ADDITIVE SOLUTIONS FOR PLATELETS

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HISTORICAL CONTEXT

- Red Cells
  - 1914-1930s: Stored in citrate without glucose and at room temperature
  - 1940s – 1950s: Acid citrate dextrose developed which has a low pH to prevent carmelization of the glucose with steam sterilization
    - Low temperature storage
  - 1960s: CPD, and CPDA1 and CP2D
    - Red cells stored as a concentrate in plasma anticoagulant preservative
  - 1970s: Europe: SAGM (Saline-adenine-glucose-mannitol)
  - 1980s: US: AS-1, AS-3 and AS-5

HISTORICAL CONTEXT

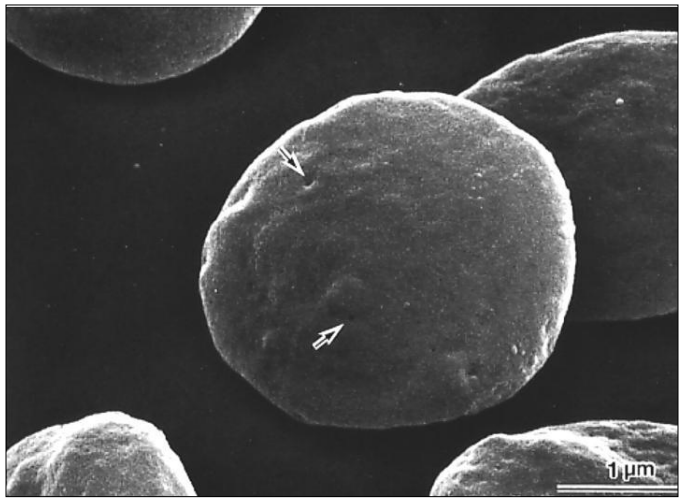
- Platelets:
  - 1960s –1970s: Concentrates derived form whole blood by PRP in ACD, CPD, CPDA1,CP2D or by apheresis in ACD-A or ACD-B
- Europe:
  - 1980-1990s: Whole blood concentrates derived from single buffy-coats in plasma, then prepooled buffy-coats in plasma then pooled buffy coats in additive solution
  - 2000s- .more recently pathogen inactivated pooled buffy coats in additive solution (France, Norway)
- US and Canada:
  - 1980-1990s: Whole blood concentrates derived from PRP in plasma: prestorage leukoreduced in late 1990s
  - 2000s: prepooled leukoreduced WBD Platelets in plasma
  - 2008 - 2011:
    - InterSol for apheresis platelets in US
    - BC-WBD pools in Canada

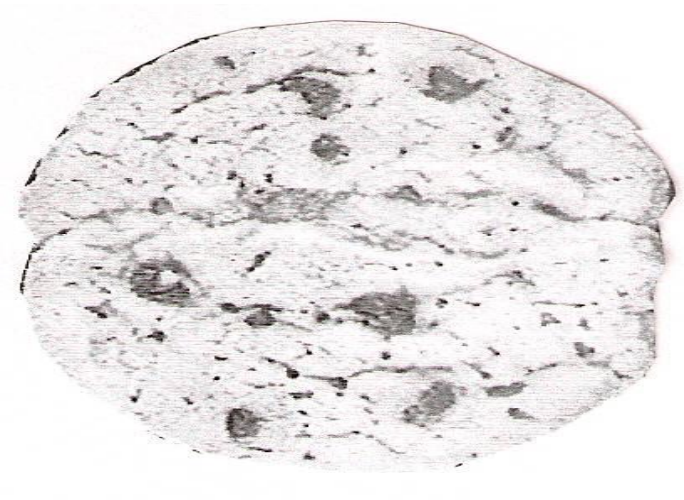
ADVANTAGES OF PAS

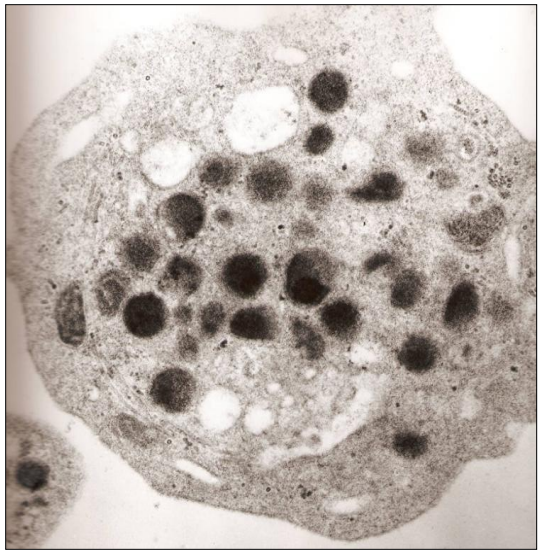
- Clinical advantages:
  - Manipulate the chemical composition in order to optimize viability or even functionality
  - Decrease allergic reactions, isoagglutinin mediated hemolytic reactions and possibly TRALI by removal of plasma
  - PAS environment may facilitate bacterial detection
- Manufacturing advantages:
  - Increase in the availability of plasma as a transfusable product or for fractionation
    - World demand for IVGG (7%), albumin (3%) increasing
  - PAS environment may facilitate pathogen reduction
  - Developments in automation for whole blood platelets may streamline the process of platelet separation, plasma removal and PAS replacement

Manipulation the chemical composition of the PAS in order to optimize viability or even functionality

