Red Cell Storage

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WHO Expert Panel, Blood Transfusion Medicine,
2006-2012

Conflict of interest

I am the U.S. Government inventor of an improved red cell storage solution variously called EAS-81, AS-7 or Sol-X®. I receive patent royalty payments from the government under U.S. law. The solution is now licensed by Haemonetics but is not in production.

I receive writers royalties from UpToDate for the chapter on “massive transfusion.”

Blood Transfusion at St. Vitesse – 18 July 1918

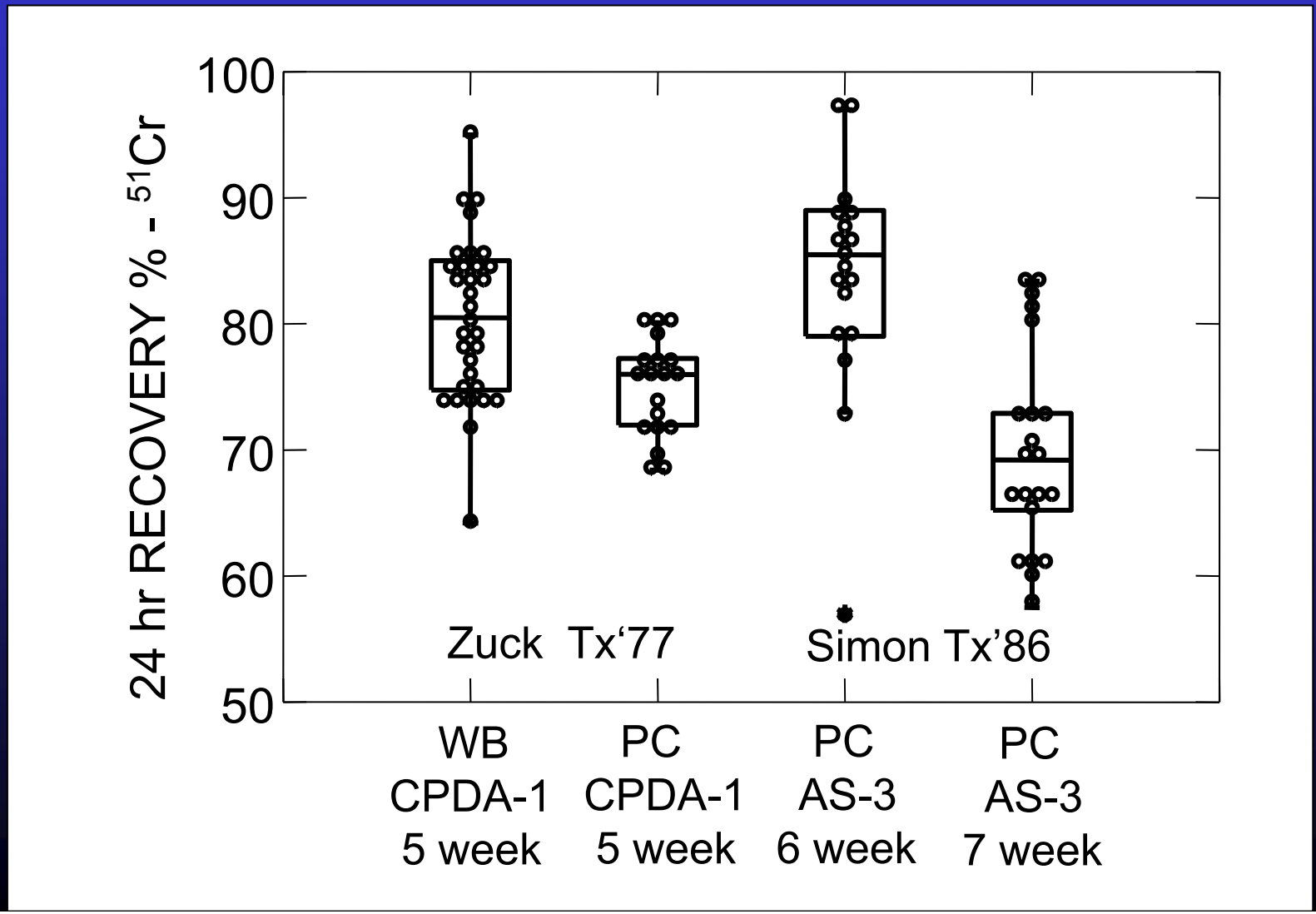
O H Robertson

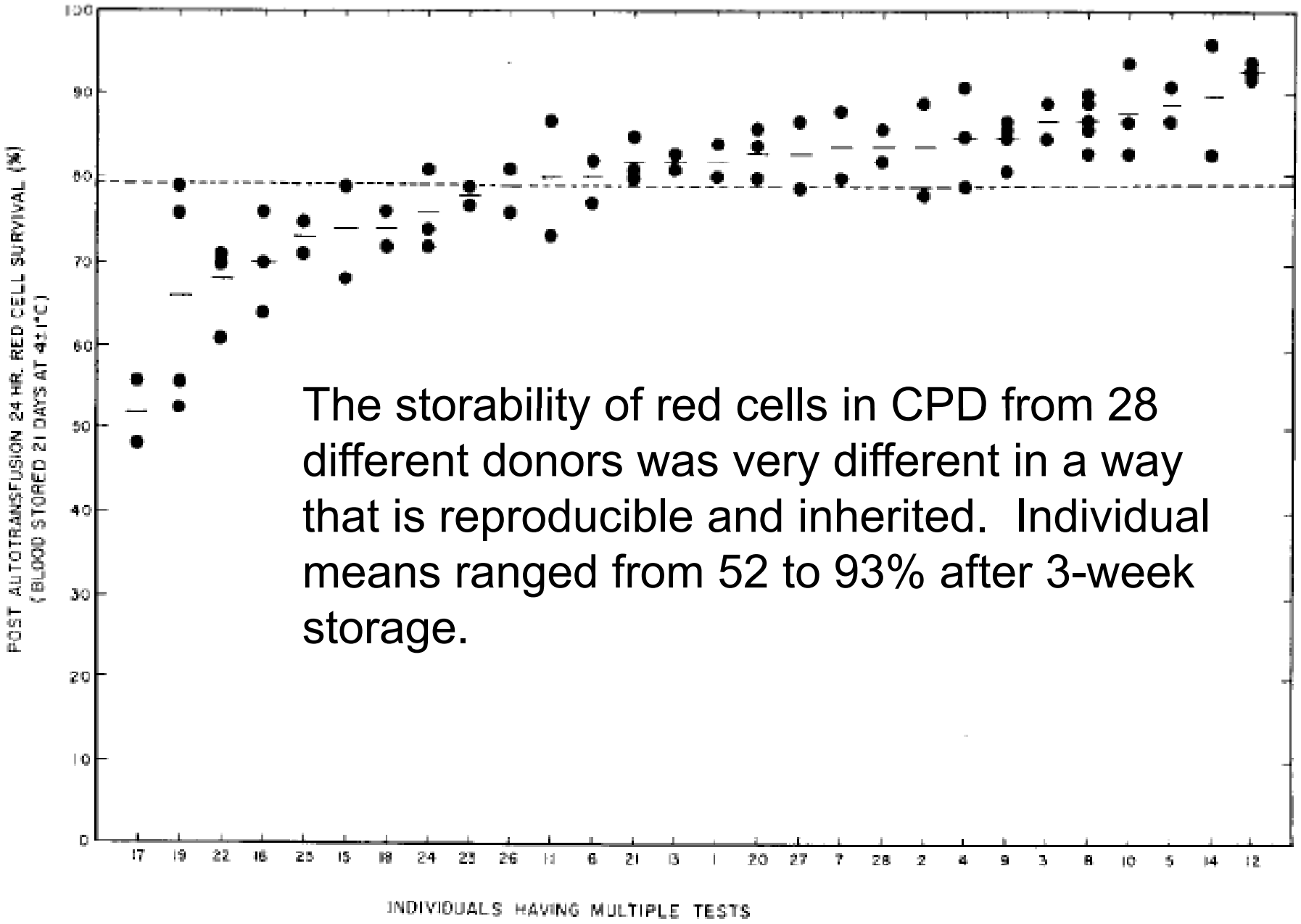
Canadian Army Painter – Pvt. Arthur Elias - 1918

History of RBC storage systems

1914 - Citrate
1917 – Citrate & Glucose
1940s - Acid citrate & dextrose (ACD)
1950s - Phosphate (CPD)
1960s – Plastic bags & components
1970s – Adenine
1980s – Additive solutions and mannitol
1990s - Leukoreduction
2000s - Bicarbonate

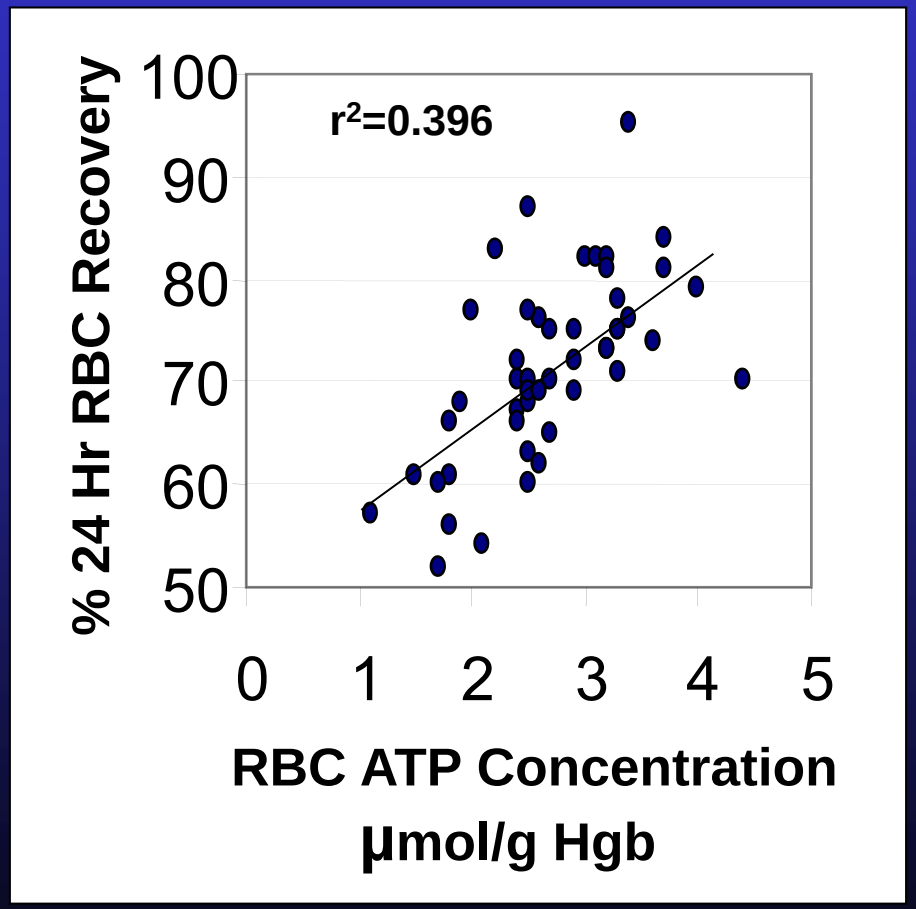
Additive solutions maintain storage volume and RBC viability





