BLOOD SUBSTITUTE

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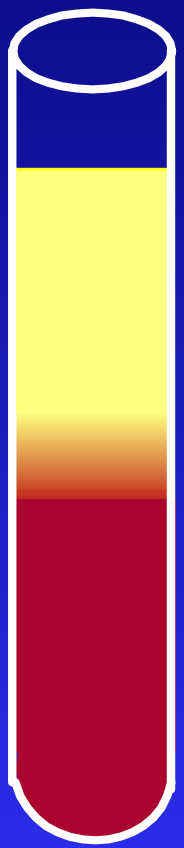
HEM/91000P

William Harvey (1578-1657)

Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus

"... Door een bloedvat af te binden, kunnen we aantonen dat het bloed van de slagaders naar de aders stroomt, reden om aan te nemen dat het bloed continu circuleert met het hart als aandrijfkracht. De vraag is nu of dit is bedoeld voor de voeding of voor het behoud van bloed en longen door warmte te transporteren , zodat het in armen en benen afgekoelde bloed in het hart opnieuw wordt opgewarmd. ..."

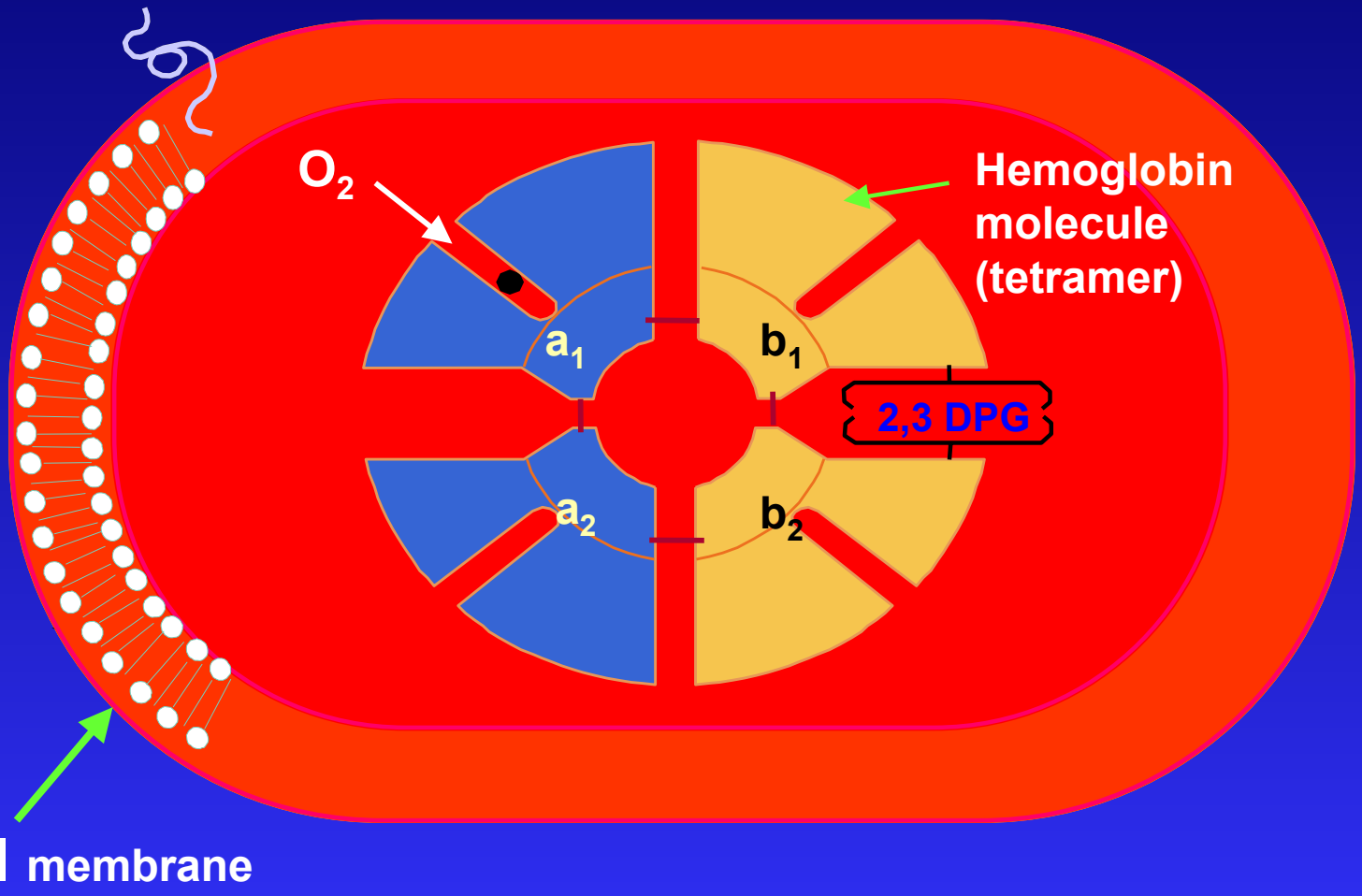
BLOOD



Transport and communication system

Host Defence
Coagulation
Oncotic pressure
Nutrient & waste
Thermoregulation
Oxygen carrier

RED BLOOD CELL



Bloodtransfusion

- Goal:
  - increase in D02 and VO2
- Strategy:
  - fluid ---> to optimalise the fillingpressure
  - RBC-mass ---> to increase the DO2

Oxygen delivery

Pulmonary factors
diffusion
Pa02 Ventilation
Perfusion
FiO2 (altitude)

(=220-age)
Sinus node dysfunction
Drugs (B-blockers)

HB concentration

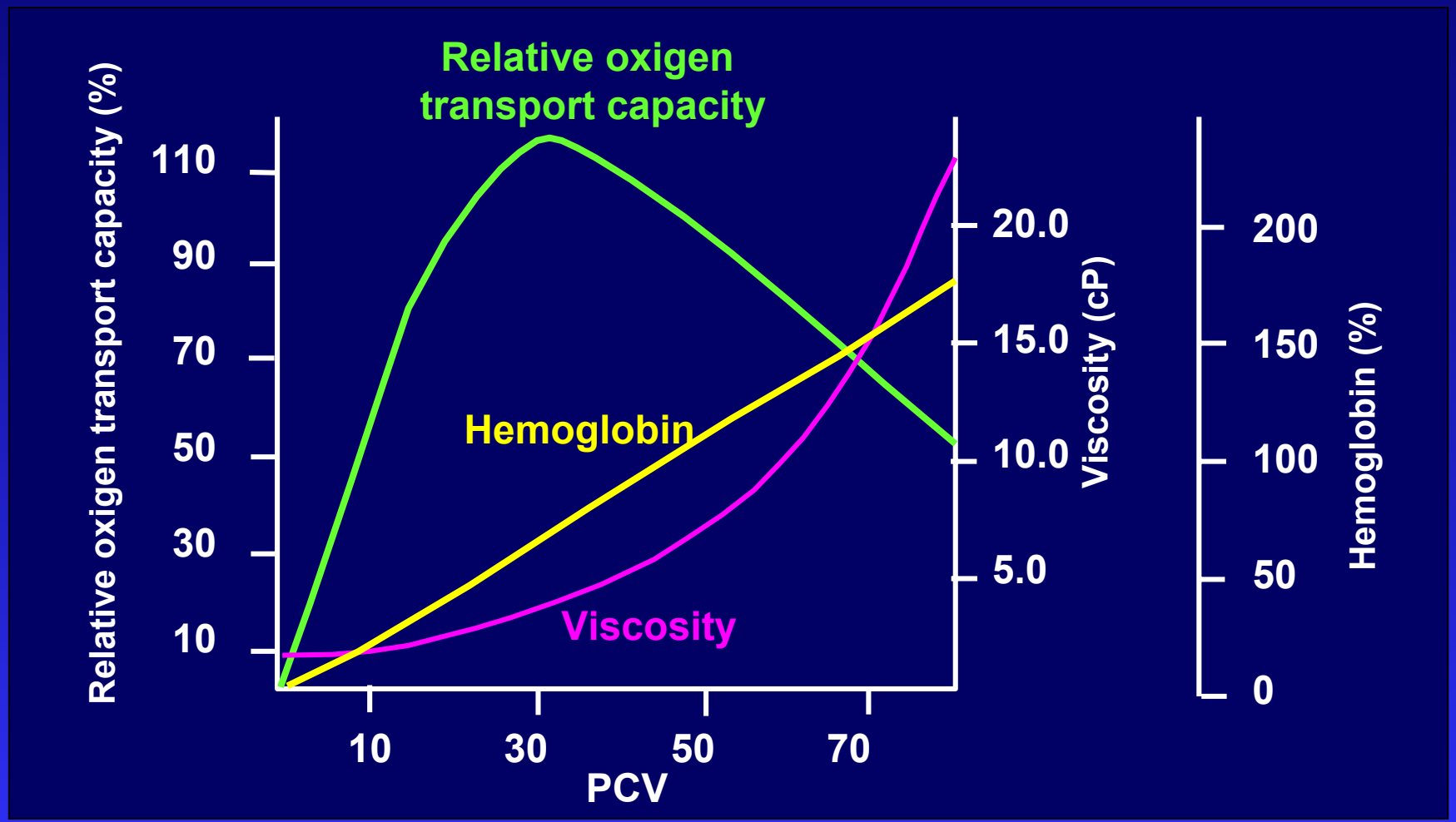
$$D02 = (Hr \times Sv) \times Ca02$$

Sa02

Genetic factors
Heart size
Conditioning factors
Contractility
After Load
Preload
Disease factors
Wall motion
Ventricular function
Valve stenosis or regurgitation

500 ml/min/m^2

Influence of packed cell volume (PCV) on the relative oxigen transport capacity of blood



HEM/90221P

Oxygen Consumption

Pulmonary factors
- diffusion
- Ventilation
- Perfusion

Pa02

FiO2 (altitude)

(=220-age)
Sinus node dysfunction
Drugs (B-blockers)

HB concentration

Sa02

$$V02 = (Hr \times Sv) \times (Ca02 - CvO2)$$

Genetic factors
- Heart size

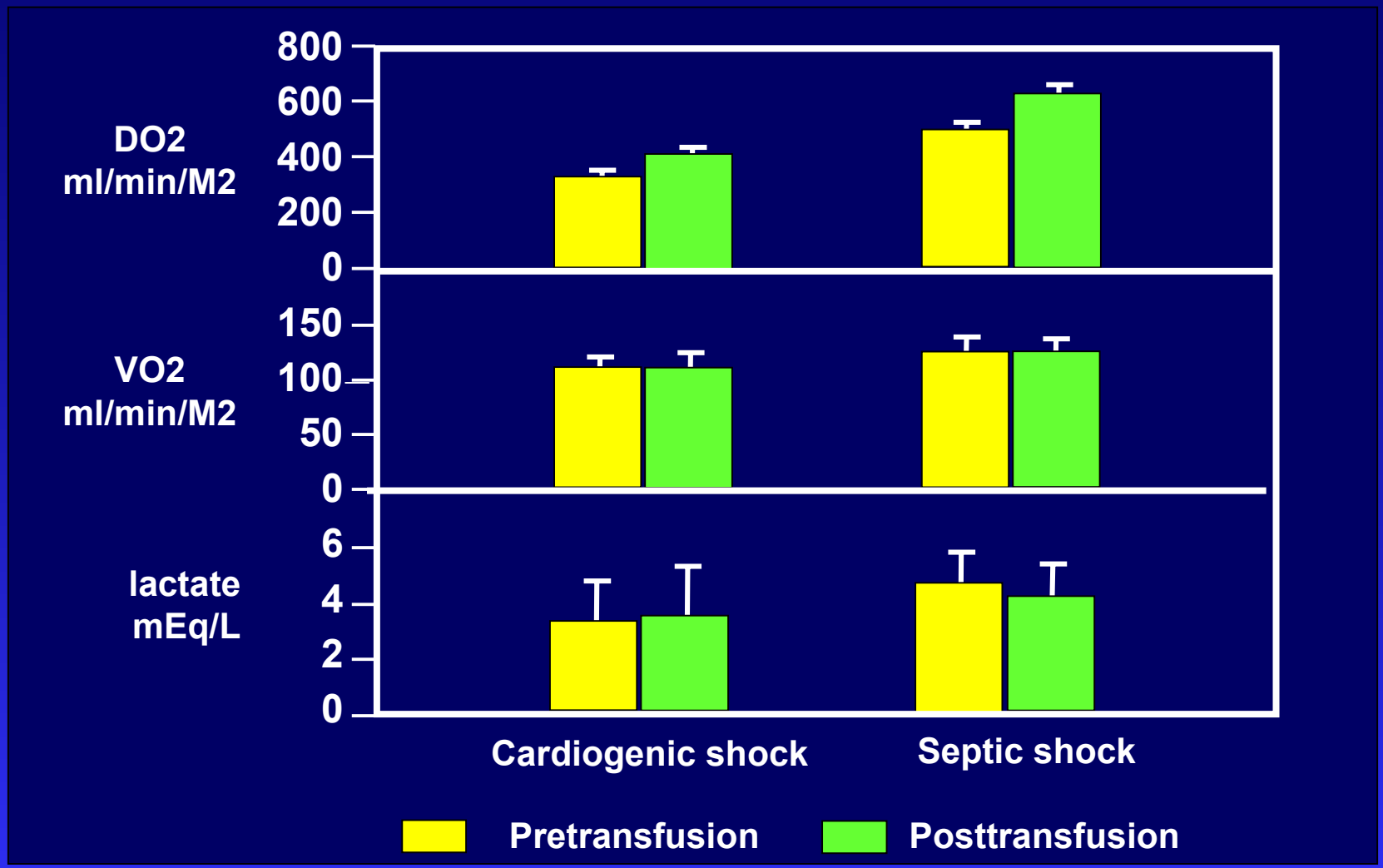
Conditioning factors
- Contractility
- After Load
- Preload