The Quest for the Platelet Shangri-la

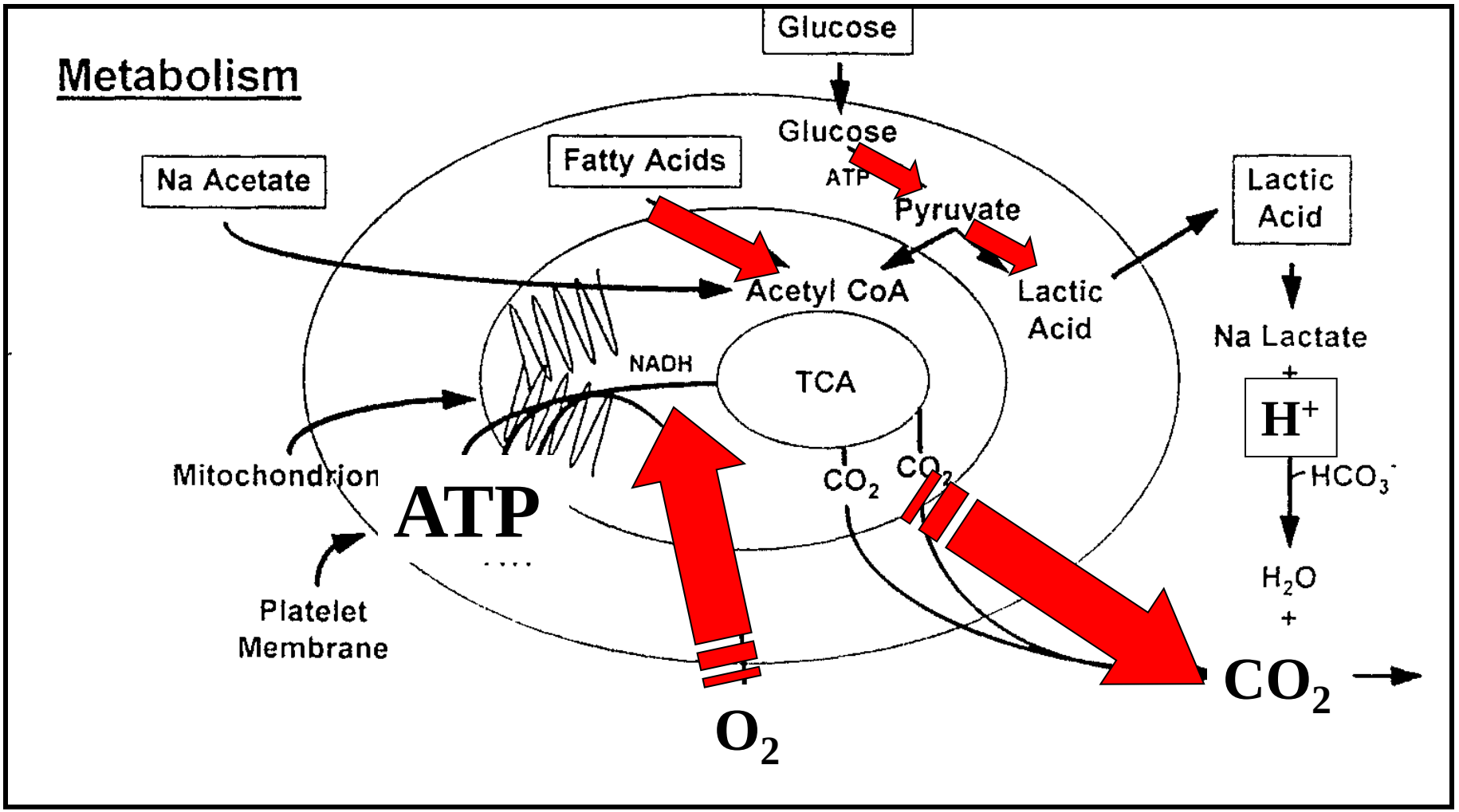






ASFA2011

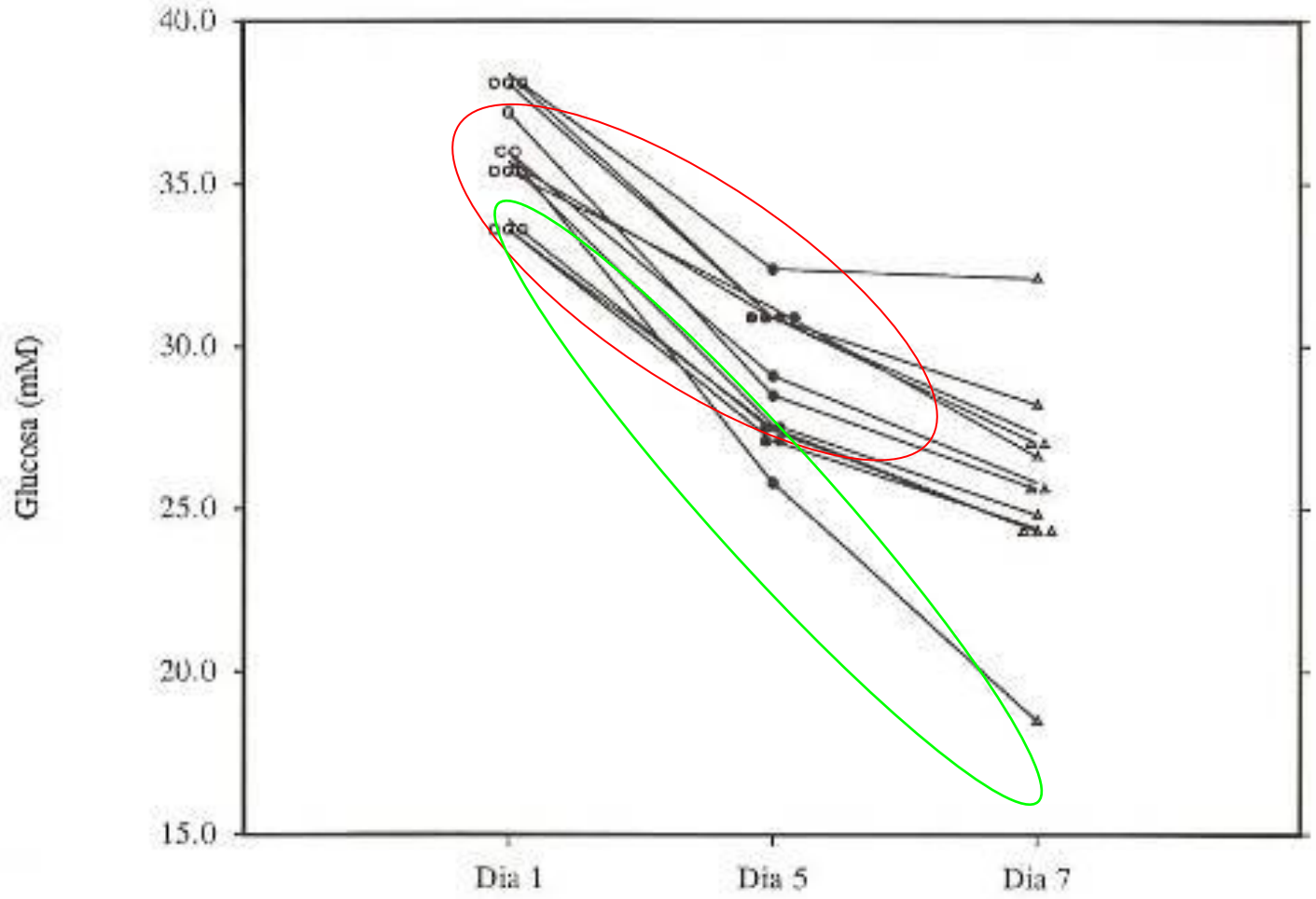
PLATELET ENERGY METABOLISM



PLATELET ENERGY METABOLISM

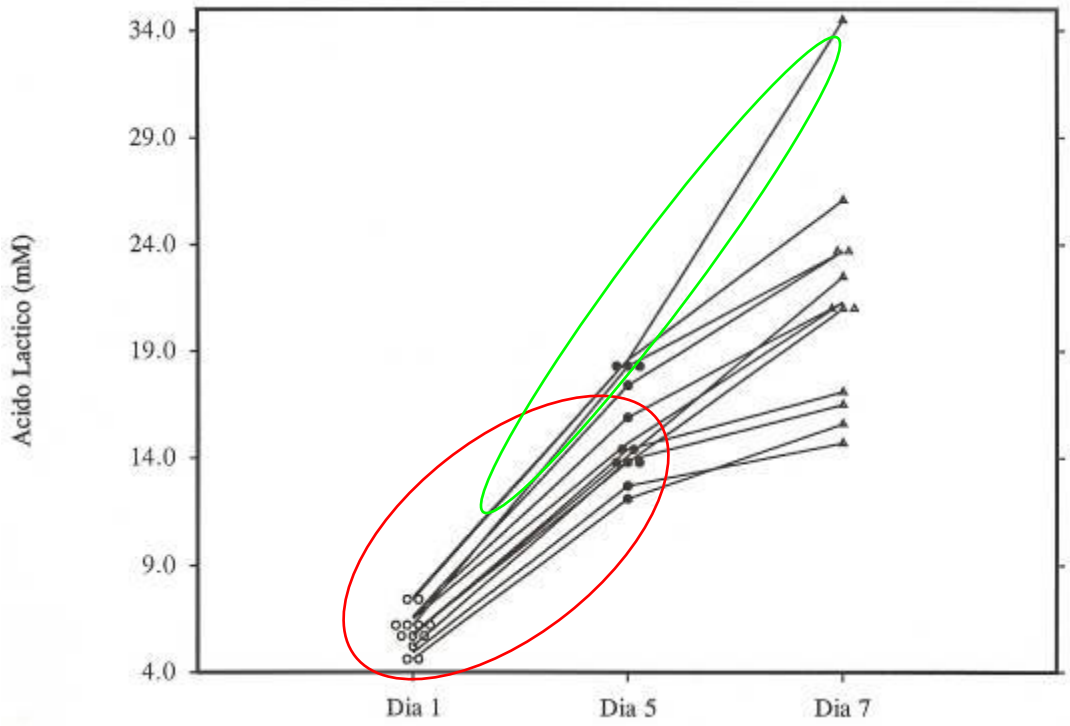
- 85 % of ATP production is by the Krebs (TCA) cycle
  - Nutrients
    - Fatty acids
    - Amino acids
    - Carbohydrates (glycogen)
- 15 % of ATP production by glycolysis

Glucose

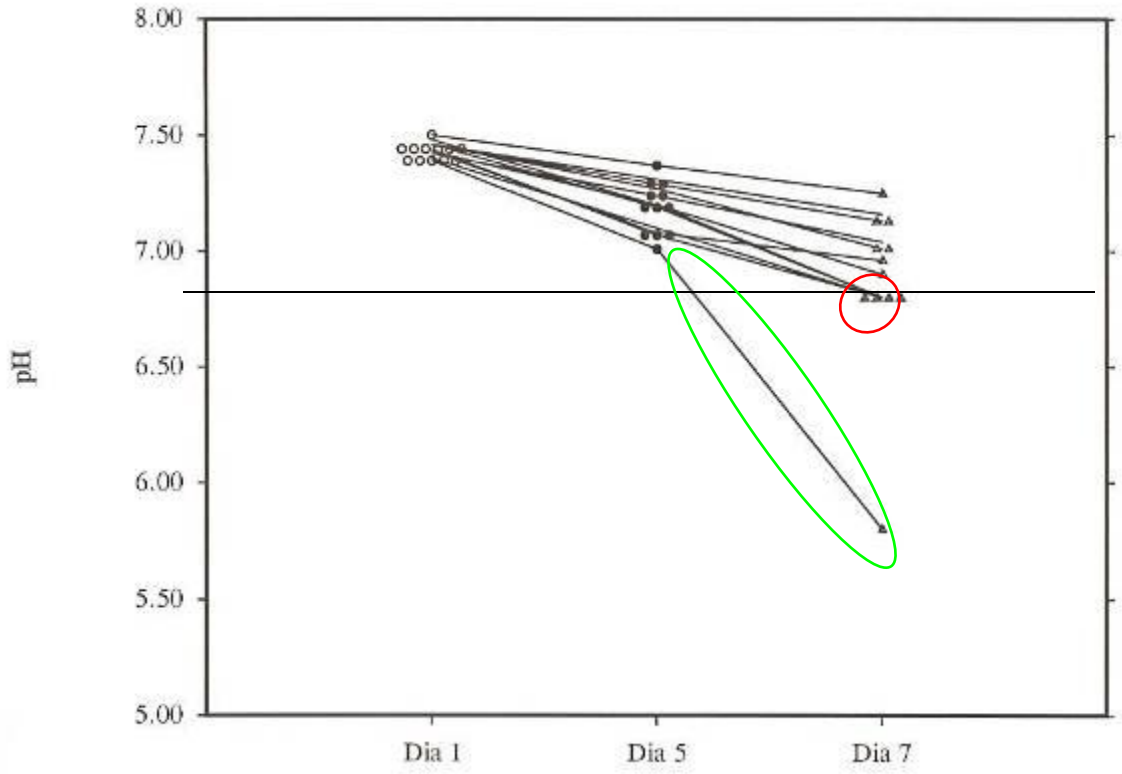


LACTIC ACID

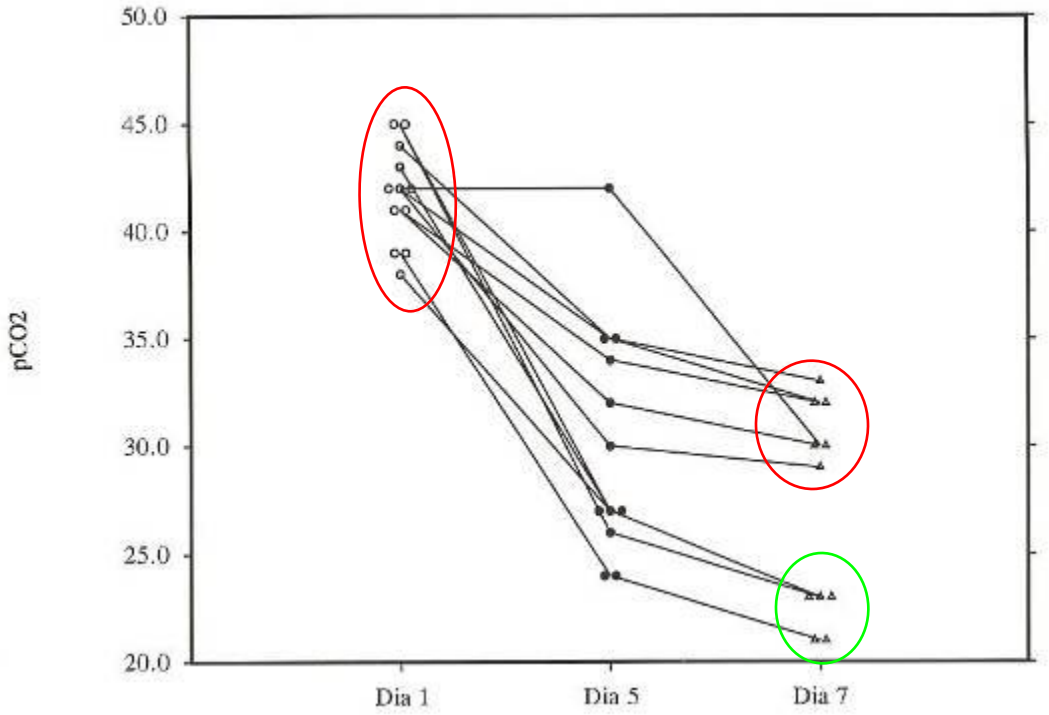
Glucose is metabolized primarily to lactic acid



