h for 3-6 days
   - blocks δALA synthetase
   - half-life ~ 4 hrs
   - unstable, ∴stored at 4°C under vacuum & must be used immediately

d. pain control - chlorpromazine
   ± opioids

e. IPPV may be required for respiratory failure

Methaemoglobin

- caused by the ***oxidation*** of ferrous to ***ferric*** iron in the haem moeity ($Fe^{++} \rightarrow Fe^{+++}$)
- unable to bind O_2 and therefore inactive, but ***increases*** the affinity of adjacent (unaffected) haem moeities, with a resultant reduction in the P_{50}
- production normally prevented by 2 mechanisms,

1. reduced glutathione & ascorbic acid → e^- donors
2. enzymatic reduction
   i. NADH methaemoglobin reductase
      - transfers an electron from cytochrome b_5
   ii. NADPH methaemoglobin reductase
      - no endogenous electron donor, requires ***methylene blue*** or similar
      - ~ 10x more efficient, than NADH system

- ***Causes***

1. congenital
   i. methaemoglobin reductase deficiency
   ii. cytochrome b_5 deficiency
   iii. M haemoglobins
2. acquired
   i. chemicals - sodium nitrite, amyl nitrite, ethyl nitrite, silver nitrate
   - potassium chlorate / permanganate, alanine dyes,
   - aminobenzenes, nitrotoluenes, phenylenediamine
   ii. drugs - sulphonamides, GTN, phenacetin
   - benzocaine, prilocaine, lignocaine

- ***Clinical Features***

a. < 10% MetHb - minimal or no symptoms
b. ~ 30-40% MetHb - dyspnoea, tachycardia, headaches & fatigability
c. > 70-80% MetHb - lethal levels, patients appear "black"

NB: ***cyanosis*** is the principal manifestation
→ out of proportion to clinical signs

• clinical cyanosis begins at MetHb **~ 1.5 g**/100ml (~ 10% MetHb / [Hb] = 15 g/dl)

- ***Investigations***

a. ABG's - normal P_{aO2} in the presence of severe cyanosis
b. SpO_2 - trends toward ~ **85%** with normal P_{aO2}
c. co-oximetry → [MetHb] ~ 0.2-0.5% normal
> 1.0% = ***methaemoglobinaemia***

- ***Management***

• in the absence of symptoms, no treatment is required
• physiological mechanisms correct the anomaly within 24-48 hrs
• in severe cases, ***methylene blue*** ~ 1-2 mg/kg will correct cyanosis in ~ 1 hr
• if the patient is G6PD deficient this will be ineffective and may precipitate a crisis
• factors of limited value,

a. high dose vit.C
b. supplemental O_2

Glucose 6 Phosphate Dehydrogenase (G6PD) Deficiency

Def'n: inherited rbc enzyme deficiency resulting in ***haemolytic anaemia***
- ***sex-linked*** chromosomal disorder → affecting predominantly ***males***

- more common in certain ***racial groups***,
  a. Negroes, West Africans
  b. Mediterraneans
  c. S.E. Asians

- clinical presentations,
  a. acute drug-induced haemolytic anaemia
  b. chronic haemolytic anaemia
  c. jaundice
  d. neonatal jaundice and kernicterus

- trigger factors include,
  a. acute illness of any type
  b. infections - viral and bacterial
  c. diabetic ketoacidosis
  d. ***drugs***
     i. antimalarials - primaquine, pamaquine, etc
     ii. antibiotics - sulphonamides
     - nitrofurantoin
     - chloramphenacol
     iii. analgesics - high dose aspirin
     - phenacitin, PAS
     iv. others - dimercaprol (BAL)
     - vitamin K
     - probenecid
     - phenothiazines

- generally, drugs either,
  a. result in ***oxidation*** of Hb, or
  b. impair reduction of met-Hb

NB: → intravascular & extravascular haemolysis

THE ANAEMIAS

Classification

1. ***microcytic***
   - i. abnormal ***iron metabolism*** - iron deficiency anaemia
   - ii. anaemias with 2° iron loading
     - sideroblastic anaemias, thalassaemia minor
     - anaemias with ***abnormal haemoglobin synthesis***
     - transfusional haemochromatosis
2. ***macrocytic***
   - i. ***megaloblastic*** anaemias - cobalamin deficiency
     - folate deficiency
   - ii. non-megaolblastic anaemias - alcoholism, chronic liver disease
     - myxoedema
     - scurvy
     - ± haemolysis (2° reticulocytosis)
3. ***normocytic***
   - i. anaemia of ***chronic disease*** - chronic infection / inflammation
     - CRF, RA, SLE, PAN
     - malignancy
     - endocrine failure - Addison's, panhypopituitarism
   - ii. ***haemolytic*** anaemias
   - iii. primary ***marrow*** failure & the myeloproliferative disorders

NB: the use of the terms ***hypochromia*** and ***normochromia*** have decreased, as MCHC (R: 30-35 g/dl) remains almost ***constant*** in most conditions

hereditary spherocytosis is an exception to this with a MCHC ≥ 36 g/dl

- ***Common Causes***

1. blood-loss, iron deficiency, microcytic anaemia
2. B_{12} / folate macrocytic anaemia
3. normocytic anaemia
   - i. CRF
   - ii. chronic diseases
   - iii. haemolytic anaemias

Iron Deficiency Anaemia

- ***Causes***

1. increased utilisation - postnatal & adolescent growth spurts
2. physiological iron loss - menstruation & pregnancy
3. pathological iron loss
   i. GIT or GUS blood-loss
   ii. hereditary telangectasia, parasitic infections
   iii. pulmonary haemosiderosis
   iv. intravascular haemolysis
4. decreased iron intake / absorption
   i. cereal-rich, meat-poor diets, food faddists
   ii. elderly & indigent persons
   iii. achlorhydria
   iv. malabsorption syndromes
   v. post-gastrectomy

• daily iron requirements,

a. male ~ 1.0 mg/day
b. females ~ 1.5 mg/day
c. dietary intake ~ 10-15 mg/day
   ~ 10% absorption
d. RES breakdown of rbc's ~ 25-35 mg/day

• transported bound to ***transferrin*** and stored as ***ferritin***

a. Hb ~ 2500 mg
b. storage ~ 100-1000 mg
c. tissue enzymes ~ 300 mg
d. plasma pool~ 4 mg

• iron stores fall first, then serum iron, then [Hb]
• iron deficiency can deplete cytochromes, myoglobin & Fe-containing enzymes, but there are no associated clinical syndromes

- ***Clinical Features***

a. lassitude, weakness
b. angina, SOBOE, LVF
c. hyperdynamic CVS
d. pica - especially for ice
e. dysphagia, anorexia, vomiting
f. pallor
g. angular stomatitis, atrophic glossitis
h. koilonychia (18%), brittle nails, longitudinal ridging

- ***Investigation***

a. FBE
b. feces for occult blood
c. serum iron studies - Fe, ferritin, transferrin, TIBC
   - usual picture - ↓ Fe / ↑ transferrin & TIBC
   - serum ferritin < 100 µg/ml → depleted iron stores
   - but, serum ferritin can be normal/elevated with reduced tissue stores
   - thus, if deficiency suspected then need to do ***bone marrow***
   - raised serum ferritin can be caused by conditions other than iron overload

- ***Treatment***

a. dietary inadequacy → ferrous sulphate ~ 2 x 300 mg tds for 8-10 weeks
   ~ **35 mg** iron / 300 mg
   - if stores + rbc's = 1000 mg + 2500 mg, then replacement → 100 days
b. IV iron/dextran complex
   - total deficit, ~ 1-2g , can be given after test dose ~ 1-5 mg
c. transfusion
   - 1 ABP contains ~ **250 mg** iron
   - indicated only if surgery planned or CVS symptoms

NB: if B_{12} / folate adequate → reticulocytosis, leukocytosis & thrombocytosis

[Hb] usually increases ~ 1g / dl / week

- ***Sideroblastic Anaemias***

1. hereditary or congenital sideroblastic anaemia
2. acquired sideroblastic anaemia
   - i. drugs / toxins - isoniazid, chloramphenacol
     - alcohol, lead
   - ii. neoplasia & inflammatory disease
   - iii. alkalating agent chemotherapy - cyclophosphamide

Haemochromatosis

Def'n: an iron storage disease, characterised by an inappropriate increase in ***GIT absorption***, resulting in,

1. excess iron deposition ~ 20-25g (N: 1-1.5g)
2. functional abnormalities of liver, heart & pancreas

Clinical Features

- may be inherited as an ***autosomal recessive*** disorder, or acquired as ***transfusion siderosis***
- 5-10x more common in ***males***
- becomes clinically evident ~ 40-60 yrs

a. skin pigmentation
b. diabetes
c. liver dysfunction ~ 30% develop ***hepatocellular carcinoma*** untreated
d. cardiomyopathy
e. arthropathy
f. hypogonadism

Investigation

a. serum iron studies ↑ ferritin
b. CXR / AXR
c. ***liver biopsy***

Management

a. weekly ***phlebotomy*** ~ 500 ml for 2-3 years
   - followed by phlebotomy 1-3 monthly
b. desferrioxamine
   - ineffective, as only removes ~ 10-20 mg/day
     cf. ~ 250 mg by venesection

Megaloblastic Anaemias

1. ***cobalamin deficiency***
   - i. inadequate intake — vegetarians, rarely
   - ii. malabsorption
     - ↓ ***intrinsic factor*** — pernicious anaemia; post-gastrectomy; congenital absence or dysfunction (rare)
     - terminal ileal disease — tropical sprue, non-tropical sprue; regional enteritis, Crohn's; surgical resection; neoplasms & granulomatous disorders (rare); selective B_{12} malabsorption
     - competition for B_{12} — tapeworm; bacteria, blind loop syndrome
     - drugs — PAS, cholchicine, neomycin
     - other — **N_2O**, transcobalamin II deficiency
2. ***folic acid deficiency***
   - i. inadequate intake — alcoholics, teenagers (fads), some infants
   - ii. increased requirements — infancy, pregnancy; malignancy; increased erythropoiesis (chronic haemolysis); chronic exfoliative skin disorders; haemodialysis
   - iii. malabsorption
     - intestinal disease — tropical sprue, non-tropical sprue
     - drugs — phenytoin, ethanol, barbiturates
   - iv. impaired ***metabolism***
     - ↓ dihydrofolate reductase — ***methotrexate***; pyrimethamine, triamterene, pentamidine, etc.
     - alcohol
     - congenital enzyme abnormalities
3. ***other causes***
   - i. drugs which impair DNA metabolism
     - ***nitrous oxide*** — ↓ methionine synthase, ***10-formyl-THF***
     - purine antagonists — 6-mercaptopurine, azathioprine
     - pyrimidine antagonists — 5-FU, cytosine arabinoside
     - miscellaneous — acyclovir, zidovudine, hydroxyurea
   - ii. metabolic disorders — rare
   - iii. unknown aetiology
     - refractory megaloblastic anaemia
     - Di Guglielmo's syndrome (atypical acute non-lymphocytic leukaemia)
     - congenital dyserythropoietic anaemia

Vitamin B_{12}

- structurally similar to porphyrins, with ***cobalt*** in the central position
- minimum daily requirement → ~ 2.5 µg/day
- total body stores ~ 2 mg → ~ 3-6 years supply
- present as ***cobalamin*** and ***hydroxycobalamin***, the later being more persistent
- both are converted to physiologically active forms → methyl & 5-desoxyadenosylcobalamin
- neither may be used therapeutically as chemically unstable
- intestinal absorption in ***terminal ileum*** at specific receptors
- bound to glycoprotein ***intrinsic factor*** secreted by gastric parietal cells
- carried in plasma by ***transcobalamin II*** and stored in liver & tissues with transcobalamin I

Folic Acid

- common name for ***pteroylmonoglutamic acid***
- absorbed in duodenum & jejunum, then converted to ***5-methyltetrahydrofolic acid***
- minimum daily requirement → ~ 50 µg/day
  ~ 200-500 µg/day in pregnancy / disease
- total body stores ~ 5-20 mg → ~ 3 month supply
- in critically ill patients without supplementation, relative deficiency may develop in 3-4 days

  → thrombocytopaenia, hypersegmented neutrophils, macrocytosis

Folate | B_{12} Reactions

- only two important reactions, each using B_{12} as the coenzyme,

1. L-methylmalonyl-CoA ⟶ ***succinyl-CoA*** *methylmalonyl-CoA mutase*
2. homocysteine ⟶ ***methionine*** *methionine synthase*
   - uses 5-methyl-THF as the methyl donor
   - methionine synthase is inhibited by N_2O: Co^{+} → Co^{++}
   - oxidised cobalt is unable to act as a methyl carrier

- ***methionine*** is a dietary constituent, however daily requirements are ~ 2 times the average intake
- in addition to its role in protein synthesis, methionine acts as a precursor to ***S-adenosylmethionine*** (SAM), which is a direct methyl donator in a number of important reactions,

a. noradrenaline → adrenaline

b. synthesis of arachidonic acid

c. myelination of nerves
   ? decreased SAM → subacute combined degeneration of the cord

d. SAM → active formate, + THF → ***10-formyl-THF***

• the product ***10-formyl-THF*** is a precursor to 5,10-methylene-THF which is required for the production of the essential DNA base ***deoxythymidine***
• after administration of N_2O the first detectable changes are a reduction in methionine synthase activity, followed soon after by an interference with DNA synthesis
• the later is manifest by an abnormal ***deoxyuridine suppression test***
• following very prolonged administration, (≥ 4 days), ***agranulocytosis*** is an almost universal result

> ***NB:*** "interference with ***thymidine synthesis*** is to be expected in man after 12 hrs of exposure to N_2O, but may appear within 2h or even less" (Nunn BJA 1987)

• replacement R_X with ***methionine***, providing SAM for methyl transfer should theoretically help
• replacement R_X with ***folinic acid***, (5-formyl-THF), ***cannot*** restore methionine levels, or its products (SAM), but it can restore ***deoxythymidine synthesis***

> ***NB:*** in the presence of B_{12} deficiency, administration of folate will reduce methionine, further reducing ***myelination*** with possible precipitation of neurological sequelae
> → SACD & neuropathy

• the conversion: desoxyuridine → thymidine
requires 5,10-methylene-THF → ***dihydrofolate***
• this is then reduced to THF by ***dihydrofolate reductase***, which is inhibited by,

a. selective bacterial enzyme inhibitors
   i. trimethoprim
   ii. pentamidine
   iii. pyrimethamine
b. methotrexate

• ***folinic acid*** (5-formyl-THF) can be administered orally or parenterally to provide reduced folate, without the requirement for *dihydrofolate reductase*

■ ***Clinical Features***

a. weakness, lassitude
b. sore, atrophic tongue, angular stomatitis, diarrhoea
c. pallor, weakness, jaundice
d. neurological signs
   i. classically posterior columns - joint position & vibration
      + Romberg sign (usually sensory)
   ii. peripheral neuropathy
   iii. ataxia
   iv. weakness
   v. dementia

- ***Investigation***

  a. FBE
  b. serum folate & B_{12}
  c. bone marrow Bx
  d. ***intrinsic factor Ab*** - absorption tests are no longer required

- ***Management***

  a. B_{12} deficient states: hydroxycobalamin 1000 µg monthly, IM
  b. folate deficiency: folate 5-15 mg/day, oral or IV
  c. folate inhibitors: folinic acid 30-60 mg/day

Anaemia of Chronic Disease

1. chronic inflammatory disorders
   i. infection > 1 month
   ii. connective tissue disorders
   iii. malignancy
2. endocrine failure - thyroid, adrenal, pituitary, hypogonadism
3. hepatic failure

- usual [Hb] ~ 9-11 g/dl
- reticulocyte count is normal
- serum iron & transferrin levels are reduced, saturation is normal
- serum ferritin is raised
- hepatic transferrin synthesis is depressed & iron is less readily released from the RES
- the decreased availability of iron stores inhibits erythropoeisis
- also decreased rbc survival ~ 85% normal

■ ***Uraemia***

• multifactorial,

1. major factors
   i. ↓ erythropoeitin
   ii. mild haemolysis
2. minor factors
   i. uraemic toxins
   ii. hyperparathyroidism
   iii. hypersplenism
   iv. folate & iron deficiencies

• rbc morphology → distorted, fragmented cells (schistocytes, burr/helmet/tear-drops)
• linear relationship between haematocrit and creatinine clearance
• ***recombinant erythropoeitin*** results in,

a. improved well-being and physical capacity
b. ↑ VO_2 maximum
c. ↓ LV mass ~ 30% after 12 months

• however, may lead to increased risk of ***thrombosis***, ∴ aim to increase Hb gradually

■ ***Anaemia & Alcoholism***

a. macrocytosis in the absence of anaemia or folate/B_{12} deficiency
b. folate or iron deficiency
c. hypersplenism
d. pyridoxal phosphate deficiency - sideroblastic anaemia
e. haemolysis - Zieve's syndrome
f. blood loss