Disease factors
- Wall motion
- Ventricular function
- Valve stenosis or regurgitation

Skeletal muscles
- Aerobic enzymes
- Fiber type
- Muscle disease

Capillary density

Oxygen utilization



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Why increase in DO2 and not in VO2?

- Low 2,3-DPG in packed cell
- reduction in RBC deformability
- Increase in blood viscosity
- dysfunction in the microcirculation
- Heterogenicity in vascular conductivity
  - High flow (vasodillatation) increase in DO2 when high HT
  - Low flow (vasoconstriction) increase in DO2 when low HT

BLOOD substitution Clinical situation

- Preoperative status
- Chronic anaemia
- Acute anaemia
  - Bleeding
  - Acute operation
  - Sepsis ICU patiënt

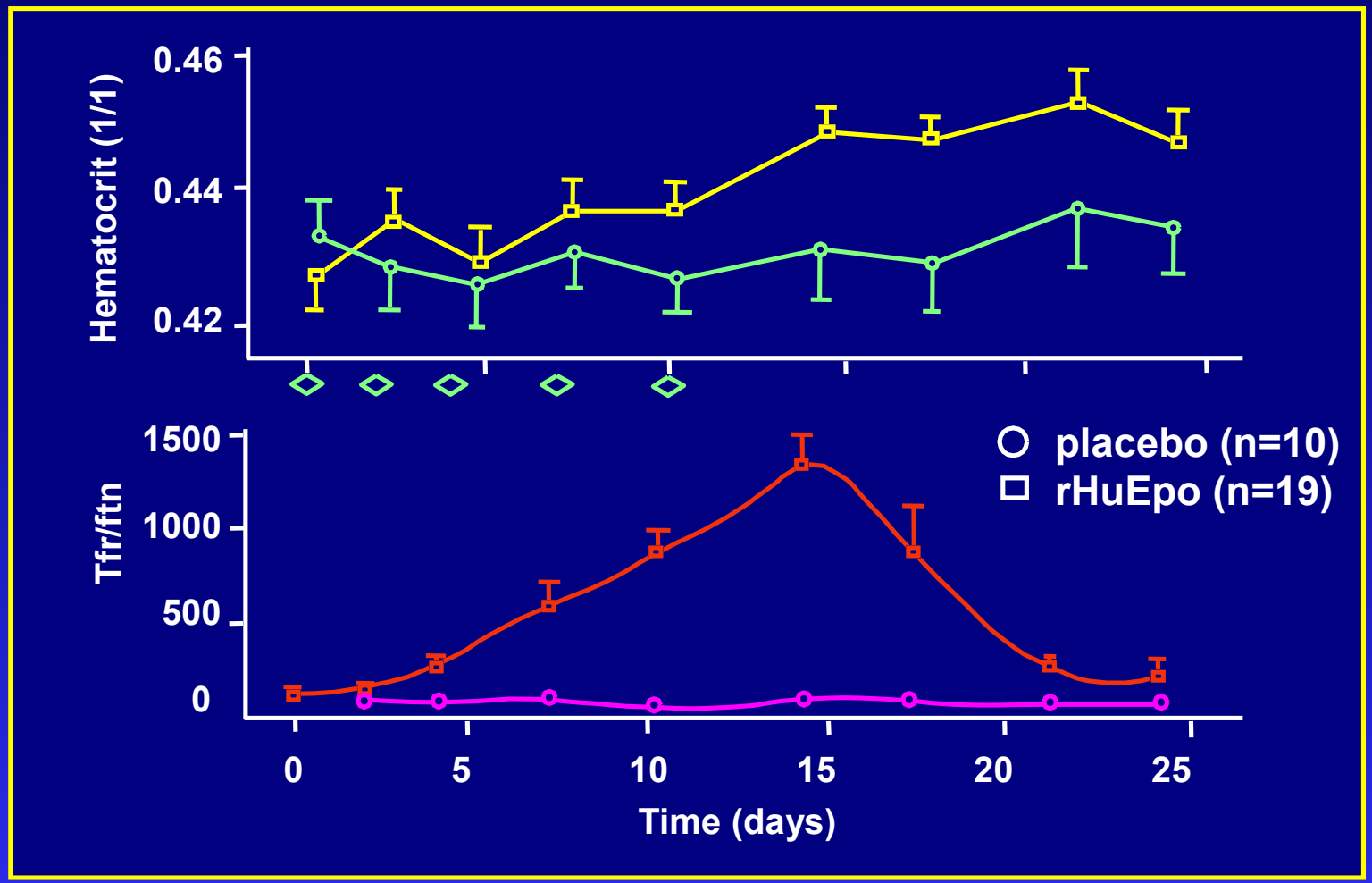
Jehovah's Witnesses

- Agree with EPO, IV Fe, folinic acid, PFC, human HBOC, recombinant HBOC, PFC
- Not agree with transfusion allo or auto, mammalian HBOC

- General rule: avoid risk situations for your patients

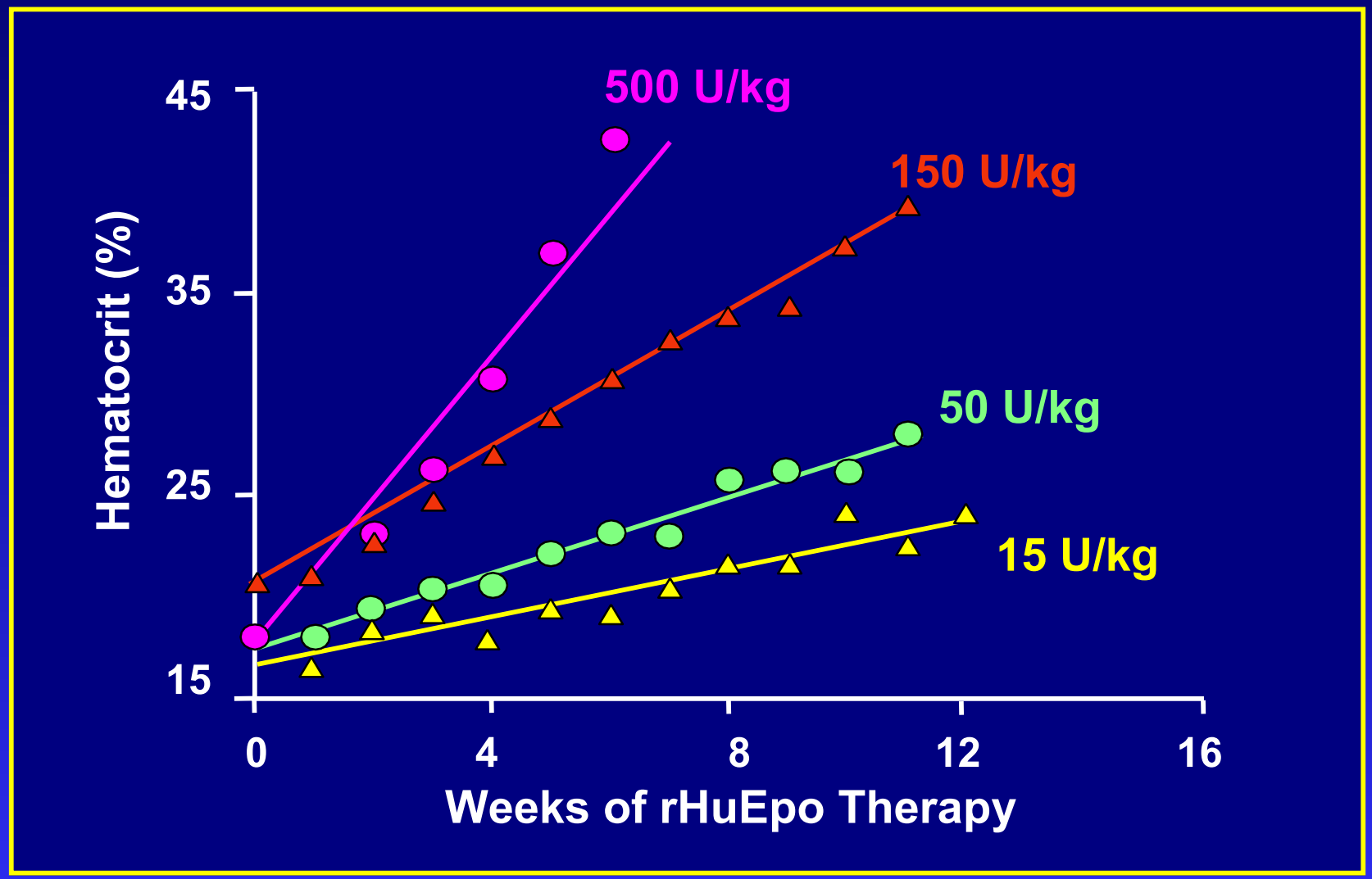
Preoperative status

- Cardiopulmonary evalutation
- Correction of anaemia before operation
- Correction of coagulation disorders: anamnesis, PTT, APTT, fibrinogen, PFA, platelet count
- If large bloodloss is expected: 4 U PC
  - Pre-op IV EPO 300-500U/Kg IV D-3 to D+2
  - folinic acid 15 mg IV /d D-3 to D+5
  - IV iron 200 mg D-3 to D0