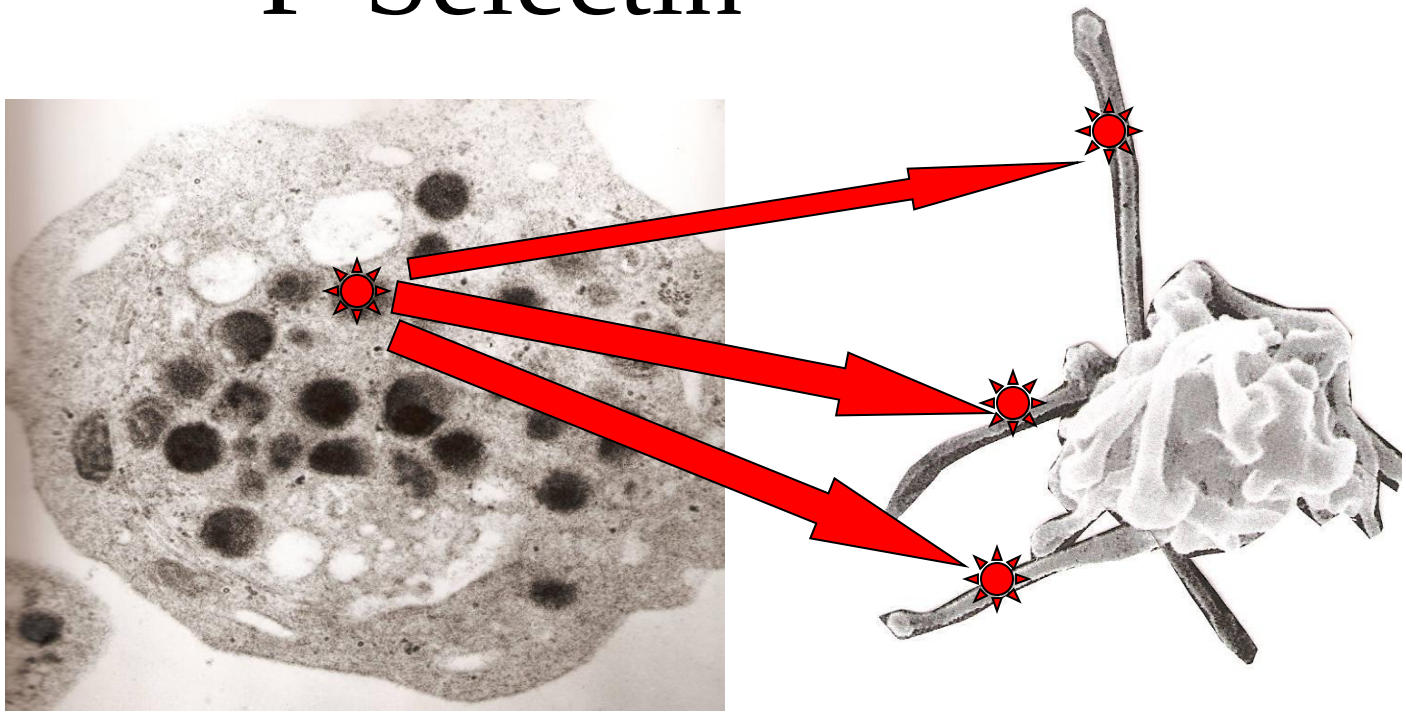
pH



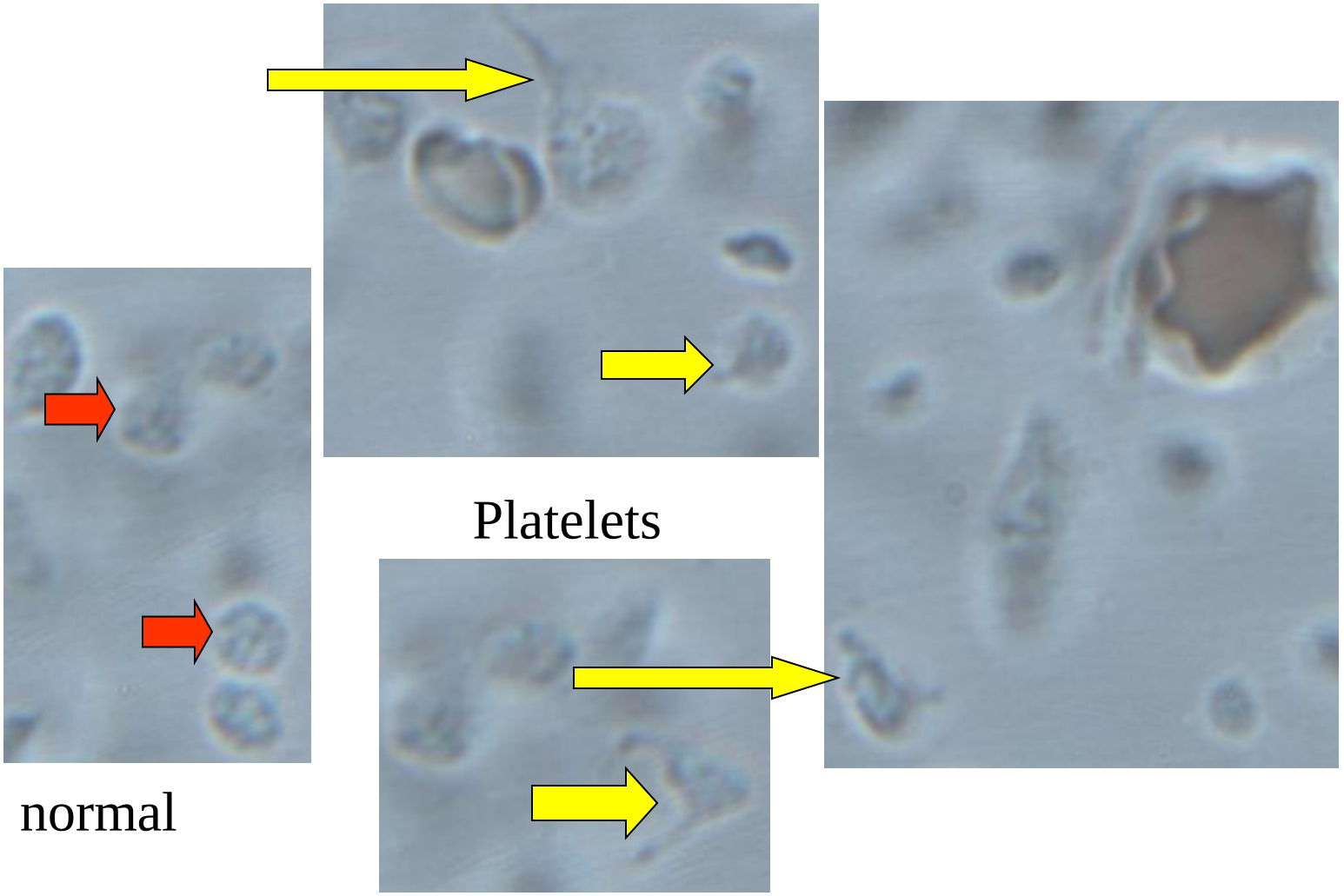
CO_2



P-Selectin



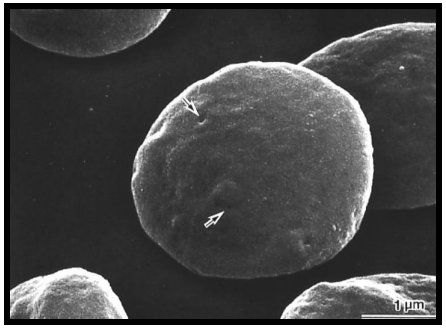
LIGHT MICROSCOPY



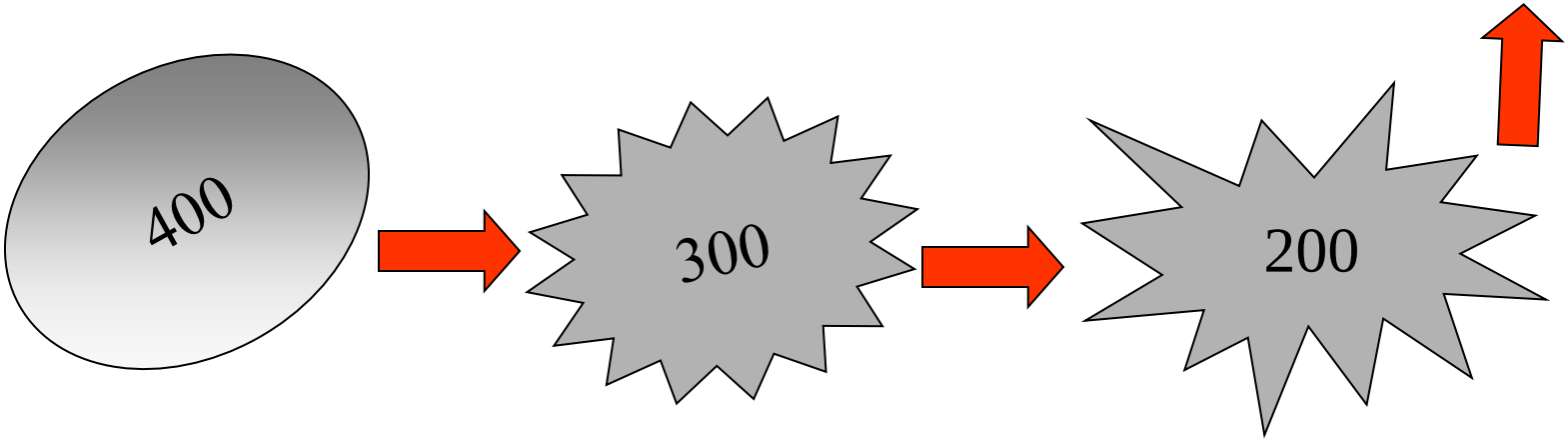
Platelets

normal

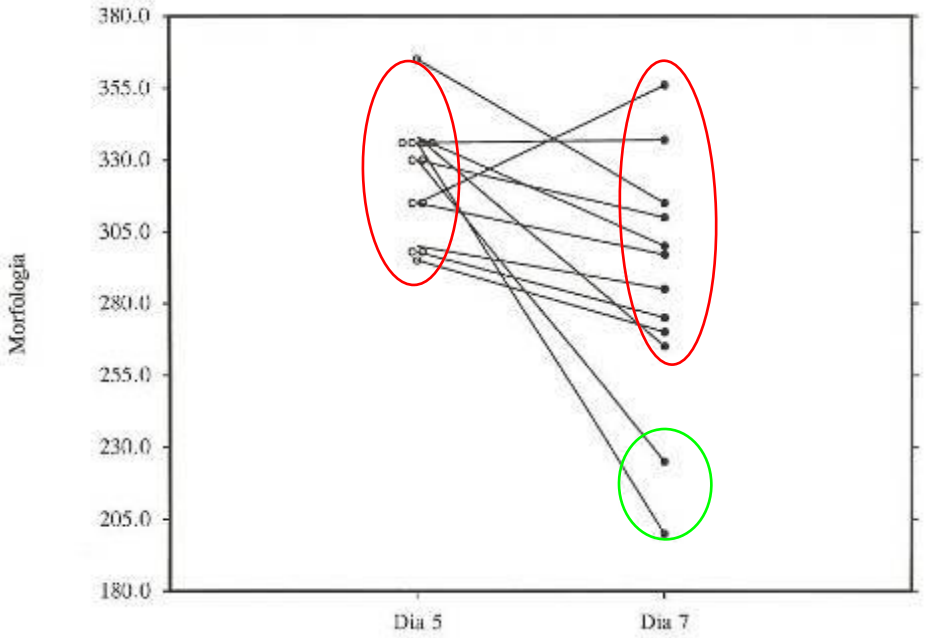
MORPHOLOGY



100



MORPHOLOGY SCORE

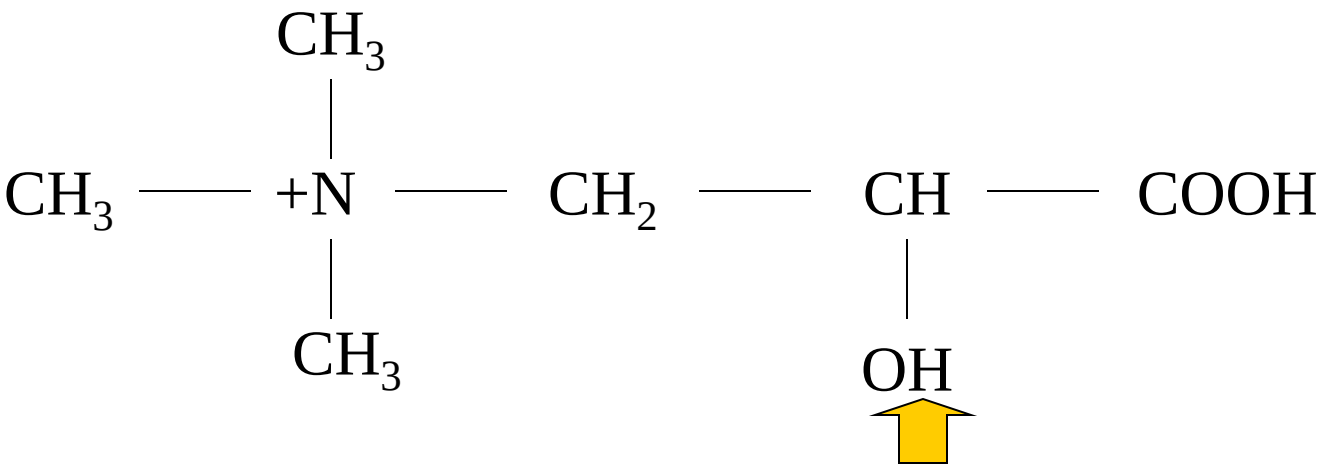


METABOLIC ADDITIVES

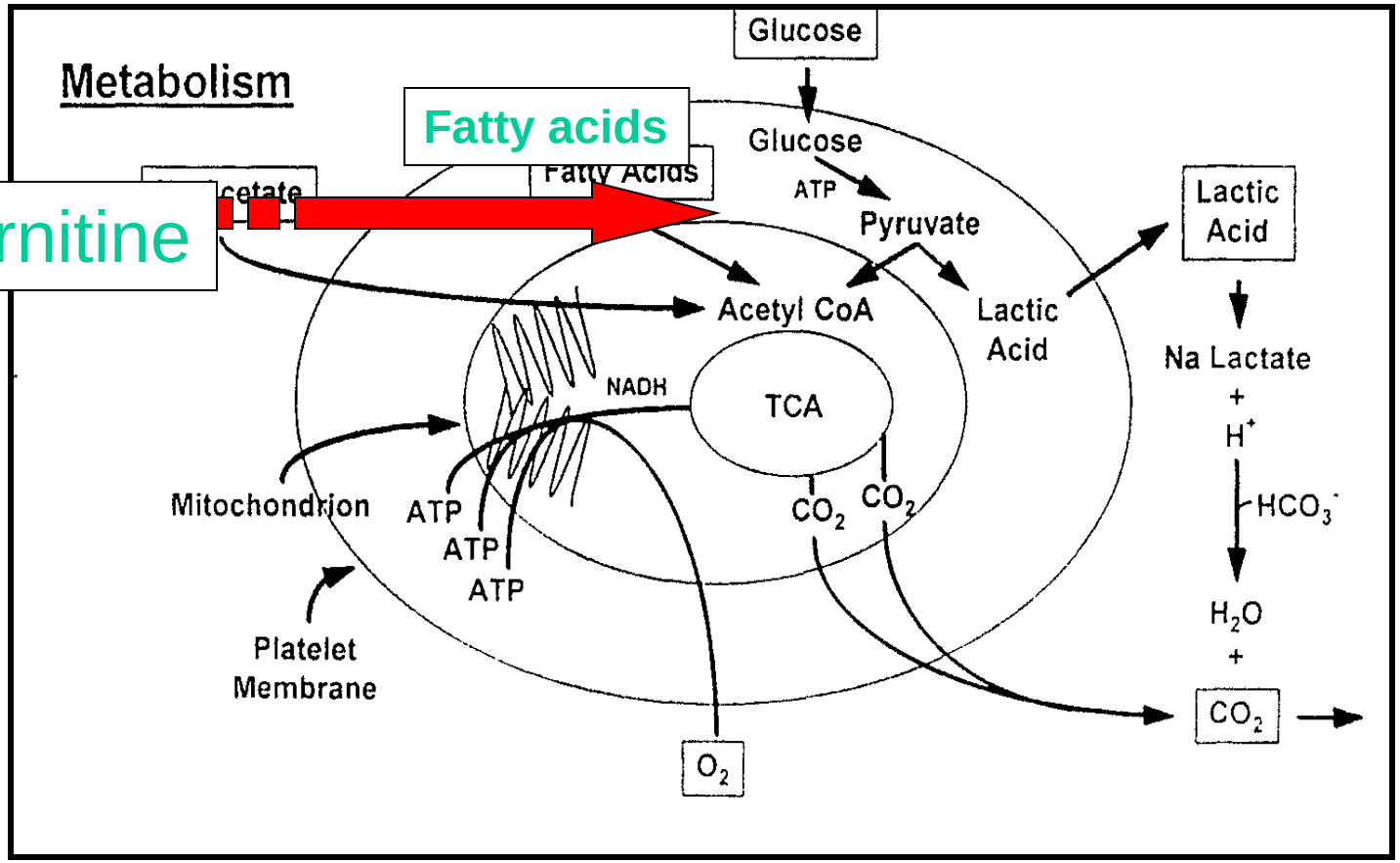
What can we add to improve the metabolism of platelets?

L-Carnitine and Platelets