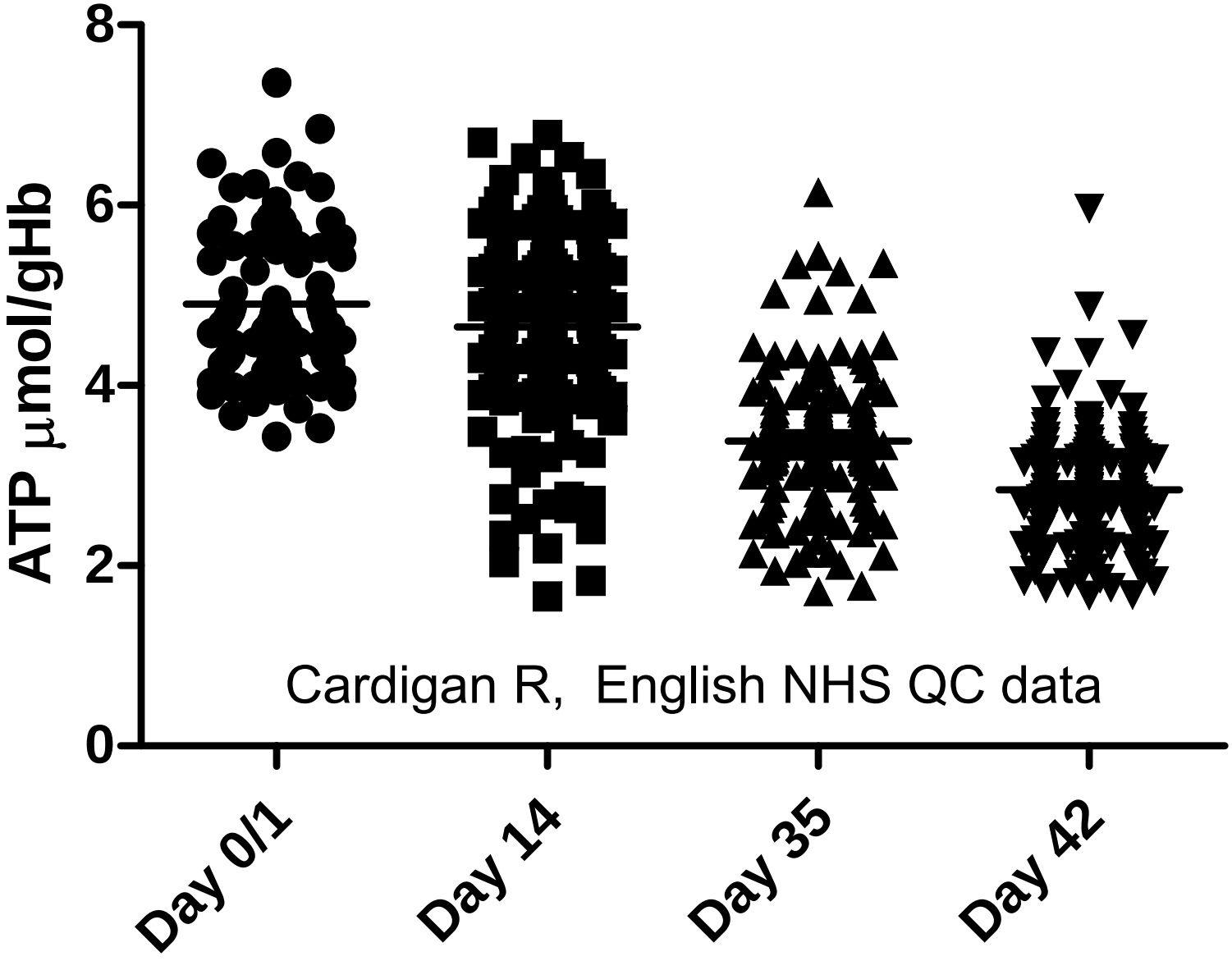
The storability of red cells in CPD from 28 different donors was very different in a way that is reproducible and inherited. Individual means ranged from 52 to 93% after 3-week storage.

Correlation of RBC ATP concentration and viability

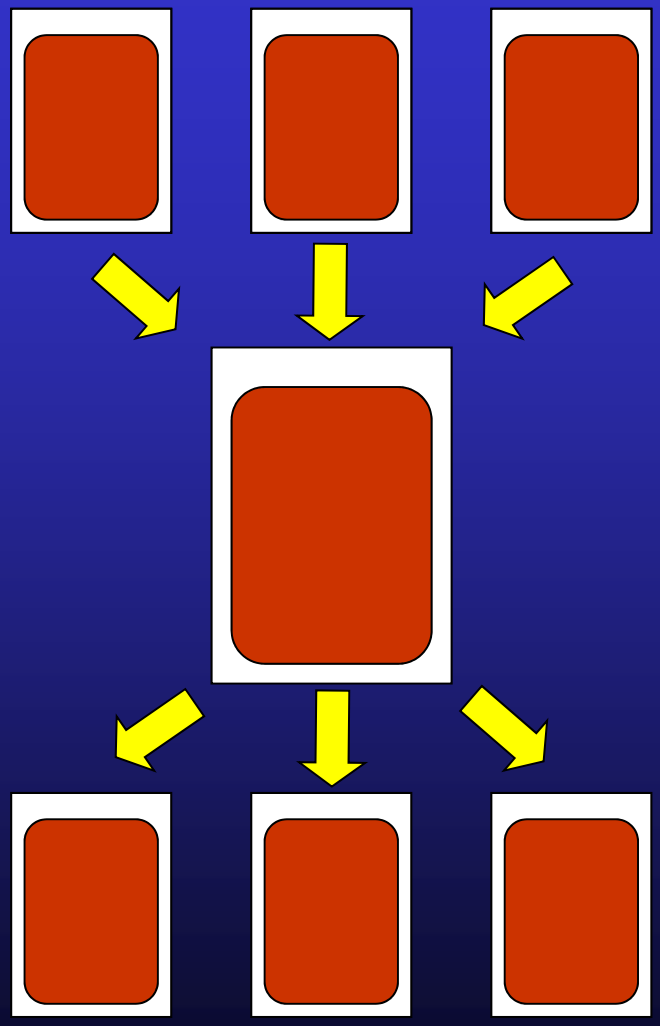


RBC ATP concentration correlates significantly with viability, but the predictive value of the correlation is not good. However, neither measure is precise. Repeat assays of ATP concentration differ by 5%. Repeat measures of RBC recovery also differ with a SD of 5%.

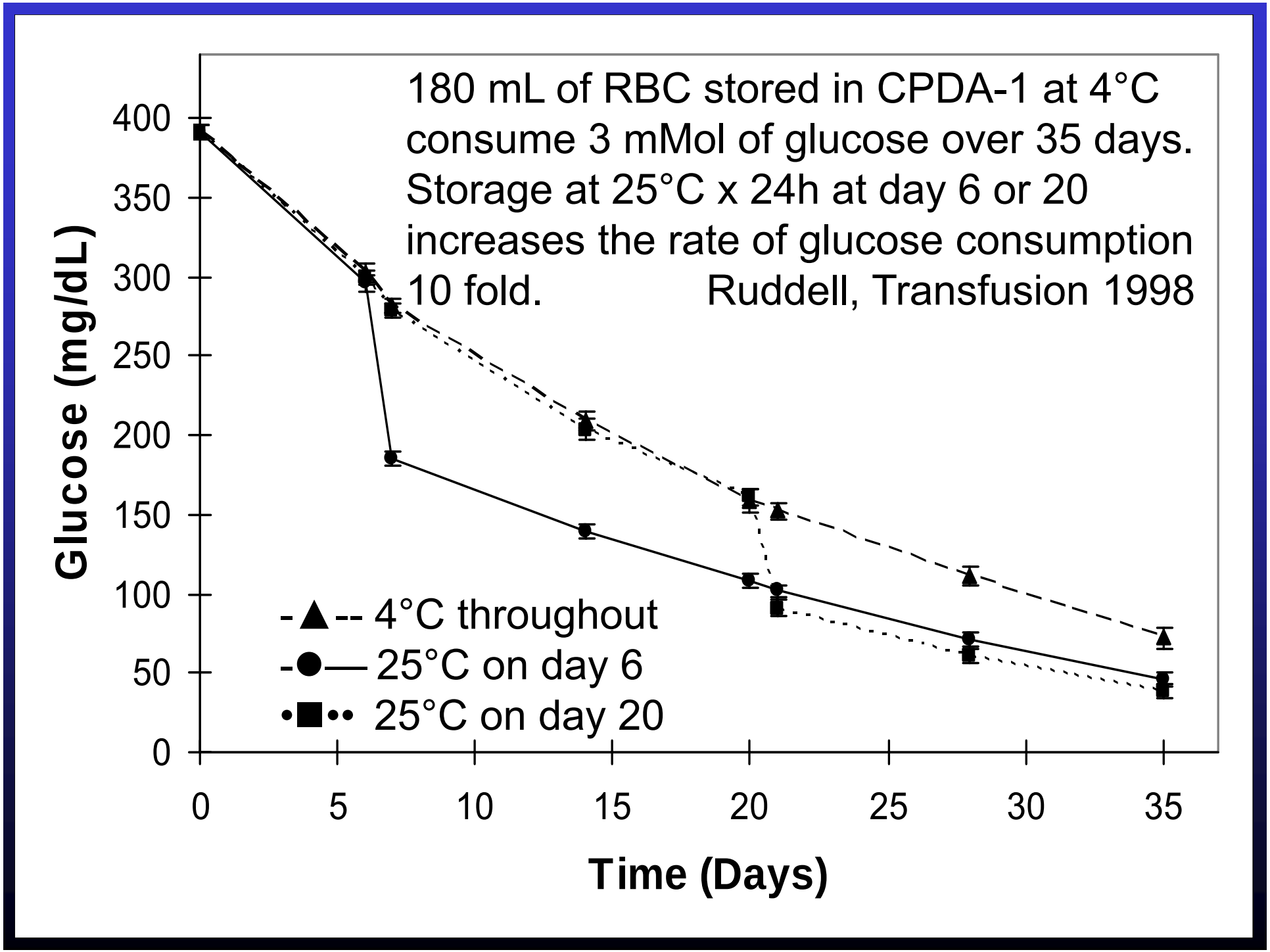
ATP reference data (n=140)