Gareau et al, 1996

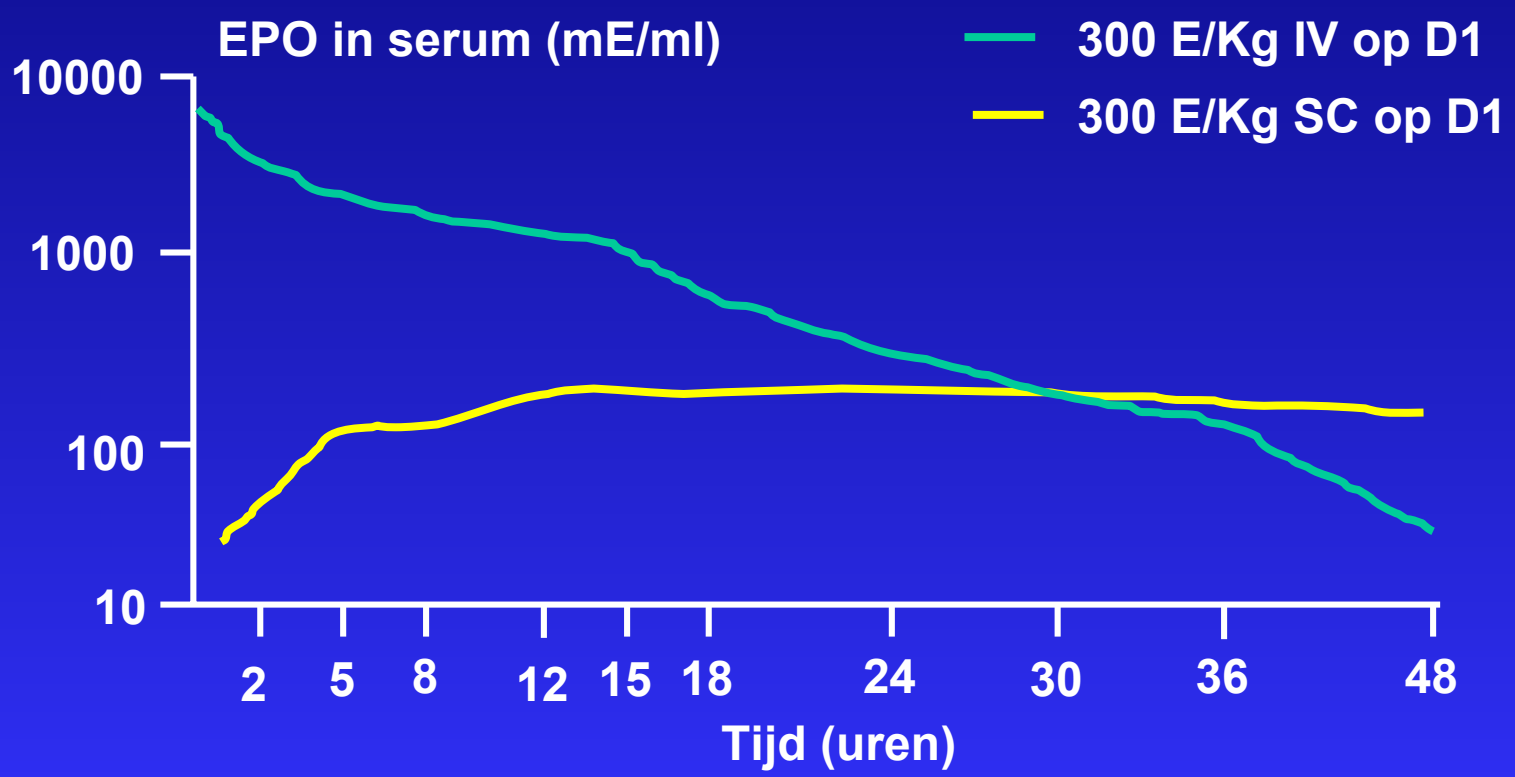
HEM/70675M



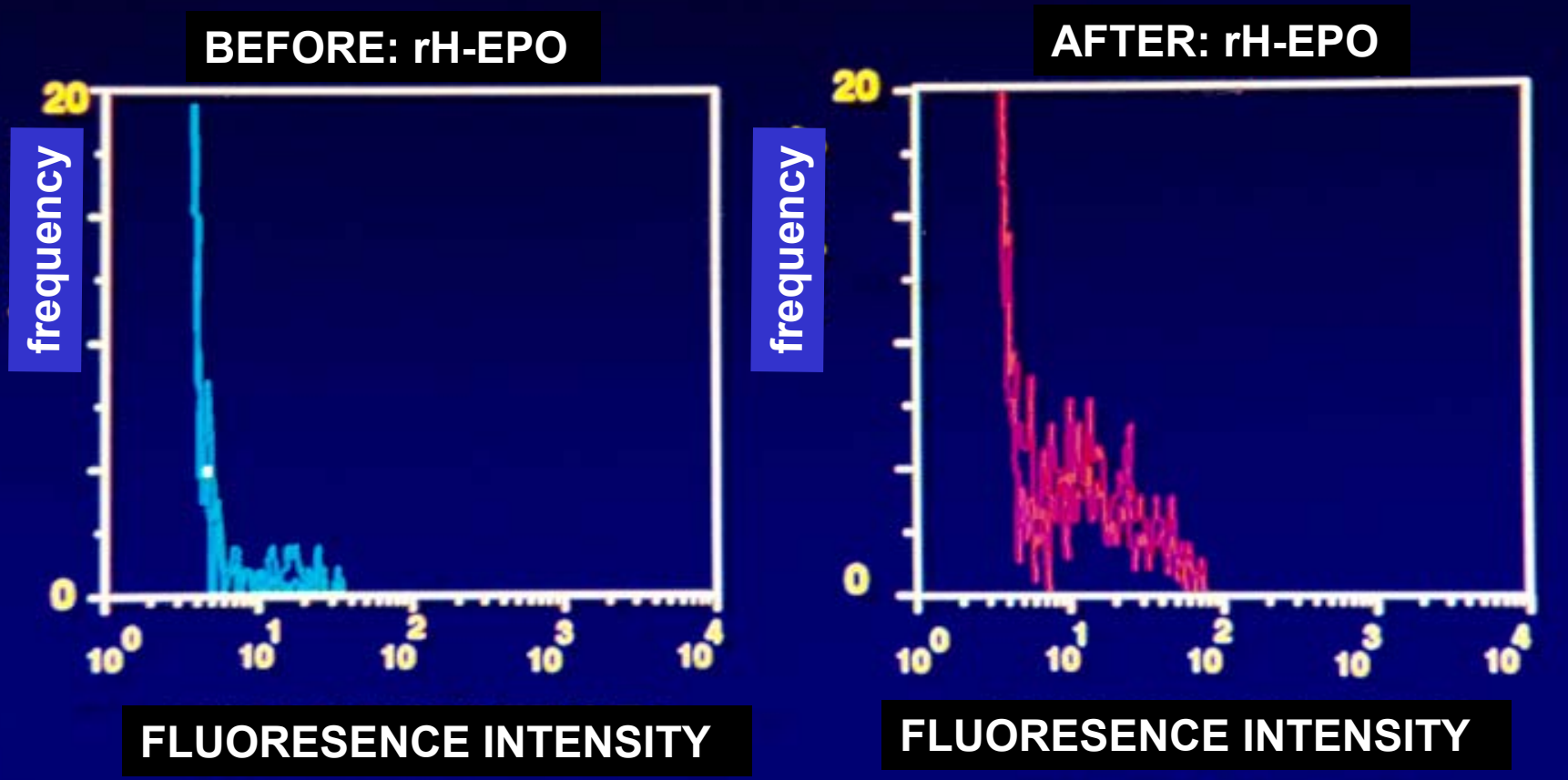
Brown et al, 1993

ACZA 2 PZ

Pharmacokinetics of EPO



Effect of EPO on reticulocyte age in CHD patients



RBC enzymes during EPO therapy

	Nl Limits	Before EPO	After 1 mth	During EPO
Hx U/gHb	0.64-0.90	1.09	1.52	1.71
PK U/gHb	3.80-12.0	7.89	9.09	9.84
G6PD U/gHb	4,10-7.90	5.65	5.95	7.19
ATP umol/gHb	1.97-3.29	3.6	3.5	3.36
DPG umol/g Hb	10.6-16.2	10.72	14.67	16.95
Retic $X10^9/L$	88 +/- 70	37.5	67.6	100

Chronic anaemia

- Hematological consult + adapted treatment
- Hematological growth factors, substitution of deficiencies
- Pharmacological agents for prophylaxis and treatment of bleeding (e.g tranexamic acid, epsilon-aminocaproic acid, aprotinine, desmopressin)
- Restricted phlebotomy for laboratory tests
- Hormone manipulation to control menstrual blood loss
- Supplemental oxygen to alleviate symptoms of anaemia
- (Oxygen-carrying red-cell substitutes) no effect

Oxygen Consumption

Pulmonary factors
- diffusion
- Ventilation
- Perfusion

Pa02

FiO2 (altitude)

(=220-age)
Sinus node dysfunction
Drugs (B-blockers)

HB concentration+ P50

Sa02

$$V02 = (Hr \times Sv) \times (Ca02 - CvO2)$$

Genetic factors
- Heart size

Conditioning factors
- Contractility
- After Load
- Preload

Disease factors
- Wall motion
- Ventricular function
- Valve stenosis or regurgitation

Skeletal muscles
- Aerobic enzymes
- Fiber type
- Muscle disease

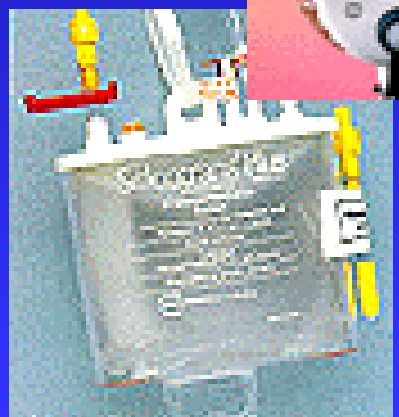
Capillary density

Acute Anaemia

- Maintain Q, peroperativ hemodilution
- Reduction of transfusion trigger
  - except: cardio&pulmonary disease, old age, betablockers
- Normovolemic hemodilution
  - Pre-op 750-1500 ml, compensation with colloïd:cristaloid
  - decrease in bloodviscosity, increase of Q, increase of coronary flow, increase in O2 extraction
  - When 20% loss of RCM is expected
  - Except: cardio&pulmonary disease, old age, betablockers, renal disease, coagulation disorders, fever, pain, or when VO2 is increased.

Acute Anaemia

- Blood cell salvage per and post operatively
  - Except in the presence of infection
- Reduction in bleeding
  - Anesthesical and surgical techniques: controled hypotension, loco-regional, endoscopic, operation field on the top, disection with laser or electro-cauter, bone waks.
  - Pharmacological agents for prophylaxis and treatment of bleeding
- Resticted phlebotomy for laboratory tests
- Hormone manipulation to control menstrual blood loss



Acute Anaemia

- Supplemental oxygen to alleviate symptoms of anaemia
- IV EPO 300-500U/Kg IV
- folinic acid 15 mg IV /d
- IV iron if needed (cave infection)