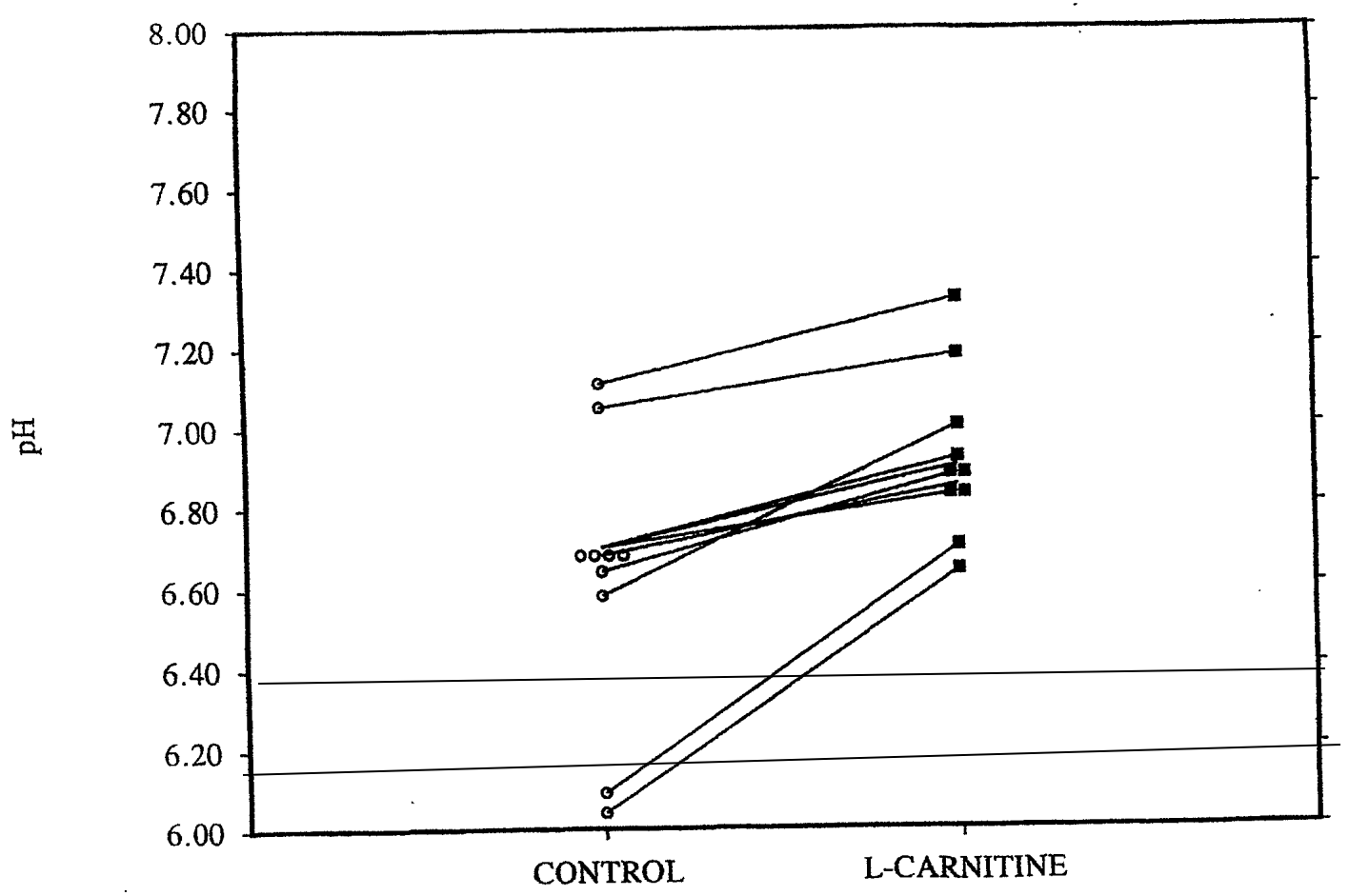
L-carnitine facilitates the transport of long chain fatty acids across the outer mitochondrial membrane



L-Carnitine


Metabolism
Glucose
Fatty acids
Carnitine
Glucose
ATP
Pyruvate
Fatty Acids
Lactic Acid
Acetyl CoA
Lactic Acid
Na Lactate
+
H^+
HCO_3^-
NADH
TCA
Mitochondrion
ATP
ATP
ATP
CO_2
CO_2
H_2O
+
Platelet Membrane
O_2
CO_2

Day 7 pH in Apheresis Platelets in Plasma: Control vs L-Carnitine



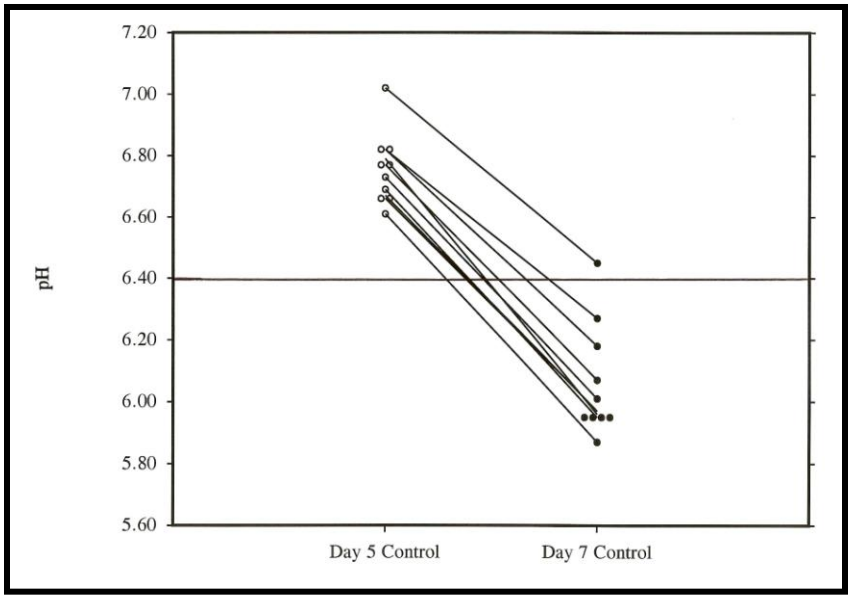
Unpublished observation.