Haemolytic Anaemias

1. ***extrinsic*** abnormalities
   - i. red cell antibodies - ***immunohaemolytic anaemias***
   - ii. microangiopathic - HUS / TTP, pre-eclampsia, DIC
   - iii. hypersplenism
   - iv. mechanical trauma
     - impact - march haematuria, CPB pump
     - turbulence - artificial valves, calcific stenoses
   - v. direct toxic effect - malaria, clostridial infection
   - vi. hypotonic IV fluids
2. ***membrane*** abnormalities
   - i. hereditary spherocytosis - β-spectrin abnormality
   - ii. spur cell anaemia
   - iii. paroxysmal nocturnal haemoglobinuria
   - iv. rare causes - hereditary elliptocytosis, stomatcytosis
3. ***intrinsic*** red cell abnormalities
   - i. enzyme deficiency
     - hexose-monophosphate shunt - ***G6PD***
     - Embden-Meyerhof (glycolytic) - pyruvate kinase, hexokinase
   - ii. haemoglobinopathies
   - iii. thalassaemias

NB: alternatively, LIGW divides them into intravascular | extravascular

Hypotonic IV Fluids

- normal rbc's do not haemolyse in solutions > 160 mosmol/kg (~ 0.5% saline)
- complete haemolysis occurs at ~ 110 mosmol/kg
- clinically,
   a. solutions > 143 mosmol/kg (0.45% saline) can be infused peripherally
   b. sterile water can be infused by CVC

- ***Arteriopathies Microangiopathic***

1. TTP
   - unknown aetiology
   - may follow Rx with chemotherapeutic agents - mitimycin, cyclosporin
   - characterised by fibrin deposition on surface of damaged endothelium
   - clinical features,

   i. thrombocytopaenia < 20,000

   ii. microangiopathic haemolytic anaemia < 5.5 g/dl in 30%
      - fragmented and nucleated rbc's

   iii. renal failure

   iv. neurological
      - fluctuation in neurological status early
      - later predominant symptoms - confusion, disorientation
        - seizures, hemiparesis, aphasias

   v. ***normal*** coagulation screen

   vi. positive ANA ~ 20%

   vii. diagnosis is ***clinical***

   - most effective management → ***plasmapheresis*** (7 x FFP - XΔ)
   - variable success with steroids, aspirin, FFP, prostacyclin, cyclophosphamide
2. HUS
   - variant of TTP, really a spectrum of disease
   - more common in children & may follow *E.coli* or *Shigella* GIT infection
   - less CNS involvement, predominantly renal failure & haemolysis
3. "TTP-like" syndrome
   - seen with pre-eclampsia, malignant hypertension, scleroderma, transplanatation

- ***Investigation: Intrvascular Haemolysis***

a. FBE - anaemia, reticulocytosis
   - altered rbc morphology
   - marrow can ↑ rbc production 8x, ∴ don't see anaemia until rbc $t_{½\beta}$ < 20 days
   - by this stage reticulocyte count ~ 30%

b. ↓ haptoglobin
   - an alpha-globulin acute phase reactant, normal $t_{½\beta}$ ~ 4 days
   - binds specifically & tightly to ***globin*** moeity → rapid removal by RES
   - levels progressively decline & are undetectable with $t_{½\beta}$ < 17 days

c. ↓ haemopexin - beta-globulin which also binds free Hb

d. ↑ methaemalbumin - formed when Hb combines with albumin
   - occurs when haptoglobin/haemopexin depleted

e. ↑ plasma bilirubin, LDH
- predominantly ***unconjugated hyperbilirubinaemia*** ≤ 2x normal
- associated acholuria & increased urobilinogen excretion
- $LDH_{1/2}$ isoenzymes

f. rbc survival studies
- chromium-51 labelled rbc's

Immunohaemolytic Anaemias

1. ***warm antibody*** immunohaemolytic anaemia
   - usually IgG, occasionally IgA

   i. idiopathic

   ii. lymphomas - Hodgkin's, non-Hodgkin's lymphoma
   - chronic lymphocytic leukaemia

   iii. SLE

   iv. tumours - rarely

   v. drugs
   - α-methyldopa type → warm Ab type
     - Coomb's (+) IgG in ~ 10% taking 2g/d
   - penicillin type → hapten mediated
     - IgG to penicillin-rbc complex
   - quinidine type → "innocent bystander"
     - IgG, IgM to drug-plasma protein complex
     - complex settles on rbc surface (or platelets)

2. ***cold antibody*** immunohaemolytic anaemia
   - IgM rbc Ab's which are associated with acute disease
   - result in agglutination at temperatures < 32 °C, and disagglutination with warming
   - most IgM Ab's fix complement poorly, ∴ haemolysis is ***mild***

   i. cold agglutinin disease
   - acute - mycoplasma infection
     - infectious mononucleosis
   - chronic - idiopathic
     - lymphoma

   ii. paroxysmal cold haemoglobinuria

Investigation AIHA

a. direct Coomb's test - washed patient rbc's versus anti-IgG + C'

b. indirect Coomb's - patient serum versus commercial marker rbc's

- ***Management***

1. removal of precipitating cause
2. corticosteroids
   - ↑ rbc survival time
   - no change in Ab production
   ~ 1-2 mg/kg prednisolone / day
3. immunosuppressive agents
   - cyclophosphamide, azathioprine
   ~ 40% are steroid resistant
4. splenectomy
   - last resort
   - post-splenectomy ***sepsis*** a major concern
5. plasmapheresis is relatively ***ineffective***
6. Mx of associated CVS compromise | Tx as required

- ***Abnormal Haemoglobins***

1. sickle syndromes
   i. sickle cell trait - AS
   ii. sickle cell anaemia - SS
   iii. double heterozygous states
      - sickle β-Thalassaemia
      - sickle C disease - SC
      - sickle D disease - SD
2. unstable Hb variants
   - congenital Heinz body haemolytic anaemia
3. variants with high O_2 affinity
   - familial erythrocytosis
4. M haemoglobins - familial cyanosis

- ***RBC Enzyme Defects***

• the mature rbc retains non-O_2 metabolic pathways,

a. glycolytic pathway → ATP

b. hexose-monophosphate shunt → reduced NAD
→ reduced glutathione
  - acts to protect Hb and membrane lipids from oxidation

c. Rapaport-Luebering shuttle

• glycolytic pathway defects (***pyruvate kinase***) present in early childhood with haemolytic anaemia
• HMP shunt defects (***glucose-6-phosphatase***) decrease available reduced ***glutathione***
• this results in oxidation of Hb sulphhydryl groups, with condensation as ***Heinz bodies***
• ingestion of oxidants may result in acute haemolytic anaemia,

a. sulphonamides, chloramphenacol
b. primaquine, chloroquine, quinine, quinidine
c. methylene blue
d. vit. K
e. nalidixic acid, nitrofurantoin, nitrates

Hereditary Spherocytosis

NB: haemolysis and "prehepatic" hyperbilirubinaemia

- ***Pathogenesis***

1. ***autosomal dominant*** with variable penetrance
2. rbc membrane is abnormally permeable to sodium
   - defect of protein b-***spectrin***
3. increased metabolic work to expel sodium
4. glucose deprivation ∴ leads to rbc destruction

- ***Clinical Features***

1. malaise, abdominal discomfort
2. jaundice, anaemia, splenomegaly
3. spherocytosis, increased osmotic fragility of rbc's
4. raised MCHC > 36 g/dl
5. negative Coomb's test

- ***Hypersplenism***

Def'n: applied to any clinical condition where the spleen removes excessive quantities of circulating cellular elements, criteria for diagnosis,

1. splenomegaly
2. splenic removal of one or more cellular elements
3. normal, or hyperplastic bone marrow
4. evidence of increased turnover of the element concerned

- ***Splenomegaly***

a.	infections	- EBV, CMV, HIV, viral hepatitis - septicaemia, endocarditis, TB, malaria, typhoid, paratyphoid - brucellosis, leishmaniasis, histoplasmosis, trypanosomiasis
b.	infiltrations	- amyloidosis, lipid storage disease - leukaemia, lymphoma, myelofibrosis, polycythaemia rubra vera
c.	autoimmune	- RA, SLE, AIHA, serum sickness
d.	portal hypertension	- cirrhosis, CCF - hepatic, splenic, or portal venous obstruction
e.	rbc disease	- thalassaemia, sickle-cell disease
f.	miscellaneous	- thyrotoxicosis, sarcoidosis

- ***Massive Splenomegaly***

1. common
   i. chronic myeloid leukaemia
   ii. myelofibrosis
2. rare
   i. malaria
   ii. kala azar - visceral Leishmaniasis
   iii. 1° lymphoma of spleen

- ***Moderate Splenomegaly***

1. portal hypertension
2. lymphoma | leukaemia
3. thalassaemia
4. storage diseases

Myeloproliferative Disorders

1. ***chronic myeloid leukaemia***
   - massive splenomegaly & leukocytosis ~ 50,000 - 200,000
   - chronic, relatively indolent phase & the blastic phase which is rapidly fatal
   - characteristic chromosomal abnormality, ***Philadelphia chromosome***

2. ***polycythaemia rubra vera***
   - polycythaemia → PCV > 52% 18 g/dl males
     PCV > 47% 16.5 g/dl females
   - increased rbc mass with ↑ WBC's and platelets ~ 50%
   - pruritis, plethoric facies, retinal vein engorgement
   - symptoms of impaired cerebral blood flow
   - accelerated atherosclerotisis
   - ***thrombotic***, or ***haemorrhagic*** disease
   - splenomegaly ~ 75%
     ± hepatomegaly
   - survival ~ 2 yrs without Rx
     → ~ 10-12 years with
   - Rx: phlebotomy, myelosuppressive therapy (DXRT, hydroxyurea)

3. ***myelofibrosis***
   - fibrosis of bone marrow resulting in ***extramedullary erythropoiesis***
   - mainly the liver and spleen → hepato-splenomegaly
   - thrombotic tendency, haemorrhage is uncommon

4. ***essential thrombocytosis*** ***thrombocythaemia***
   - excessive megakaryocyte proliferation, with platelets ≥ 800,000
   - symptoms resemble PRV, with haemorrhagic or thrombotic complications

- ***Secondary Polycythaemia***

1. chronic ***hypoxaemia***
   - pulmonary disease
   - obstructive sleep apnoea
   - carboxyhaemoglobinaemia, eg. smoking
   - cyanotic congenital heart disease
   - haemoglobinopathies with "left-shift"
2. ectopic ***erythropoeitin*** production
   - renal cell carcinoma
   - hepatoma
   - cerebellar haemangioma
3. reduced plasma volume - diuretics

BLOOD TRANSFUSION

- ***Indications for Transfusion***

1. increase the O_2 carrying capacity of blood → ↑ DO_2
2. increase circulating blood volume, when DO_2 is low

NB: Hct at which transfusion indicated is ***age & disease*** dependent, otherwise healthy patients rarely require transfusion at Hct > 30%, whereas transfusion is usually required at Hct < 21% (RDM)

- ***Compatibility Testing***

1. ABO-Rh typing
   - i. **rbc's** tested with commercial anti-A, anti-B and anti-D (***direct Coomb's***)
   - ii. **serum** tested against A-rbc's and B-rbc's (***indirect Coomb's***)
   - iii. ABO: O ~ 45%; A ~ 41%; B ~ 10%; AB ~ 4%
   - iv. Rh(D): positive ~ 85%; negative ~ 15% ~ 60-70% ***anti-D-positive***
2. antibody screening
   - i. trial transfusion between ***recipient serum*** and commercially supplied rbc's
     - looking for commonly occurring rbc antigens other than ABO-Rh
     - same 3 phases and similar length to cross-match
   - ii. also performed on the ***donor serum*** shortly after collection
     - primarily preventing reactions with subsequently transfused units
3. cross-matching
   - trial transfusion between ***donor rbc's*** and ***recipient serum***
   - i. ***immediate phase***
     - donor rbc's mixed with recipient serum
     - conducted at room temperature, complete in ~ 5 minutes
     - detects ***ABO***, plus MN, P, and Lewis incompatibilities
   - ii. ***incubation phase***
     - incubation of first phase reactions at 37°C in albumin for 30-45 minutes, then in low ionic strength saline for 10-20 minutes
     - promotes aggregation of surface Ag, and reduction in surface (-)'ve charge
     - aids detection of ***incomplete antibodies***, especially ***rhesus***, by the 3rd phase,
   - iii. ***antiglobulin phase***
     - polyvalent ***antihuman antiglobulin*** reacts with incomplete antibodies
     - detects most of Rh, Kell, Kidd and Duffy

- ***Effectiveness of Matching***

1.	ABO-Rh typing	~ 99.8%	compatible	1:500-1000
2.	+ antibody screening	~ 99.94%	compatible	1:1700
3.	+ cross-matching	~ 99.95%	compatible	1:2000

- ***Emergency Transfusion***

1. type O Rh-negative blood
   - universal donor, uncrossmatched blood
   - some type O donors produce high titres of anti-A,B immunoglobulins

     → ***packed cells*** better than whole blood
   - transfusion of > 2 units of whole type O requires continued use until the blood bank determines levels of anti-A/B have declined (theoretically !)
   - continued use of type O results in minor haemolysis & hyperbilirubinaemia
2. type specific, partially cross-matched blood
   - ABO-Rh typing plus immediate phase X-match ~ 5-10 minutes
   - only **1:1000** patients has an unexpected Ab found in full X-match
   - greater risk in previously transfused patients ~ 1:100 unexpected Ab

Effects of Blood Storage

- ***Citrate Phosphate Dextrose + Adenine***

a. Citrate - prevents clotting by binding Ca^{++}

b. Phosphate - pH ~ 5.5, acts as a buffer against the large fall in $[H^+]$ at 1-6°C
? also may increase 2,3-DPG levels

c. Dextrose - allows continued glycolysis & maintenance of ATP

d. Adenine - improves rbc survival by adding substrate for ATP synthesis
- ↑ survival from ***21*** ® ***35 days***

NB: duration of storage set by requirement for ³ **70%** rbc survival 24 hours post-T_X
storage at 1-6 °C slows the rate of glycolysis by ~ 40x

i.	whole blood	~ 430 ml blood & 70 ml preservative	Hct ~ 40%
ii.	packed cells	~ 230 ml blood & 70 ml preservative	Hct ~ 70%

1. ***metabolic effects***
   - ↓ glucose / dextrose / ATP / 2,3-DPG, and ↑ lactate
   - ↑ P_{aCO2}, ↓ pH, ↓ HCO_3^-
   - ↓ Na^+ / ↑ K^+
   - oxidant damage to membranes with ***spherocyte*** formation
   - ↓ 2,3-DPG → ↑ O_2 affinity
   - changes occur earlier & to greater extent in whole blood cf. packed cells
2. ***microaggregates***
   - conventional filters remove particles > **170 µm**
   - aggregates of platelets/fibrin/leukocytes range from 20 to > 170 µm
   - clinical significance of microaggregates debated
   - most would no longer use a micropore filter
   - ***no change*** in the incidence of ARDS

Frozen Storage

- rbc's stored with ***glycerol*** at -79°C survive well
- all glycerol must be removed prior to use & this is difficult and expensive

1. long-term storage of rare blood types
2. safer in patients susceptible to allergic reactions
   - freezing & washing process decreases ***HLA antigens***
3. reduced risk of hepatitis infection ? since questioned
4. low levels of leukocyte & fibrin aggregates safer for massive transfusion
5. normal levels of 2,3-DPG retained, therefore better O_2 capacity

Adsol

- shelf-life extended to 42 days
- contains adenine, glucose, mannitol, and NaCl

Heparin

- used for priming CPB pumps etc.
- anticoagulant, not preservative as lacks glucose
- antocoagulant effect decreases with time due to liberation of thrombogenic substances from the cellular elements during storage, therefore must be used within 24-48 hours

Classification

1. ultrafresh < 24 hours
2. fresh < 7 days
3. stored > 7-35 days

Complications

- ***Hazards of Rapid or Massive Transfusion***

1. impaired O_2 transport
   i. fluid overload / underload
   ii. defective rbc function
   iii. impaired Hb function
   iv. DIC
   v. ARDS
   vi. MOSF
   vii. microaggregates
2. haemostatic failure
   i. dilution - especially platelets
   ii. depletion / consumption
   iii. decreased production
   iv. DIC
3. electrolyte & metabolic disturbance
   i. hyperkalaemia / delayed hypokalaemia
   ii. sodium overload
   iii. acid-base disturbances
   iv. citrate toxicity
   v. hypothermia
   vi. metabolic acidaemia
4. vasoactive reactions
   i. kinin activation
   ii. damaged platelets & granulocytes
5. serological incompatibility
   i. immediate generalised reaction
   ii. delayed transfusion reaction
6. impaired reticuloendothelial function

NB: the majority are related to the type and time of storage
massive transfusion $\geq$ 1 times the patients blood volume

?? over what time-frame $\rightarrow$ 1BV per 24 hours
½BV per 4 hours

▪ ***Oxygen Transport***

• HbO_2 dissociation ∝ pH, Temp., P_{aCO2} and 2,3-DPG

1. ***citrate*** is metabolised to HCO_3^- → ***L***-shift
   - WB & FFP have the greatest effect
2. hypothermia → ***L***-shift
3. stored blood deficient in ***2,3-DPG*** → ***L***-shift
4. CO_2 / H^+ load → ***R***-shift