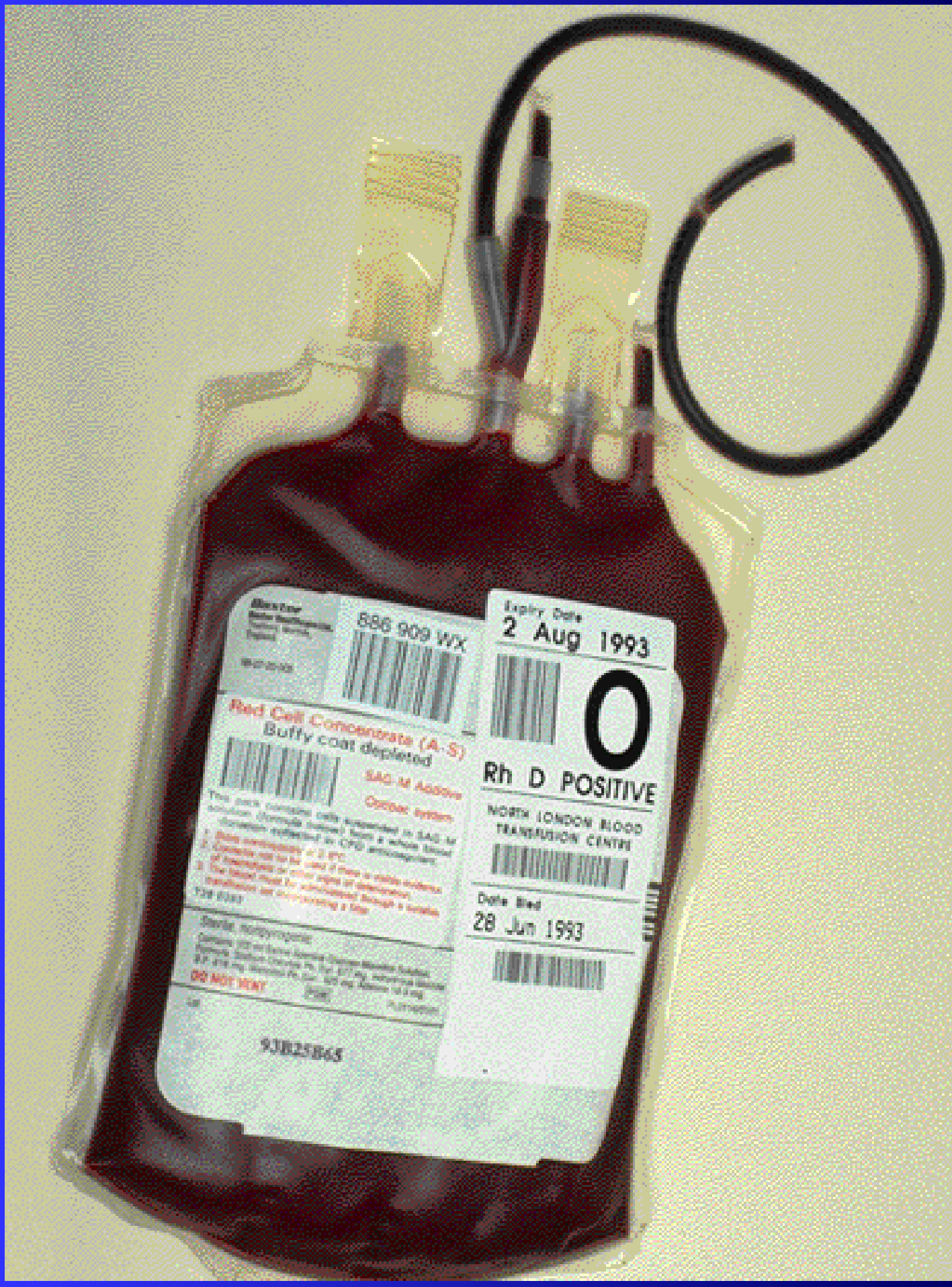
- Oxygen cariers

Aims for an oxygen-carrying solution

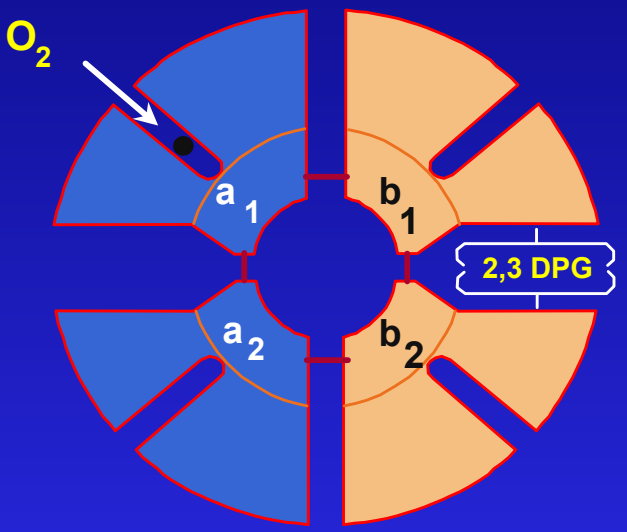
- Can be used immediately
- Can be stored in a ready-for-use
- formulation for years
- Without transfusion reactions (non-antigenic, non-toxic, non-pyrogenic, virus free)
- The same O_2 carrying capacity and delivery to the tissues as RBC's
- Adequate colloid oncotic pressure
- Adequate survival in the circulation

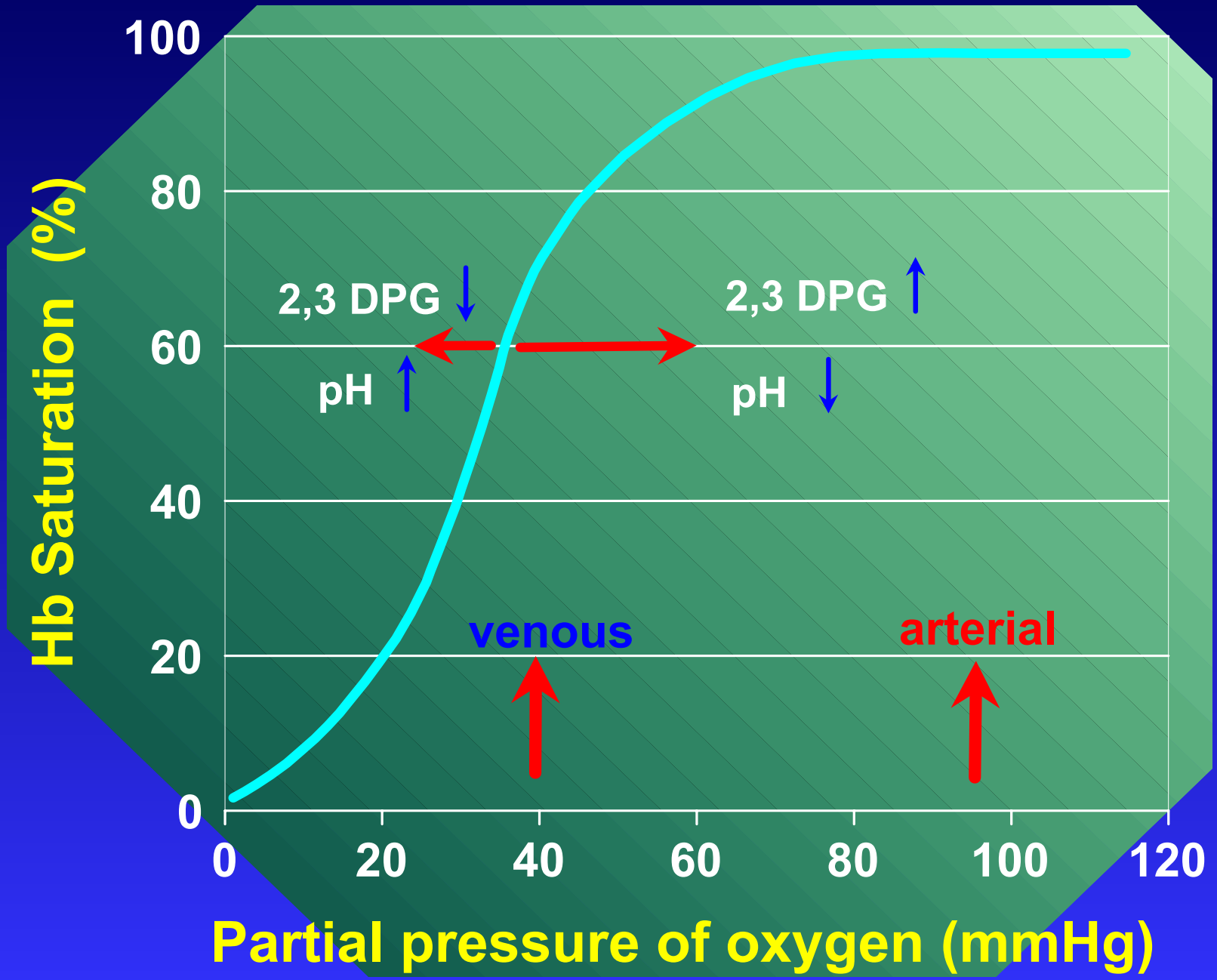
Artificial oxygen-carrying blood substitutes

- Modified haemoglobins
- Perfluorochemicals


886 909 wx
Expiry Date
2 Aug 1993
O
Rh D POSITIVE
NORTH LONDON BLOOD TRANSFUSION CENTRE
Date Bled
28 Jun 1993
Red Cell Concentrate (A-S)
Buffy coat depleted
SAG-M Additive
Cpdda system
DO NOT HEAT
93B25865

HAEMOGLOBIN

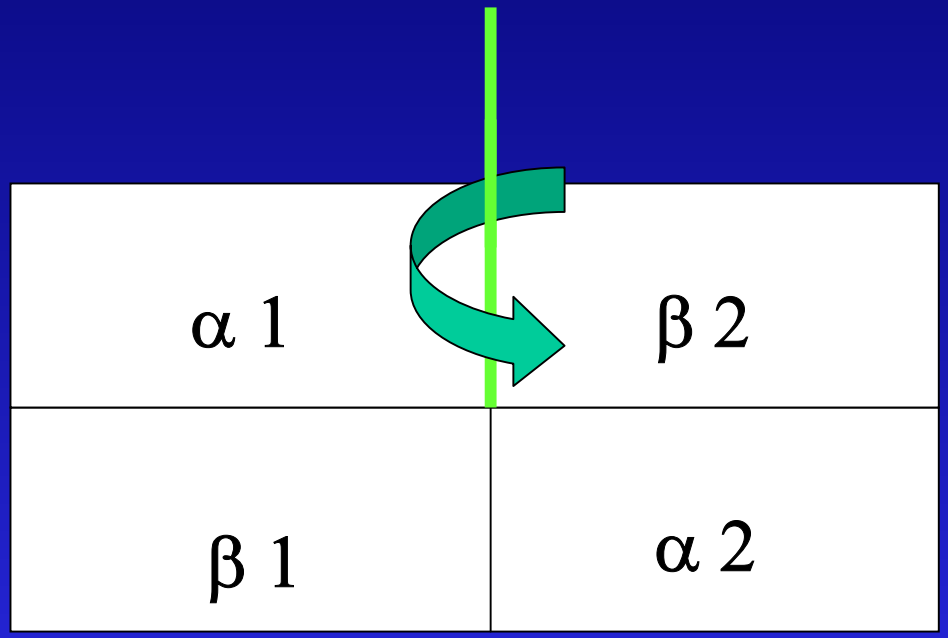



100
80
60
40
20
0
Hb Saturation (%)
2,3 DPG
pH
2,3 DPG
pH
venous
arterial
0
20
40
60
80
100
120
Partial pressure of oxygen (mmHg)