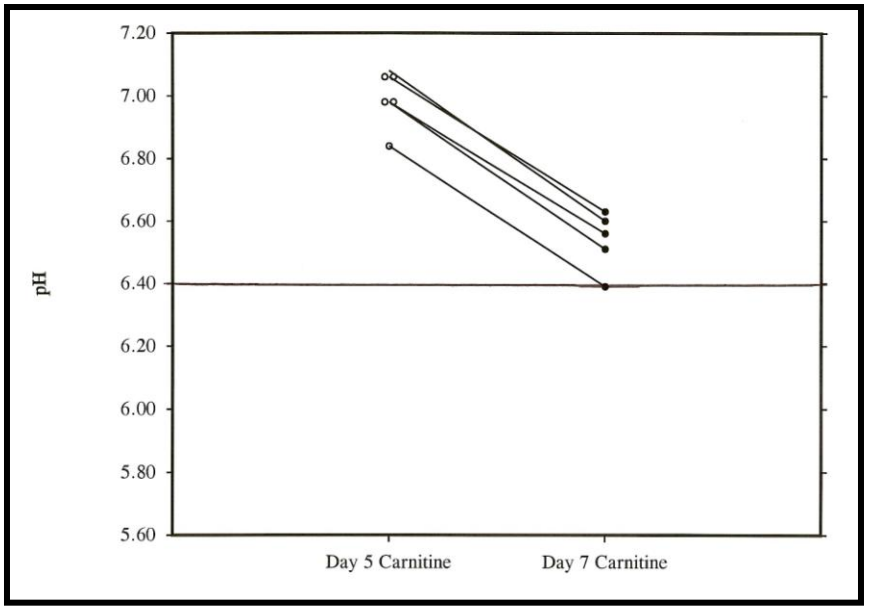
Hyperconcentrated Platelets in Plasma

	Controls	L-Carnitine	p
Platelet Count (10^9/L)	2252 ± 226	2304 ± 185	0.66
pH	6.1 ± 0.18	6.53 ± 0.1	<0.01
Glucose consumption (mM/10^{12} platelets/day)	1.5 ± 0.24	0.94 ± 0.19	<0.01
Lactate generation (mM/10^{12} platelets/day)	2.43 ± 1.0	1.11 ± 0.26	<0.01

Sweeney J, et al. *Vox Sang*. 2007;93(suppl 1):109.

Dot Plot Distribution of pH in Hyperconcentrated Platelet Pools: Day 5 and Day 7 Controls vs Carnitine





Sweeney J, et al. *Vox Sang*. 2007;93(suppl 1):109.

HISTORICAL ASPECTS

Tullis JL, et al. Preserved platelets: their preparation,storage and clinical use .Blood 1959;14:456-75

-Salt solution fortified with sodium acetate and gelatin

Baldini M, et al. The viability of stored human platelets Blood 1960;16:1669-92

-Phosphate buffered saline with glucose and plasma

Rock G, Swenson SD, Adams GA. Platelet storage in a plasma free medium. Transfusion 1985 25;551-6

-Modified Tyrode solutions with citrate and glucose.

Issue: Fall in pH unless a buffer is added (histidine, bicarbonate, phosphate)

Cause: Continuing production of lactic acid by the platelets despite storage in an oxygen environment

ADDITIVE SOLUTIONS

- Without Glucose

Difficulty in maintaining pH beyond day 5

- With Glucose

Better maintenance of pH at day 7

ADDITIVE SOLUTIONS

- Without glucose
  - PAS - I
  - PAS – II
  - PAS –III
  - PAS- 3M
  - T- Sol
  - Composol
- Residual plasma (35%)
- Na acetate(25mM)
- NaCl, KCl, $MgCl_2$, Na_2HPO_4, Na Citrate

- With Glucose
  - M Sol
  - PAS-G
  - Seto-Sol
- Residual plasma(2-25%)
- Na acetate(25mM)
- NaCl, KCl, $MgCl_2$, Na_2HPO_4, Na Citrate

NOMENCLATURE FOR PAS

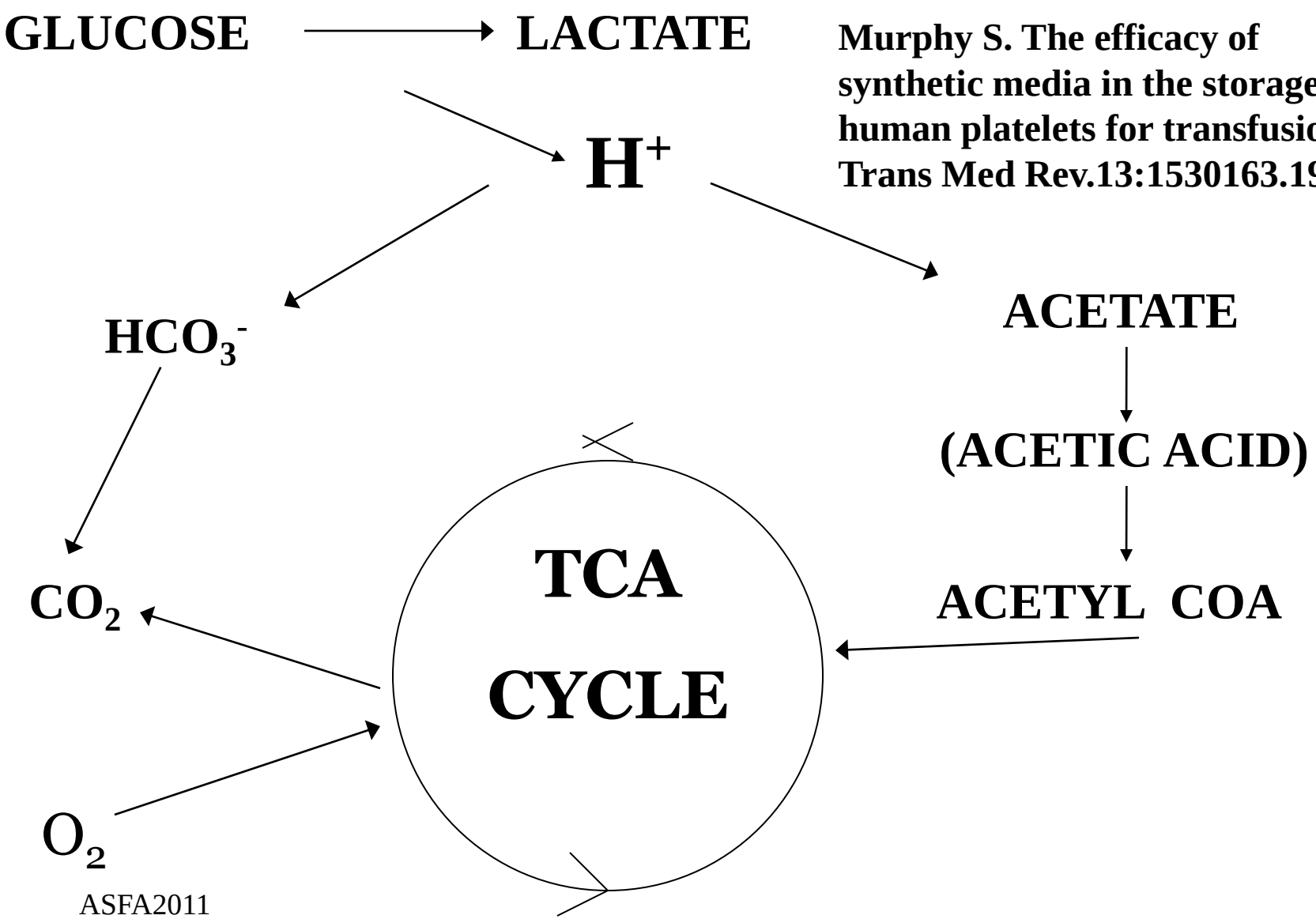
	Citrate	PO_4	Acetate	Mg	K	Gluconate	Glucose	Other
PAS								
PAS-A	X	X			X			PAS-1
PAS-B	X		X					PAS-II, T-SOL, SSP
PAS-C	X	X	X					PAS-111, Intersol
PAS-D	X		X	X	X	X		Composol
PAS-E	X	X	X	X	X			PAS-IIIM, SSP+
PAS-G	X	X	X	X	X		X	

Ashford, Gullikson et al: Vox Sang 2010. 98: 577-578

HELPFUL INGREDIANTS IN PLATELET ADDITIVE SOLUTIONS

- Glucose
  - Energy substrate
- Sodium acetate
  - Provides energy and buffering capacity
- Citrate
  - Low citrate (8mM) is associated with less glucose consumption (TCA cycle)
- Potassium and magnesium
  - Reduces rate of metabolism and decrease storage related activation
  - Decreases ADP responsiveness, fibrinogen binding in response to ADP and P-Selectin expression
- Phosphate
  - Buffer and stimulates glycolysis (balanced effect)
- Bicarbonate
  - Buffer
- Plasma carry over (35% - 2%)
  - Provides glucose, citrate, ions, buffer, fatty acids etc

ROLE OF ACETATE



Murphy S. The efficacy of synthetic media in the storage of human platelets for transfusion. Trans Med Rev.13:1530163.1999

VARIOUS APPROACHES FOR PAS

1) Use of glucose, but with addition of extra buffering capacity (separately) to neutralize the lactic acid

   Issue: Manufacturing

2) Storage in a glucose free medium with high plasma carry over to provide nutrients and buffering capacity

   Issue: Extended platelet storage

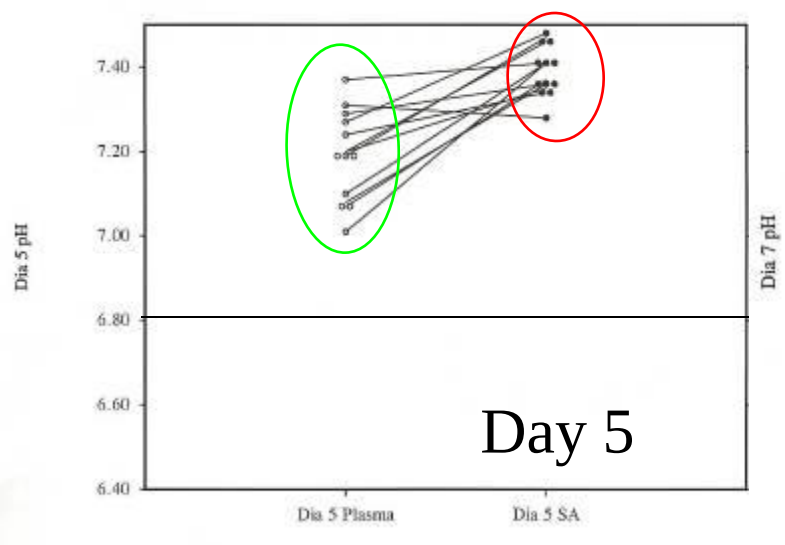
3) Use of metabolic modifiers to reduce lactic acid production