• good correlation between decrease in rbc 2,3-DPG and $\mathbf{P_{50}}$ after 7 days storage,

i. 2,3-DPG 4.8 µmol/l → 1.2 µmol/l

ii. P_{50} 26.5 mmHg → **18 mmHg**

NB: specific organ hypoxia ***has not*** been demonstrated from low P_{50} transfusion; however, washed rbc's depleted of 2,3-DPG given to patients with anaemic hypoxia, showed ***no change*** in mixed venous P_{vO2} or cardiac output

• recommendations,

1. warm all blood products
2. avoid HCO_3^- administration
3. attempt to use fresh blood in hypoxic, low CO patients
4. use frozen blood if available

• ***microaggregates*** progressively accumulate with storage & potentially decrease gas exchange
• reduced^{++} with micropore filters, however, incidence of ARDS is ***unaffected***

▪ ***Transfusion Coagulopathy***

NB: most important factors are ***volume of transfusion & duration of hypotension differential diagnosis***,

1. dilutional thrombocytopenia
2. low factor V & VIII activity
3. DIC
4. haemolytic transfusion reaction
5. preexisting coagulopathy
   i. aspirin, NSAID's
   ii. anticoagulant therapy
   iii. haemophilia, von Willebrand's
6. hypothermia

■ ***Dilutional Thrombocytopenia***

• total platelet activity in stored whole blood ~ 60-70% after 6 hrs
~ **5-10%** after 48 hrs

• effects of dilution depend upon,

1. initial platelet count
2. risk of haemorrhage depends upon acute versus chronic,
   i. acute loss < 50,000-75,000
   ii. chronic disease < 10,000-15,000
3. volume transfused ~ 2 BV's in children
   - thrombocytopathy with massive transfusion

NB: → baseline & subsequent ***clotting studies***

• Vietnam war studies & experimental data support,

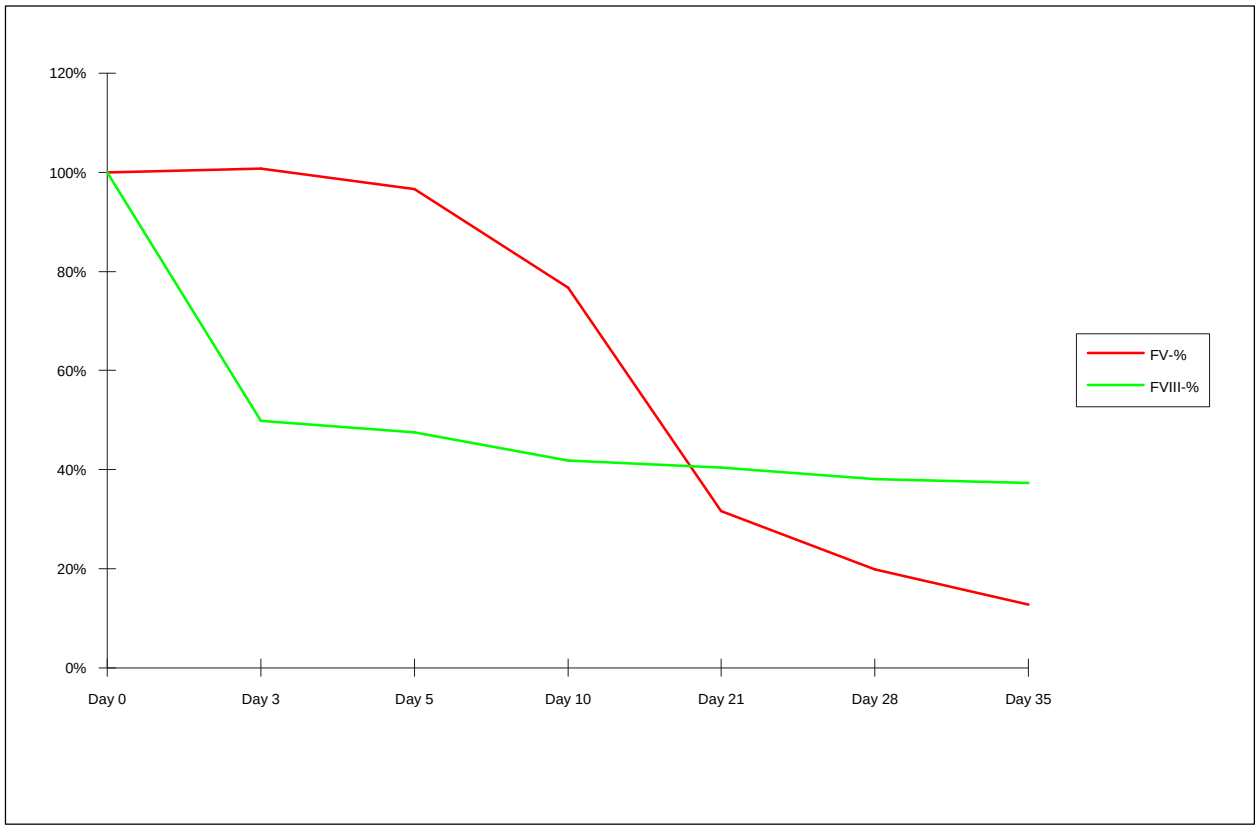
1. ↑ likelihood of a platelet count < 100,000 with > 10-15 unit transfusion
2. bleeding becomes increasingly likely at platelets **< 75,000**

• however, counts do not fall as predicted by haemodilution alone, ? release from marrow & RES
• there is ***no benefit*** in prophylactic administration of platelets in massive transfusion
• therapy should be assessed by laboratory data & clinical evidence of disordered coagulation
• higher counts are required in surgery and trauma
• ***platelet concentrates*** ~ 50 ml and contain ~ 70% of the platelets of a unit of whole blood
• in a 70 kg adult each unit will raise the platelet count ~ 7,000-10,000 / mm^3
• paediatric doses 0.1-0.3 units/kg → ~ 20,000-70,000 / mm^3

■ ***Low Factor V & VIII Activity***

• respectively, these decrease to ~ 15% and 50% of normal activity in whole blood after 21 days
• packed cells contain minimal quantities
• however, only 5-20% F_V and 30% F_{VIII} activity are required for normal haemostasis
• therefore, these factors ***rarely*** decrease below those levels required for coagulation
• concomitant reductions may increase coagulopathy from other sources, ie. platelets
• RDM study giving FFP to 15^+ unit transfusions with disordered coagulation, resulted in ***no improvement*** in coagulopathy, ie. other causes are usually responsible
• criteria for ***FFP administration*** in massive transfusion,

1. generalised bleeding uncontrollable by surgical means
2. APTT > 1.5x normal
3. platelet count > 70,000 ie. correct the platelets first !



NB: data from actual quality control on Red-Cross banked ***whole blood***, Feb '89
F-VIII falls first, but F-V falls furthest

- ***Disseminated Intrvascular Coagulation***

1. relatively uncommon entity
2. microvascular ***thrombosis*** occurs rarely
3. rarely results in specific organ damage or infarction
4. accompanying large vessel thrombosis is not uncommon,
   but is probably ***not*** directly a result of DIC → ie. low flow
5. ***bleeding*** is common, but usually originates from sites of local pathology
6. ***heparin*** is seldom useful and frequently worsens bleeding
7. DIC is associated with a ***high mortality***, 2° underlying disease severity

NB: ? may be regarded as an incidental preterminal event in many patients

- ***Metabolic Effects***

1. ***citrate toxicity***
   - citrate itself is nontoxic → ***hypocalaemia***
     ∝ to citrate content of unit
     ∝ rate of infusion, hyperventilation
   - ≤ 1.5-2.0 ml/kg/min rarely a problem (≤ 1^U/5 min in average adult)
   - FFP has higher % citrate than WB → ≤ 1.0 ml/kg/min
   - decreases in Ca^{++} are ***transient*** and are restored immediately following T_X
   - RDM → $CaCl_2$ ***very rarely*** required
     monitor by ***ECG*** at higher rates
   - factors ↑'g citrate toxicity - hypothermia (↓ metabolism ~ 50%, 37→31°C)
     - hypovolaemia
     - liver disease, transplantation

2. ***hyperkalaemia***
   - usually with whole blood ∝ to the shelf-life of the unit
     ≤ 19-30 mmol/l after 21 days
   - rate of infusion important ≤ 1.5-2.0 ml/kg/min
   - again, $CaCl_2$ administration rarely required & should be based on biochemistry
   - ABP's better for ***neonates*** - check unit [K^+] for neonates
     - monitor by ECG at higher rates

3. ***hypothermia*** → L-shift of HbO_2 curve
   - all banked products stored at ~ 2-6°C and T_X should be warmed 38-40°C
   - ↓ core T < 30°C → ↑'s ***cardiac irritability*** and impairs coagulation
   - decreases of 0.5-1.0°C may induce postoperative ***shivering*** & ↑ VO_2 ~ 400%
   - ≥ 42°C results in rbc destruction
   - warming with ***radiofrequency*** warmers is OK, microwaves result in rbc damage

4. ***acid-base*** * depends upon reason for T_X
   - CPD → **pH ~ 5.5**
   - freshly collected blood pH ~ 7.0-7.1, decreasing to pH ~ 6.9 after 21 days
   - most acid in WB is **CO_2 ~ 150 mmHg** → lungs
   - metabolic acidosis is still present when this is removed by adequate ventilation
   - however, metabolism of citrate generates HCO_3^- and acidosis is rarely a problem providing ***hypovolaemia*** is avoided and liver function is adequate
   - $NaHCO_3$ may have be harmful → use according to AGA's only

Transfusion Reactions

- ***Classification***

1. time of onset → immediate vs. delayed
   - as actual mechanisms are uncertain in many cases, the terms anaphylactic / anaphylactoid are not used → ***immediate generalised reaction***
2. aetiology → immune vs. non-immune

- ***Immune Reactions***

1. ***donor rbc*** serological incompatibility
   i. acute incompatible transfusion reaction / immediate generalised reaction
      → high titre anti-A or anti-B in recipient plasma
      acute haemolytic transfusion reactions
   ii. delayed (X-match compatible) transfusion reaction
2. reactions against ***donor plasma protein*** antigens (eg. F_{VIII} Ab's)
   i. anti-IgA antibodies - selective IgA deficiency
      - IgA deficiency **~ 1:900** / anti-IgA **~ 20-60%**
      - not all patients will have an IGR, but those who react will do so ***repeatedly***
      - use either autologous blood or IgA deficient donors
      - may also have subclass specific anti-IgA, with milder symptoms
   ii. anti-IgG antibodies
   iii. reactions to exogenous donor antigens - dietary, drugs
   iv. serum sickness
3. high titre ***alloantibody*** in donor plasma against recipient
   i. ABO incompatible donor plasma
   ii. high titre atypical rbc alloantibody in donor plasma
      - pregnancy or previous transfusion
      - usually Rhesus or Kell & results in lysis of recipient rbc's
      - interdonor incompatibility
        → screen all plasma for high anti-A/B, or atypical Ab's
        refrain from using ABO incompatible plasma unless unavoidable
   iii. delayed reactions to donor reaginic IgE Ab's (transfer of allergy)
   iv. leukoagglutinins → ***transfusion associated lung injury (TRALI)***
      - plasma from multiparous females, frequently use of FFP post-CPB
4. reactions due to contaminants
   i. plasma "activation" → complement and kininogen/kinin systems
   ii. histamine release in stored blood
   iii. generation of cytokines
   iv. chemical additives

- ***Non-Immune Reactions***

  i. incorrectly stored or out-of-date blood
  ii. inadvertently frozen blood
  iii. overheated blood
  iv. infected blood
  v. mechanical destruction - infusion under pressure

- ***Acute Haemolytic Transfusion Reactions***

1. incidence ~ 1:4000-14,000
2. mortality ~ 1:100,000 (2.5-10%)
3. aetiology ~ 23% **anti-Fy^a** (mainly IgM)
   ~ 18% anti-A
   ~ 12% anti-D
   * complement fixing with direct ***intravascular haemolysis***
4. symptoms & signs
   - fever & chills, nausea, flushing
   - chest pain, dyspnoea, apprehension
   - bleeding diathesis[§]
   - hypotension[§] [§]may be the only signs ***under GA***
   - haemoglobinuria[§]
5. complications
   - anaemia, thrombocytopaenia, DIC
   - haemoglobinuria (? ***acid haematin*** precipitate → ***ARF***)
   - ARDS, MOSF
6. investigations
   i. FBE & film - Hb, platelets, helmet cells, ghosts
      - free Hb, ↓ haptoglobin, urine [Hb]
   ii. APTT, INR, FDP/XDP's
   iii. fibrinogen - not ↓'d with storage, \ ↓ = DIC most likely
   iv. return used unit for re-crossmatch, Ab screen & direct antiglobulin test
   v. sample for culture
   vi. MBA_{20} - K^+, renal function
7. management
   i. cease T_X immediately
   ii. ABC - ↑ F_IO_2 ± IPPV as required
      - maintain BP, volume loading ± inotropes
   iii. maintain urine output ≥ 1.0 ml/kg/hr
      - IV fluids ± mannitol 12.5-50 g
      ± frusemide
   iv. alkalinise urine → pH > 8.0
      • HCO_3^- ~ 0.5-1.0 mg/kg
      • acetazolamide

- ***Delayed Haemolytic Transfusion Reaction***

1.	incidence	~ 1:6000 - F:M ~ 3:1
2.	aetiology	- anti-Jk^a, anti-e, anti-c * non-complement fixing Ab, with removal in ***RES***
3.	symptoms & signs	- may be asymptomatic - usually ~ 1 week - may occur at 2-3 days, or after 1 month - fever & chills, jaundice, haemoglobinuria
4.	complications	- mortality rare - may result in anaemia, ARF
5.	investigations	- anaemia, jaundice, hyperbilirubinaemia - (+)'ve direct Coomb's test
6.	management	- usually no active management required - rare severe reactions managed as above - determine rare or low titre Ab's for future

- ***Nonhaemolytic Transfusion Reactions***

1.	incidence	**~ 2-3%** of all units and up to 8% of patients
2.	aetiology	- Ab's against donor WBC's (HLA or "leukoagglutinins") ~ 2.5 x 10^9 WBC's / unit of blood - Ab's against other plasma protein components
3.	symptoms & signs	- fever, chills, myalgias, nausea, non-productive cough - resembles early onset of haemolytic reaction
4.	investigations	- as for haemolytic reaction - return remaining blood to check matching - rule out occurrence of ***haemolysis***
5.	prophylaxis	- washed rbc's (7-10 days old) - microfiltration - frozen / thawed cells - dextran sedimentation - WBC filters - antihistaminics (H_1 & H_2), antipyretics, steroids

- ***Post-Transfusion Jaundice***

1. ***haemolysis*** - free Hb → ***unconjugated***
   - stored rbc's
   - immunological
2. haematoma reabsorption / associated injuries
3. ***liver disease*** - hypoxia, hypotension → ***conjugated***
   - drugs
   - sepsis
   - post-transfusion hepatitis
   - pre-existing liver disease (Gilbert's ~ 7-10%)
4. ***post-hepatic obstruction***

Infective Complications

NB: donor blood tested for → HBV, HCV
HIV, HIV-2
syphilis (only room temperature storage)
malaria excluded by ***donor history***

- ***Human Immunodeficiency Virus***

- except for triple-washed red cells, the transmission rate from an infected component is **100%**
- 123 cases of transfusion-acquired HIV ***prior*** to testing in May 1985
- **78%** of a cohort of severe haemophilia A patients tested HIV positive in NSW

1. declaration form & private interview - late 1984
2. heat treatment of F_{VIII} by CSL - late 1984
3. ELISA screening of all donors - ***May 1985***

NB: ***no documented*** case of transfusion-acquired HIV since then in Australia

- first 5 years, 1985-90 → 46 positive donors
  overall incidence ~ **1:120,000**
  NSW incidence ~ 1:70,000

NB: USA estimated risk from ***screened products*** ~ 1:40,000

- theoretical risk of donation within the ***"window"*** period remains
- transmission also reported from ***organ donation*** from seronegative donors
- theoretically, seronegative transmission may be detected by antigen (**p24**) testing
- however, large studies have not supported the cost-effectiveness of this method
- presently used in Thailand in an attempt to curb the spread in that country

Hepatitis Viruses

NB: ***most common*** post-transfusion infection,
likely to remain so despite introduction of hepatitis C testing

1. ***hepatitis A***
   - potentially transmissible by transfusion and cases have been reported
   - there is no carrier state and the window of infectivity is small
   - the only effective means of prevention is a screening ***history*** from donors

2. ***hepatitis B***
   - Australia was the first country to test all donors for HBsAg, introduced in 1970
   - prior to HCV screening, still accounted for **~ 5-10%** of post-transfusion hepatitis, despite sensitive screening test
   - ↓ non-A non-B hepatitis with HCV screening will ↑ percentage of HBV cases
   - infective donors are missed due to,

   i. low titre HBsAg

   ii. donation during the "window" period,
   where donor has lost detectable HBsAg but remains clinically infective