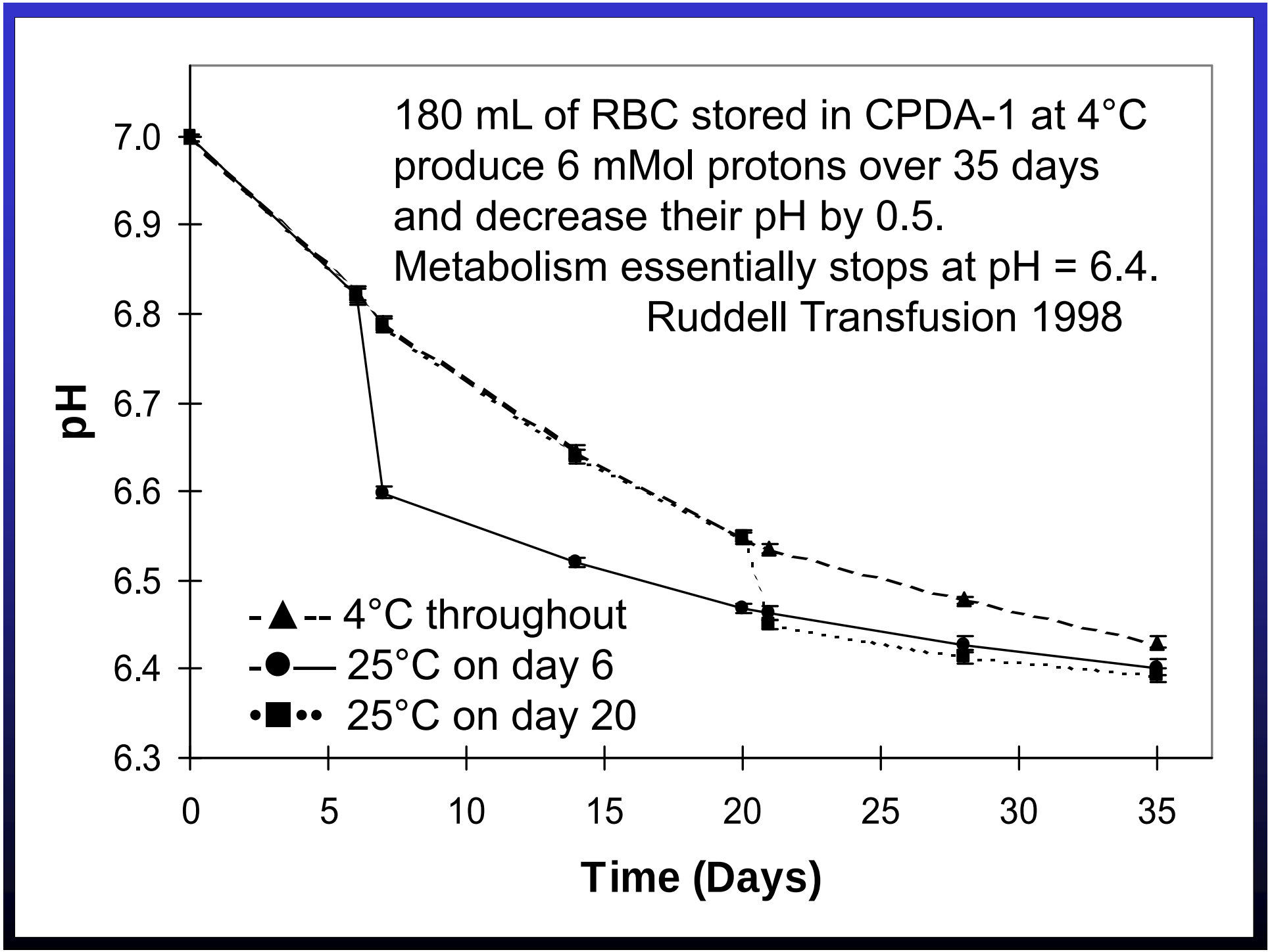


Pooling studies reduce inter-donor variability



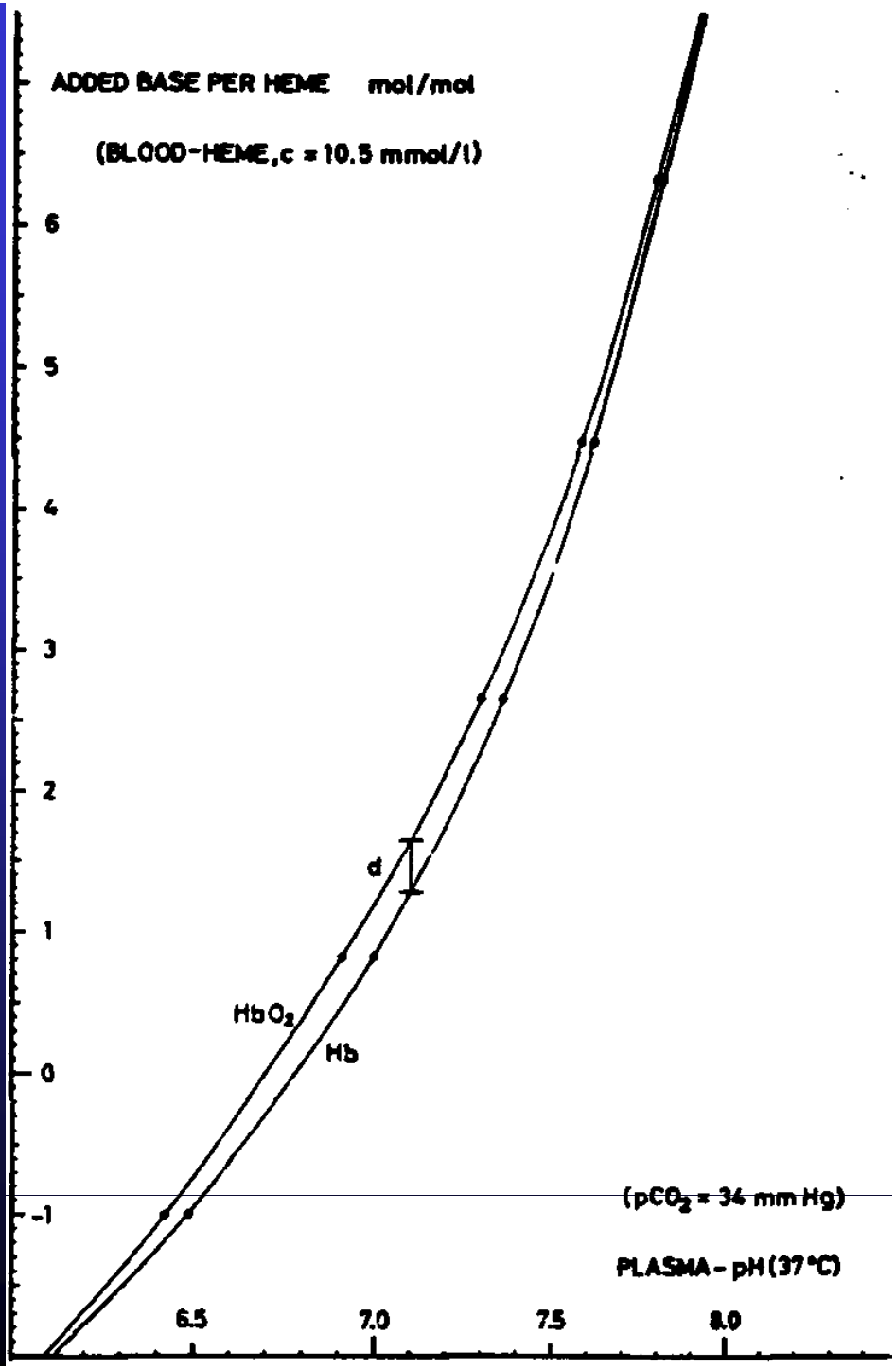
ABO compatible units of PRBCs were pooled in sets of three or four and the pool was aliquoted to form an equal number of mixed units. The units contained RBCs from all the donors in the set, diluting unusual RBC behavior, such as more rapid metabolic rates or increased hemolysis, within the units and allowing allocation of RBCs from each donor to all arms of the study.

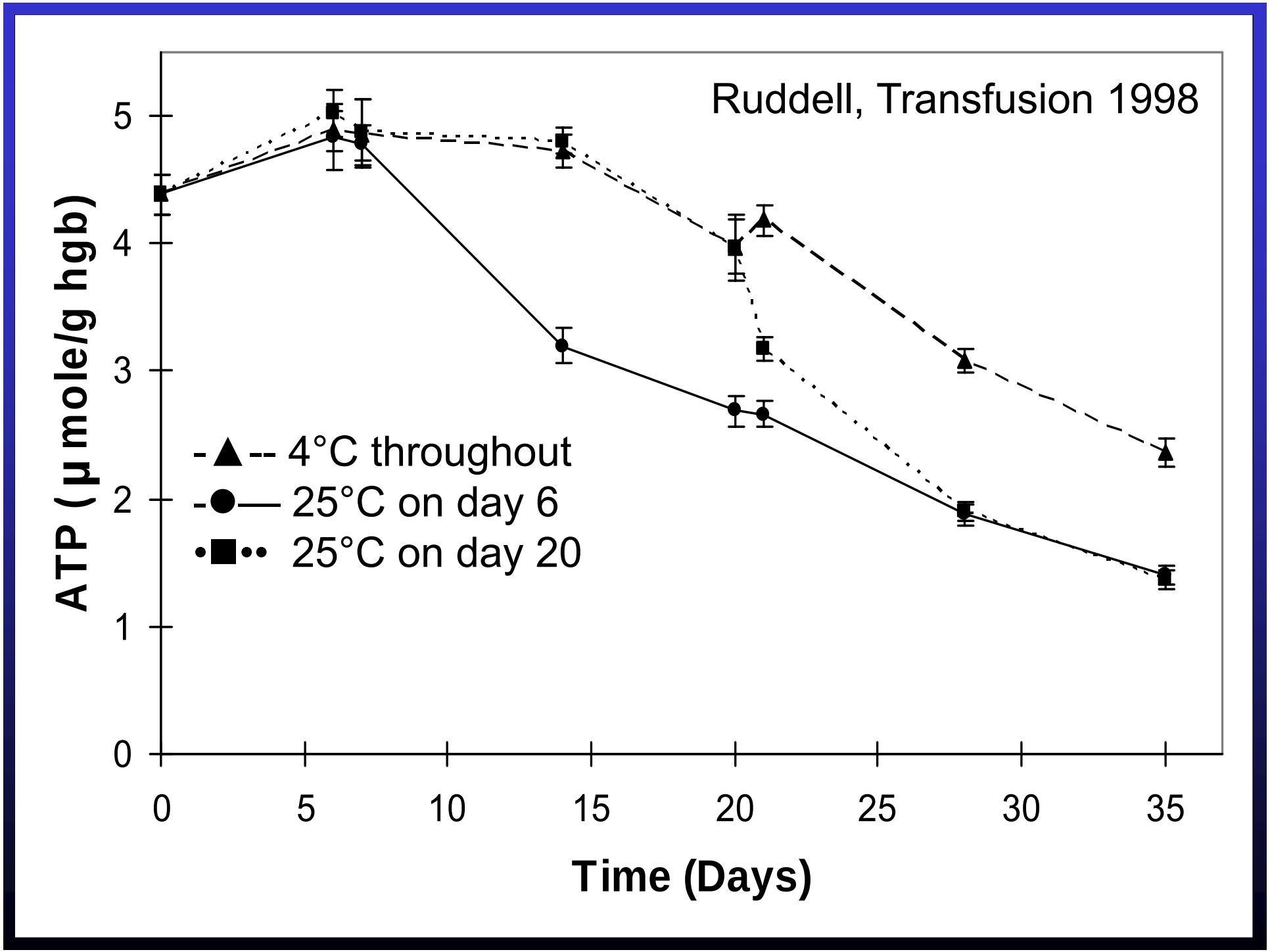

180 mL of RBC stored in CPDA-1 at 4°C consume 3 mMol of glucose over 35 days. Storage at 25°C x 24h at day 6 or 20 increases the rate of glucose consumption 10 fold.
Ruddell, Transfusion 1998
Glucose (mg/dL)
400
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
Time (Days)
4°C throughout
25°C on day 6
25°C on day 20


180 mL of RBC stored in CPDA-1 at 4°C
produce 6 mMol protons over 35 days
and decrease their pH by 0.5.
Metabolism essentially stops at pH = 6.4.
Ruddell Transfusion 1998
pH
7.0
6.9
6.8
6.7
6.6
6.5
6.4
6.3
0
5
10
15
20
25
30
35
Time (Days)
4°C throughout
25°C on day 6
25°C on day 20

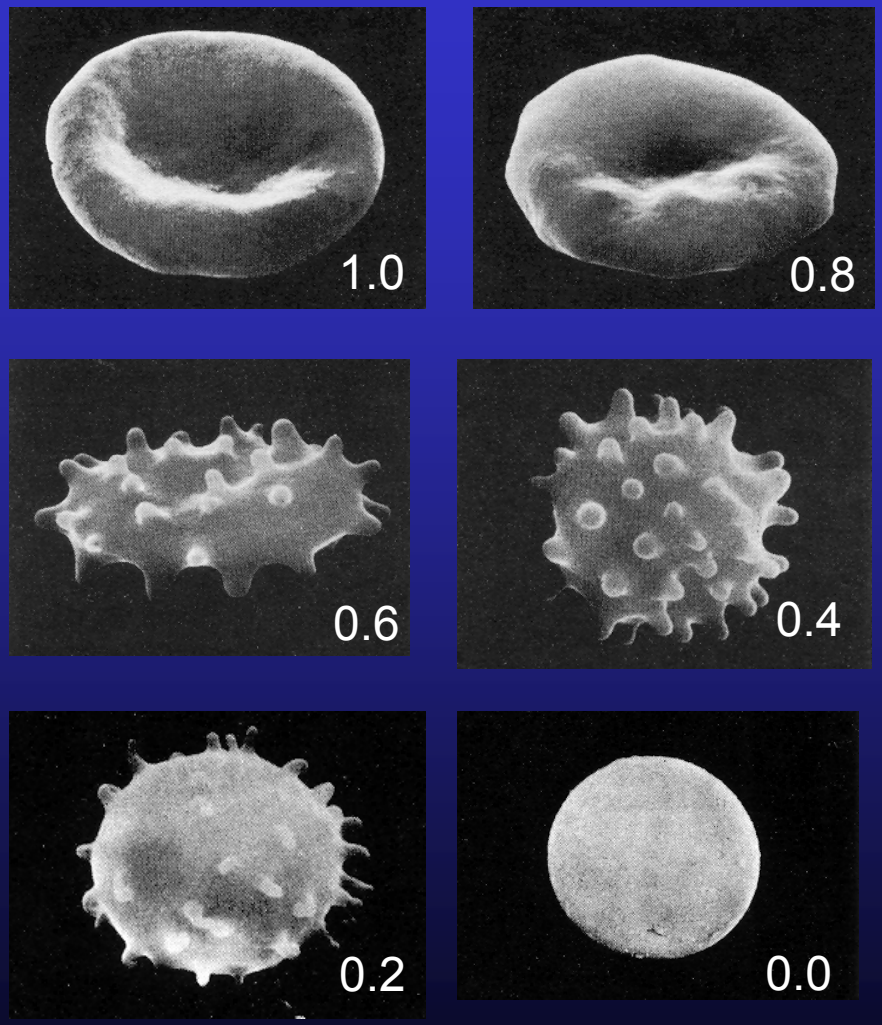
One millimole of oxyhemoglobin, 69 g, will buffer 15.2 mM of protons between pH 7.2 and 6.2.