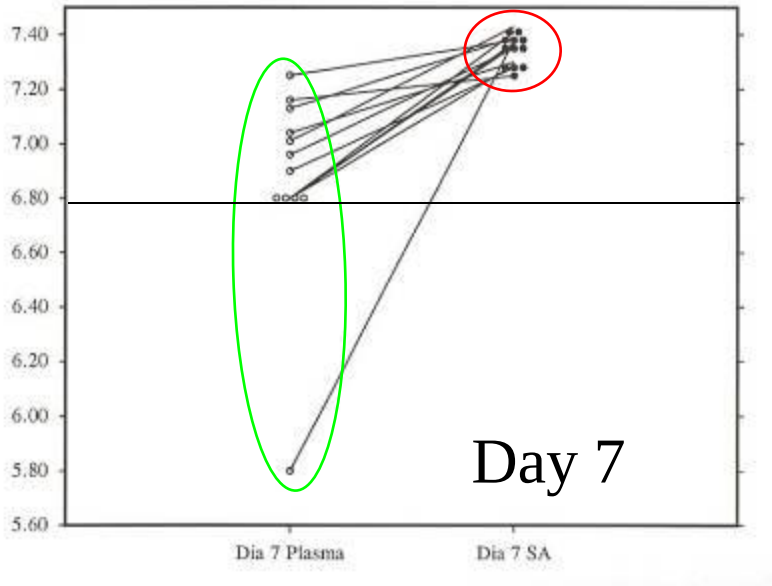
   Issue: Regulatory – introduction of new ingredients

GLUCOSE CONTAINING PAS

		SetoSol	M-SOL	PAS-G	
NaCl		**110**	**77**	**110**	
KCl		**4**	**3**	**5**	
Mg Cl_2		**3**	**1.6**	**3**	
Na phosphate		**7.5**		**4**	
Na citrate/acid			**4.8**	**10**	
Na acetate		**15**	**21**	**15**	
Maltose		**29**			
Na HCO3		**10**	**44**	**12**	
Glucose		**14**	**15**	**30**	
pH				**7.0-7.2**	

Platelet Additive Solution with Added Glucose: pH effects



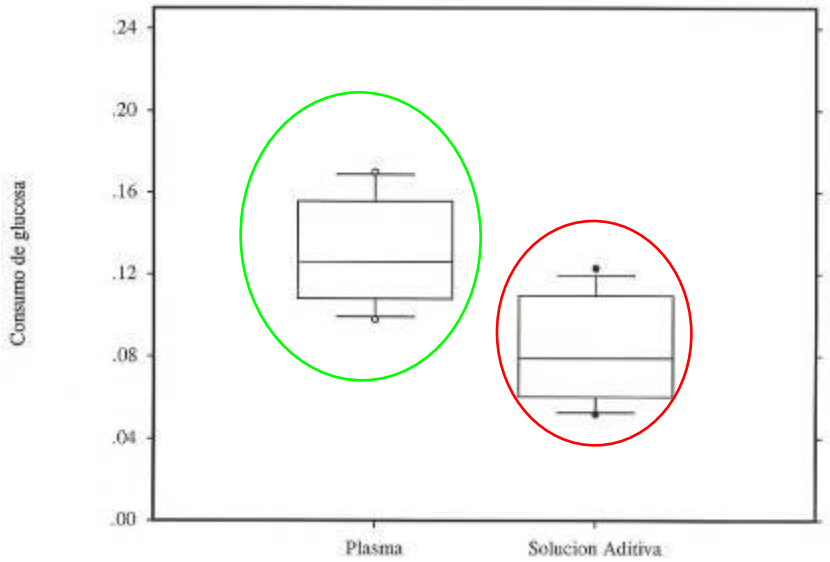


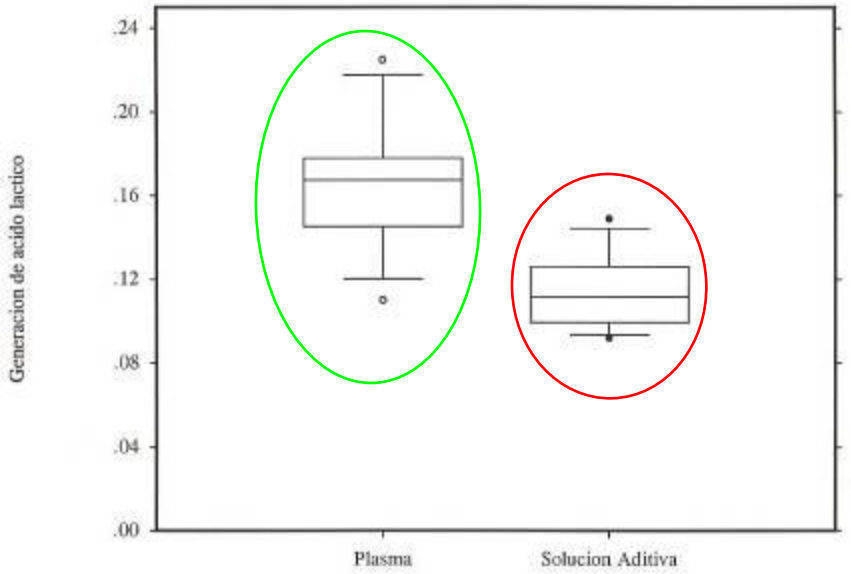
Using glucose, an acceptable pH is maintained to day 7

Plasma

Additive Solution

Platelet Additive Solution with Added Glucose





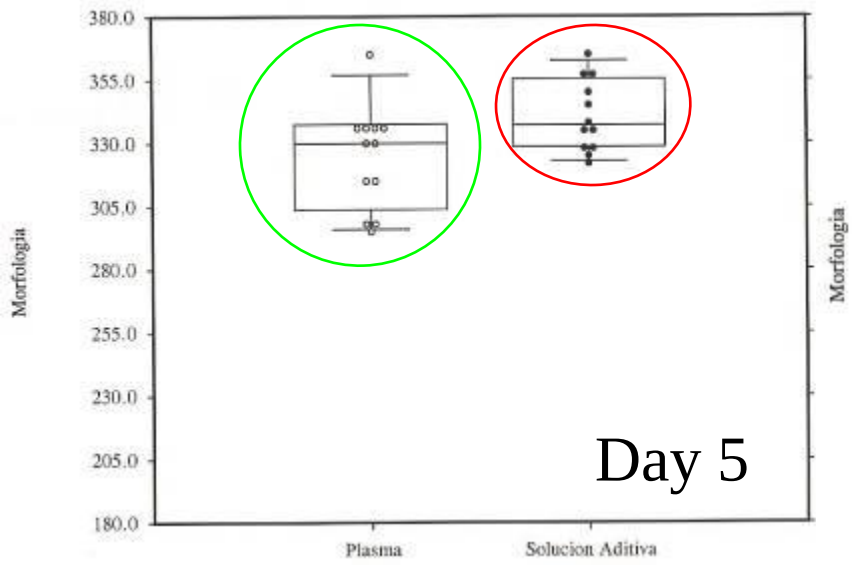
mM/10^{11}platelets/Day

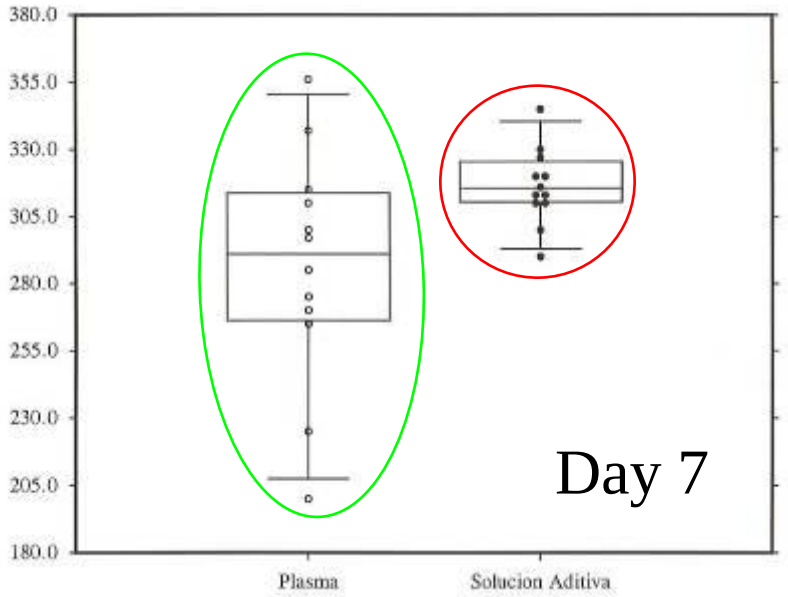
The additive solution decreases glucose consumption and lactate generation

Plasma

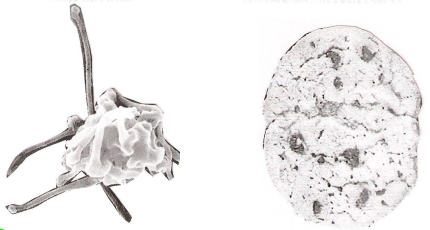
Additive Solution

Platelet Additive Solution with Added Glucose





The morphology score is higher with the additive solution which predicts better in vivo survival (longer lifespan)



Plasma

Additive Solution

Decrease allergic reactions, isoagglutinin mediated hemolytic reactions and possibly TRALI by removal of plasma

REDUCTION IN ALLERGIC REACTIONS

- De Wildt-Eggen et al: Transfusion 2000. 40: 398-403
  - 192 transfusions of PCs in plasma to 12 patients = 12% reaction rate
  - 132 transfusions to of PCs in AS to 9 patients = 5.3% reaction rate
- Kerkhoffs JLH: Transfusion Med. 2006. 108: 3210-3215
  - 17/354 reactions to plasma stored platelets (4.8%)
  - 9/411 reactions to AS stored platelets (2.2%)

However, both studies showed inferior 1 hour and 24 hour CCIs In the platelets stored in AS

REDUCTION IN ALLERGIC REACTIONS

- Herve F. Vox Sang 2007; 93 (suppl) : 267
  - Prepoooled platelets in plasma – 1: 640
  - Prepooled platelets in AS – 1: 4100
  - Apheresis platelets in plasma – 1: 168
  - Apheresis platelets in AS: 1: 190
- Azuma et al: Transfusion
  - Reduction in reaction rate in 12 patients with recurrent allergic reactions from 42% in plasma to 0.63% in M-Sol
  - > 90% reduction in plasma
  - CCIs were equivalent or better than the plasma stored platelets

REDUCTION IN ALLERGIC REACTIONS

Transfuse PAS stored platelets only

Isoagglutinin Mediated Hemolysis

Patient: 45-year old autologous BMT female

- Group A neg: receives an apheresis product, Group O neg
- 5 minutes later: severe lower backache
  - Rx with morphine
  - Pain gets better
- Platelet transfusion continues to completion
- 1 hour later: fever (102^{o}F), chills, nausea, passes red urine
- Hb drops from 9.5 to 6.5 g/dL over two hours
- Anti-A titer was 8!!
  - Received two O neg apheresis units on each of the preceding two days

Fung MK et al. *Arch Pathol Lab Med.* 2007.131;909-916.