   - testing for HBcAb has been advocated, but low specificity and controversial
   - currently in NSW **~ 3:10,000** donations are HBsAg positive
   - incidence increasing with immigration from S-E Asia

3. ***hepatitis C***
   - non-A non-B hepatitis ***commonest*** post-transfusion infection for the past 20 years
   - NSW mid-80's → ~ 1.7% of CABG's transfused got biochemical hepatitis
   - incidence fell by ~ 50% with introduction of ***donor declaration form***
   - HCV identified in **1989**, ? responsible for ~ 90% of non-A non-B hepatitis
   - 2nd generation ELISA tests → ~ 0.3% of donations positive (NSW)
     ~ **0.1%** confirmed by RIBA test

NB: risk is now ***unknown***, but "likely to be so low that it will be difficult to carry out a large enough study for it to be established" AIC 1993

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4. ***delta hepatitis***
   - defective RNA virus, dependent upon HBV for replication
   - may occur concurrently with HBV, ***coinfection***, or ***superinfection*** in a carrier
   - management is through prevention of HBV

5. *hepatitis E*
   - endemic form of non-A non-B hepatitis
   - mode of spread similar to HAV, ie. fecal-oral
   - theoretically transmissible through blood but no reported cases

Cytomegalovirus

- member of the herpes virus family
- geographical prevalence varies from ~ 40-100%
- primary infection usually unnoticed, unless the host is ***immunocompromised***
- most frequent cause of ***death*** in bone marrow transplantation → ***pneumonia***
- may contribute to disease progression and/or activation in ***HIV***
- at risk patients include,
    i. low birth weight & premature neonates
    ii. congenital immunodeficiency syndromes
    iii. splenectomised patients
    iv. those on immunosuppressive chemotherapy
    v. transplant recipients

- managed by transfusion with CMV negative blood, but limited supply due to high prevalence
- ***leukocyte filters*** have been shown to be effective in neonates but are expensive

HTLV-1

- retrovirus related to HIV → T-cell leukaemia ~ 1% of infections
  tropical spastic paraparesis

- endemic within some Aboriginal groups within Australia, and in areas of the Western Pacific
- screening is carried out for donors having been to high risk areas
- pilot study in the NT screening all donors
- no proven transmission in Australia, but 4 donors (+)'ve in the NT and 1 of 212 haemophiliacs found to have evidence of infection
- problems as ELISA screens also get HTLV-II, the pathogenicity of which is unknown

Syphilis

- *Treponema pallidum* is more likely to be present in the serum during the ***seronegative phase***
- routine screening therefore offers limited protection, however it does act as a surrogate test for HIV infectivity
- the organism is destroyed by storage at 4°C, thus ***platelets*** are the likely medium
- there has been no recorded transmission in Australia in the past 20 years

Malaria

- Australian donors are excluded for ***12 months*** following overseas travel
- this is increased to ***24 months*** if chemoprophylaxis was taken
- a recent case of *P. falciparum* malaria in Victoria is believed to be the first case in 20 years
- in transfusion transmitted disease, the ***exoerythrocytic phase*** in the liver is bypassed

    → ∴ relapses ***do not*** occur

- frozen red cells and cell-free blood components have been associated with infection

- ***Other Transmissible Diseases***

1. Chagas' disease - *Trypanosomiasis cruzi*
2. Lyme disease - *Borrelia burgdorferi* (spirochaete)
3. Jakob-Creutzfeldt - 'prion' particles, spongiform encephalopthy
4. toxoplasmosis
5. brucellosis
6. filariasis
7. salmonellosis, typhus, measles

- ***Methods to Reduce Infection Transmission***

1. exclude donors from high risk groups
   - donor declaration form & interview
2. screen all donors for HIV, HBV, HCV & CMV Ab's, VDRL
3. avoid homologous transfusion & transfuse minimal unit requirement
4. avoid multiple donor components unless absolutely required
5. use autologous blood where possible

Leukocyte Transfusion Effects

- ***Beneficial Effects***

1. longer renal graft survival
   - inactivation of alloreactive clones by high-dose immunosupressive therapy
   - induction of suppressor cells
   - induction of anti-idiotypic antibodies
   - improved by donors sharing one HLA-DR Ag
   - largely abandoned following the advent of ***cyclosporin*** therapy
2. graft versus leukaemia effect
   - increase in bone marrow transplant remission rates
   - 1 study only, not supported by subsequent study

- ***Adverse Effects***

1. HLA alloimmunisation
   i. non-haemolytic febrile transfusion reactions
      - most common effect ~ 1% of all transfusions
        ≤ 50% in multi-transfused patients
   ii. refractoriness to random donor platelets transfusions
      - occurs in 30-70% of multiple donor recipients
      - refractoriness may be nonimmunologic → consumption
      - HLA-Ab's present in ~ 50% of multiple donor recipients
      - ***critical immunogenic leukocyte load (CILL)*** for alloimmunisation
2. graft versus host disease in immunosuppressed
3. transmission or reactivation of CMV
4. transmission of HTLV-1
5. generalised immunosupression *suggestive evidence
   i. ↑ postoperative infection rate - including 1 prospective study
   ii. ↑ tumour recurrence - all retrospective studies
   - 5 studies ↑ incidence, 3 equivocal
   - 3 studies no relationship

NB: studies pending assessing effects of leukodepleted blood products

- ***Methods of Leukocyte Depletion***

1. prestorage leukodepletion → centrifugation, washing, freezing & thawing
2. bedside filtration → clinically equally effective to date

- ***Recommendations for Leukodepleted Blood Products***

1. to prevent ***recurrent NHFTR*** $< 5 \times 10^8$
2. prevent/delay ***alloimmunisation*** to HLA-Ag's $< 5 \times 10^6$
3. those presently under investigation
   i. prevention of refractoriness to platelets
   ii. recurrence of febrile reactions to platelets
   iii. CMV infection
4. those where leukodepleted products are ***not recommended***,
   i. GVHD
   ii. acute lung injury due to donor anti-leukocyte Ab's
   iii. reactions or alloimmunisation in patients with limited transfusion exposure
   iv. reactions or alloimmunisation in patients receiving acellular components

METHODS OF HOMOLOGOUS TRANSFUSION REDUCTION

1. reduction of blood loss
   i. surgical techniques
      - diathermy & ligature
      - limb torniquets
      - local vasoconstrictor

   ii. ***anaesthetic techniques***
      - regional anaesthesia
      - controlled hypotension
      - haemodilution
      - pharmacotherapy
2. toleration of a lower haematocrit
3. autologous transfusion
   i. preoperative donation & autologous transfusion
   ii. acute venesection, isovolaemic haemodilution & autologous transfusion
   iii. intraoperative cell salvage
4. dedicated "homologous" transfusion

Toleration of a Lower Haematocrit

- historically a Hct < 30% has been an indication for perioperative transfusion
- O_2 carrying capacity decreases ***linearly*** with Hct, however physiological DO_2 may be maximal at a Hct ~ 30%
- Fortune *et al.* (J.Trauma 1987) conducted a prospective study of trauma patients managed at either a Hct ~ 30 or a Hct ~ 40
   1. no improvement in cardiopulmonary function with a higher Hct
   2. ↑ ***shunt fraction*** in higher group due to greater number of transfusions

- animal data suggest a ***critical Hct* ~ 10%**, below which cardiovascular reserve is exhausted
- Tremper (ASA 1992),
   1. healthy patients with good CVS function tolerate **Hct ~ 20** and below if adequately volume resuscitated
   2. in patients with impaired myocardial function, Hct ~ 30% may be required
   3. signs of CVS decompensation require assessment of need for transfusion

Controlled Hypotension

Def'n: deliberate induction of a MABP ~ 50-65 mmHg

1. reduction of intraoperative ***blood loss***
   - first controlled study by Eikenhoff & Rich 1966
   - most studies → **~ 50% *reduction***
   - variable response, some patients do not respond as expected
   - effects appear to be independent of changes in cardiac output
   - more effective than haemodilution in reducing transfusion requirement
2. improved ***visibility*** of the surgical field
   - may be better monitor than absolute pressure reduction

NB: absolute pressure reduction may be less important than hypotension plus positioning & venous drainage

- ***Indications***

a.	neurosurgery	- aneurysm - tumour resection
b.	orthopaedic	- joint replacement - bone transplant - scoliosis & other extensive back surgery
c.	oncology	- large tumours & exenteration procedures
d.	plastic surgery	- large tumours - head and neck procedures
e.	ENT	- middle ear surgery, rhinoplasty - head and neck tumours
f.	patient refusal of transfusion & anticipated major blood-loss	

- ***Monitoring***

1.	routine	- F_IO_2, S_pO_2, $ETCO_2$, NIBP, ECG, temperature, spirometry
2.	IABP	* ***radial*** not dorsalis pedis - inaccuracies at low MABP with vasodilatation
3.	CVP / PAOP	∝ estimated blood loss & presence of CVS disease
4.	mixed venous P_{vO2} where higher doses of SNP used	
5.	investigational	
	i.	EEG, processed EEG, SSEP's
	ii.	gastric mucosal pH

- ***Methods of Hypotension***

1. controlled haemorrhage
2. regional anaesthesia
3. inhalational anaesthetics
4. vasodilators
   - i. nitrovasodilators - SNP, GTN, hydrallazine
   - ii. ganglionic blocking agents - trimethaphan
   - iii. adrenergic blocking agents - α, α/β
   - iv. adenosine
   - v. PGE_1
   - vi. calcium channel blockers & Mg^{++}
5. central α_2-agonists - clonidine, dexmedetomidine

- ***Organ System Effects***

NB: end-organ effects depend upon,

i. the ***method*** of hypotension (hypovolaemia $\rightarrow \downarrow$ perfusion)
ii. the ***duration*** & ***magnitude*** of hypotension
iii. preexisting end-organ dysfunction

1. ***neurological***
   - assessed by ^{133}Xe clearance, EEG changes, jugular venous P_{vO2}
     $\rightarrow$ no permanent changes in cerebral function
   - current rationale for lower limit for MABP ~ 50-65 mmHg based upon the lower limit of ***cerebral autoregulation***
   - curve shifted to the right in chronic hypertensive patients
   - possibly some advantage using SNP at lower levels of MABP
     $\rightarrow$ better preservation of CBF and BBB function
   - deep isoflurane anaesthesia results in better preservation of cellular P_{O2} values
   - at MAP ~ 50 mmHg, CVO_2 is favourably influenced
   - ***all*** agents may result in increased CBV & ICP, thus should not be used prior to opening of the cranium, unless ICP is monitored
2. ***respiratory***
   - i. $\uparrow$ dead space $\propto$ $\downarrow$ MAP, $\uparrow$ mean P_{AW}, $\uparrow$ head-up tilt
     - prevented by maintenance of CO with ***volume loading***
   - ii. $\uparrow$ shunt $\propto$ $\downarrow$ HPV
     - effects are greatest in ***normal*** subjects, cf. CAL patients $\rightarrow$ little change
     - SNP > GTN >> isoflurane
   - controlled ventilation preferred

3. ***cardiovascular***
   - deep halothane was associated with ↓↓ CO → SNP, GTN, trimethaphan
   - IV agents ***are not*** associated with regional ischaemia in the absence of ***severe stenosis*** → > 40% reduction in resting CBF
   - ***trimethaphan*** may offer some advantage in the presence of severe IHD
   - ***isolflurane*** → ↓ SVR & minimal change in CO
   - Reiz *et al.* 1983 → isoflurane induced coronary steal
   - retrospective & outcome studies show no significance of "steal" during CABG, but ? no direct data relating induced hypotension doses
   - further, episodes of clinical "steal" have usually been ascribed to concurrent hypotension, (Merin, Adv.Anesth.1989)
   - ***adenosine*** also appears effective & safe but requires further testing in the presence of IHD
4. ***renal***
   - RBF/GFR decrease but readily return following hypotension
   - no adverse effects & renal dysfunction is infrequently seen
5. ***gastrointestinal***
   - no portal venous autoregulation & minimal hepatic autoregulation
   - no changes in LFT's at MABP ~ 50-65 mmHg
   - severe changes and centrilobular necrosis seen at MABP < 25 mmHg
6. ***eye***
   - uveal and retinal arterial supplies
   - no precapillary sphincters in the uveal circulation, \ ***pressure passive*** flow
   - changes in MAP directly transmitted to IOP
   - transient visual impairment & rarely blindness may result

- ***<u>Contraindications</u>***

1. longstanding uncorrected hypertension
2. major end-organ dysfunction
   i. cerebrovascular disease
   ii. severe ischaemic heart disease
   iii. hepatic or renal disease
3. peripheral vascular disease
4. uncorrected hypovolaemia
5. severe anaemia

NB: most of these are relative contraindications, depending upon severity, eg. hypotension via GTN is used in the R_X of severe angina !

- ***Complications***

1. mortality ~ 2-10:10,000
   ~ 0.01-0.007% directly related to anaesthesia
   ~ same as for other general anaesthesia (USA figures)
2. CNS - dizziness, prolonged awakening
   - cerebral venous thrombosis
   - cerebral, cerebellar infarction
3. retinal thrombosis
4. renal dysfunction, ARF
5. postoperative bleeding into the operative site

Pharmacological Reduction in Blood-Loss

1. inhibitors of fibrinolysis - epsilon aminocaproic acid
   - tranexamic acid (~ 7x as potent)
   - bind to the same site & inhibit ***plasmin*** activity
   - demonstrated to reduce blood loss post-CABG ~ 10-20%
   - possible fatal ***thrombotic complications***, but none seen in CABG studies
   - contraindicated in suspected DIC or with thrombotic tendency
2. ***aprotinin***
   - naturally occurring ***protease inhibitor*** → plasmin, trypsin, kallikrein
   - high dose therapy may also have a platelet protective effect during bypass
   - exact doses / timing of therapy uncertain, but must be given pre-bypass
   - substantially increases the ACT, \ require ACT > 750s on bypass (N > 400)
   - one study showed reduction from ~ 1500 ml → 300 ml
3. ***DDAVP***
   - synthetic anologue **1-deamino-8-*d*-arginine vasopressin** (ADH)
   - increases both VIII:vWF and VIII:C activity
   - nonspecific increase in platelet activity
   - early reports showed reduced blood loss post-CABG, later reports ***no change***
   - indicated for ***haemophilia A*** and ***type I von Willebrand's disease***
   - not effective in vWD types II & III
   - dose 0.3-0.4 µg/kg - ampoules 4.0 µg/ml
4. ***epogen*** - recombinant DNA ***erythropoietin***
   i. renal failure and other chronic anaemia states
   ii. in combination with preoperative autologous donation programmes
   - efficacy in perioperative haemorrhage requires evaluation
   - significant elevation of reticulocyte count not evident for ~ 1 week
   - very expensive & major side effect is ***hypertension* ~ 50%**

Autologous Transfusion

1. preoperative donation & storage
2. acute preoperative phlebotomy & haemodilution
3. perioperative salvage from the surgical site

▪ *Preoperative Donation & Storage*

1. minimisation of transfusion reactions - excluding ***clerical errors***
2. minimal disease transmission - bacteraemia is an absolute C/I
3. stimulation of ***erythropoiesis*** - hidden benefit
4. long-term frozen storage in patients with unusual antibodies

• requires ~ 72 hours to normalise ***plasma proteins***, therefore last donation should be at least 3 days prior to surgery
• all patients should receive ***iron supplements***
• "high risk" patients are not necessarily unable to donate

NB: it is ***not recommended*** to use a unit of autologous blood unless transfusion actually indicated, due to small incidence of clerical error etc.