Siggard-Anderson & Salling. Scand J Clin Lab Invest 1971; 27:361-366



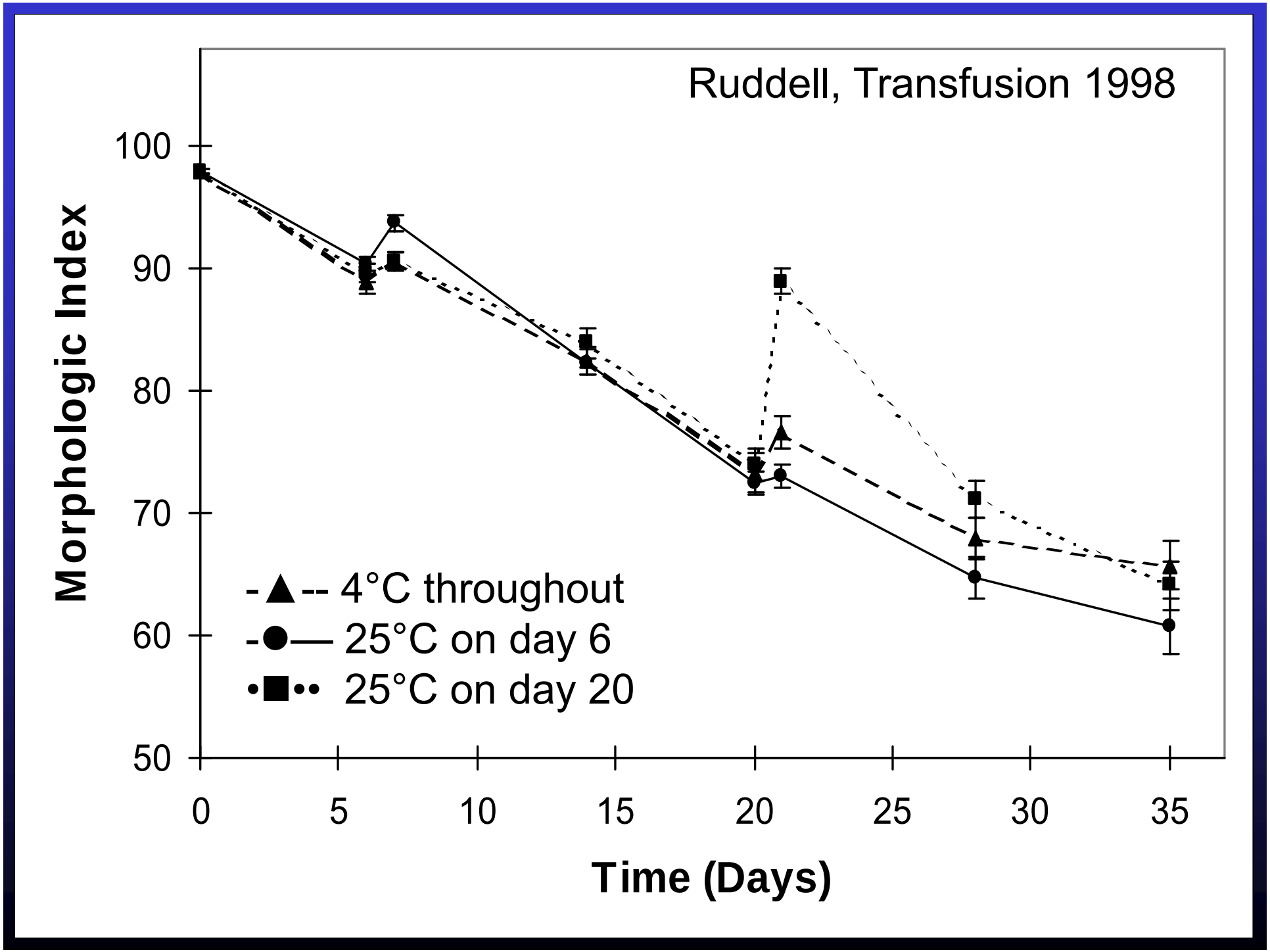

Ruddell, Transfusion 1998
ATP (µ mole/g hgb)
0
1
2
3
4
5
0
5
10
15
20
25
30
35
Time (Days)
4°C throughout
25°C on day 6
25°C on day 20

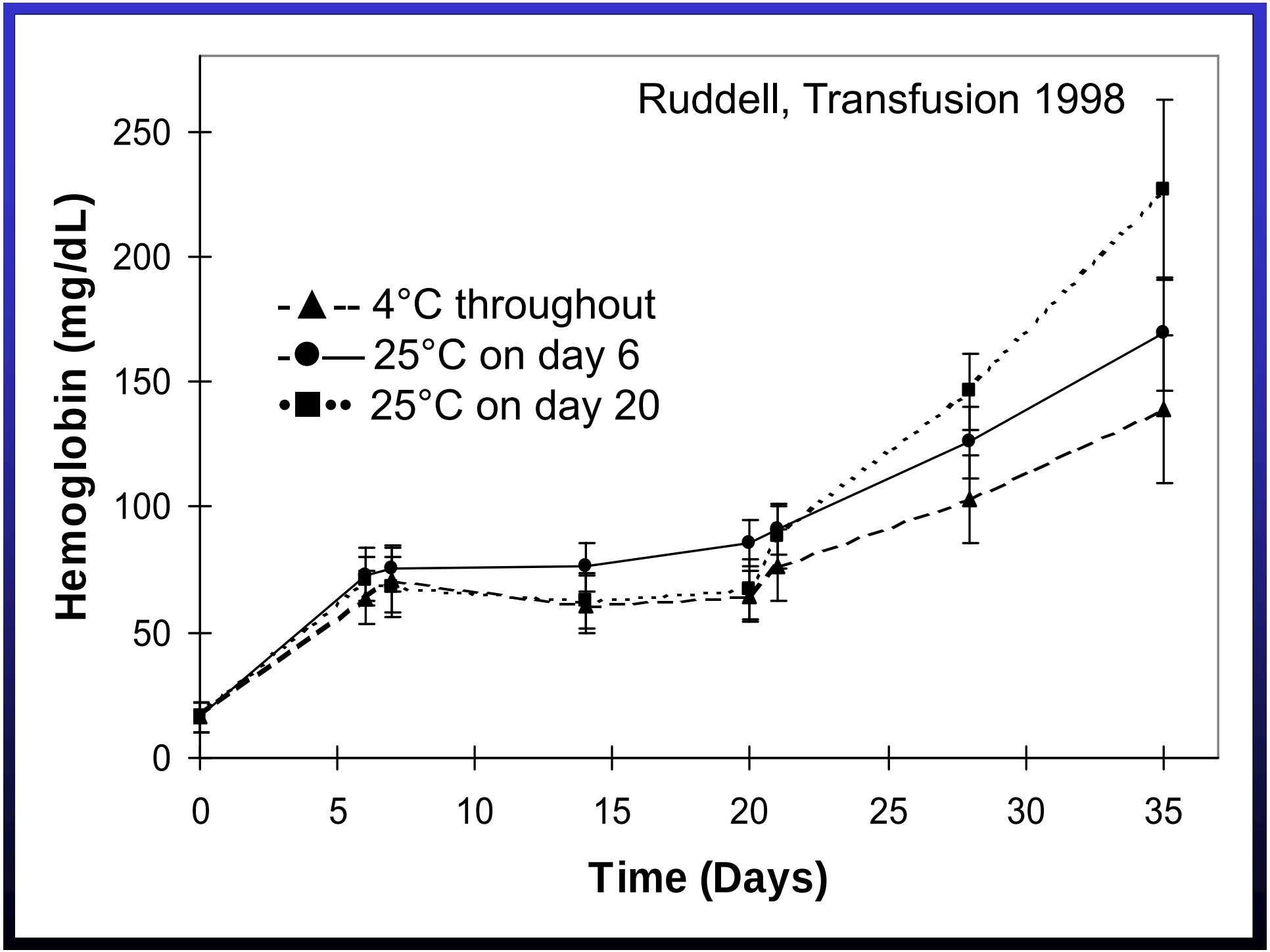
RBC shape change during storage



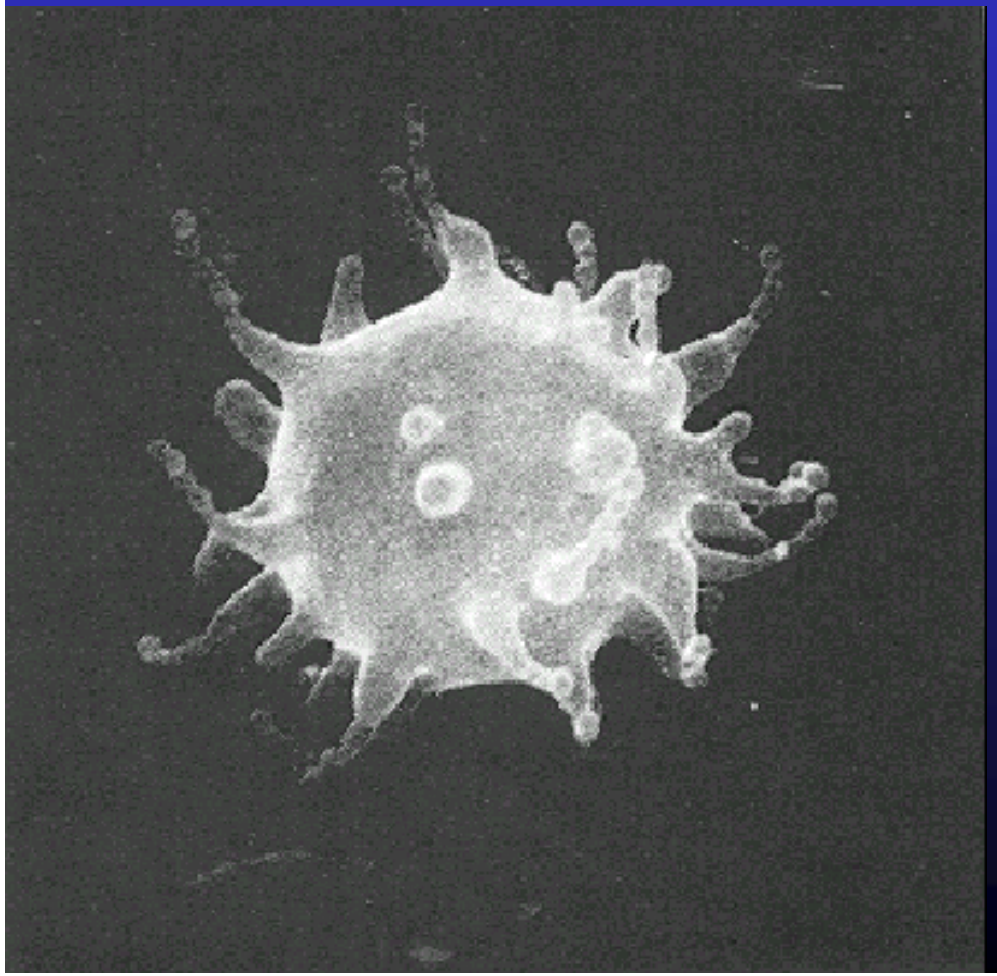
- Red cell shape is a function of Ca^{++} concentration, pH, and ATP concentration
- Red cell shape degrades linearly during storage
- The early phases largely repair with rejuvenation
- Membrane loss by microvesiculation is not repairable.

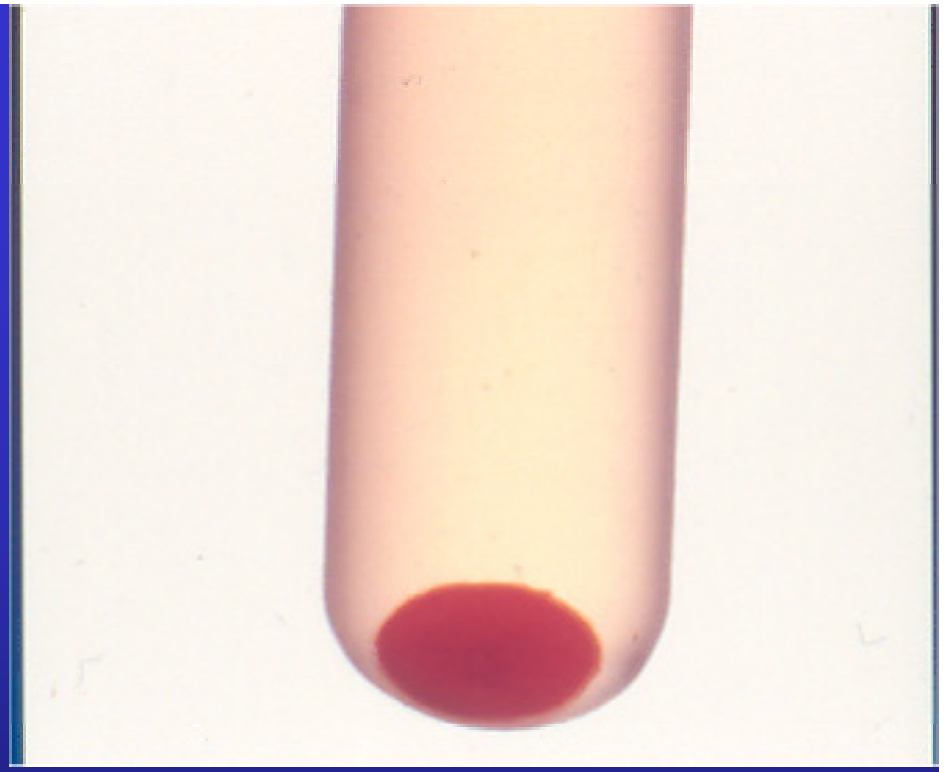
Red Cell Shape, Bessis M, Weed RI, Leblond PF, 1973