17 cases: Apheresis; 8 cases: Whole blood derived-platelets

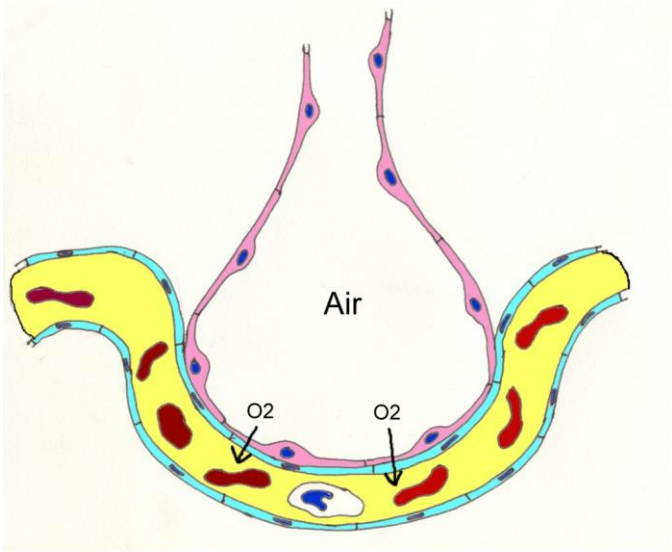
BMT = Bone marrow transplant

Isoagglutinin Mediated Hemolysis

- Exclude Group O apheresis products for non-Group O recipients
- Measure the titer of anti-A and anti-B in all Group O apheresis donors
- Volume reduction immediately before transfusion (hyperconcentrate the platelets)
- Use of PAS

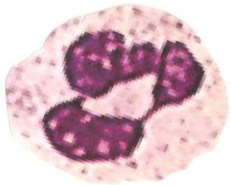
Will PAS help in any way with attenuation of TRALI reactions ?

NORMAL LUNG



Healthy adult- 36% of neutrophils are circulating and of this about 28% are in the pulmonary pool but are increased in systemic inflammatory conditions

Neutrophils are considered to exist in any one of three states- quiescent, primed or activated



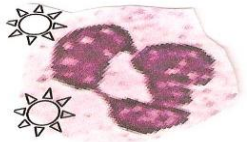
Neutrophils in healthy adults are mostly if not exclusively quiescent.

TRANSFUSION RELATED ACUTE LUNG INJURY: ONE HIT

Donor IgG

+

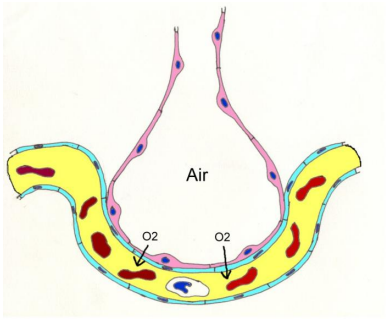
Cognate antigen on recipient Neutrophils

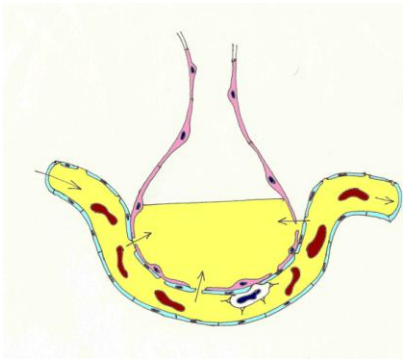


Lung Sequestration

Proteases, lipids, O_2 radicals

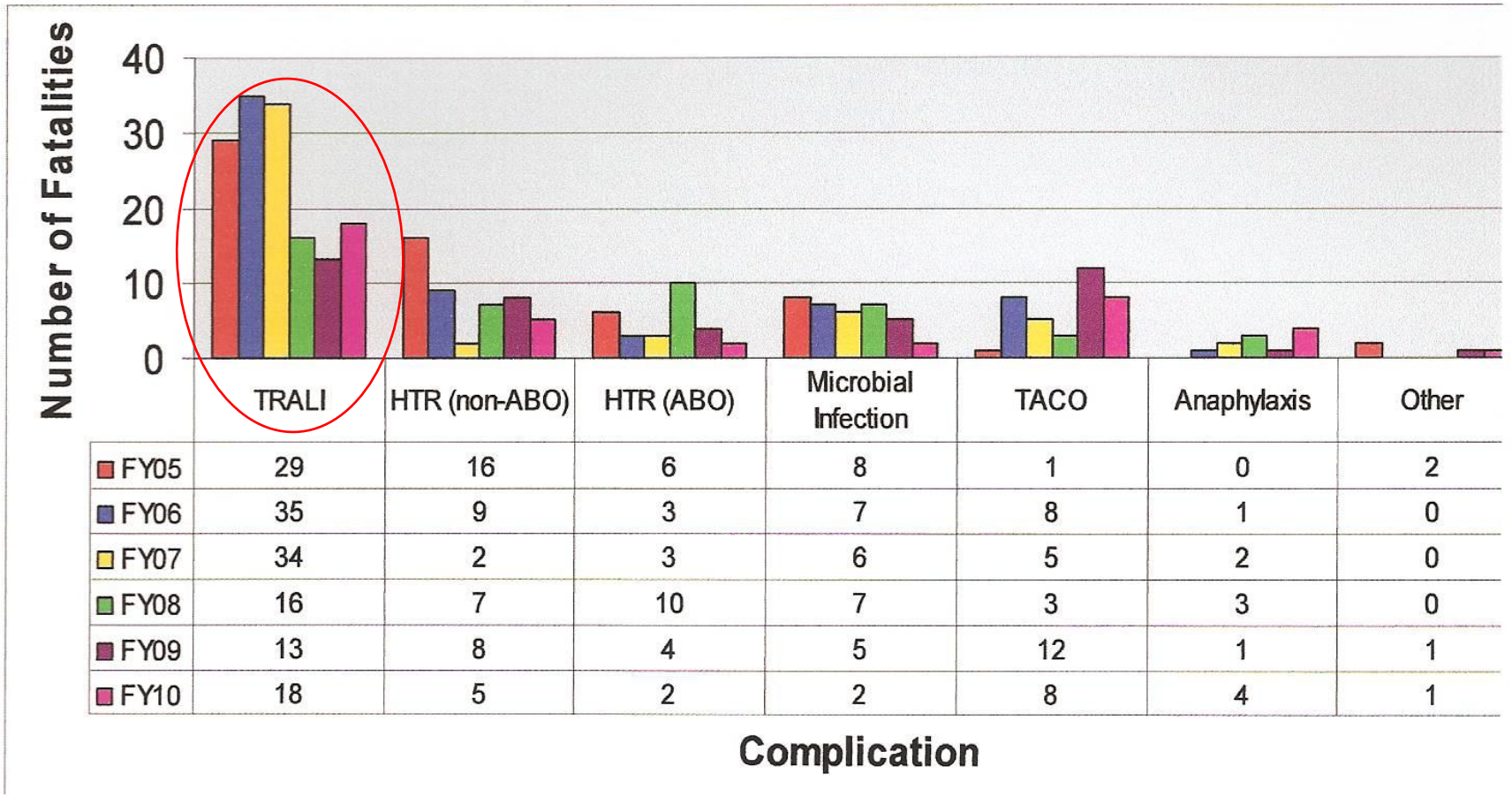
Damaged Alveolus





FDA FATALITIES: 2005-2010

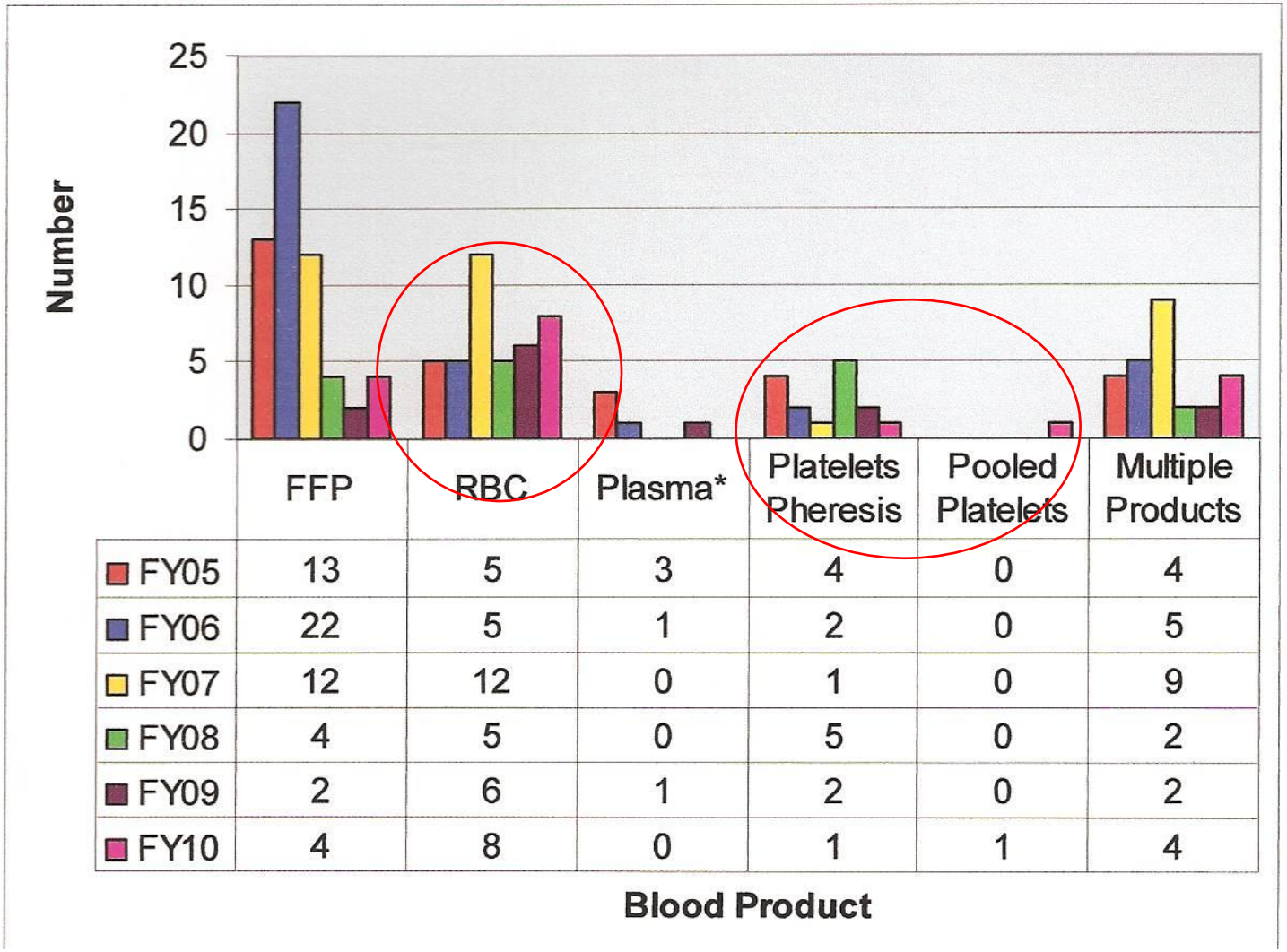
Figure 1: Transfusion-Related Fatalities by Complication, FY2005 through FY2010



	TRALI	HTR (non-ABO)	HTR (ABO)	Microbial Infection	TACO	Anaphylaxis	Other
FY05	29	16	6	8	1	0	2
FY06	35	9	3	7	8	1	0
FY07	34	2	3	6	5	2	0
FY08	16	7	10	7	3	3	0
FY09	13	8	4	5	12	1	1
FY10	18	5	2	2	8	4	1

FDA FATALITIES: 2005-2010

Figure 2: Reports of TRALI Cases by Implicated Blood Product, FY2005 through FY2010



	FFP	RBC	Plasma*	Platelets Pheresis	Pooled Platelets	Multiple Products
FY05	13	5	3	4	0	4
FY06	22	5	1	2	0	5
FY07	12	12	0	1	0	9
FY08	4	5	0	5	0	2
FY09	2	6	1	2	0	2
FY10	4	8	0	1	1	4

Is There a Plasma Volume Threshold for TRALI?