▪ *Acute Preoperative Phlebotomy & Haemodilution*

• fast, easy and inexpensive
• less planning than pre-donation
• limited number of units, with decreasing Hct in each
• not suitable for patients anaemic preoperatively
• will also dilute platelets and coagulation factors, therefore avoid with coagulopathy
• volume replacement either with crystalloid (3:1) or colloid
• the estimated ***withdrawal volume*** is given by the estimated blood volume and Hct,

$$V_W \sim EBV \times \frac{H_I - H_E}{H_{AV}}$$

where H_I = initial Hct, H_E = endpoint and H_{AV} = the average

• blood is collected into standard anticoagulant bags, requiring thorough mixing
• may be kept safely,

a. at room temperature ~ 6 hrs
b. refrigerated ~ 24 hrs

- ***Intraoperative Blood Salvage***

1. semicontinuous flow centrifuge → washed cells with a **Hct ~ 60-70%**
2. cannister collection & disposable liner
3. single use, self-contained revision

NB: 2 & 3 → unwashed cells, little data re Hct

- none of these techniques will have functioning ***platelets*** or ***coagulation factors***
- all are relatively contraindicated in the presence of malignant cell or bacterial contamination

Red Blood Cell Substitutes

1. stroma-free haemoglobin SFH
   - i. free Hb → P_{50} ~ 12-14 mmHg
     - prepared by filtration of outdated, lysed rbc's
     - small size of free α/β chains results in ready ***glomerular filtration***
     - plasma half-life ~ 3-4 hours, ∴ limited use
   - ii. ***modified rDNA Hb***
     - 1 amino-acid change on α-chains maintains tetrameric structure
     - longer plasma half-life
     - P_{50} ~ 32 mmHg
     - a solution of 7 gm% has an oncotic pressure ~ 25 mmHg
2. perfluorochemical emulsions PFC
   - inert, immiscible liquids with an O_2 solubility ~ 20x normal plasma
   - emulsified forming suspensions ~ 0.1 µm, but problems with stability
   - content ***linear*** with P_{aO2} therefore require high F_IO_2
   - fluorocrits ~ 2% with a P_{aO2} ~ 500 mmHg → C_{aO2} ~ 1.5 ml%
   - "Fluosol DA 20%" trialed in Japan

NB: both of these solutions are cleared by the reticuloendothelial system, and have effective plasma half-lives of ~ 24 hours

COMPONENT THERAPY

▪ ***Platelets***

1. ***random donor platelets*** - concentrate from a single unit of blood
   - each bag contains ~ 40-70 ml → > 5.5 x 10^{10} platelets
   - stored at 20-24°C and are viable for ***~ 3-5 days***
   - filters with pore sizes < 170 µm remove significant numbers
2. ***single donor platelets*** - collected by plateletpheresis
   - requires HLA matched donor to minimise antigenic differences

• causes of thrombocytopaenia,

a. ***reduced production*** - marrow failure (aplastic), marrow infiltration
   - deficient substrate (B_{12}, folate)

b. ***sequestration*** - splenomegaly, hypothermia

c. ***dilution*** - massive transfusion (≥ 1 BV)

d. ***accelerated destruction***

   i. consumptive - coagulopathy (DIC, PIH, TTP), splenomegaly

   ii. autoimmune - ITP, SLE, lymphoma, HIV

   iii. drug induced - aspirin, heparin (HITS I&II)

NB: → 2 groups, gradual vs. rapid reduction in platelet numbers

• requirement for platelets depends upon ***cause*** and ***rate*** of development
• effects of transfusion variable, depending upon cause & preceding transfusion, $t_{½\beta}$ ~ 10 days,

a. 1 unit of platelets ~ 7,000-11,000 / mm^3 / m^2 SA increase

b. 0.1-0.3 units/kg ~ 20,000-70,000 / mm^3 (standard dose)

• indications,

1. platelet count < 10,000 x 10^9/l * varies between institutions
2. platelet count < 50,000 x 10^9/l + spontaneous bleeding or surgery
3. platelet dysfunction, ***irrespective*** of count + spontaneous bleeding or surgery

• important points,

a. antibody production is ∝ to units transfused

   → limited effectiveness of future transfusions

b. not all hospitals have platelets readily available

c. they should be administered immediately preoperatively

d. they should ***not*** be run through a micropore filter

■ ***Fresh Frozen Plasma***

• 200 ml standard volume contains ***all factors***, including,

1. VIII:C ~ 200^{U} - may be harvested prior to freezing
   - noted on unit label
2. IX ~ 200^{U}
3. fibrinogen ~ 400 mg

• prepared within ***8 hrs***, after which the ***labile factors*** (V/VIII) begin to diminish, stored **-30°C**
• for same reason should be used ASAP upon thawing
• contains proportionally more ***citrate*** than whole blood
• administered as ABO compatible transfusion, volume ~ 200 ml/unit
• ***indications*** for use,

1. isolated factor deficiencies - laboratory proven
2. massive blood transfusion - rarely, when V/VIII activity < 25%
   + INR > 1.8 / fibrinogen > 0.8 g/l
3. reversal of warfarin effect
4. antithrombin III deficiency - thrombotic state
5. immunodeficiency states - source of globulins, IgG not available
6. thrombotic thrombocytopenic purpura
7. haemophilia A - rarely, as require 10-15 U/kg for an acute bleed
   ~ 4-5 units of FFP / 70 kg
8. von Willebrand's disease

■ ***Cryoprecipitate***

• fresh plasma frozen & thawed at 1-6°C → ~ 3% fails to redissolve, the cryoprecipitate
• then warmed to room temperature with **20-50 ml** of supernatant plasma
• ***single donor*** preparation, stored for up to 6 months at **-30°C**
• contains,

1. **VIII:C** ~ 20-85% of the original levels
   ~ 80-120 units / 10-15 ml of plasma, or
   ~ ½ VIII:C activity of FFP in $1/10^{th}$ the volume
   → ~ 120 ml for R_X acute bleed in ***haemophilia A***
2. **VIII:vWF** ~ 40-70% original plasma
3. ***fibrinogen*** ~ 3-10x original plasma / ml
   ~ 150 mg / 10-15 ml of plasma, cf. 200 ml of FFP
   - may result in ***hyperfibrinogenaemia*** in haemophiliacs
   → paradoxical bleeding
4. F-XIII ~ 3-10x original plasma / ml
5. ***fibronectin*** - opsonin

• indications,

1. ***haemophilia A***
   - factor VIII:C deficiency → principal use
   - ***not*** indicated for haemophilia B, as minimal content of factor IX
2. ***fibrinogen deficiency***
   - preferrable to commercial fibrinogen preparations, which are pooled from 500-5000 donations and carry a high infection risk
   - massive transfusion → plasma fibrinogen < 0.8 g/l
   - ***10 units*** increase plasma levels ~ 1 g/l in an adult (N:1.5-4.0 g/l)

■ ***Haemophilia B***

• patients with haemophilia B (IX deficiency) are managed with commercial concentrates which contain F-VII, IX and X
• concentrates are from pooled donor sources and have a greater risk of ***transmissible disease***
• this has now been reduced by heat treating, or ***monoclonal*** production

■ ***Prothrombinex***

• contains factors **II, IX** and **X** → ~ 250^{U} / 10 ml for each factor
• has low levels of VII
• prepared from human donor plasma
• presented as a freeze dried powder, requiring reconstitution with water
• screened for HBV, HBC and heat treated for HIV
• average dose ~ 1 ml/kg for acute haemorrhage, then 0.5 ml/kg each 24 hours

Von Willebrands Disease

• heterogeneous disorder of factor **VIII:vWF** function, three types

1. type I - ↓ VIII:vWF ***concentration***
2. type II - ↓ VIII:vWF ***function***
3. type III - rare, combined disorder with severe clinical symptoms

NB: all are ***autosomal dominant*** except for type III, ***incidence*** **~ 1:800-1,000**

• coagulation studies vary with time and may be ***normal*** when tested,

1. ↑ skin bleeding time
2. normal platelet count
3. may have a small increase in APTT

PLASMA & COLLOIDS

Haemaccel

- synthetic polypeptide plasma volume expander
- **3.5%** ***gelatin*** solution, with the mean MW ~ 35,000-45,000
- gelatin prepared from hydrolysis of ***animal collagen***, cross linked by ***urea bridges***
- plasma expansion by **~ 70%** of infused volume
- renal excretion by GFR complete by 48 hours
- useful as a synthetic plasma substitute & as an insulin carrier

• gelatin	~ 35 g	
• Na^+	~ 145	mmol/l
• Cl^-	~ 145	mmol/l
• K^+	~ **5.1**	mmol/l
• Ca^{++}	~ **6.25**	mmol/l
• HSO_4/HPO_4	~ small amounts	
• pH	~ 7.3	
• osmolality	~ 300-306	mosm/l

- advantages,
  a. cheap, safe, reliable synthetic colloid
  b. low incidence of adverse reactions
  c. renal excretion
  d. long shelf half-life ~ 8 yrs at 15°C
     ~ 3 yrs at 30°C

- disadvantages,
  a. allergic reactions ~ 0.146% **~ 1:650**
     - skin rashes, pyrexia
     - anaphylactoid reaction ? due to ***hexamethylene diisocyanate***
     - renal failure rare
  b. short $t_{½\beta}$ ~ 1.5-6 hrs (x' ~ 3-4 hrs)
  c. renal excretion
  d. Ca^{++} related complications

■ ***Dextrans***

- polysaccharides produced by fermentation of sucrose by ***Leuconostoc mesenteroides*** bacteria
- these are then hydrolysed and fractionated into different molecular weights
- advantages,
    a. stable, cheap, non-toxic
    b. non-pyrogenic plasma substitutes & expanders

■ ***Dextran 40 Rheomacrodex***

- 10% (100g/l) solution in normal saline or 5% dextrose
- average MW ~ 40,000, osmolality ~ 350-370 mosm/kg, ie. ***hypertonic***
- plasma $t_{½\beta}$ ~ 2-3 hrs with ~ 5% being metabolised (70 mg/kg/day)
    i. plasma volume expansion **~ 1.5-2x** infused volume
    ii. thromboembolic prophylaxis ~ 38% ↓ DVT
    iii. rheological microcirculatory benefit
    iv. CPB pump priming

- contraindications,
    i. thrombocytopaenia
    ii. coagulopathy
    iii. hypersensitivity

- problems,
    i. hypervolaemia, circulatory overload, CCF
    ii. anaphylactoid / anaphylactic reactions ~ 0.07% **~ 1:1500**
        - reduced by Promit (0.001%)
    iii. renal failure - renal tubular obstruction

- does ***not*** interfere with blood cross-matching or Coomb's testing, cf. high MW dextran
- maximum dose ~ 30 ml/kg/day

■ ***Dextran 70 Macrodex***

- 6% (60g/l) solution in normal saline or 5% dextrose
- average MW ~ 70,000, osmolality ~ 335 mosm/kg, ie. mildly ***hypertonic***
- plasma $t_{½\beta}$ ~ 6 hrs with ~ 5% being metabolised (70 mg/kg/day)
- problems are the same as for dextran 40, plus, interference with ***haemostasis*** with large volumes
    a. fibrinogen coating
    b. interferes with factor VIII
    c. decreased platelet adhesion and aggregation

    NB: ***does not*** interfere with normal X-match & indirect Coomb's, only enzyme assays

■ *NSA-5% Albuminex*

- heat treated plasma protein solution, was mainly albumin, now marketed as NSA-5%
- prepared from fractionated plasma from pooled human donors
- ***pasteurised*** to kill HBV, HCV, HIV etc.
- shelf-life → **5 yrs** at 2-8°C
  → 1 yr at 25°C
- ***Na^+-octanoate*** is added to stabilise the short chain FFA and heat stabilise albumin
- acetate and citrate 1-2 mmol/l are added
- NaOH is added to bring the pH to 7.0
  - human albumin ~ 50 g/l
  - Na^+ ~ 140 mmol/l
  - Cl^- ~ 125 mmol/l
  - octanoate ~ 8 mmol/l
  - pH ~ 7.0
  - osmolality ~ 300 mosm/kg

- main problem was ***anaphylactoid reactions*** (~ 0.02%), ? heat labile ***pre-kallikrein factor***
- other plasma substitutes include,

a. hydroxy ethyl starch - $t_{½\beta}$ ~ 24 hrs
   - reactions ~ 0.08%

b. fluosol DA

c. FFP

d. NSA-20% *cf. old HSA-conc. which was 25%

Common Intravenous Solutions [1]

Solution	Na^+	Cl^-	K^+	Ca^{++}	Glu	Osm.	pH	Lact.	kJ/l
D_5W	0	0	0	0	278	253	5	0	840
NaCl 0.9%	150	150	0	0	0	300	5.7	0	0
NaCl 3.0%	513	513	0	0	0	855	5.7	0	0
D_4W / NaCl 0.18%	30	30	0	0	222	282	3.5-5.5	0	672
Hartmans	129	109	5	0	0	274	6.7	28	37.8
Plasmalyte	140	98	5			294	5.5	(27)	84
Haemaccel	145	145	5.1	6.25	0	293	7.3	0	0
NSA-5%	140	125	0	0	0		7	0	?
NSA-20%									?
Mannitol 20%	0	0	0	0	0	1,098	6.2	0	0
Dextran 70	154	154	0	0	0	300	4-7	0	0

[1] values in mmol/l, irrespective of common presentation volume

PLASMA EXCHANGE

- ***Rationale***

1. ***removal*** / reduction of circulating toxic factor
   - i. antibodies - monoclonal
     - autoantibodies
     - alloantibodies
   - ii. immune complexes
   - iii. mediators of inflammation
   - iv. chemicals/drugs - where these are highly protein bound
2. ***replacement*** of deficient plasma factors
3. ***potentiation*** of drug action
4. ***immunoregulation***
5. enhanced RES function
6. potentiation of other modes of therapy

- ***Acute Diseases***

1. ***immunoproliferative diseases*** with monoclonal Ab's
   - i. hyperviscosity syndrome - Waldenstrom's macroglobulinaemia
   - ii. cryoglobulinaemia
   - iii. renal failure in multiple myeloma
2. ***autoimmune diseases***
   - i. myasthenia gravis
   - ii. GBS
   - iii. Goodpasture's syndrome
   - iv. SLE
   - v. TTP | HUS
   - vi. rapidly progressive GN
   - vii. coagulation inhibitors
   - viii. autoimmune haemolytic anaemia
   - ix. pemphigus
3. plasma ***factor replacement*** → FFP replacement
   - i. DIC
   - ii. SIRS
   - iii. immunodeficiency states

4. Reye's syndrome - mechanism unknown
5. ***toxin removal***
   i. paraquat poisoning
   ii. envenomation
6. rapid plasma removal & rbc replacement in severe anaemia with CCF/IHD

- ***Complications***

1. ***technical***
   i. vascular access - pneumothorax, arterial puncture
   ii. air embolism
   iii. acute hypo/hypervolaemia - unilateral pump failure
   - incorrect setting
   iv. heat loss - especially children
2. ***circulatory***
   i. hypo/hypervolaemia - need fluid balance chart, daily weight
   ii. vasovagal reactions
   iii. vasoactive reactions
   iv. immediate generalised response
3. ***haemostasis***
   i. require heparinisation unless existing coagulopathy
   ii. altered procoagulant / anticoagulant protein levels
   → variable effects, both haemorrhagic & thrombotic
   iii. decreased antithrombin III & altered response to heparin
4. ***immunology***
   i. frequently pre-existing immunosuppression
   ii. reduction in immunoglobulin & complement levels with repeated exchange
   iii. bacteriacidal & opsonic properties impaired unless FFP used as replacement
   → use 2 units after large or frequent exchange
   iv. risk of post-transfusion infection - hepatitis
5. ***metabolic effects***
   i. disequilibrium syndrome - less than with haemodialysis
   ii. alterations of COP & oedema formation
   iii. altered transport & binding protein levels

HAEMOSTATIC FAILURE

- there are 4 main processes which arrest bleeding post-vascular injury,
    1. smooth muscle constriction / vascular spasm
    2. platelet adhesion / aggregation - primary haemostasis
    3. coagulation - secondary haemostasis
    4. finbrinolysis & re-endothelialisation

Platelet Function

- non-nucleated cytoplasmic fragments derived from ***megakaryocytes*** ~ 2-4 µm diameter
- average lifespan ~ 8-10 days, with about 30% sequestered in the spleen
- platelet factor nomenclature is essentially obsolete, but,
    a. PF3 - platelet ***phospholipid*** procoagulant activity
    b. PF4 - cationic alpha-granule protein (neutralizes heparin)

- platelet haemostatic function is divided into 3 phases,
    1. ***adhesion***
        - binding of vWF to GPIb → ***GPIb-vWF complex***
          \+ vWF to exposed collagen
        - some additive effect from GPIIb / GPIIIa
    2. ***aggregation***
        - contact with collagen & thrombin → ADP & serotonin
          → formation of TXA_2
        - TXA_2 → vasoconstriction
          fibrin deposition
          platelet aggregation
        - aggregation is mediated by GPIIb / GPIIIa and a fibrinogen link
        - aggregation ***does not*** occur in the absence of ***fibrinogen*** or divalent cations
    3. ***secretion***
        - release of procoagulants and ligands from alpha and dense granules results in further activation and platelet adhesion

        i. granule contents - PF4 (heparin inhibitor)
        - fibronectin, thrombospondin
        - platelet derived growth factor
        - fibrinogen, plasminogen, factors V, VIII, and vWF

        ii. arachidonic acid - PGG_2, PGH_2, TXA_2

Platelet Disorders

- a satisfactory platelet plug will not be formed if,
  1. there are ***too few*** platelets
  2. they are ***functionally inert*** - storage > 3 days
     - CPB
     - aspirin, uraemia, alcohol
     - congenitally impaired
     - low fibrinogen, F-VIII

- causes of ***thrombocytopaenia***,
  a. reduced production
     - marrow failure/infiltration - aplastic anaemia, neoplasia, severe sepsis
     - folate / B_{12} deficiency
     - drugs, chemicals, radiation
  b. reduced survival
     i. Ab induced - ITP, SLE, CLL, haemolytic anaemias
        - drugs: quinine, quinidine, sulphonamides, β-lactams
     ii. Ab independent - prosthetic valves, DIC, TTP, hypersplenism
  c. dilution - massive transfusion
  d. sequestration - hypothermia, hypersplenism