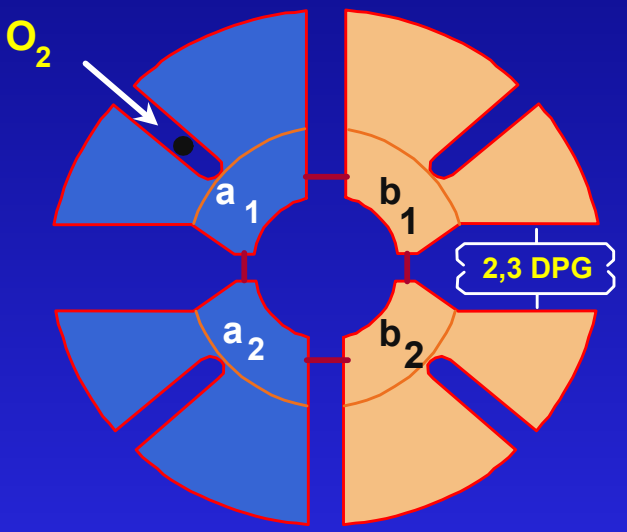
P50 (30 mmHg)
OXY-Hb <---> DEOXY-Hb

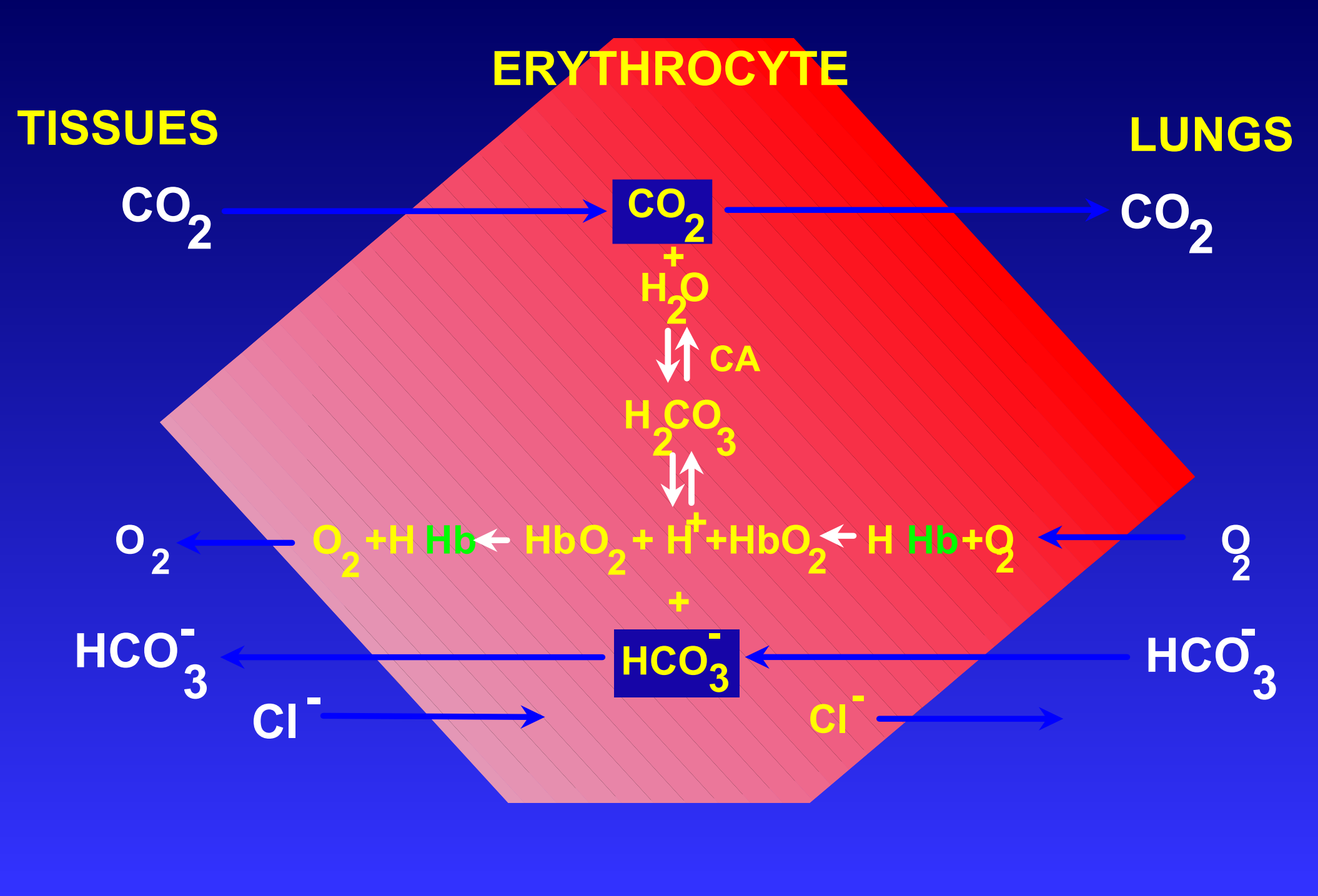
-COOPERATIVITY (heme/heme interaction)
    - 16 mmHg
-2,3 DPG
-BOHR effect :
    -CO_2
    -Cl^-
    -PH
-HILL number (log function of the slope at P50)
    -2,7-3,1
-TEMPERATURE

COOPERATIVITY

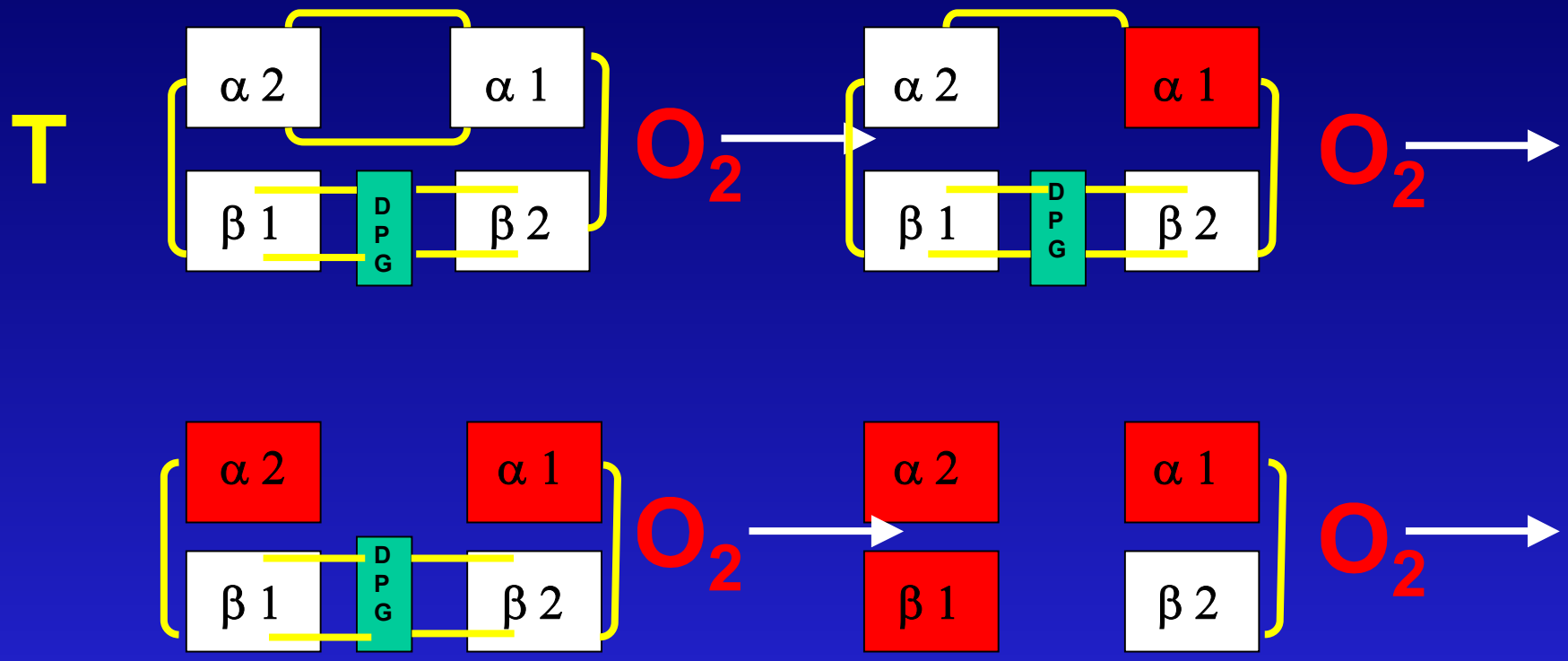


HAEMOGLOBIN




ERYTHROCYTE
TISSUES
LUNGS
CO_2
CO_2
CO_2
+
H_2O
CA
H_2CO_3
O_2 +H Hb
HbO_2 + H^+ +HbO_2
H Hb+O_2
O_2
O_2
+
HCO_3^-
HCO_3^-
HCO_3^-
Cl^-
Cl^-

HILL



NL= 2,7 – 3,1

1= high affinity Hb

OXY-Hb --> DEOXY-Hb

P50 > 30 mmHg

-COOPERATIVITY

<16 mmHg

-2,3 DPG

-BOHR effect

-Low PH

-High CL^-

-High C02

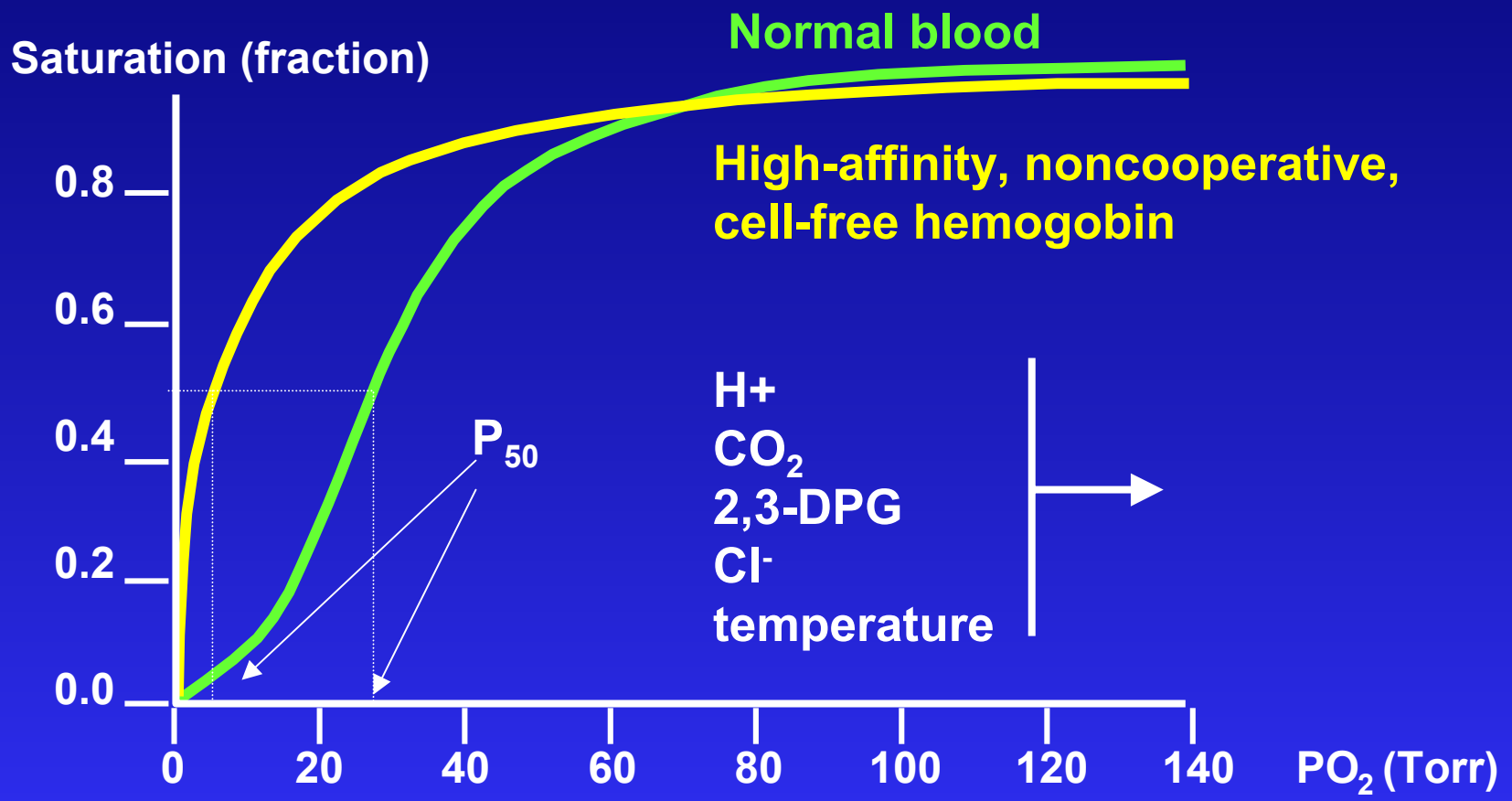
-HILL number (log function of the slope at P50)