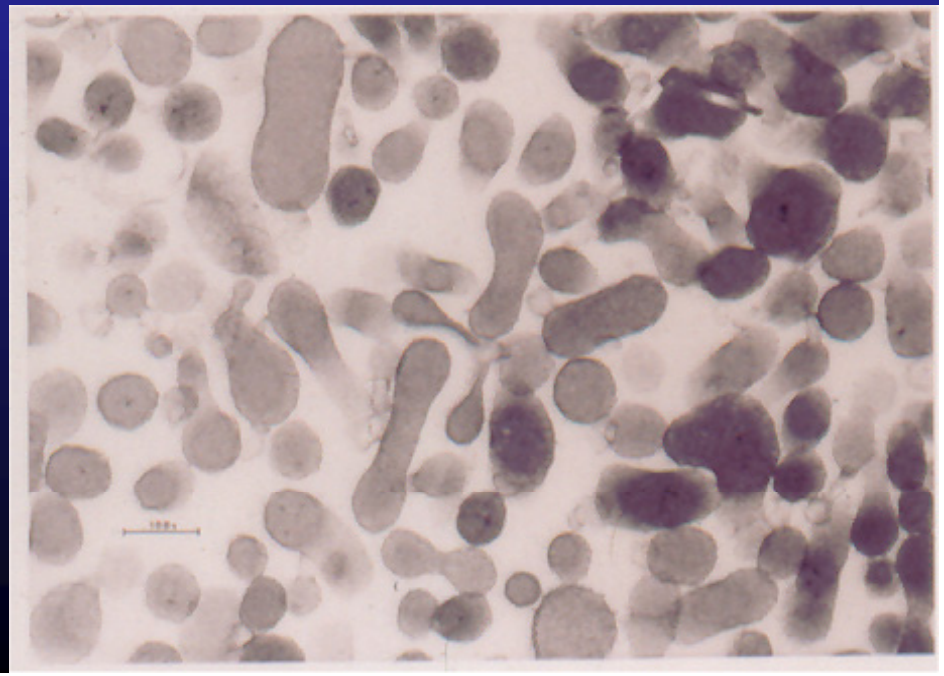

Ruddell, Transfusion 1998
Morphologic Index
100
90
80
70
60
50
0
5
10
15
20
25
30
35
Time (Days)
4°C throughout
25°C on day 6
25°C on day 20


Ruddell, Transfusion 1998
Hemoglobin (mg/dL)
250
200
150
100
50
0
4°C throughout
25°C on day 6
25°C on day 20
0
5
10
15
20
25
30
35
Time (Days)

RBC microvesiculation during storage







RBC recovery falls during storage as ATP declines, 1

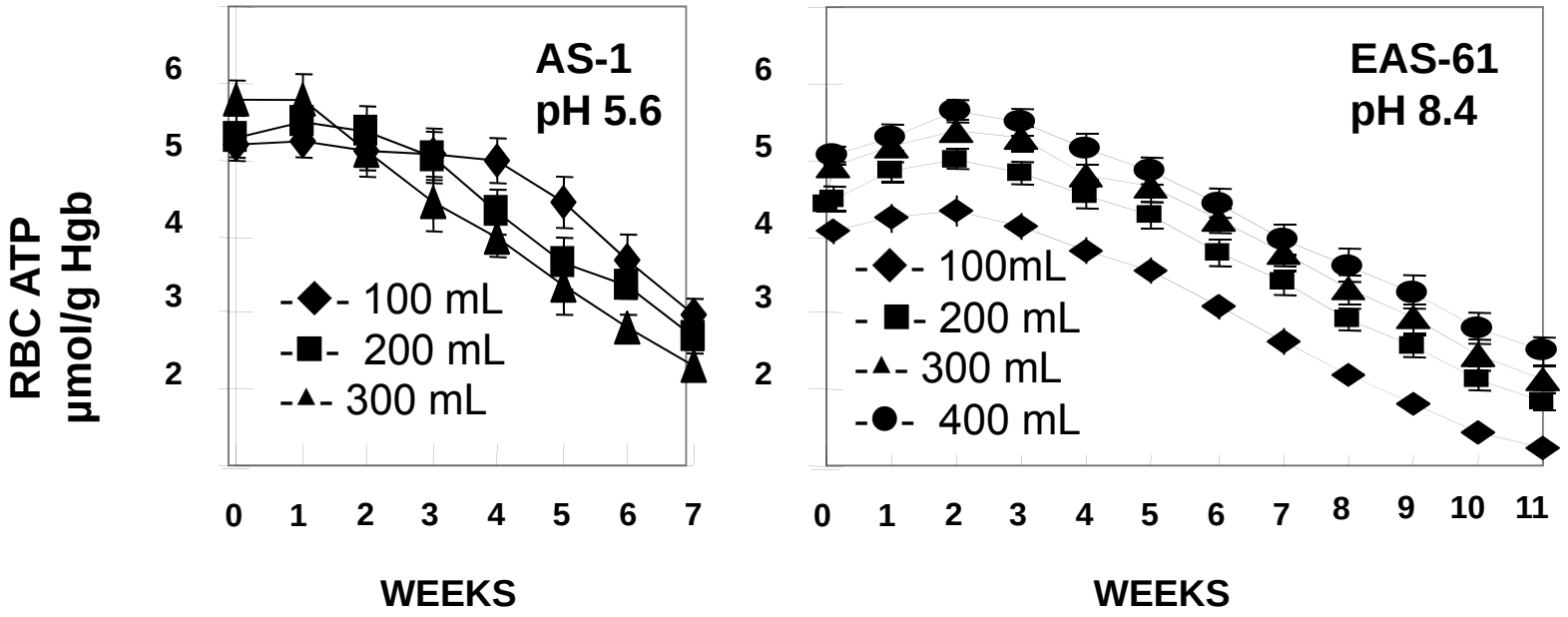
- Recovery can be maintained for months under conditions that support normal ATP concentrations
  - Meryman, Transfus Sci 1994; 15:105
- Recovery can be improved by rejuvenation at the end of storage increasing ATP and recovery 77% to 89%
  - Hogman, Vox Sang 1985; 48:257

RBC recovery falls during storage as ATP declines, 2

- Phosphotidyl serine exposure increases as RBC ATP concentrations fall because aminophospholipid translocase activity is highly ATP dependent.
  - Gleiss, FEBS Lett. 2002;519:153-8.
- RBC microvesiculation increases as ATP falls through a calpain related mechanism
  - Kamp, Biochemistry 2001;40:9438-46

Effects of increasing volumes of acidic and alkaline storage solutions.

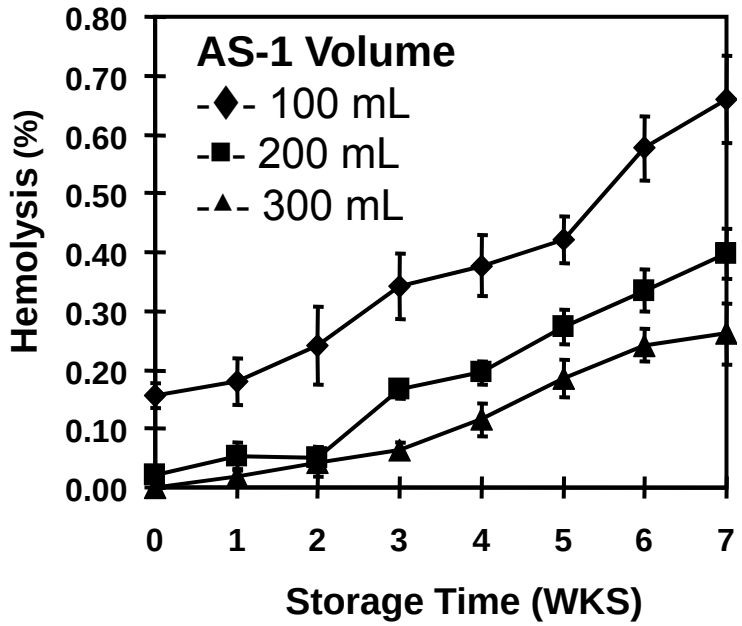
Effect of storage solution volume on RBC ATP

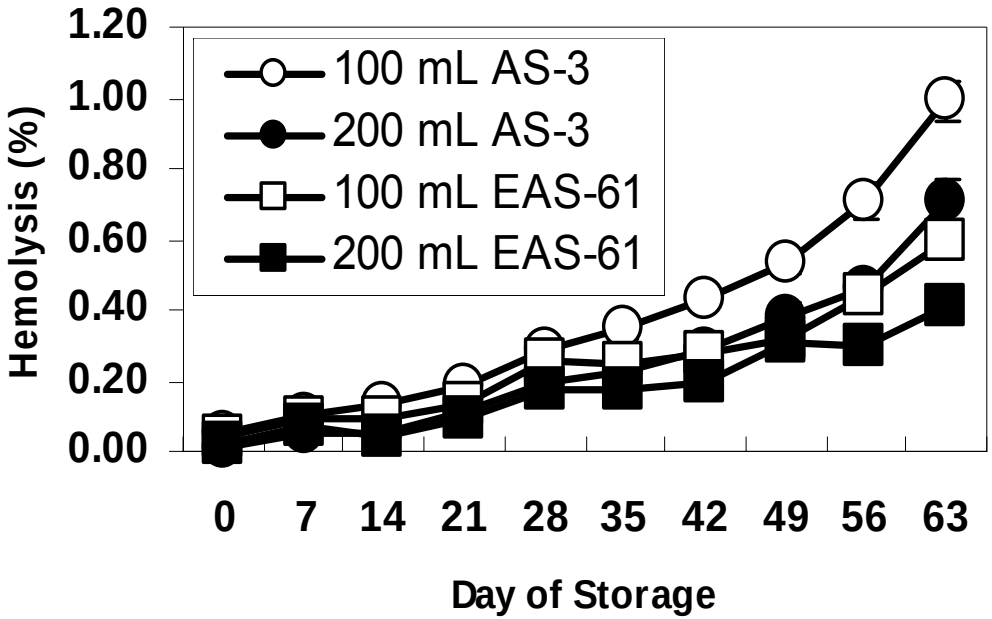


Hess et al. Transfusion 2000 Aug: 40:1000-1006

Effects of increasing volumes of acidic and alkaline storage solutions.

Effect of Storage Solution Volume on Hemolysis



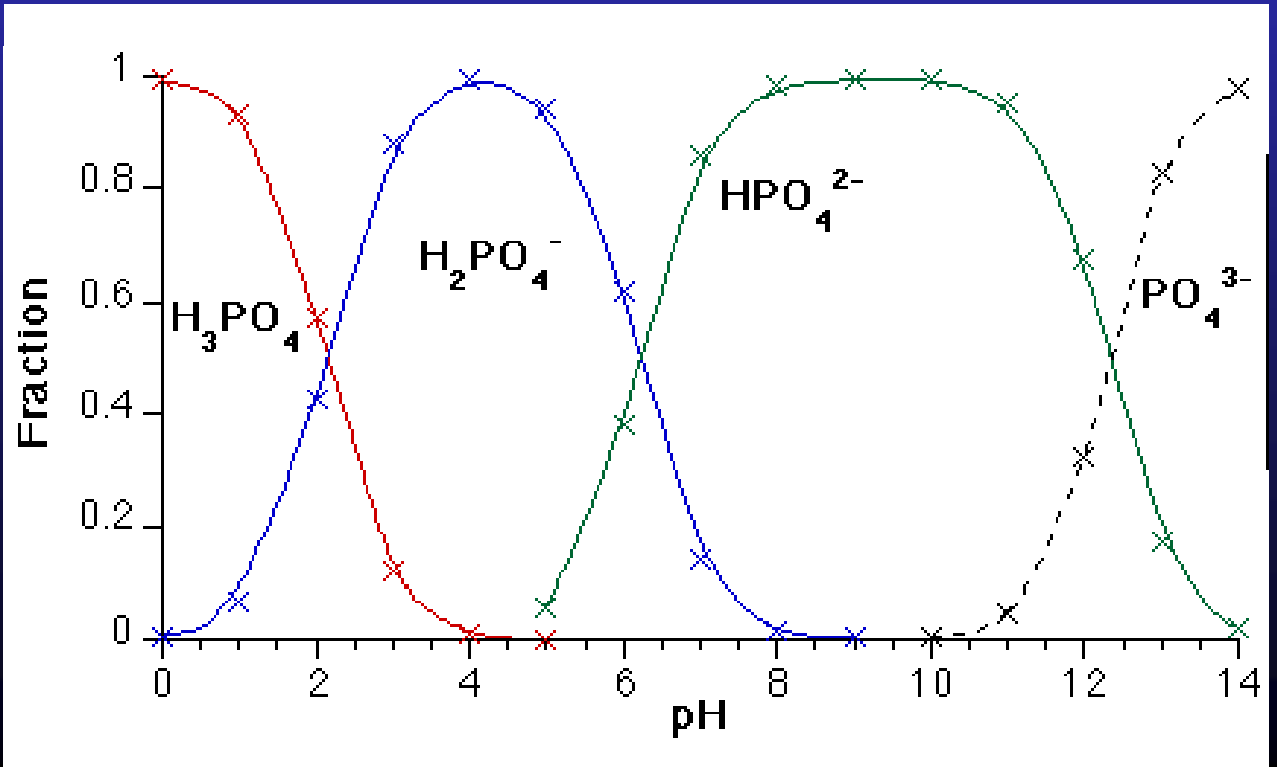


Hess et al. Transfusion 2000 Aug: 40:1000-1006

Phosphate in RBC storage solutions

There is 1.3 mMol of phosphate in 63 mL of CPD (20 mM/L), it adds 1 mMol of protons going from pH 5.5 to 7.2