- ***Clinical Sequelae***
  1. < 100,000 - abnormal bleeding time
     - abnormal Hess Test
  2. < 80,000 - prolonged bleeding with trauma or surgery
  3. < 40,000 - spontaneous ***purpuric*** lesions
  4. < 20,000 - spontaneous bleeding (haematemasis, epistaxis, ICH)

  NB: the characteristic feature is bleeding immediately following injury

- ***Assessment***
  a. FBE / platelet count
  b. bone maorrow biopsy
  c. ***Hess test*** - torniquet at MAP for 5/60
     - petechiae within 3 cm area of forearm (N < 10, ABN > 20)
  d. bleeding time
     - has not been shown to be an accurate predictor of surgical bleeding
  e. platelet aggregation studies / secretion studies

- ***Management***

a. platelet concentrate ~ 2-3 day half-life
- 6 units ~ 30-40,000/µl

b. DDAVP - high affinity for V_2 receptors (V_1 = smooth muscle)
- clinical uses - CRF, cirrhosis, vWD, platelet defects
- postop. cardiac and orthopaedic
- increases - factor VIII/vWF complex ~ 3-5x
- tissue plasminogen activator
* released from endothelial stores, ∴ ***ceiling effect***

c. fibrinolytic inhibitors
i. amicar ~ 15 mg/kg/hr (EACA ~ 1 g/hr)
ii. tranexamic acid ~ 10 mg/kg q8h
- these may be given following DDAVP to reduce the effects of tPA
- NB: tPA results in platelet activation

d. ***treat underlying cause***

e. ***adequate surgical haemostasis***

- ***Renal Failure***

- abnormalities include,

a. platelet adherence

b. platelet aggregation

c. vasoconstriction

d. mild thrombocytopaenia

- DDAVP will correct the abnormality, the effect lasting ~ 4-12 hrs
- cryoprecipitate is also effective, lasting ~ 24-36 hrs
- conjugated oestrogens, ***Premarin***, 0.6 mg/kg/d for 5 days may improve platelet function for up to 3-14 days

Antiplatelet Agents

▪ ***Aspirin***

• ***irreversibly*** acetylates and inactivates platelet and megakaryocyte cyclo-oxygenase
• inhibits TXA_2 production and subsequent,

a. platelet aggregation
b. vasoconstriction

• effect on the bleeding time lasts up to 5-7 days
• its effects on ***endothelial cyclooxygenase*** are transient, lasting only 2-4 hrs, due to,

1. lower affinity of aspirin for endothelial isoenzyme
2. rapid regeneration of the enzyme

• clinical indications,

a. prevention of myocardial infarction - unstable angina
- post-AMI
b. prolong patency of CAVGs following surgery
c. prevention of thromboembolic complications of cardiac valve disease
d. reduction in incidence of CVA/TIA's in patients with carotid/vertebrobasilar disease
e. prevention of vascular occlusive disease in the limbs

• usual dose range ~ 100-325 mg/day
• 30-75 mg/day is equally efficacious in prevention of TIA/CVA as higher doses
• side-effects,

a. peptic ulceration / GIT haemorrhage
b. asthma
c. angioneurotic oedema

▪ ***Other Agents***

1. dipyridamole
   - PDE inhibitor which increases platelet cAMP
   - often combined with aspirin due to additive effect
   - "little evidence to support use of this agent alone, or in combination with aspirin"
2. dazoxiben
   - along with other ***thromboxane synthase*** inhibitors, less effective than aspirin

3. ***ticlopidine***
   - potent inhibitor of ADP induced platelet aggregation
   - also inhibits collagen, adrenaline, arachidonic acid and thrombin platelet effects
   - primary and secondary prevention of CVA's and thromboembolic complications
   - in previous CVA/TIA patients, reduces subsequent stroke, AMI and death
   - currently used for CVA prevention in ***aspirin intolerant*** patients
   - side effects - neutropaenia, thrombocytopaenia, pancytopaenia
     * readily reversible
     - cholestatic jaundice

Coagulation Disorders

- sequential activation of the coagulation cascade results in the formation of ***thrombin***, with the generation of ***fibrin*** from fibrinogen
- this self-polymerising species is then converted by cross-linking of strands by ***factor* $\mathbf{XIII_a}$**
- abnormalities of this step may be due to,

1. congenital deficiencies - haemophilia A & B
2. acquired deficiencies
   i. anticoagulant therapy/overdose
   ii. vitamin K deficiency
   iii. liver disease, malnutrition
   iv. complex acquired coagulopathies - DIC, massive transfusion, dilution
     - CPB
     - liver transplantation

Normal Coagulation

NB: the "classical" division of coagulation into ***intrinsic & extrinsic systems*** is not applicable to humans *in vivo*,

1. no coagulopathy, nor disease state, is associated with deficiencies of several of the proteins of the ***intrinsic system***
2. ***thrombin*** generation is via
   i. tissue factor, factor VII, factors IX and X
      - note, VII_a activates both IX & X, $\therefore$ IX deficiency clinically significant
   ii. an absolute requirement for ***platelet phospholipid***, ***VIII:C*** and ***V*** as cofactors
3. activation of ***factor XII*** to its fragments (α-XII_a & β-XII_a) does not primarily promote clotting via the activation of XI to XI_a, rather β-XII_a maintains vessel ***patency*** by,
   i. activates prekalikrein $\rightarrow$ kallikrein, with formation of ***bradykinin***
   ii. activates plasminogen $\rightarrow$ ***plasmin***

- ***Critical Events***

1. the binding of ***von Willebrand Factor*** to the exposed ***subendothelium***
   - this may be deficient due to,
   i. diminished levels of vWF (vWD - type I)
   ii. structural abnormality of vWF, or (vWD - type IIa, IIb)
   iii. abnormality of collagen
2. subendothelial bound vWF exposes & binds multiple glycoprotein platelet receptors (**GPIb receptors**)
   - the **vWF-GPIb** interaction is probably central to many surgical coagulopathies
   - manipulation of this event is the likely 1° role of ***aprotinin***
   - this step fails when,
   i. too few platelets $< 50{,}000 \rightarrow$ impairment of surgical haemostasis
   ii. circulation failure - demargination is seen at PCV < 20%
   - functional dilution by blood flow
   iii. lack of GPIb - arises during CPB due to ***proteolytic degradation***
   - platelet ***storage*** > 3 days
   - ***Bernard-Soulier*** syndrome
   iv. GPIb dysfunctional
   - abnormal compound - myeloma, ITP
   - dextran infusion
   - hypofibrinogenaemia

NB: the next 2 steps of haemostasis,

1. generation of the ***platelet plug***, and
2. solidification of that plug by ***coagulation***,

are completely dependent upon ***adhesion*** of platelets to the site of injury

NB: Murphy *et al*. (BJA 1993) state that the ***bleeding time*** is the only practicable test of this axis, although it has poor predictive value as a ***screening test***, in the patient with clinically manifest coagulopathy it may be a useful indicator (??)

- ***Anticoagulant Mechanisms***

1. antithrombin pathways
   i. ***antithrombin III***
      - α_2-globulin synthesized by the liver, $t_{½\beta}$ ~ 70 hrs
      - principal antagonist of the serine proteases - XII_a, XI_a, X_a, IX_a, VII_a
        - thrombin, plasmin & kallikrein
      - accounts for ~ 85% of the plasma inhibition of ***thrombin***
      - ***heparin*** binds to lysine on ATIII $\rightarrow$ $\uparrow$ protease inhibition, especially $\mathbf{X_a}$
      - ATIII $t_{½\beta}$ is reduced markedly by heparin, as complex removed by RES
      - this probably accounts for resistance to anticoagulation with prolonged therapy
   ii. ***proteins C & S***
      - protein C activated on endothelium, with prothrombin & ***thrombomodulin***
      - factors V_a & VII_a are rapidly inactivated by C_a
      - protein S acting as a cofactor to protein C
2. extrinsic pathway inhibition $\rightarrow$ VII_a-thromboplastin complex inhibitor
3. fibrinolytic system
   i. tPA released by endothelial cells & incorporated into fibrin clot
   ii. fibrinogen-bound plasminogen $\rightarrow$ ***plasmin***
   iii. plasmin cleaves several proteins - fibrinogen & fibrin
        - factor VIII:C and platelet GPIb

Routine Tests of Coagulation

1. ***bleeding time***
   - i. Simplate II - modified Ivy technique
     - torniquet @ 40 mmHg & standard template incision
     - normal range **< 9 *minutes***, operator dependent
   - ii. Duke or Ivy - less reproducible than Simplate II
2. ***platelet count*** ~ 150-400 x 10^9/l
3. ***thrombin time*** - normal range 14-16s
   - tests final conversion of ***fibrinogen*** ® ***fibrin***
   - bypasses intrinsic & extrinsic systems, and is abnormal in,
   - i. afibrinogenaemia, hypofibrinogenaemia, dysfibrinogenaemia
   - ii. heparin therapy - corrects with protamine
   - iii. elevated FDP's - partially corrects with protamine
4. ***international normalised ratio*** / prothrombin time
   - tests the ***extrinsic pathway***, normal range ~ 13-17s
   - platelet poor citrated plasma is recalcified & brain ***thromboplastin*** added
   - time taken to clot is measured as a ratio of control reagent
   - standardised control reduces inter-laboratory variation
   - recommended Australasian Reference Thromboplastin, ART
   - i. VII deficiency
   - ii. liver disease, warfarin therapy, vitamin K deficiency
5. ***activated partial thromboplastin time***
   - normal range ~ 25-35 s
   - screens for coagulation factor deficiency, except **VII & XIII**
   - recalcified, platelet poor citrated plasma, plus an activator & platelet substitute
   - varies with reagents used and laboratory
   - interpret with clinical findings and prothrombin time
   - i. factor deficiency → corrected by the addition of normal plasma
   - ii. factor inhibitor → not corrected by normal plasma
   - iii. heparin therapy → therapeutic range ~ 1.5-2.5 x baseline
6. ***fibrin/fibrinogen degradation products***
   - blood collected into a tube containing thrombin & a fibrinolytic inhibitor
   - latex agglutination test against ***fibrinogen-related Ag*** in serum
   - standard FDP's don't differentiate between 1° and 2° fibrinolysis
   - XDP's measure ***D-dimer*** which indicates fibrinolysis after fibrin formation
   - i. ↑ FDP & XDP - local lysis of fibrin, DIC
     - malignancy, systemic infection, SIRS
   - ii. ↑ FDP - primary fibrinolysis
   - iii. normal XDP's help exclude ***pulmonary thromboembolic disease***

7. ***fibrinogen*** - N: 1.5-4.0 g/l
   - based on either thrombin clotting time, heat precipitation or immunological methods
   - discrepancies between ***functional*** and ***immunological*** methods found in the presence of FDP's and dysfibrinogenaemia

   i. ↓ production
      - hereditary a/hypo-fibrinogenaemia
      - liver disease
      - severe malnutrition syndromes

   ii. ↑ consumption
      - DIC
      - fibrinolysis

8. ***factor VIII / vWF***

   i. VIII:C - biological ***activity*** of factor VIII in procoagulant assay

   ii. VIII:Ag - antigenic determinants of VIII by immunoradiometric assay

   iii. vWF:Ag - antigenic determinants of vWF by immunoradiometric assay

   - vWF forms the dominant portion of circulating VIII:vWF/C **~ 50:1**
   - circulates in large ***multimeric*** forms, which are essential for platelet adhesion
   - ***ristocetin*** facilitates binding of vWF to platelets & aggregates normal platelets
     → no effect in vWD

9. ***thromboelastography***
   - functional assessment of the entire coagulation cascade & fibrinolytic system
   - results may take up to several hours
   - requires multiple samples run sequentially throughout procedure
   - frequently require treatment prior to availability of results

10. euglobulin lysis time
    - normal range > 90 minutes
    - ↓ time reflects the presence of activators of the ***fibrinolytic system***

Common Coagulation Disorders		
APTT -	**INR** -	• usually acquired • liver disease, oral anticoagulants, DIC • ¯ **II, V, X**
APTT -	**INR** «	• ↓ VIII:C, IX - haemophilias • ↑ ATIII - heparin • ↓ VIII:vWF
APTT «	**INR** -	• mild liver disease • early in oral anticoagulant use • ↓ VII - rare congenital deficiency

Coagulation Defects

1. ***congenital***
   - i. x-linked recessive - haemophilia A
     - haemophilia B
   - ii. autosomal dominant - von Willibrand's disease
     - factor XI deficiency
     - disfibrinogenaemias
   - iii. autosomal recessive - factor I, II, V, VII & X deficiencies
2. ***acquired***
   - i. massive transfusion - dilution
   - ii. DIC - consumption
   - iii. vitamin K related
     - dietary deficiency & malabsorption syndromes
     - inhibition - oral anticoagulants
       - salicylate intoxication
   - iv. liver disease
   - v. heparin
   - vi. heparinoids

Surgical Acquired Coagulopathies

- ***Predisposing Factors***

1. inadequate haemostasis
2. sepsis
3. hypoxia
4. hypothermia
5. severe tissue damage
6. massive blood loss or prolonged hypotension
7. cardiopulmonary bypass CPB
8. pre-existing liver disease, liver transplantation
9. obstetric complications - AFE
   - abruption
10. pre-existing bleeding diathesis- vWD, thrombocytopaenia
    - anticoagulation, aspirin

- ***Hypovolaemic Shock / Massive Transfusion***

• diagnosis is based mainly upon ***clinical grounds***, with supporting laboratory data
• 2 underlying mechanisms,

1. dilution of platelets and coagulation factors
2. consumption 2° activation by tissue factor & tPA released from traumatised tissues

NB: ***dilutional thrombocytopaenia*** is the most frequent cause,
often becoming apparent at transfusions > 1 BV and platelets < 100,000 x 10^6/mm^3
the platelet count ***does not*** determine the functional integrity of platelets

• ↑ INR and APTT in the absence of DIC is usually due to ***hypofibrinogenaemia***
• the presence of DIC leads to loss of other factors (V & VIII:C)
• RDM states that fibrinogen is not low in stored blood, \ ↓ fibrinogen = consumption / DIC
• this is supported by data from Red Cross BB, virtually no loss of fibrinogen with storage of whole blood, however if transfused large quantities of ***packed cells*** + crystalloid then this may become significant

NB: all agree the use of prophylactic FFP or platelets in ***massive transfusion***,
in the absence of clinical & laboratory evidence of coagulopathy, is ***not justified***

▪ ***Disseminated Intravascular Coagulation***

- non-localised activation of the coagulation and fibrinolytic systems
- trigger varies, but the universal pathology is circulating ***phospholipid*** → coagulation activation
- this may be manifest primarily as a,

1. haemorrhagic disorder - loss of platelets & soluble clotting factors
   - especially ***fibrinogen***, V and VIII:C
2. thrombotic disorder - distal gangrene & organ infarction
3. mixture of both

- ***heparin therapy*** is based on the premise that inhibition of ***thrombin*** will,

1. reduce the consumption of fibrinogen, other clotting factors and platelets
2. reduce both the thrombotic tendancy and the haemorrhagic disorder

NB: there have been ***no trials*** which support this view,
in several studies the heparin treated group have had a ***worse outcome***

- treatment is therefore aimed at,

1. correcting the ***underlying pathology***, ie. removing circulating phospholipid, and
2. replacement component therapy

NB: there is no compelling evidence that administration of clotting factors & platelets ***increases*** the incidence of thrombotic complications with DIC

- other treatments which may become viable include recombinant antithrombin III and protein C

▪ ***Liver Transplantation***

a. complex coagulopathy from procedure itself

b. preoperative ***liver dysfunction*** → ↓ II, V, VII, IX, X, XI and fibrinogen
   → ↓ plasminogen, α_1-antiplasmin
   → ↓ proteins C & S, antithrombin III

c. ***hypersplenism*** - some patients

d. ***massive transfusion*** - some patients

NB: a low grade DIC or ***consumptive coagulopathy*** frequently exists,
due to decreased hepatic clearance of activated coagulation factors

- significant ***fibrinolysis*** may occur during the ***anhepatic phase*** due to,

1. increased release of tPA from hypoperfused distal tissues (?? why)
2. lack of hepatic α_1-antiplasmin

- ***aprotinin*** is effective in limiting the coagulopathy with orthoptic liver transplantation
- earlier studies suggesting reduced blood-loss with antithrombin-III have not been supported

Cardiopulmonary Bypass

• recent studies have shown large doses of ***aprotinin*** reduce blood-loss associated with CPB
• originally studied in the 60's & 70's with no significant effect, but using much smaller (~ 50%) doses than present studies
• Royston 1987 reported a significant reduction in blood-loss associated with CPB for repeat valve replacement procedures
• the aim of this study was to assess the effects upon postoperative ***pulmonary*** function, the results on blood-loss were unexpected
• other studies have extended these findings to patients with,

1. septic endocarditis
2. recent aspirin ingestion

• detrimental effects of CPB on haemostasis include,

1. platelet dysfunction / consumption
   i. loss of membrane structure & granule contents
   ii. generation of activation markers on the cell surface
2. activation of the fibrinolytic & contact systems
3. activation of granulocytes → degranulation