>2,7-3,1

-TEMPERATURE

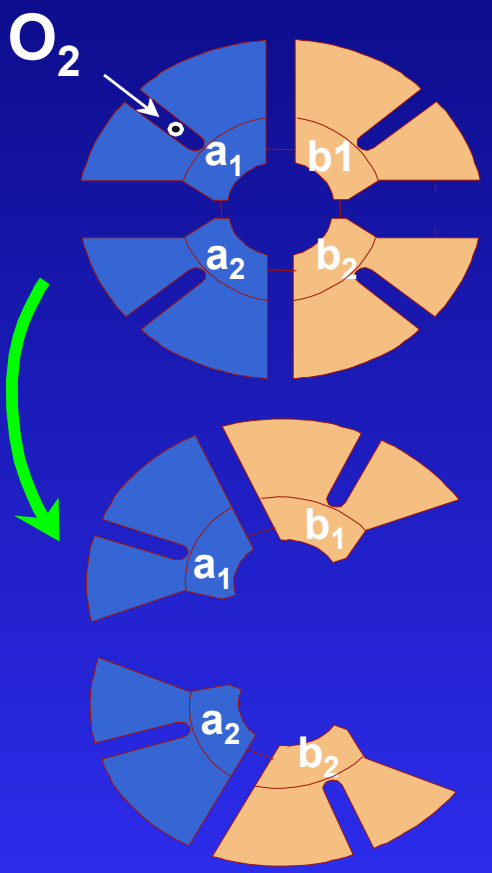
-High temperature

Oxygen equilibrium curves



ACZA 1PZ

Problems with the use of SF-Hb-solutions

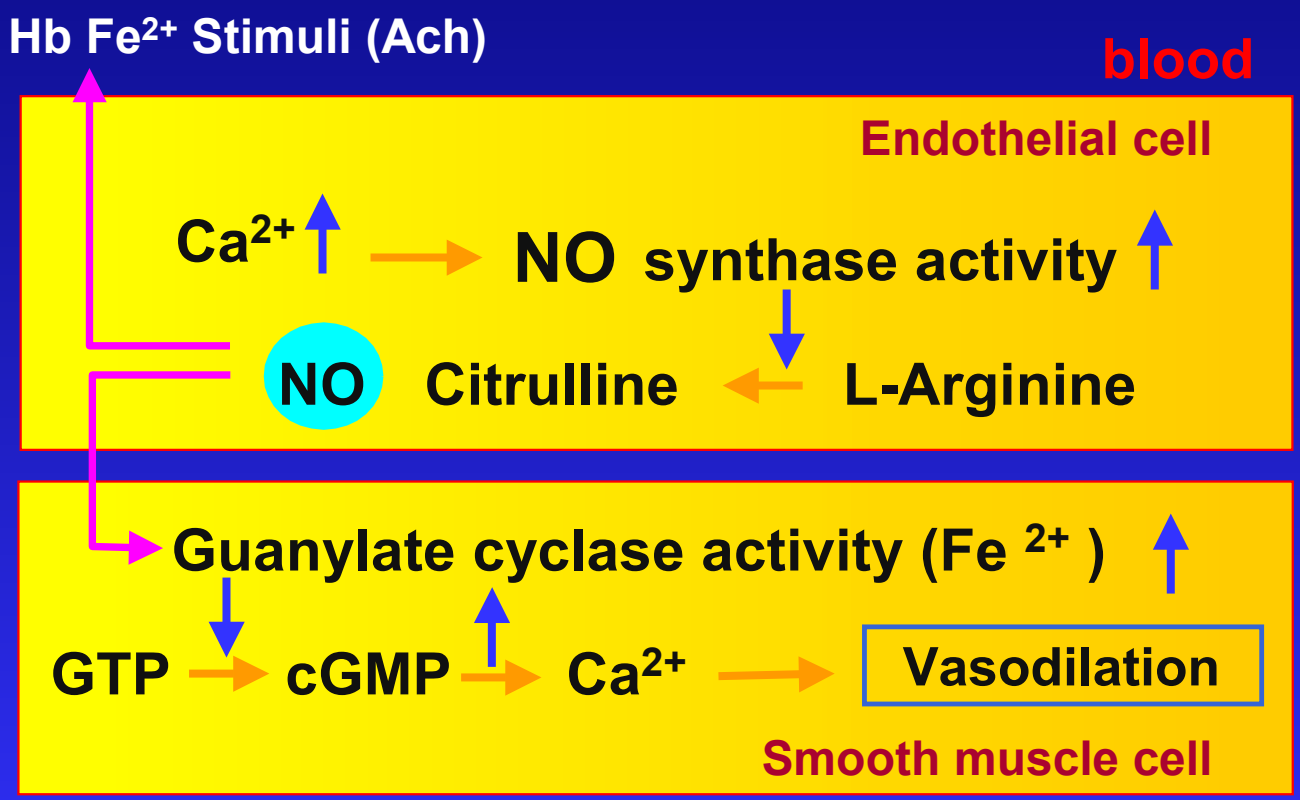


- Low P50 (no 2,3-DPG)
- Tetramer to Dimers
- Low half-life (1-2hrs)
- Iso-oncotic at 7 g/dl
- Renal toxicity
- Vasoconstriction

Vasoconstriction

Hypertension & gastrointestinal

- Stroma
- NO

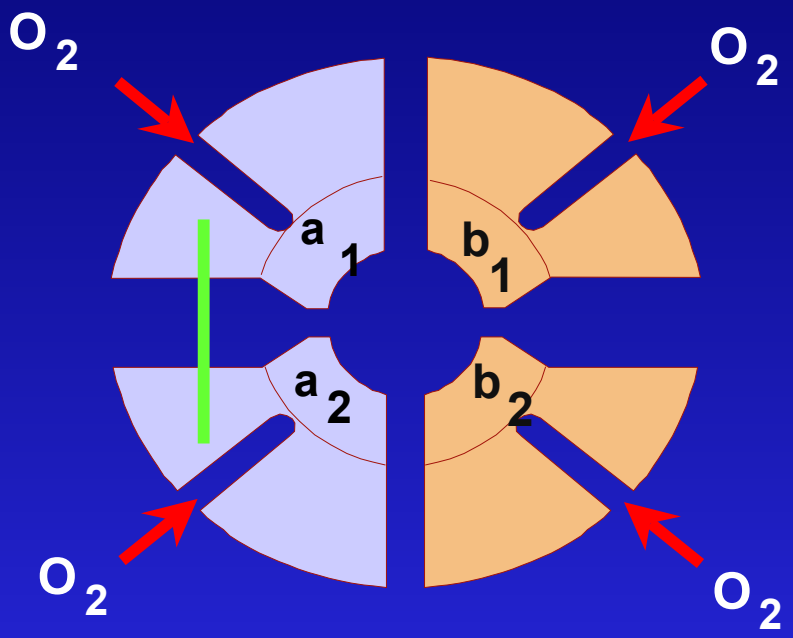


HEM/90872P

Hb modifications

- To decrease oxygen affinity
- To prolong intravascular retention
- To decrease colloid osmotic activity
- To prevent toxicity (renal & extravasation & hypertension & gastrointestinal)

Crosslinked Tetrameric Hb

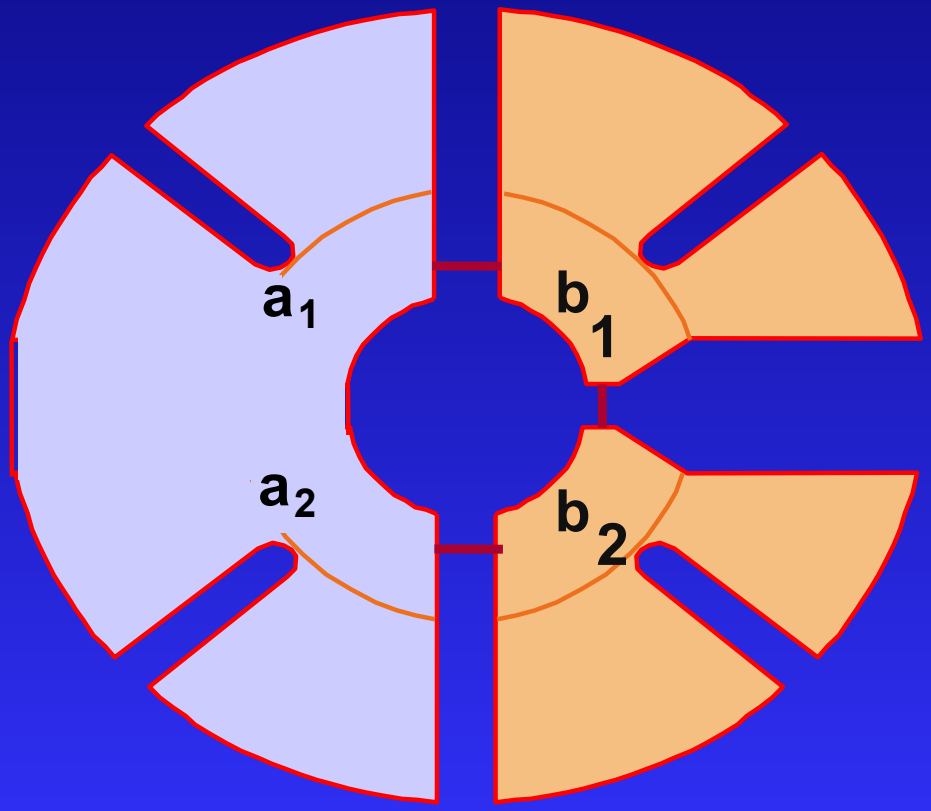


bis-(3.5-dibromosalicyl) fumarate

- cross-link two a subunits
- modifying the 2.3-DPG pocket (increase in P_{50})
- Diaspirin cross-linked tetrameric Hb Phase III trials

Recombinant Hb

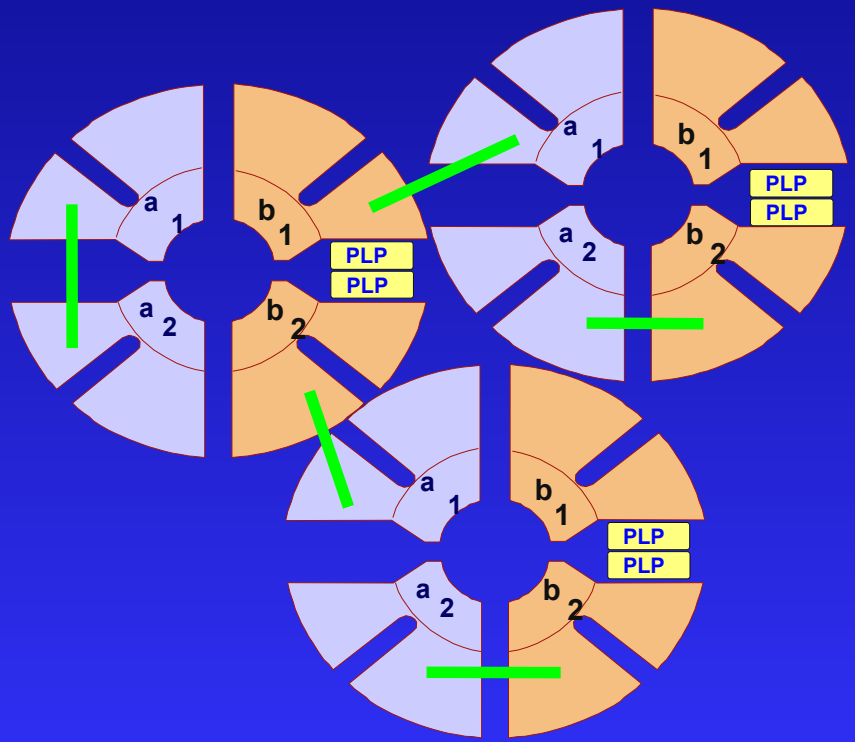
- engineered in E-coli
- prevent breakdown of the tetramers
- high P_{50}



HEM/90858P

Crosslinked polymerized Hb

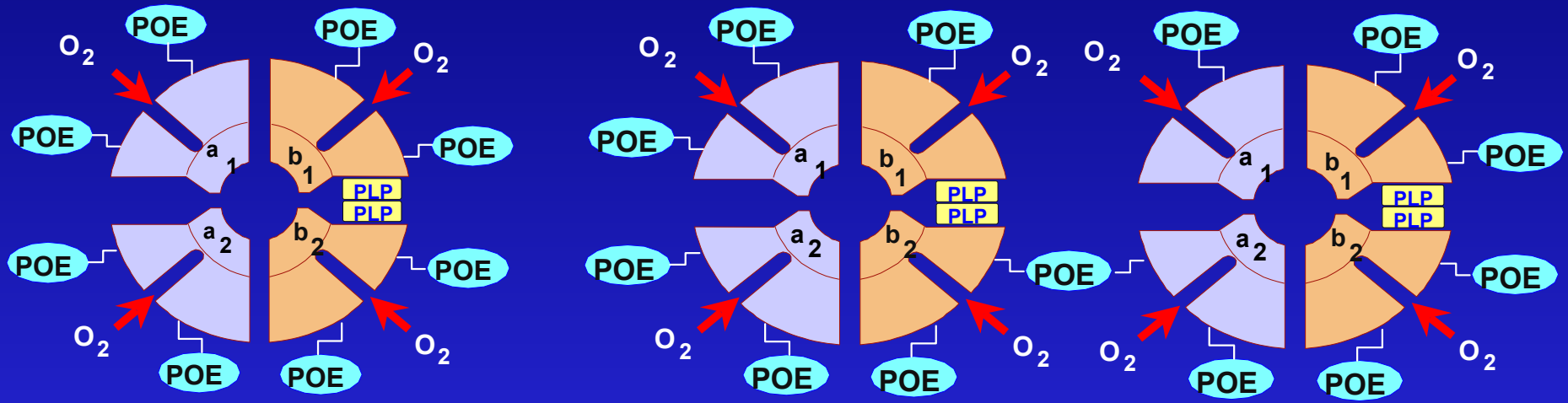
- glutaraldehyde cross-linking
- pyridoxal phosphate increase the P_{50}
- bovine poly Hb P_{50} is controlled by Cl