There is 1 mMol of phosphate in 110 mL of EAS (9 mM/L), it removes 0.05 mMol of protons going from pH 8.6 to 7.2 and 0.30 mM going from pH 7.2 to 6.5



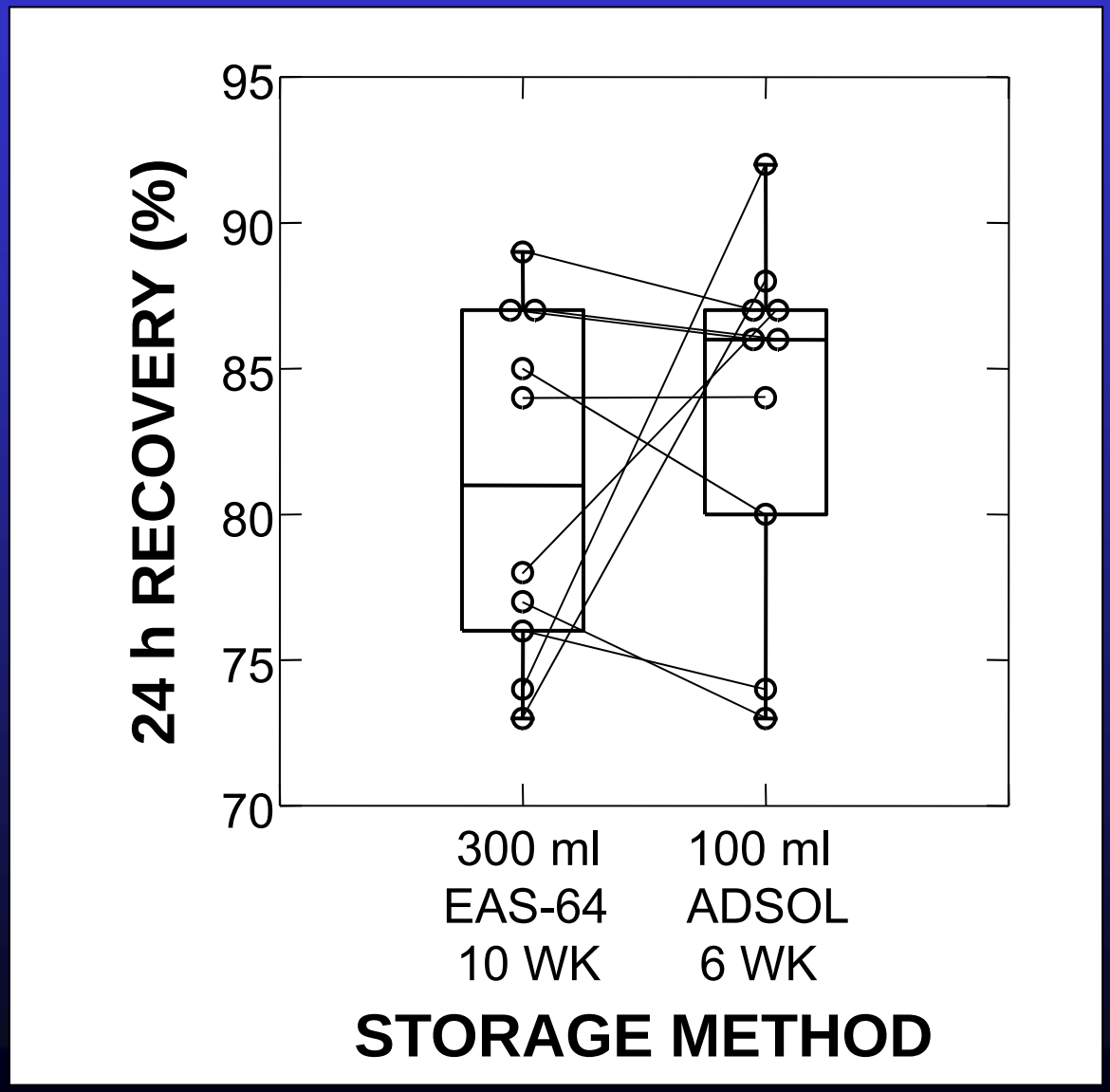
Binary Additive Solutions

- Manufacturers will need to make additive solutions in two parts, one containing the sugar and the other the alkaline salts so the sugar will not caramelize when Pasteurized.
- Pall has done this with AS-17
- Baxter has done this with ErythroSol

Formulation of Additive Solutions

	EAS-64	Adsol
Adenine	2	2
Dextrose	50	122
Mannitol	20	41.2
Na_2HPO_4	9	0
NaCl	75	154
mOsm, kg/L	244	471.2
mOsm supernatant @ 1h	270	350
pH (22°C)	8.4	5.8

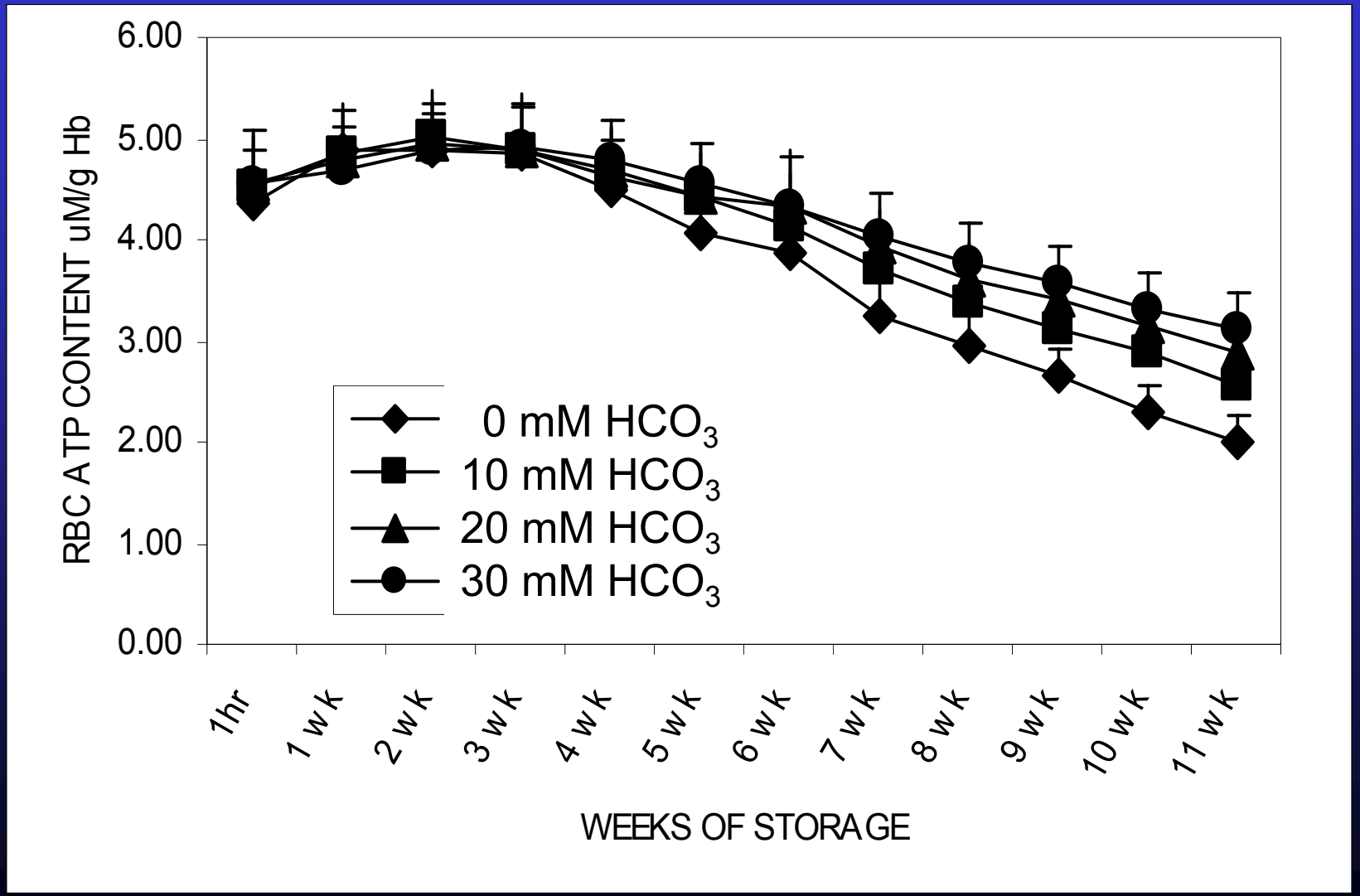
10-Week RBC Storage



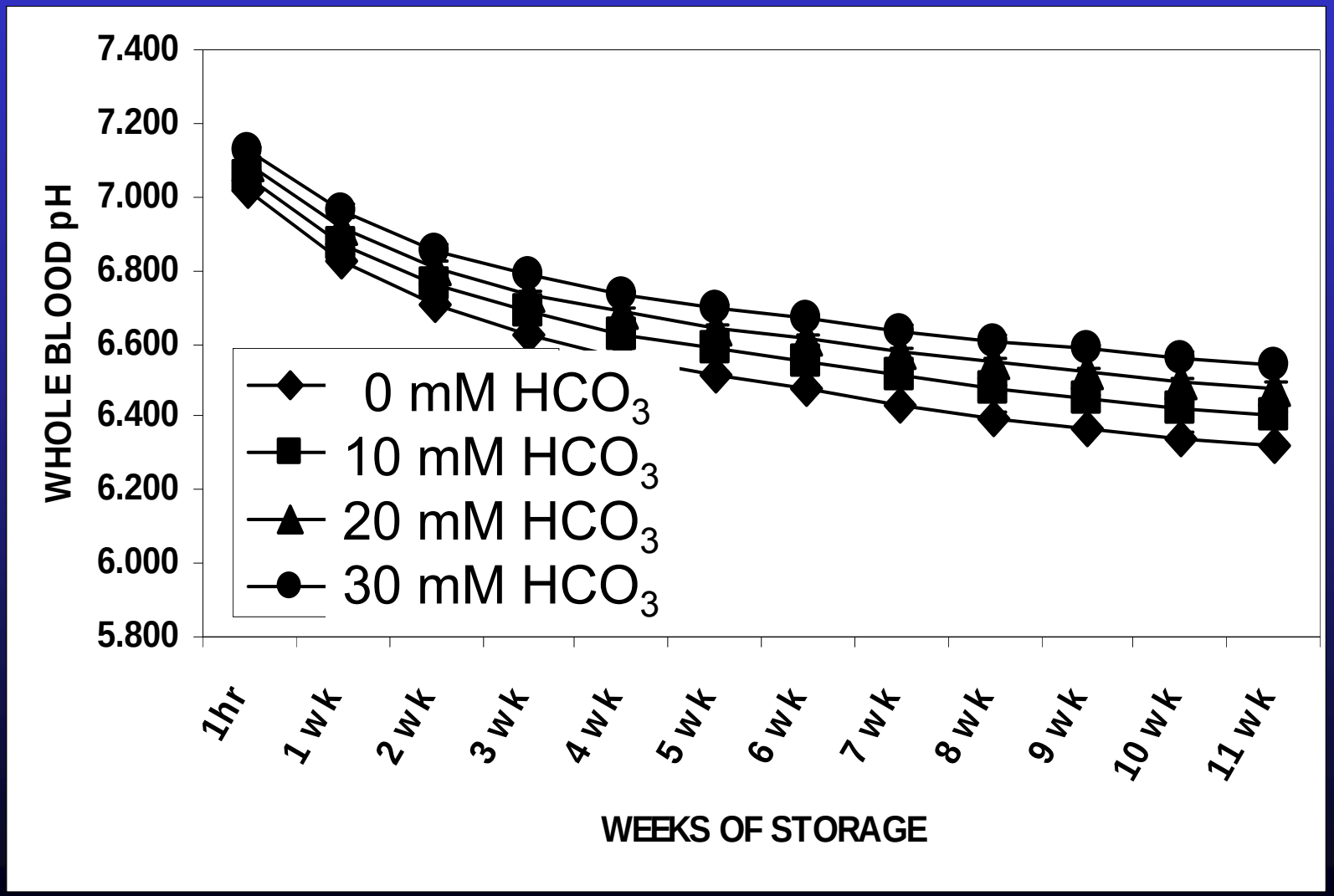
24-hour *in vitro* RBC recovery was 84 ± 6 % in both groups (n=10).