• the likely, not proven, site of action of aprotinin is platelet ***membrane GPIb***

a. loss of GPIb is one of the early events during CPB which is prevented by aprotinin
b. GPIb contains the binding site for ***thrombin***-induced platelet activation
c. enzymatic hydrolysis of GPIb may result in platelet activation

• GPIb is a transmembrane hetrodimer, readily cleaved by plasmin, elastase and calpain
• all of these are direct ***platelet agonists*** and are inhibited by aprotinin,

1. ***plasmin*** - activity 2° tPA or contact system activation
2. ***elastase*** - generated from activated neutophils during CPB
   - inhibition requires greater concentrations cf. plasmin
3. ***calpain*** - cysteine protease present on thrombin stimulated platelets
   - ? also plasmin stimulated platelets

NB: inhibition of tPA-induced plasmin on the platelet surface could account for much or all of the observed effect

Ruptured Aortic Aneurysms

• ***mortality*** is strongly associated with coagulopathy and uncontrollable haemorrhage
• of those who reach hospital the mortality ~ 21-70%, mean ~ **50%**
• postoperatively, haemorrhage and MOSF are the major causes of death
• coagulopathy *per se* is associated with other factors which increase mortality,

1. increased time for resuscitation
2. more extensive surgical procedures
3. larger transfusion requirement
4. renal failure

NB: however, coagulopathy itself increases risk, being due to either,

i. DIC
ii. dilution of platelets and procoagulant factors
iii. a combination of both

• patients presenting appear to fall into 2 groups, one with a relatively good prognosis, the other with a mortality ~ 70-100%
• Bell *et al.* (Transfusion Med.1991) in a prospective study, took admission coagulation screens on 23 consecutive acute AAA's,

a. 6 of 13 patients with abnormal screens died
b. 0 of 10 with normal screens died

• these findings have been supported by other studies, with 4 of 4 and 11 of 15 dying
• it ***has not*** been demonstrated that early correction of the coagulation abnormality in these patients will improve survival
• previous attempts to avert the coagulopathy of massive transfusion with platelets & FFP have been unsuccessful

NB: early & aggressive attempts to reverse ***tissue hypoxia*** probably offer the best chance of preventing the coagulopathy and improving survival in this patient group

Fibrin Glue

• prepared as a 2-part solution of ***fibrinogen*** and ***thrombin***
• direct application onto the bleeding site bypasses the physiological requirements for haemostasis
• may delay nerve and bone repair
• other complications, viral transmission, adhesion formation and unwanted thrombosis remain theoretical
• evidence of efficacy best demonstrated in the presence of congenital or acquired disorders
• recent large prospective trial comparing fibrin with conventional topical haemostasis showed 90% success cf. 12.4%

ANTICOAGULANTS & THROMBOLYTICS

Heparin

• physiological effects include,

1. anticoagulation
2. lipoprotein lipase release
3. aldosterone antagonism ? due to drug carrier, not heparin itself

• heterogeneous sulphated mucopolysaccharide, MW ranging from 3,000 to 30,000 (x ~ **15,000**)
• no inherent anticoagulant activity in the absence of functional ATIII
• binds the ***lysyl*** residue of ATIII, rendering the ***arginine*** at the active site more accessible to the ***serine*** residues of the active serine proteases of the coagulation system

→ accelerates the ***rate*** of formation of the serine protease/ATIII complex

a. predominant action on $\mathbf{II_a}$, $\mathbf{X_a}$ and $\mathbf{IX_a}$
   - inhibition of thrombin requires binding of both thrombin & ATIII to heparin
   - inhibition of X_a requires binding of only ATIII to heparin
   - the ***LMW heparins*** are unable to bind both thrombin & ATIII,
     ∴ they are only able to catalyse the inhibition of X_a by ATIII

b. effects on XII_a and XI_a are weak

c. minimal effects on VII_a

d. factors I, V, VIII, are ***not*** directly affected
   - VIII levels may actually rise with heparin Rx due to,

   i. reduced ***thrombin*** activation of VIII

   ii. destruction of $VIII_a$ by thrombin-thrombomodulin activated ***protein C***

• plasma ATIII levels fall ~ 30% with infusion, due to reduced plasma $t_{½\beta}$
• has no effect upon ***fibrinolysis***

• releases tissue-bound ***lipoprotein lipase*** into the blood-stream

→ ↑ triglyceride hydrolysis of chylomicrons

• aldosterone suppression is 2° to the ***antiseptic*** in the comercial preparation, not due to heparin itself
• other effects include,

1. inhibition of ***platelet function*** & prolonged bleeding time
2. increased ***endothelial permeability***
3. inhibition of delayed hypersensitivity

- ***Indications***

1. deep venous thrombosis
2. anticoagulation in first 12 weeks of pregnancy
3. prevention of thromboembolic disease
   i. AF
   ii. CCF
   iii. prosthetic heart valves, mitral stenosis
   iv. post-op. - major orthopaedic or abdominal surgery
4. prevention of arterial (or mural) thrombus
   i. large AMI
   ii. post-thrombolysis for AMI
   iii. unstable angina
   iv. post-embolectomy
5. prevention of extra-corporeal thrombus
   i. haemodialysis, haemoperfusion, haemofiltration, plasmapheresis
   ii. balloon counterpulsation
   iii. cardiopulmonary bypass

- indications for ***low dose heparin***,

1. post-operative
   - major orthopaedic or abdominal surgery
2. prolonged bed rest
3. previous history of thromboembolism
4. age > 40 years and
   i. obesity
   ii. CCF
   iii. neoplasia

- ***Administration***

1. ***unfractionated*** heparin
   - i. loading dose ~ 70 U/kg
   - ii. infusion ~ 20 U/kg/hr
   - iii. APTT ~ 1.5-2.5x control
   - resistance occurs in - acute PTE
     - inflammatory & malignant disorders
     - infusions of GTN
   - platelet counts monitored if used for > 7 days
   - half-life is dose-dependent, predominantly cleared by RES, plus liver ***heparinase***
2. ***low-dose*** unfractionated heparin
   - review of multiple randomised trials in different surgical groups,
     - overall postoperative incidence of DVT **~ 20%**, reduced by 66%
     - incidence of PTE **~ 2%**, reduced by 50%
     - significant reduction in deaths 2° to PTE
   - both bd and tds 5000^{U} regimens appear equally effective, ∴use lower dose
   - due to circadian alteration of coagulation, bd at 0600 & 1400 may be more effective
   - inhibitory effect on X_a occurs at a lower dose & may be mechanism of effect
   - has ***not*** been shown to be effective for elective joint replacement surgery

     → oral warfarin regimens, INR ~ 1.5-2.0
3. low molecular weight heparin
   - mixture of heparins, MW ~ **3000-9000**
   - enhanced inhibition of X_a with relatively little thrombin inhibition
   - ∴minimal effect on APTT, effect measured by ***anti-X_a activity***
   - elimination $t_{½\beta}$ ~ 18 hrs, ∴single daily dose 2,500-5,000^{U} sc is therapeutic
   - *in vivo* haemorrhagic side-effects are similar cf unfractionated heparins
   - like HMW, LMW heparin has been associated with HITS

- ***Side Effects***

1. haemorrhagic complications
   - **~ 4%** of patients receiving anticoagulant doses
   - rapid reversal with ***protamine*** 1 mg / 100^{U} heparin activity
2. ***heparin-induced thrombocytopaenia***
   - i. non-immune < 15% of patients
     - especially MW > 20,000, induces platelet ***aggregation*** in disease states
     - thrombocytopaenia is usually mild and transient

ii. ***immune*** ~ **3-5%** of therapeutic patients
~ 0.3-1.0% of low dose patients

- heparin-dependent platelet membrane **IgG** Ab
- rarely IgM, IgA-IgG
- rarely seen if treated < 7 days
- higher incidence with heparin extracted from ***bovine lung*** (16%), cf. porcine intestinal heparin (1-5%)
- circulating heparin may bind ***all*** IgG, ∴aggregation tests may be normal
- some suggest repeating tests > 4 days following cessation of heparin
- platelet count usually returns within 4-7/7, but Ab persists for up to 6/12
- has been reported with use of LMW heparins, ∴these are unacceptable
- Rx: cease ***all*** heparin
- if associated with significant ***thrombotic*** complications, then aspirin / plasmapheresis / dextran 40

3. anaphylaxis
4. abnormal LFT's - mild elevation of AST, ALT
5. alopecia - usually transient, seen with prolonged use
6. osteoporosis

Warfarin

- dicumarin derivative, inhibits the hepatic ***gamma-carboxylation*** of K-dependent clotting factors
- this is required for binding Ca^{++}/phospholipid
- warfarin inhibits ***vitamin K reductase*** and ***vitamin K epoxide reductase***
- thus, prevents vit.K → **vit.KH_2** which is the cofactor for N-terminal-γ-carboxylation
- the target proteins are still produced but are unable to be activated in circulation

- oral bioavailability ~ 100%
- plasma $t_{½\beta}$ ~ 35 hrs
- the rate of decrease of,

1. factor VII and protein C - is rapid and dose-dependent
2. factors II, IX and X - slower and responsible for ongoing effect

- anticoagulation may be achieved with 15 mg loading dose, then 5 mg/day, in ~ 2-3 days
- standard dose ~ 5 mg/day takes ~ 8 days
- some recommend the later to avoid the mild ***hypercoagulable state*** which occurs with loading
- therapeutic levels require INR levels,

a. venous thrombosis ~ 2.0-3.0
b. arterial thrombosis ~ 2.5-3.5

- low dose warfarin (1-2 mg/d) has been recommended post gynaecological surgery
- ineffective following orthopaedic joint replacement surgery

• recent controlled trial showed similar rate of recurrence following thromboembolism using either ***4 weeks*** or ***6 months*** therapy
• standard recommendations following PTE,

a. 6 weeks - patients with no persistent venous thrombosis risk factors

b. 3 months - other patients

Side Effects

1. haemorrhage ~ 8%
   - effects can be reversed by FFP or vit.K
   - Rx vit.K usually have factor levels ~ 30% by 4 hrs and normal by 24 hrs
2. microvascular thrombosis
   - usually in patients with protein S/C deficiencies
   - Rx vit.K and heparinisation
3. teratogenic effects
   - should not be administered in first 12 weeks of pregnancy
4. rare effects - alopecia, dermatitis, urticaria

Drug Interactions

1. warfarin ***potentiation***
   i. decrease GIT vitamin K absorption - antibiotics, cholestyramine
      - malabsorption, diarrhoea, vomiting
   ii. displacement of warfarin from albumin - sulphonamides, chlofibrate
      - indomethecin
   iii. competition for metabolic breakdown - tolbutamide, phenytoin
2. bleeding potentiation
   - NSAIDs, heparin, penicillins, cephalosporins
3. warfarin ***antagonism***
   i. increase procoagulant synthesis - oestrogens
   ii. hepatic enzyme induction - barbiturates, chloral hydrate
      - rifampicin, carbamazepine

Selective Thrombin Inhibitors

▪ *Thrombin Activity*

• cleaves fibrinopeptides A & B from ***fibrinogen*** to yield ***soluble fibrin***
• both free and fibrin bound thrombin are able to cleave fibrinogen, allowing propogation of thrombus at the site of injury
• thrombin activates Factor **XIII**, which cross-links fibrin, increasing mechanical stability & reducing susceptibility to lysis
• thrombin binds to ***thrombomodulin***, on the endothelial surface, initiating activation of ***protein C***
• protein C, in the presence of ***protein S***, inactivates Factors $\mathbf{V_a}$ and $\mathbf{VIII_a}$
• thrombin stimulates release of both,

1. tissue plasminogen activator (tPA), and
2. plasminogen activator inhibitor type 1

from endothelial cells → ***endogenous thrombolysis***

• thrombin therefore plays an integral role in the balance of thrombosis / thrombolysis
• thrombin is also an ***effector*** molecule,

1. presence of ***inducible receptors*** for thrombin on endothelial & vascular smooth muscle cell surfaces
2. direct effects on cell proliferation
   - ↑ smooth muscle cell proliferation
   - ↓ endothelial cell proliferation
3. influences cellular mechanisms for matrix protein and collagen production
4. direct effects on WBC's
   - ↑ IL-1 from macrophages
   - promotes neutrophil degranulation

▪ *Hirudins vs Heparins*

• ***hirudin*** is a 65 amino acid peptide, isolated from the leech ***Hirudo medicinalis***
• a selective ***thrombin inhibitor***, binding directly and tightly in a stoichiometric fashion
• derivatives include,

1. r-hirudin - recombinant desulfato-hirudin, CGP-39393
2. hirulog - 20 AA synthetic peptide
   • binds both,
   i. the catalytic site of thrombin, and
   ii. an exosite required for thrombin binding to fibrinogen and thrombospondin

• potential advantages of hirudins include,

1. these agents neutralise thrombin directly
   - no need for an intermediary molecule such as ***antithrombin III***
2. heparins may be inactivated by proteins, such as ***platelet factor IV***, however this does not occur with hirudins
3. fibrin-bound thrombin is resistant to inactivation by the heparin-ATIII complex, however inhibition of clot-bound thrombin is achieved with r-hirudin
4. thrombin mediated ***platelet activation*** is not inhibited by heparin

NB: these factors are likely significant in,

1. ***rethrombosis*** following successful coronary thrombolysis
2. propogation of venous thrombosis
3. restenosis following PTCA

▪ ***Clinical Effects***

• dose-dependent ↑ APTT and INR ∝ plasma hirudin levels
• peak effect on APTT sustained for 3-6 hrs post-subcutaneous injection
• no evidence of cumulative effects with dose regimens of 8-12 hrly sc
• no increase in ***bleeding time*** was observed
• numerous animal models showing reduction in vascular thrombosis,

1. the magnitude of both platelet and fibrin deposition in the porcine carotid angioplasty site was significantly reduced cf. heparin
2. enhanced thrombolysis and reduced rethrombosis in canine acute coronary occlusion

• also inhibits neutrophil activation/degranulation in models of cardio-pulmonary bypass, and has a greater effect in inhibiting surface mediated activation of thrombin
• effects on cellular proliferation may result in reduction in late ***re-stenosis*** following angioplasty
• human trials,

1. randomised cohort, heparin vs hirudin for ***coronary angioplasty***
   - r-hirudin group had a lower incidence of acute thrombotic events
   - post-procedure ischaemic changes (24 hr Holter) less with hirudin
   - van den Bos *et al.*, Circ. 1992
2. effective prophylaxis following major orthopaedic (hip replacement) surgery
3. effective as sole anticoagulant during diagnostic coronary angiography
4. sole anticoagulant during therapeutic coronary angioplasty
   - multicentre study of 208 patients, all received aspirin, 4 dosing regimens
   - 11% acute vessel closure in lowest dose group, < 3% in higher dose groups
   - no haemorrhagic or vascular complications
   - Bonnon *et al.*, Circ. 1992

NB: ***no*** increased incidence of haemorrhagic or vascular complications in any study

- ***Other Thrombin Inhibitors***

1. argatroban - reversible, competitive thrombin inhibitor
2. argidipine
3. *d*-phenylalanine-*l*-propyl-*l*-arginyl-chloromethyl ketone - PPACK
   - an irreversible serine protease inhibitor

- ***Problems***

1. potential to result in haemorrhagic complications
2. lack of an effective ***antidote*** to rapidly terminate their systemic activity
3. variable clinical effect in some studies

- ***Dosage***

- 20 mg sc bd

Thrombolytic Therapy

- proenzyme ***plasminogen*** (MW ~ 88,000) synthesized by the liver & circulates as a β_2-globulin
- binds to ***fibrin*** during thrombus formation
- tPA activates plasminogen to ***plasmin*** by cleavage of an internal arginine-valine peptide bond
- this forms a 2 chain molecule, which rapidly undergoes further cleavages to form plasmin
- plasmin is a ***non-specific serine protease*** which inactivates,

a. fibrin | fibrinogen
   - $\rightarrow$ fragment 'X' = -D-E-D-
   - D-fragments in fibrin are cross-linked $\rightarrow$ D-dimer

b. prothrombin, factors V & VIII

c. prekallikrein & C_1

- circulating plasmin is rapidly & irreversibly inactivated by a_2***-antiplasmin*** (< 100 msec)
- the affinity of tPA is far greater for plasminogen bound to fibrin

- thrombolytics provide more rapid correction & greater resolution of pulmonary vascular abnormality following massive PTE (even at 12 months)
- preserve valvular function & reduce incidence of chronic vensous insufficiency following DVT

NB: however, there has been no demonstrated reduction in ***mortality***

- ***Indications***

1. AMI < 6 hrs
   - earlier administration → ↓ 30 month mortality (Rawles BMJ 1996)
   - though some would administer in high risk patients < 12 hrs
2. acute, within 4 days for,
   i. massive PTE
   ii. venous thrombosis
   iii. aterial thrombosis / embolism
3. specific
   i. retinal artery occlusion
   ii. priapism
   iii. AV shunt or venous cannula thrombosis