- Threshold volume of plasma is unknown
  - Probably depends on antibody concentration (titer) and multiple antibodies to recipient cognate antigens
- Most cases associated with FFP which has volume of ~225 mL
  - Since 13 million RBCs are transfused vs 4 million FFP, the volume of plasma must be important, otherwise RBCs would cause x3 the number of cases
- Recent report from the UK suggests that as little as 25 mL of plasma may cause TRALI (Win, et al. *Transfusion Med*. 2008. 18:276-2800)

TRALI = Transfusion-related acute lung injury
FFP = Fresh frozen plasma
RBC = Red blood cell

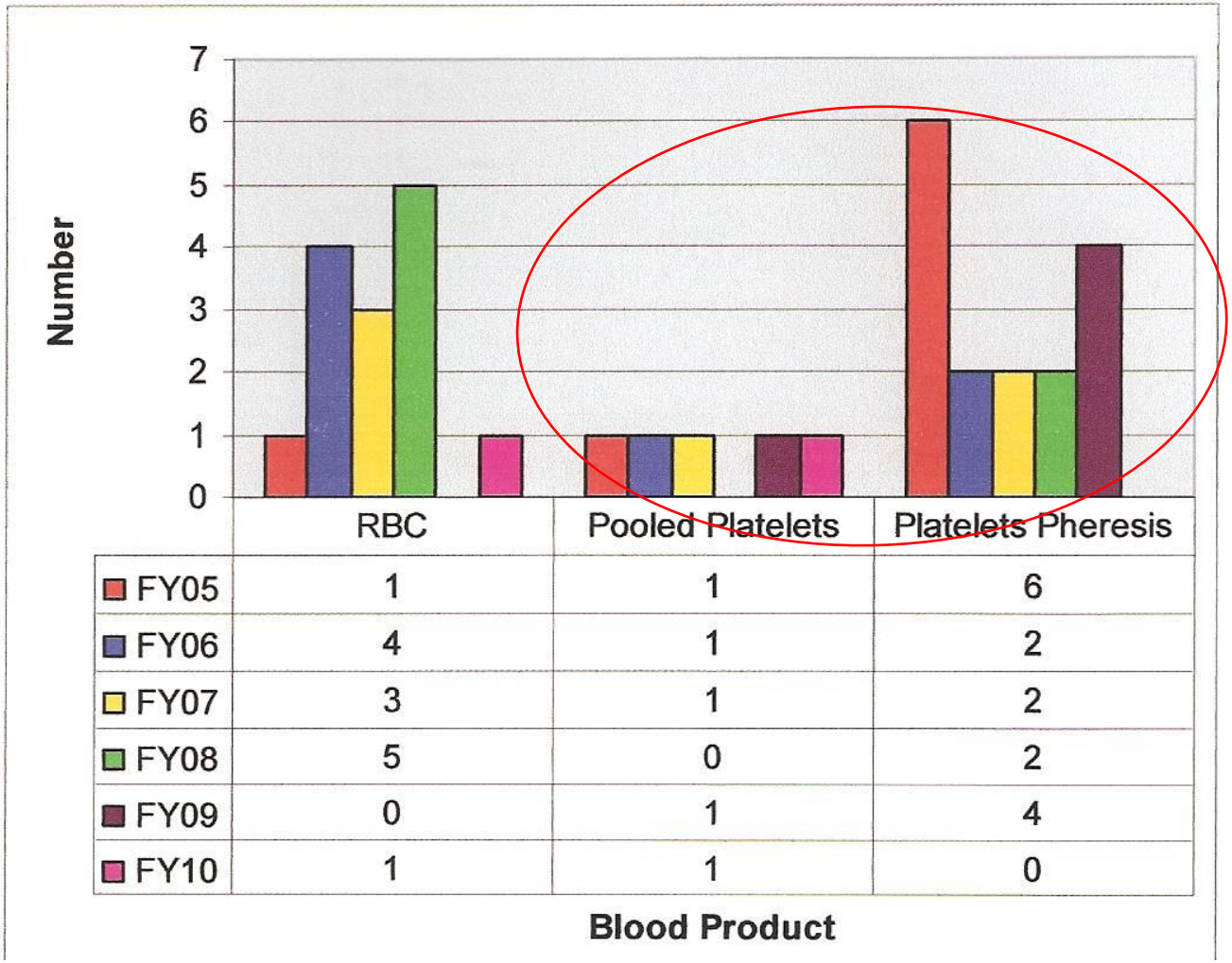
Is There a Plasma Volume Threshold for TRALI?

- Apheresis platelets:
  - Removal of 65% of plasma from apheresis platelets will leave single donor derived residual plasma between 80-130 mL
  - To achieve < 25- 50 mL residual plasma, it will be necessary to remove 80-90% of the plasma:
    - Platelets resuspended in a larger volume PAS to keep concentration <1600 X 10^9/L
    - Platelets resuspended in a fixed volume of PAS yielding hyperconcentrated platelets
- Whole blood Pools:
- Male donor plasma or PRP derived pools in AS (15 –20 mls residual plasma for each donor)

Will PAS help in any way with regard to bacterial sepsis?

FDA FATALITIES: 2005-2010

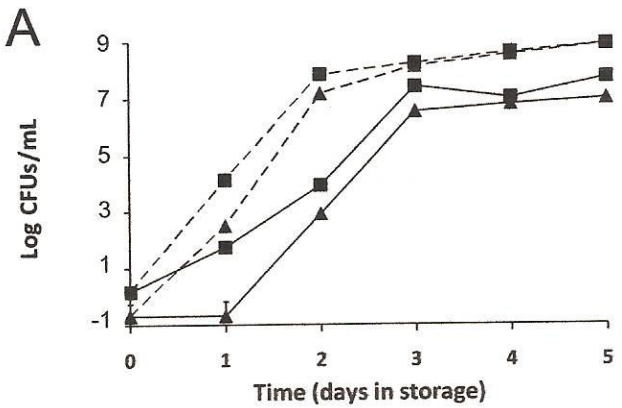
Figure 4: Microbial Infection by Implicated Blood Product, FY2005 through FY2010



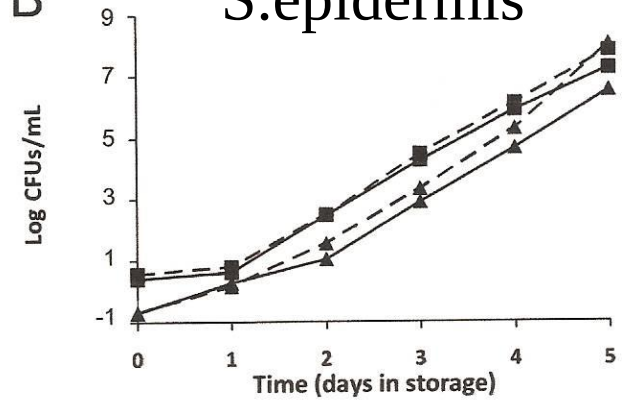
	RBC	Pooled Platelets	Platelets Pheresis
FY05	1	1	6
FY06	4	1	2
FY07	3	1	2
FY08	5	0	2
FY09	0	1	4
FY10	1	1	0

PLATELET GROWTH KINETICS IN PAS VERSUS PLASMA

S.liquefaciens



S.epidermis



- "slow" growing bacteria are missed by early in storage culture of plasma stored platelets
- This may occur because the bacteria grow attached to the surface of the bag container forming a film (biofilm)- promoted by fibrinogen, fibronectin
- PAS may discourage this growth pattern because of less plasma proteins allowing detection early in storage

Greco et al: Transfusion 2010. 50: 2344-2352

Increase in the availability of plasma as a transfusable product or for fractionation

Plasma Saving Effect

- Assuming 1.4 million Apheresis products manufactured in the US yearly with an average volume of 0.3L and 65% replacement with PAS- this would generate about 275,000L of additional plasma
- Assuming 150,000 pooled WBD-platelets with a volume of 0.27L and 65% replacement- this would generate about 26,000L
- Total about 300,000L which could be used for IVGG , albumin manufacture

Facilitation of Pathogen Reduction

- Pathogen reduction technologyis currently practiced and increasing in some locations
  - France, Norway, Kuwait
- Manipulation of the platelet product by plasma removal allows the optimization of the conditions for the PR technology to work
  - Define a range of platelet counts
  - Define a range of residual plasma

Platelets in PAS Are a New Value-Added Product for Hospitals

This platelet product will:

- Reduce allergic platelet reaction rates by at least 50% saving the product from replacement, the cost of reaction work-up, and clinical care to the patient
- Eliminate or reduce requests for “washed platelets” or “volume reduced platelets”
- Greatly simplify platelet inventory management: Group O platelets (47%) and Group A_2 platelets (8%) lack A and B antigens and become a “universal platelet product” suitable for any recipient
  - E.g., 2 units of O red cells = approximately 50-100 mL of O plasma

Sweeney JD. *Transfusion*. 2009;49:199-200.

Summary

- **Improvement in potency:**
  - **Using PAS, we can manipulate the platelet storage environment to optimize metabolism and decrease activation**
    - **Hemostatically more effective platelet**
    - **This may allow seven day storage**
- **Improvement in safety:**
  - **Using PAS, we can reduce allergic reactions by 50% at least**
  - **Using PAS, we possibly can reduce TRALI and isoagglutinin mediated hemolysis**
  - **Facilitate detection of slow growing bacteria?**
- **Augment the plasma supply:**
  - **300,000 additional liters equivalent to 600,000 source plasma apheresis procedures**

Sweeney J, et al. *Transfusion.* 2006;46:835-840.

Kerkhoffs JLH, et al. *Blood.* 2006;108:3210-3215.

Herve F, et al. *Vox Sang.* 2007; 93(suppl):267.


THE END
THANK YOU!