Transfusion 2001; 41:1006

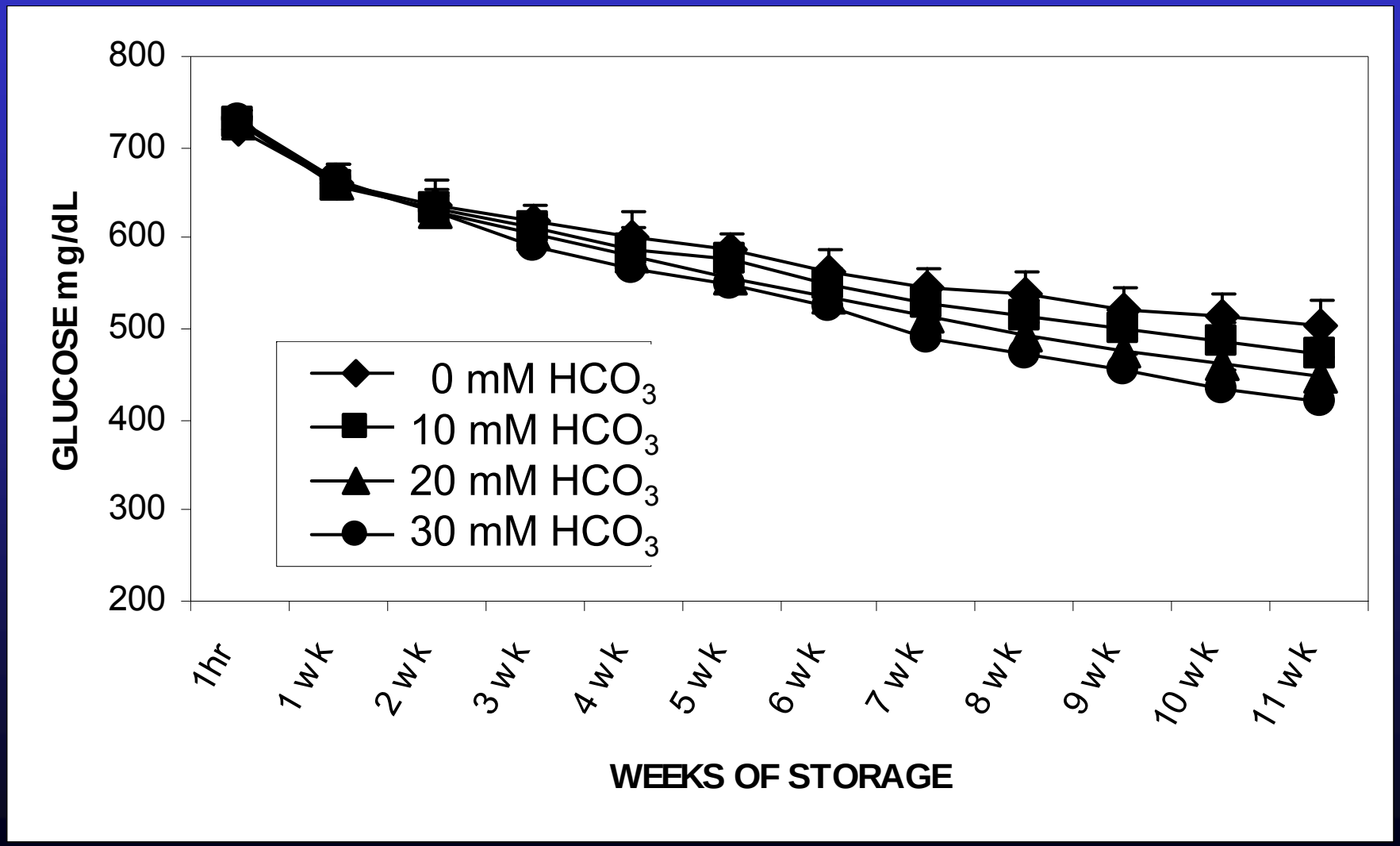
Effect of Bicarbonate on RBC ATP (solutions contain 0, 10, 20, & 30 mM)



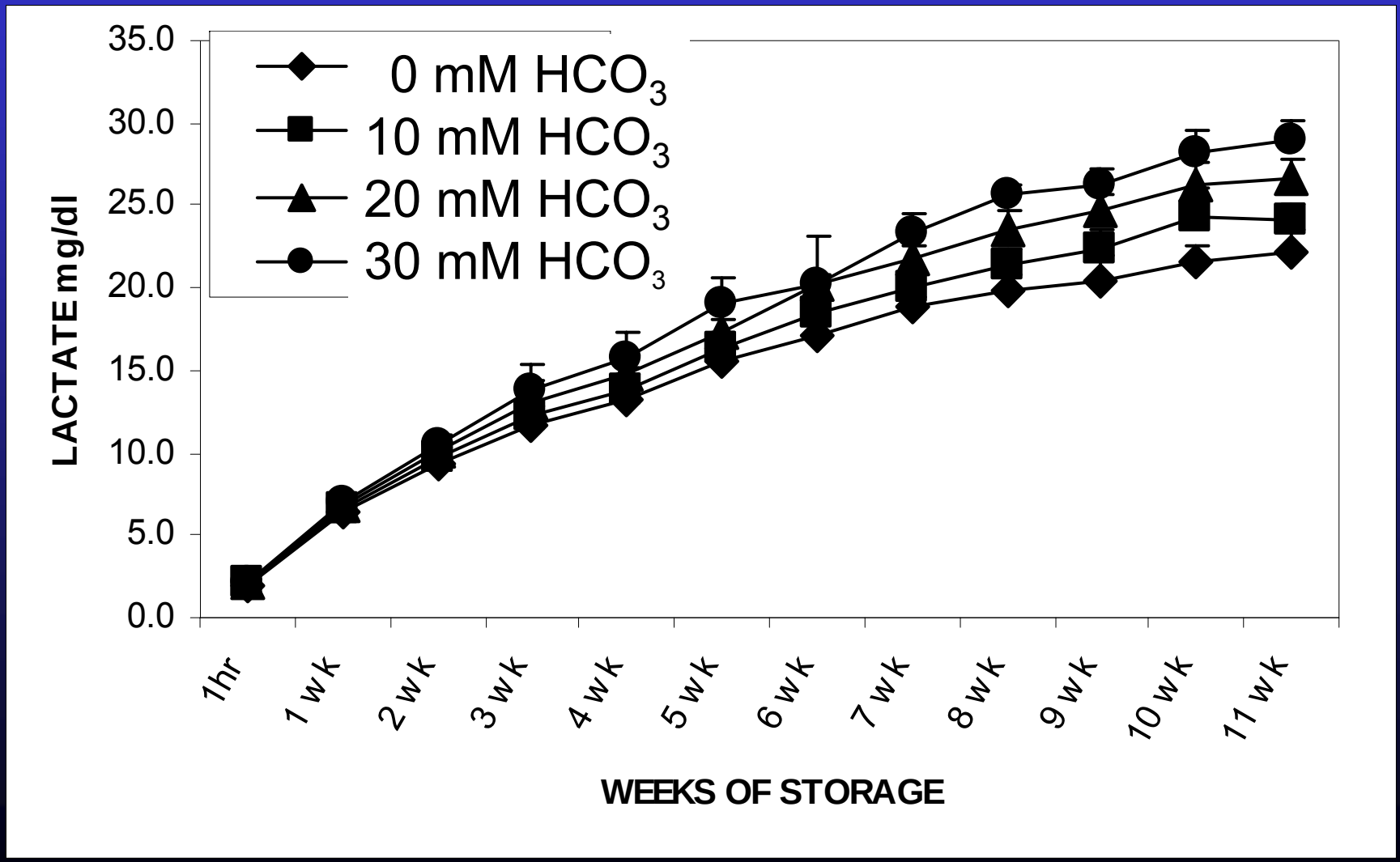
Effect of Bicarbonate on Whole Blood pH (solutions contain 0, 10, 20, & 30 mM)



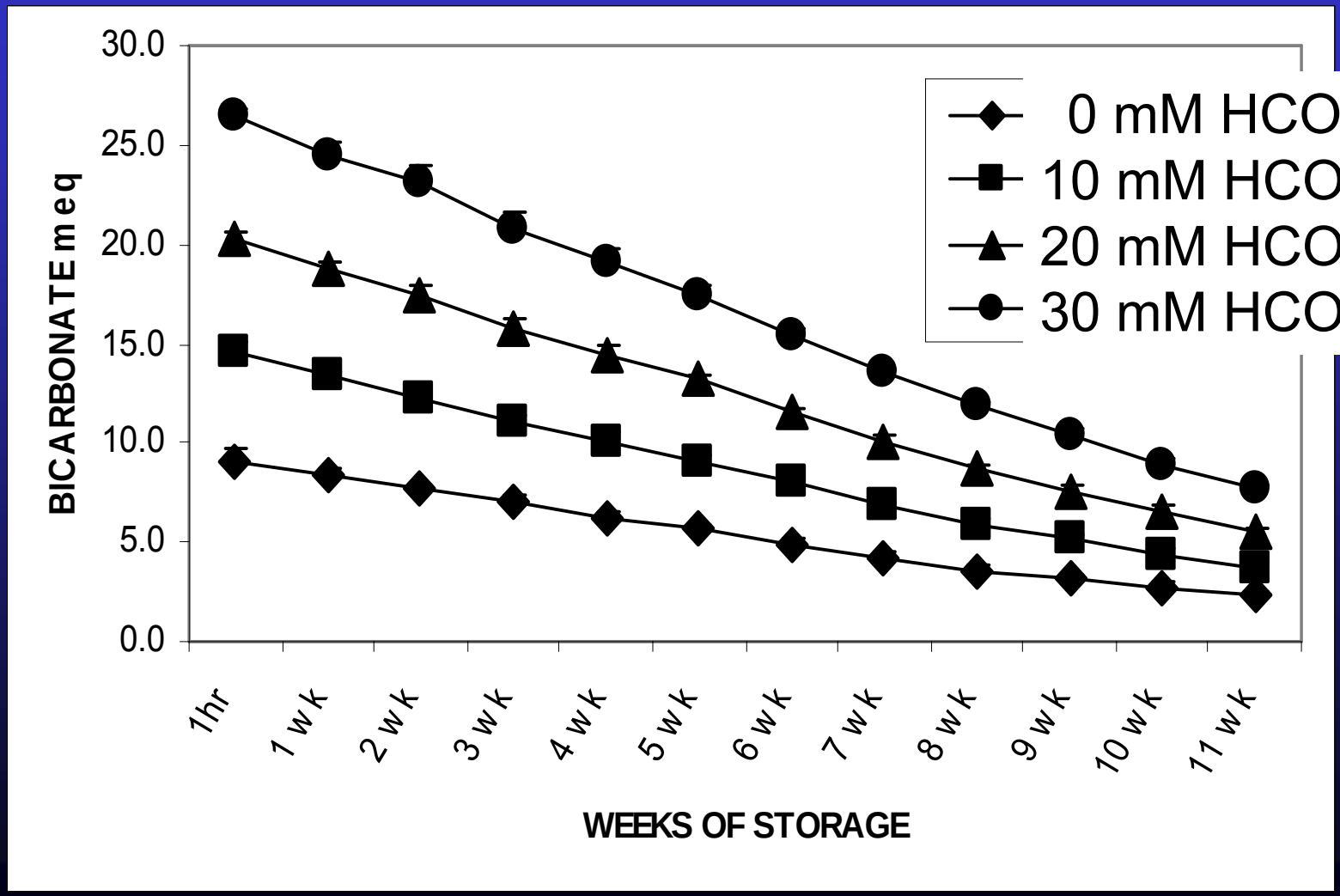
Effect of bicarbonate on solution glucose (solutions contain 0, 10, 20, & 30 mM)