Tetrameric Hb < 1%

HEM/90856P

Conjugated Hb

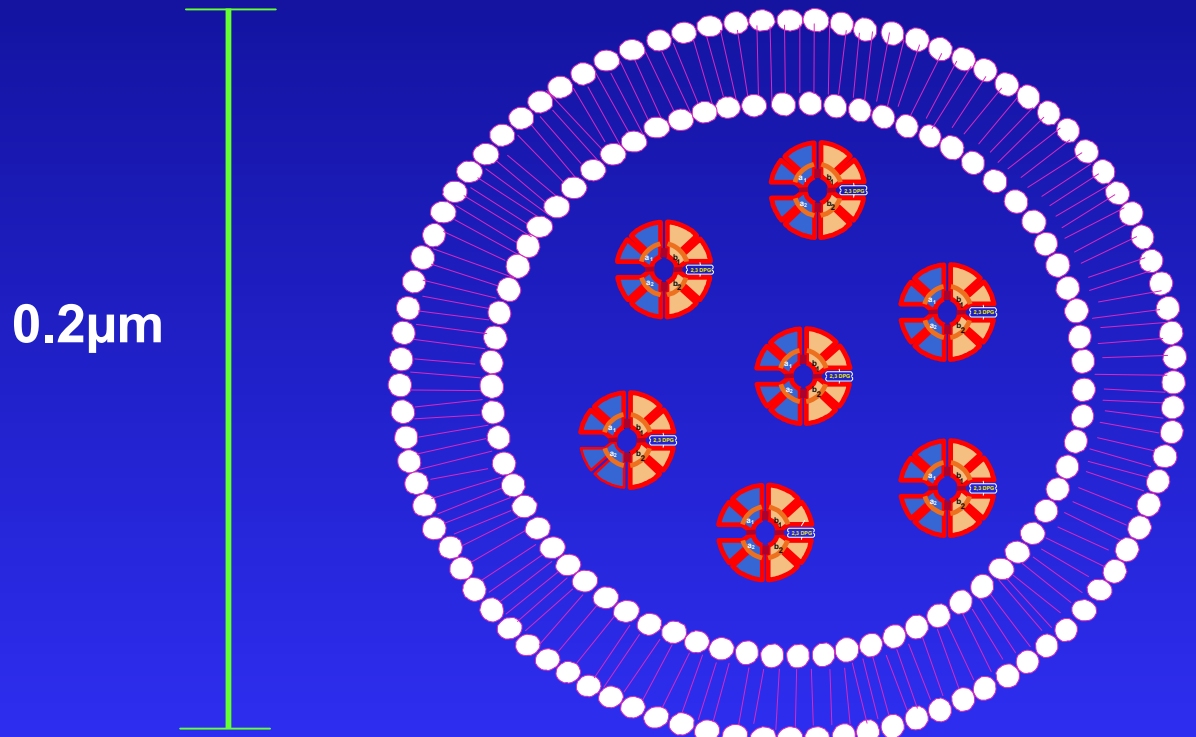


- dextran
- polyoxyethylene
- polyethylene glycol

HEM/90859P

Hb lipid vesicles

- Limiting binding of NO by extravascular Hb
- Half-life reduced due to removing by spleen and liver
- Interaction with granulocytes and immune system



HEM/90866P

P_{50} of some new hemoglobins

	Hb concentration	P_{50}
Crosslinked Hb (intramolecular)		
HemAssist® Baxter (human Hb)	10 g/dl	32 mmHg
Optro® Somatogen / Eli Lilly (rHu-Hb)	5-8 g/dl	32 mmHg
Polymerized Hb		
PolyHeme® Northfield (human Hb)	10 g/dl	28-30 mmHg
Hemolink® Hemosol (human Hb)	10 g/dl	34 mmHg
Hemopure® Biopure / Upjohn (bovine Hb)	13 g/dl	38 mmHg
Polymerized-conjugated Hb		
PEG-Hb Enzon (bovine Hb)	6 g/dl	ca. 15 mmHg
Encapsulated Hb		
Neo Red Cells® Terumo (human Hb)	5.6 g/dl	49.5 mmHg

Hill coefficient of some new hemoglobins

	Hb concentration	Hill coefficient
Crosslinked Hb (intramolecular)		
HemAssist® Baxter (human Hb)	10 g/dl	n.a.
Optro® Somatogen / Eli Lilly (rHu-Hb)	5-8 g/dl	2.27
Polymerized Hb		
PolyHeme® Northfield (human Hb)	10 g/dl	n.a.
Hemolink® Hemosol (human Hb)	10 g/dl	1
Hemopure® Biopure / Upjohn (bovine Hb)	13 g/dl	n.a.
Polymerized-conjugated Hb		
PEG-Hb Enzon (bovine Hb)	6 g/dl	1
Encapsulated Hb		
Neo Red Cells® Terumo (human Hb)	5.6 g/dl	n.a.

HEM/00492M

COP of some new hemoglobins

	Hb concentration	COP
Crosslinked Hb (intramolecular)		
HemAssist® Baxter (human Hb)	10 g/dl	43 mmHg
Optro® Somatogen / Eli Lilly (rHu-Hb)	5-8 g/dl	16 mmHg
Polymerized Hb		
PolyHeme® Northfield (human Hb)	10 g/dl	n.a.
Hemolink® Hemosol (human Hb)	10 g/dl	24 mmHg
Hemopure® Biopure / Upjohn (bovine Hb)	13 g/dl	17 mmHg
Polymerized-conjugated Hb		
PEG-Hb Enzon (bovine Hb)	6 g/dl	ca. 100 mmHg
Encapsulated Hb		
Neo Red Cells® Terumo (human Hb)	5.6 g/dl	25 mmHg

Circulation storage shelf life & half life

	Shelf life storage	Circulation
Crosslinked Hb (intramolecular)		
HemAssist® Baxter (human Hb)	n.a.	ca. 14 h
Optro® Somatogen / Eli Lilly (rHu-Hb)	18 months refrigeration	4-5 h
Polymerized Hb		
PolyHeme® Northfield (human Hb)	12 months refrigeration	24 h
Hemolink® Hemosol (human Hb)	2 years refrigeration	ca. 14 h
Hemopure® Biopure / Upjohn (bovine Hb)	2 years room temperature	ca. 24 h
Polymerized-conjugated Hb		
PEG-Hb Enzon (bovine Hb)	1 year frozen	ca. 44 h

Viscosity of some new hemoglobins

	Hb concentration	Viscosity
Crosslinked Hb (intramolecular)		
HemAssist® Baxter (human Hb)	10 g/dl	1.1 cP
Optro® Somatogen / Eli Lilly (rHu-Hb)	5-8 g/dl	n.a.
Polymerized Hb		
PolyHeme® Northfield (human Hb)	10 g/dl	n.a.
Hemolink® Hemosol (human Hb)	10 g/dl	1 cP
Hemopure® Biopure / Upjohn (bovine Hb)	13 g/dl	1.3 cP
Polymerized-conjugated Hb		
PEG-Hb Enzon (bovine Hb)	6 g/dl	n.a.
Encapsulated Hb		
Neo Red Cells® Terumo (human Hb)	5.6 g/dl	2 cP

Clinical Use

	Potential clinical use	Regulatory phase
Crosslinked Hb (intramolecular)		
Optro® Somatogen / Baxter (rHu-Hb)	Cardiac bypass	preclinical
Polymerized Hb		
PolyHeme® Northfield (human Hb)	Trauma, elective/emergency surgery	Phase III
Hemolink® Hemosol (human Hb)	Cardiac, orthopedic surgery; erythropoiesis	Phase III
Hemopure® Biopure / Upjohn (bovine Hb)	Elective surgery, sickle cell crisis	Phase III
Polymerized-conjugated Hb		
PEG-Hb Enzon (bovine Hb)	Radiosenstive, chemosensitive tumors; volume expansion	Phase II

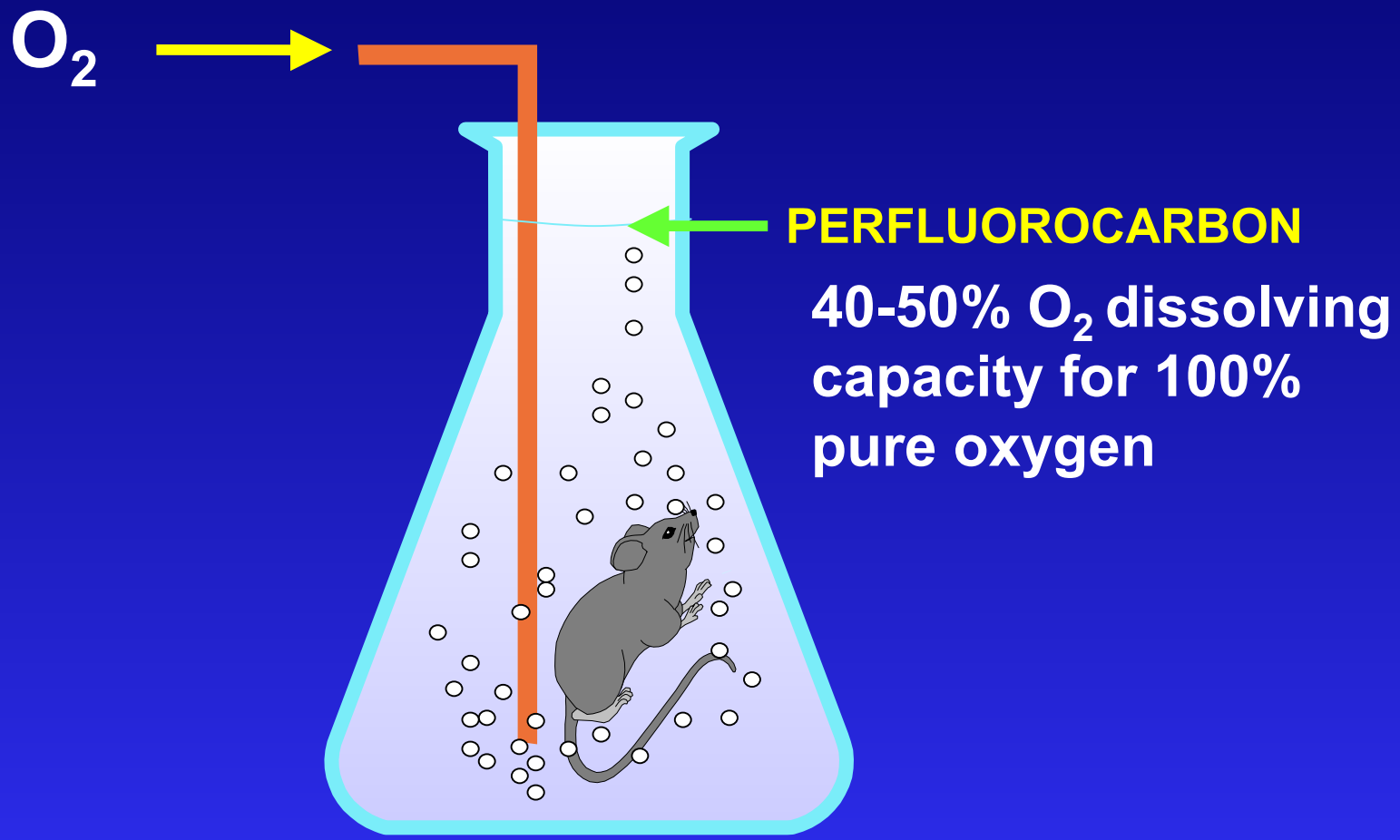
Artificial O2 carriers

The modified hemoglobin-based solutions are grouped by the source of the hemoglobin