- ***Contraindications***

1. generalized or local bleeding tendency
   i. active peptic ulcer disease
   ii. hepatic failure
   iii. intracranial neoplasm, AVM
   iv. pre-existing haemostatic deficit
2. severe uncontrolled hypertension > 180/120 mmHg
3. recent CVA < 6 months
4. recent surgery
   i. within 2 months
      - cerebral / spinal surgery or trauma
      - vascular or ophthalmic surgery
   ii. within 10 days
      - abdominal, gynaecological, thoracic surgery or trauma
      - postpartum
      - renal or hepatic biopsy

■ ***Streptokinase***

- nonenzymatic protein, MW ~ 48000, derived from group C, beta-haemolytic streptococci
- acts as a ***plasminogen proactivator***

  → combines with an equimolar amount of plasminogen to form ***plasminogen activator***

- the activator, SK-plasminogen, converts both circulating and bound plasminogen to plasmin
- Ab's to SK exist in varying amounts in virtually ***all patients***
- plasma half-life is ***biexponential*** → 18 min → clearance by SK-Ab's
  83 min → $t_{½\beta}$
- used in both low & high dose regimens

1. ***low dose***
   - commonly used for direct IA/IV clot lysis
   - requires the concomitant administration of ***heparin***
   - small amounts of SK-plasminogen formed diffuse into clot & effect lysis
   - systemic effects are neutralized by circulating antiplasmins

   i. loading dose ~ $100,000^{U}$ administered over 4 hrs

   ii. maintenance ~ $5,000^{U}$ / hr

   iii. heparin ~ $1,000^{U}$ / hr → APTT ~ 1.5-2.0 x control

   - no benefit continuing > 3 days, ∴ most Rx for 2-3 days

2. ***high dose***
   - attempt to convert ***all*** circulating plasminogen to SK-plasminogen-activator
   - this leaves only a small amount of circulating plasminogen to convert to ***plasmin***
   - SK-activator then diffuses into clot where it activates fibrin-bound plasminogen

   i. loading dose ~ $250,000^{U}$ / 30 mins

   ii. maintenance ~ $100,000^{U}$ / hr ~ 24 hrs for acute PTE
   ~ 48-72 hrs for DVT

3. ***coronary thrombolysis***

   i. single loading dose ~ $1,500,000^{U}$ / 30-45 mins

   ii. heparin ~ $1,000^{U}$ / hr → APTT ~ 1.5-2.0 x control
   - some question as to the value of heparin IV with STK

- IgG anti-SK Ab levels are usually high after 5 days, maximum at 10-14 days
- SK should be avoided for 6-12 months

- ***Side Effects***

1. ***haemorrhage*** **~ 5-8%** of patients
   → ↓ fibrinogen, V, VIII and ↑ FDP's
   - however, ~ 90% of episodes are at recent vascular puncture sites
     - more common in patients also receiving ***heparin***
   - ∴ with appropriate patient selection, bleeding should be < 5%, cf. heparin alone
   - major haemorrhage requires EACA (5g IV) and cryoprecipitate (2-4 packs)
2. febrile reaction - some Rx with ***hydrocortisone*** to reduce severity
3. allergic reactions - urticaria, flushing, pruritis, bronchospasm, headache, N&V
   * ***anaphylaxis*** per se is rare

- ***Urokinase***

- enzymatic protein, MW ~ 55,000, which is produced from human kidney tissue cultures
- directly activates plasminogen, with a plasma $t_{½\beta}$ ~ 16 min
- ***non-antigenic*** & rarely associated with febrile / allergic phenomenon

1. loading dose ~ $4{,}400^{U}$ / kg / 15 mins (~ $300{,}000^{U}$/70kg)
2. maintenance ~ $4{,}400^{U}$ / kg / hr (~ 24-48 hrs)

- ***Tissue Plasminogen Activator***

- recombinant tissue-type plasminogen activator, rTPA ~ 63,000 MW
- preferentially activates ***fibrin-bound*** plasminogen, with a plasma $t_{½\beta}$ ~ 3.6-4.6 mins
- clinically the effect lasts longer ∝ to the $t_{½\beta}$ of plasmin
- 100 mg of rTPA decreases the plasma fibrinogen ~ 20-30%, significantly less than SK
- ***non-antigenic*** & rarely associated with febrile / allergic phenomenon

1. standard coronary thrombolysis → 3 hrs Rx
   i. loading dose ~ 10 mg
   ii. maintenance ~ 50 mg / hr x 1 hr
   ~ 20 mg / hr x 2 hrs
   iii. heparin ~ $1{,}000^{U}$ / hr → APTT ~ 1.5-2.0 x control
2. ***accelerated*** coronary thrombolysis → 1.5 hrs Rx
   i. loading dose ~ 15 mg
   ii. maintenance ~ 0.75 mg/kg / 30 min (≤ 50 mg)
   ~ 0.5 mg/kg / 60 min (≤ 35 mg)
   iii. heparin ~ $1{,}000^{U}$ / hr → APTT ~ 1.5-2.0 x control
3. DVT & PTE
   i. loading dose ~ 10 mg
   ii. maintenance ~ 20 mg / hr x 2 hr
   ~ 10 mg / hr x 5 hrs

- ***Anticoagulation Post-Thrombolysis***

• generally aimed at maintaining APTT ~ 1.5-2.0 x baseline

1. post-SK ~ 4-12 hrs
2. post-UK ~ 1 hr
3. post-TPA * ***immediately***

Fibrinolytic Inhibitors

1. naturally occurring inhibitors
   i. ***alpha-2-antiplasmin***
      • produced by the liver, reduced in cirrhosis & DIC
      • levels < 50% may → unusual bleeding tendency, requiring Rx with EACA
   ii. alpha-2-macroglobulin
2. bovine subtances - ***aprotinin***
   • 58 AA polypeptide with a plasma $t_{½\beta}$ ~ 2 hrs
   • inhibitor of plasmin, trypsin, plasma & tissue kallikreins
   • inhibits fibrinolysis by preventing one of the cleavages of plasminogen
3. synthetic compounds
   i. ***epsilon amino-caproic acid***
      • amino-acid (MW ~ 131), with $t_{½\beta}$ ~ 1-2 hrs
      • loading dose ~ 5-10 g, followed by 1.0 g/hr
   ii. tranexamic acid
      • ~ 10x as potent as EACA & has largely replaced the former
      • MW ~ 157 & crosses the BBB, as does EACA, with $t_{½\beta}$ ~ 80 mins
      • dose ~ 10-15 mg/kg / q8h (~ 0.5-1.0 g/70kg)
   iii. para-amino-methylbenzoic acid

• the synthetic agents form reversible complexes with plasminogen
• saturation of ***lysine*** binding sites inhibits binding to fibrin surface & subsequent fibrinolysis

→ ***thrombotic tendency***

• however, plasmin inactivation by α_2-antiplasmin is also inhibited

THROMBOSIS & HYPERCOAGULABLE STATES

• mechanisms preventing abnormal thrombosis,

1. nonthrombogenic nature of intact ***endothelium***
2. circulating ***inhibitors*** of coagulation
3. ***clearance*** of activated factors by RES
4. ***fibrinolytic*** system

▪ *Antithrombin III Deficiency*

• usual range of ATIII in plasma ~ 85-120% of normal
• congenital deficiency is inherited as an ***autosomal dominant***
• the ***homozygote*** state is incompatible with life
• ***heterozygotes*** usually have < 60% normal ATIII activity & often present with abnormal venous thrombosis
• they are not at risk of arterial thrombosis & frequently have some additional precipitating cause
• of patients with DVT ~ **2-3%** will have low ATIII levels
• of patients with the disorder ~ 90% will have a thrombotic event prior to 55 years
• ***heparin resistance*** may or may not occur, as there is frequently enough ATIII for heparin action
• for patients suffering a thrombotic event, lifetime anticoagulation is required
• if warfarin is contraindicated, then heparin & FFP (300 ml/24 hrs $\rightarrow$ > 80% activity)
• ***acquired ATIII deficiency***,

1. nephrotic syndrome
2. cirrhosis / chronic liver disease
3. DIC
4. oestrogen therapy

▪ *Protein S / C Deficiencies*

• protein C is a vit.K dependent glycoprotein synthesized by the liver
• activated to a ***serine protease*** by endothelium-bound thrombin-thrombomodulin complex
• ***thrombomodulin*** restricts thrombosis by binding thrombin & activating protein C
• in the presence of phospholipid & Ca^{++}, protein C_a ,

a. inactivates thrombin and factors V_a & $VIII_a$
b. inhibits conversion of prothrombin to thrombin by platelet-bound V_a & X_a
c. stimulates fibrinolysis by inhibiting tissue plasminogen-activator inhibitor

NB: protein S is a cofactor for inactivation of factors V_a & $VIII_a$

• also inherited as an ***autosomal dominant***, with the ***homozygous*** state incompatible with life
• heterozygous state results in recurrent venous thromboembolic disease
• there is ***no increase*** in arterial thrombosis
• ***acquired*** reduction in protein S/C may occur in nephrotic syndrome

- ***Fibrinolytic Abnormalities***

1. hypoplasminogenaemia
2. abnormal plasminogen
3. plasminogen-activator deficiency

NB: these are all very rare

- ***Factor XII Deficiency***

• while XII activates the intrinsic coagulation cacade, also initiates ***fibrinolysis*** & ***kinin*** systems
• depending upon the balance of effect, may present with either haemorrhage or thromboembolism
• the first described case actually died of PTE

- ***Secondary Hypercoagulable States***

1. major trauma / surgery - thoracic, abdominal, orthopaedic
2. pregnancy, oestrogen therapy
3. immobility
4. neoplasia *adenocarcinoma: GIT, pancreas, prostate, lung & breast
5. nephrotic syndrome
6. dehydration, hyperviscosity syndromes
7. myelofibrosis
8. homocysteinuria
9. heparin-induced platelet Ab's
10. lupus anticoagulant
    - ~ 6-10% of SLE develop ***anticardiolipin*** Ab
    - binds laboratory phospholipid & artefactually → ↑ APTT
    - clinically arterial & venous ***thrombosis***, & thrombocytopaenia
11. Bechet's syndrome
12. CCF, AMI
13. paroxysmal nocturnal haemoglobinuria

NB: common effects → ↑ procoagulant factors &
↓ ATIII levels and plasminogen activation activity

ANAPHYLAXIS

Def'n: ***anaphylaxis:*** symptom complex following exposure of a ***sensitised*** individual to an antigen, produced by immediate or ***type I hypersensitivity*** reaction, associated with IgE mediated mast cell degranulation

anaphylactoid reactions: are indistinguishable from true anaphylaxis, however the immune nature of the reaction is either unknown, or not due to a type I hypersensitivity reaction

\ ***immediate generalised reaction*** may be a better term (AIC 1993)

▪ *Aetiology*

1. ***anaphylaxis***
   - i. prior sensitisation to an antigen, either alone or in combination with a hapten
   - ii. synthesis of antigen specific **IgE**, which attaches to mast cells & basophils
   - iii. subsequent exposure $\rightarrow$
     - mast cell & basophil degranulation
     - release of ***histamine*** + SRS-A (LT - C_4, **D_4**, E_4)
       ECF-A, NCF
       PAF, heparin
2. ***anaphylactoid reactions***
   - i. exposure & combination of antigen with **IgG, IgM** ± a hapten
   - ii. activation of ***complement*** via the classical pathway (C_{1q}, C_4, C_2)
   - iii. formation of ***anaphylatoxins*** - C_{3a}, C_{5a}
     - mast cell & basophil degranulation $\rightarrow$ ***histamine***, SRSA, etc.
3. direct release of histamine
   - classically morphine, dTC, etc.

▪ *Common Antigens*

1. antibiotics
2. blood & blood products
3. XRay contrast media
4. STP, muscle relaxants
5. sulphonamides

- ***Presentation***

NB: variable latent period, but usually within ***30 minutes*** of exposure

1. respiratory
   - dyspnoea, chest tightness
   - stridor, laryngeal oedema/obstruction
   - ***bronchospasm*** (*LTD_4)
   - ↑ peak P_{AW}, ↑ slope of alveolar plateau, ↓ $ETCO_2$
   - pulmonary oedema
2. cardiovascular
   - ***hypotension***, tachycardia ± arrhythmias
   - most common and may be sole finding
   - cardiovascular "collapse"
   - pulmonary oedema is a common finding at autopsy
   - ? existence of "myocardial depressant factors"
3. cutaneous
   - erythematous blush, generalised urticaria
   - ***angioedema***
   - conjunctival ingection & chemosis
   - pallor & cyanosis
4. gastrointestinal
   - nausea, vomiting, abdominal cramps & diarrhoea

- ***Management***

NB: multiple actions simultaneously / conclude surgery / call for experienced help

1. cease administration of the likely antigen
2. maintain oxygenation
   i. maximal O_2 via face mask
   ii. IPPV via bag/mask
   iii. intubate & 100% O_2 ASAP *cease anaesthetic agents
3. support circulation
   i. CPR if no output
   ii. ***adrenaline***
      - inhibits mast cell degranulation, ↑ SVR, venous return, ↓ bronchospasm
      - hypotension: 10-50 µg boluses prn or infusion if available
      - collapse: 0.5-1.0 mg stat, then infusion
   iii. volume expansion *"whatever is available"
      - Haemaccel, NSA-5%, CSL, N.saline
      - CVP monitoring once situation under adequate control

4. manage ***bronchospasm***
   - i. maximise F_IO_2
   - ii. slow RR, high E:I ratio ventilation
   - iii. adrenaline ~ 0.5 mg IM if no access
     - IV dependent upon MAP & ECG monitoring
   - iv. aerosol bronchodilators
   - v. aminophylline - additive effects with adrenaline
     ~ 5-6 mg/kg loading dose over 30-60
   - vi. suction ETT
   - vii. volatile agents - if isolated bronchospasm with maintenance of MAP
5. monitoring
   - i. ECG, NIBP, IABP when possible
   - ii. S_pO_2, $ETCO_2$, AGA's
   - iii. CUD, CVP ± PAOP
   - iv. transfer to ICU
6. other therapy
   - i. antihistamines - ***no benefit*** in acute episode
     - H_2 blockers contraindicated acutely
     - may be useful for ongoing ***angioedema***
     - require both H_1 & H_2 for prophylaxis
   - ii. sedation - if intubated & resuscitation successful
   - iii. steroids - marginal benefit in acute episode
     - may be useful for ongoing bronchospasm & angioedema
     - required in addition to antihistamines for prophylaxis
7. follow-up
   - i. blood specimen
     - ***tryptase*** level - released from mast-cells/basophils, stable in plasma
       - may be performed on post-mortem specimen
     - ***complement*** - levels decreased with anaphylactoid responses
       * $\mathbf{C_4}$ not usually decreased with true anaphylaxis
     - re-type screen & cross-match if due to blood reaction
   - ii. return unused blood products to the blood bank
   - iii. intradermal ***skin testing***
     - histamine releasing agents ~ 1:10,000
     - non-histamine releasing agents ~ 1:1,000
     - graded reponses of limited value, use ***absolute*** result
   - iv. medic-alert bracelet & accompanying letter(s)

Mechanisms of Immunological Injury		
Mechanism	Pathophysiology	Disease types
Type I • immediate hypersensitivity • IgE mediated	• basophil & mast cell degranulation • histamine, SRSA, ECFA, NCF • immediated wheal & flare	• anaphylaxis • atopy
Type II • cell cytotoxicity • IgG, IgM mediated	• direct phagocytosis or cell lysis • activation of ***complement***, classical • tissue deposition of complement	• blood transfusions • Goodpasteur's syndrome • autoimmune cytopaenias
Type III • immune complex • IgG, IgM, IgA mediated	• tissue deposition of Ag-Ab ***complexes*** • accumulation of PMN's, macrophages & complement	• serum sickness • SLE • necrotising vasculitis
Type IV • delayed hypersensitivity • T-cell mediated	• T-cell induced mononuclear cell accumulation • release of lymphokines & monokines • often with ***granuloma*** formation	• TB, sarcoid • Wegener's granulomatosis • granulomatous vasculitis

Multiple Myeloma

- ***Diagnosis***

1. marrow plasmacytosis > 10%
2. lytic bone lesions
3. serum or urine M component

- ***Clinical Features***

1. bone lesions
   - pain is most common symptom
   - osteolytic without osteoblastic zone
   - pathological fractures
2. infection
   - recurrent infection presenting complaint in 25%
   - may be significant hypogammaglobulinaemia (when M component excluded)
3. hypercalcaemia
4. renal failure
   - nephrocalcinosis
   - toxic effects of light chains
5. hyperviscosity syndrome
   - fatigue, headaches
   - visual disturbances, retinopathy
6. haematological
   - anaemia in 80%
   - granulocytopaenia & thrombocytopaenia rare
   - coagulopathy
   - may have cryoglobulins

- ***Investigation***

a. CBE

b. plasma electrophoresis ± urine
   - quantitative

c. plasma electrolytes - calcium, urea, creatinine
   - M component = IgG
   - IgG has +'ve charge → reduction in anion gap.
   - hyperproteinaemia → factitious hyponatraemia

d. marrow aspiration

e. skeletal radiological survey