- Modified Hemoglobin-Based Solutions
  - Derived from Outdated Human Red Cells
    - Polymerized hemoglobin (PolyHeme®, Northfield) (phase III clinical trials)
    - Polymerized and cross-linked hemoglobin (Hemolink®, Hemosol) (phase III clinical trials)
    - Pyridoxalated hemoglobin polyoxyethylene conjugate (PHP, Apex Bioscience) (phase III clinical trials)
    - Diaspirin cross-linked hemoglobin (HemAssist®/DCIHb®, Baxter) (discontinued)
  - Derived from Bovine Red Cells
    - Polymerized Purified Bovine Hemoglobin (HemoPure®, Biopure) (phase III clinical trials)
    - Polyethylene-Glycol Modified-Bovine Hemoglobin (PEG-Hgb®, Enzon) (phase Ib clinical trials)
  - Recombinant Human Hemoglobin
    - E. coli[46] (Optro® /rHb1.1, Somatogen/Baxter) (discontinued) Transgenic tobacco[47] (preclinical)

Artificial oxygen-carrying blood substitutes

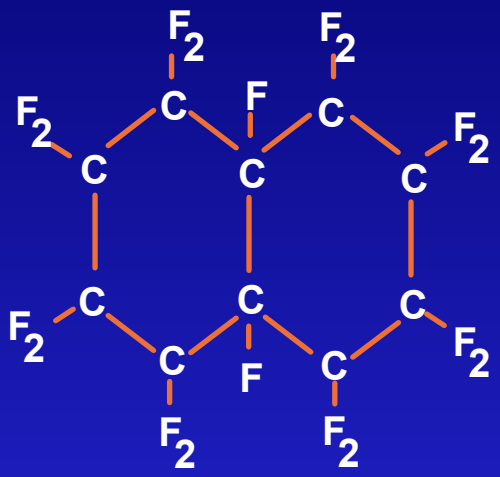
- Modified haemoglobins
- Perfluorochemicals



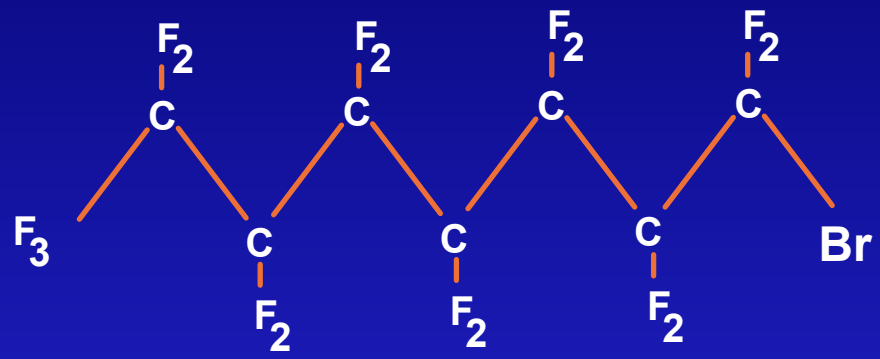
40-50% O_2 dissolving capacity for 100% pure oxygen

HEM/90211P

Fluorocarbon-based oxygen carriers



F-decalin (Fluosol-DA)



F-octylbromide (oxygent)

- High gas-dissolving capacity (O_2 and CO_2)
- Low viscosity
- Chemical and biologic inert
- Insoluble in water

Fluorocarbons are hydrophobic

Emulsion of 0.1-0.3 um
Surface active agent (surfactant)
    poloxamer:Pluronic F-68 (Fluosol-DA)
    (allergic reactions)
    egg-yolk phospholipide
T 1/2: 4 to 7 days
exhalation or phagocytosis (RES)
Problem: coalescence of the emulsion

Compared solubilities of the respiratory gases in perfluorocarbons and their emulsion

O_2 and CO_2 solubilities in fluorochemicals and their emulsions (ml/dL, 37°C, 1 atm)

	O_2	CO_2
H_2O, plasma	2.3	65
Blood	20	70
F-tributilamin N $(C_4F_9)_3$	40.3	142
F-tripropylamin N $(C_4F_7)_3$	45.3	166
F-decalin	45	130
Perfluorooctylbromide $C_8F_{17}Br$	50	
20% Emulsion of F-decalin (Fluosol)	7.5	70
50% Emulsion of perfluorooctylbromide (perflubron)	15.3	

Clark et all, 1982

HEM/00483M

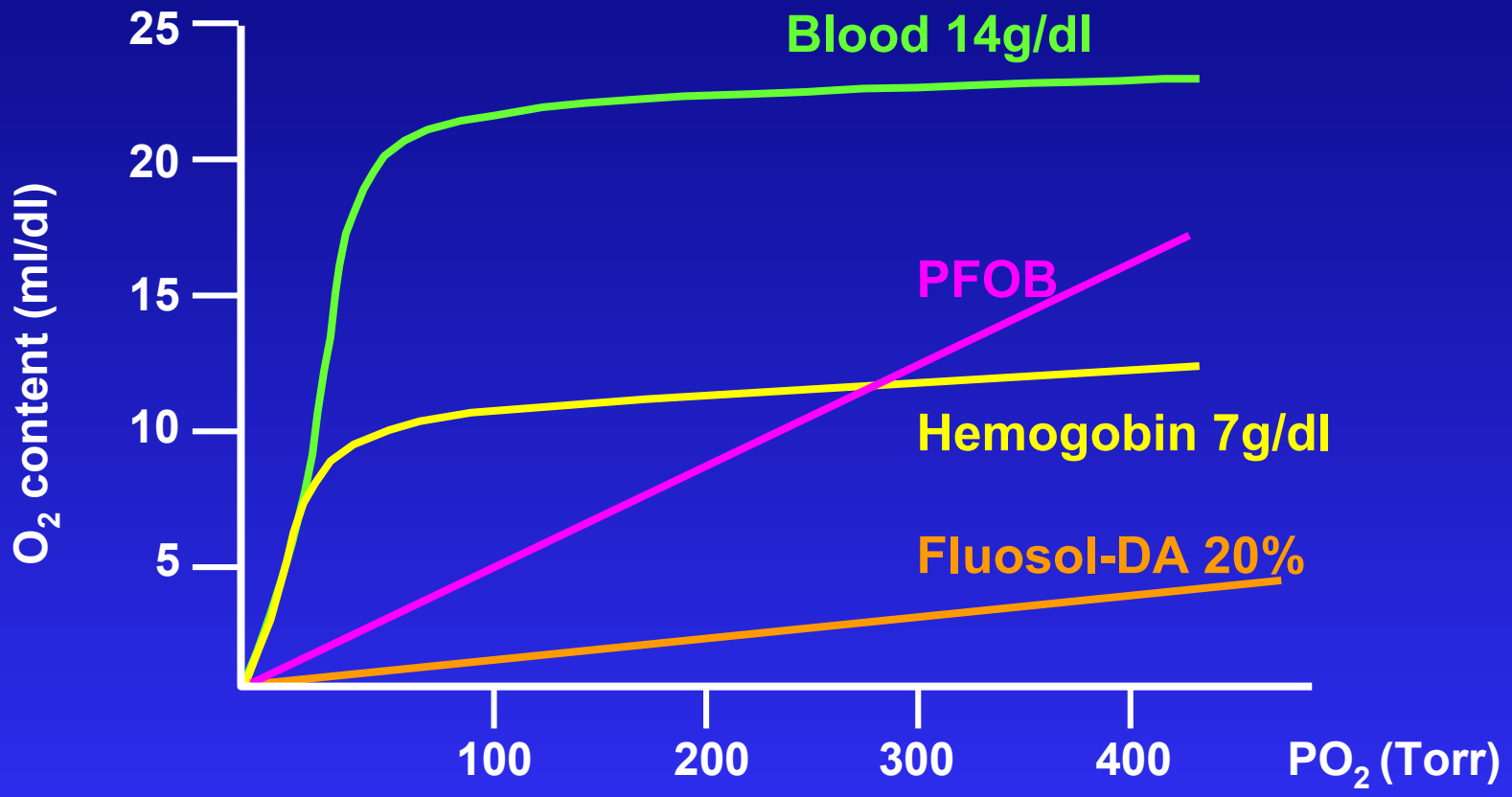
Comparisons between 1st and 2nd generation in perfluorocarbon emulsions

Fluosol DA			Perflubron
Perfluoro-decalin	Perfluoro-tripropylamide	PFC	Perfluoro-octylbromide
10-11% vol/vol		Concentration	30% vol/vol
1.96		Density	1.96
5-7 ml/dl		O_2 carrying capacity	17.2 ml/dl
Pluronic F-18 +++ Egg yolk phospholipids		Emulsifier	Egg yolk phospholipids
< 600 nM		Size of micelles	< 250 nm
7.5 (10ml/kg) 14.5 (20ml/kg) 22 (30ml/kg)		Half-life iv (hours)	4 (2.7g/kg)
7	65	Half-live tissues (days)	7 (1.8g/kg)

HEM/00485M

Oxygen equilibrium curves

Hemoglobin attains nearly complete saturation at the arterial oxygen partial pressure 100 mmHg



HEM/00489M

Artificial O2 carriers

The modified hemoglobin-based solutions are grouped by the source of the hemoglobin

- Perfluorocarbon (PFC) Emulsions
  - Perflubron (Oxygent®, Alliance/Baxter) (phase lll clinical trials)
  - Emulsified perfluorodichlorooctane (Oxyfluor® , HemaGen/PFC) (discontinued)

  - ‘First-Generation’ PFC emulsions
    - Fluosol® (Green Cross, Japan) (discontinued)
    - Perftoran (Russia)

HEM/00502M

Effects of Fluorocarbons

Future

- Blood poisoning: CO, CN-,etc.
- Converting hypoxic cells in the center of the tumor to aerobic metabolism, increasing susceptibility to radiation and chemotherapy
- Temporary vitreal replacement
- Organ preservation
- Radiology (CTscan, NMR) perfluorooctyl bromide
- Assessment of tissue oxygenation
- Ischaemia and microcirculatory disturbances
- Liquid ventillation

Advantages and disadvantages of hemoglobin-based solutions

- Advantages
  - Carries and unloads O_2
  - Sigmoidal O_2 dissociation curve
  - 100% FiO_2 is not mandatory for maximal potency
  - Easy to measure
- Disadvantages
  - Side effects
    - Vasoconstriction
    - Interference with colorimetric laboratory methods

Advantages and disadvantages of perfluorocarbon (PFC)

- Advantages
  - Carries and unloads O_2
  - Few and mild side effects
  - No known organ toxicity
- Disadvantages
  - 100% FiO_2 is mandatory for maximal efficacy
  - Additional colloid often necessary with potential side effects

The most beautiful thing we can experience, is the mysterious – it is the source of all true art and science

Albert Einstein

Oxygen Extraction

02 Extraction= (Ca02 – CvO2) / CaO2

25 to 30 %

Pharmacological disadvantages

Limit of efficacy

- intravascular persistence only a few hours & days
- colloid osmotic activity
- vasoactive
- oxidation to methemoglobin
- reperfusion injury

Toxicity

- Hemostasis: Hb binds NO → vasoconstriction and increased platelet adhesion
- Vasoactivity
  - execessive delivery of O_2 to arterioles → increase in endothelin → vasoconstriction
  - Binding of NO
- Gastrointestinal side effects (NO)
- Immunosuppression
- Interference with laboratory assays

PFC side effects

- Flu-like symptoms
- Sequestration in spleen and liver
  - Hepato & splenomegaly
  - thrombocytopenia