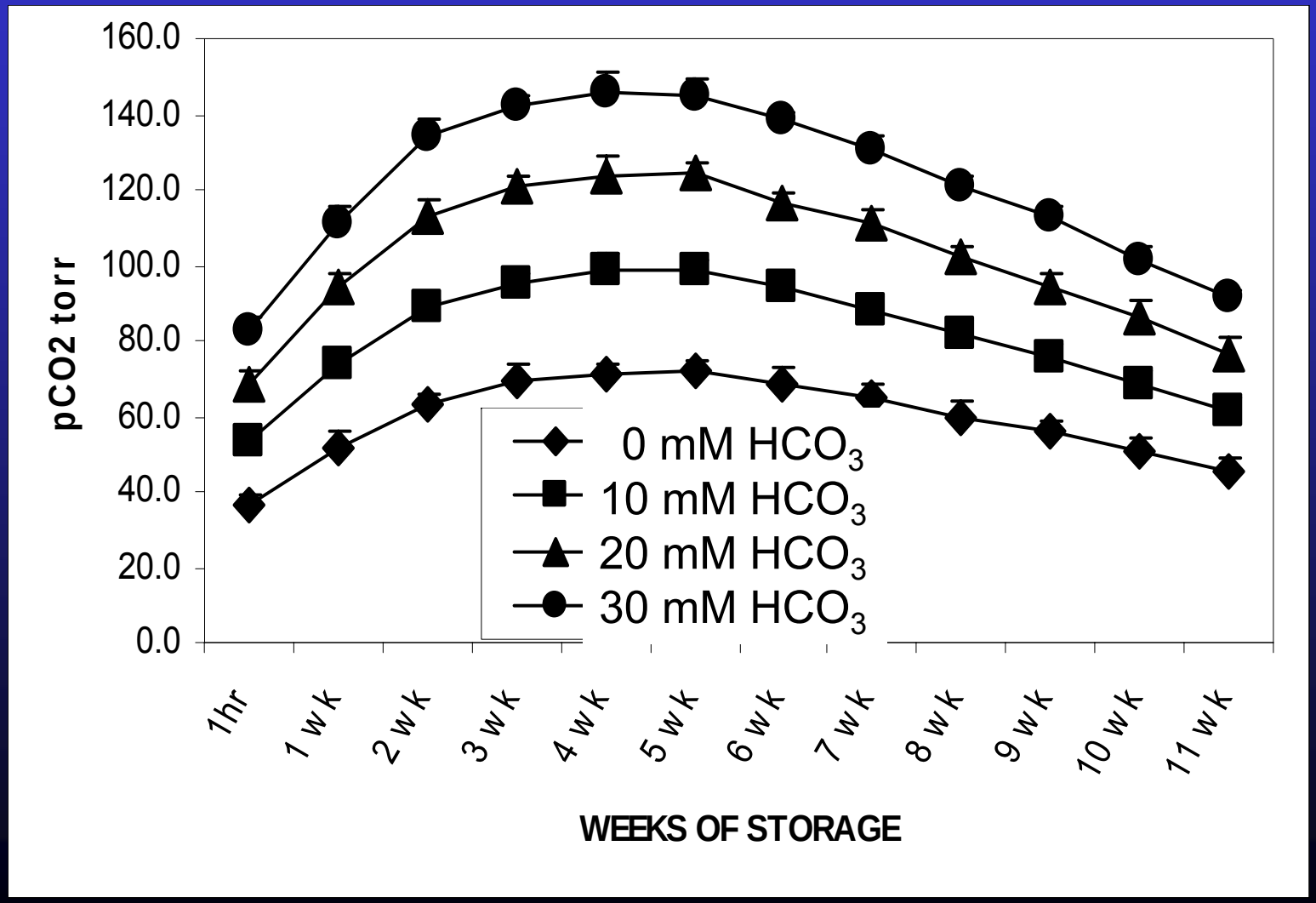
Effect of Bicarbonate on Lactate Production (solutions contain 0, 10, 20, & 30 mM)



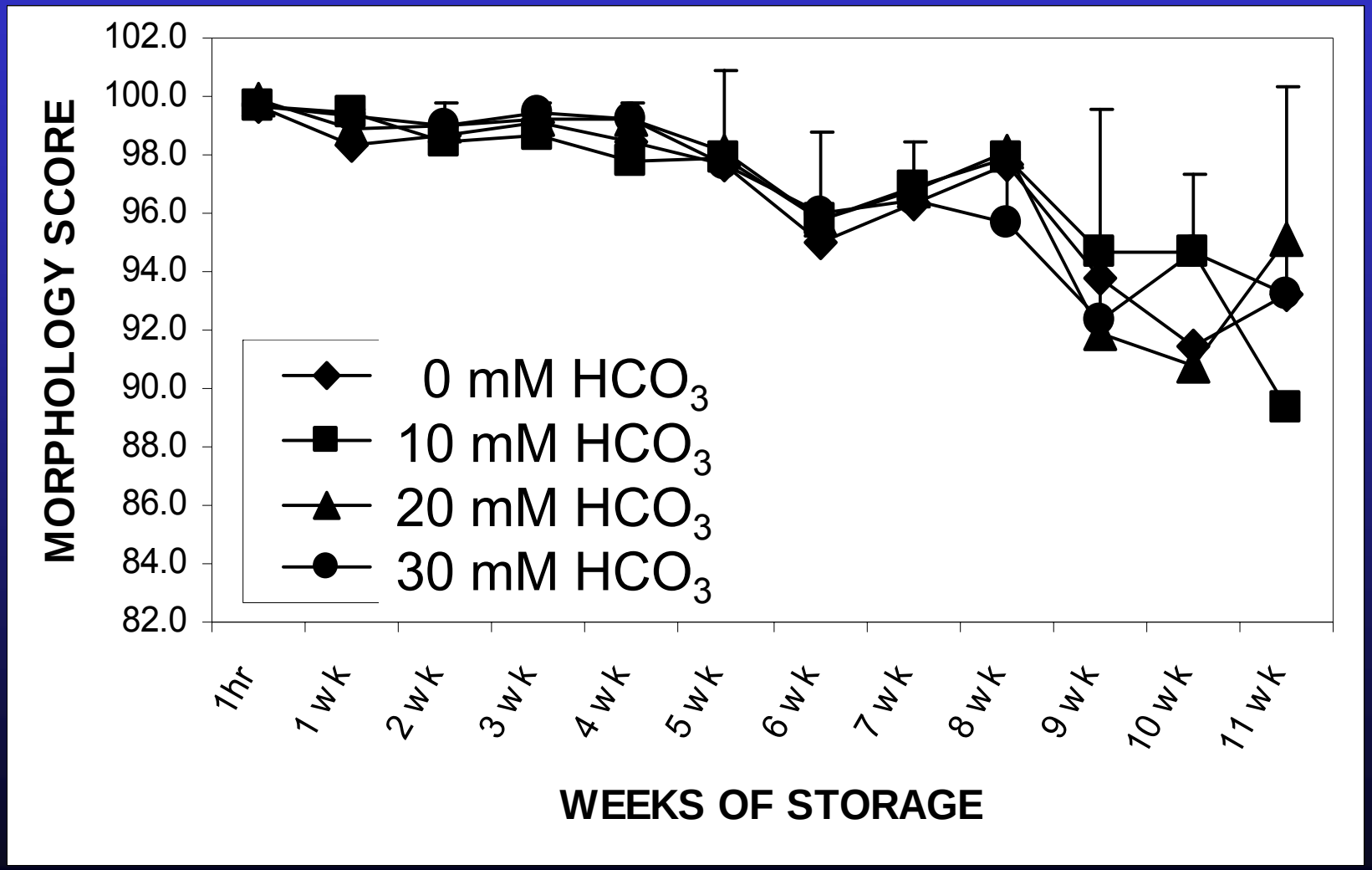
Effect of Added Bicarbonate on [HCO_3^-] (solutions contain 0, 10, 20, & 30 mM)



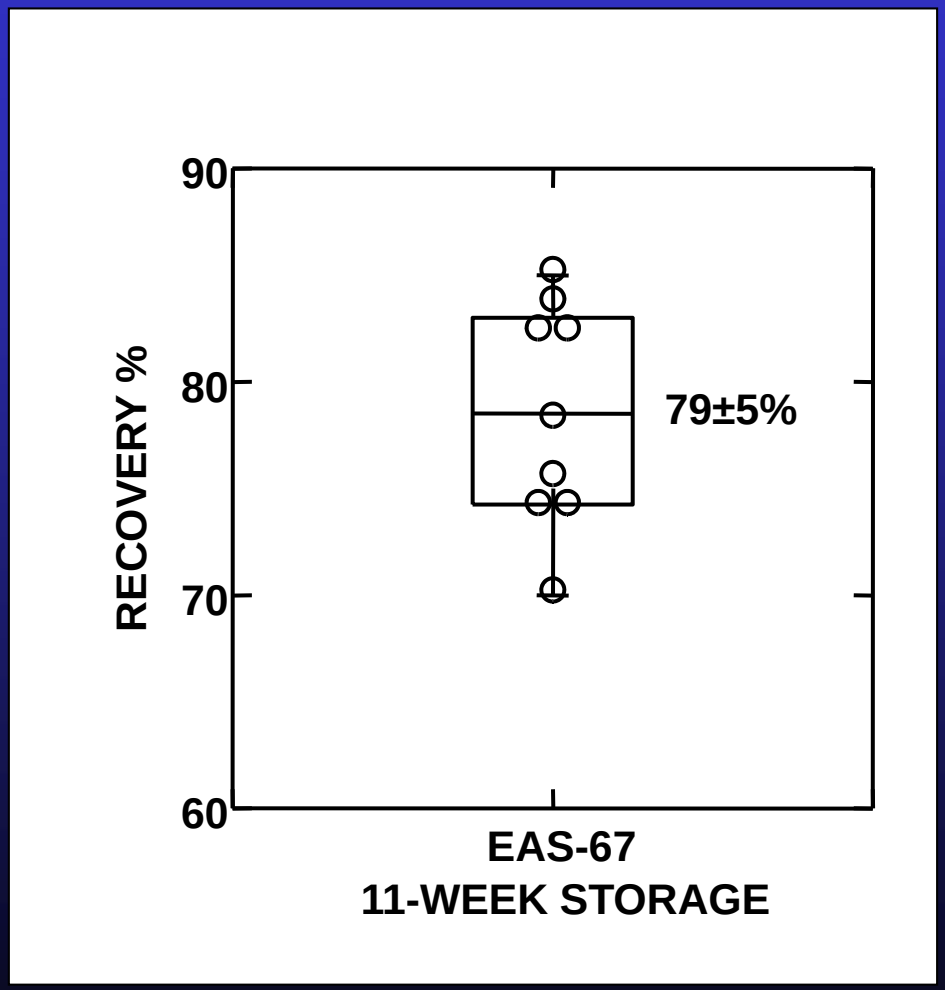
Effect of Added Bicarbonate on P_{CO_2} (solutions contain 0, 10, 20, & 30 mM)



Effect of bicarbonate on RBC Morphology (solutions contain 0, 10, 20, & 30 mM)



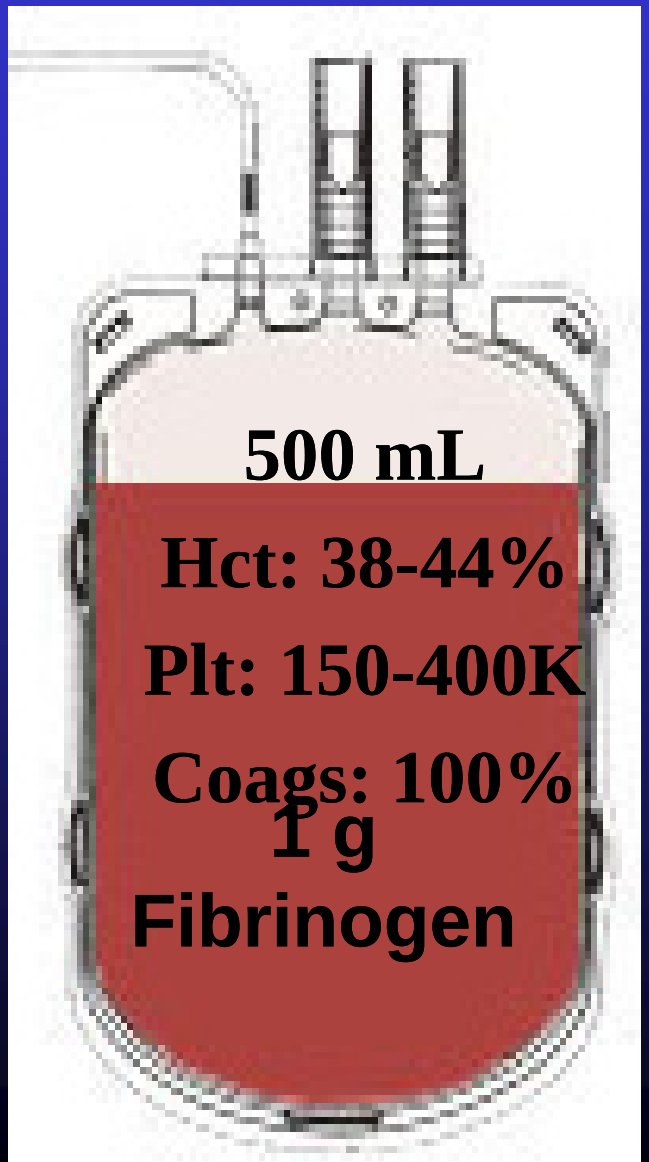
Viable RBCs after 11 weeks storage

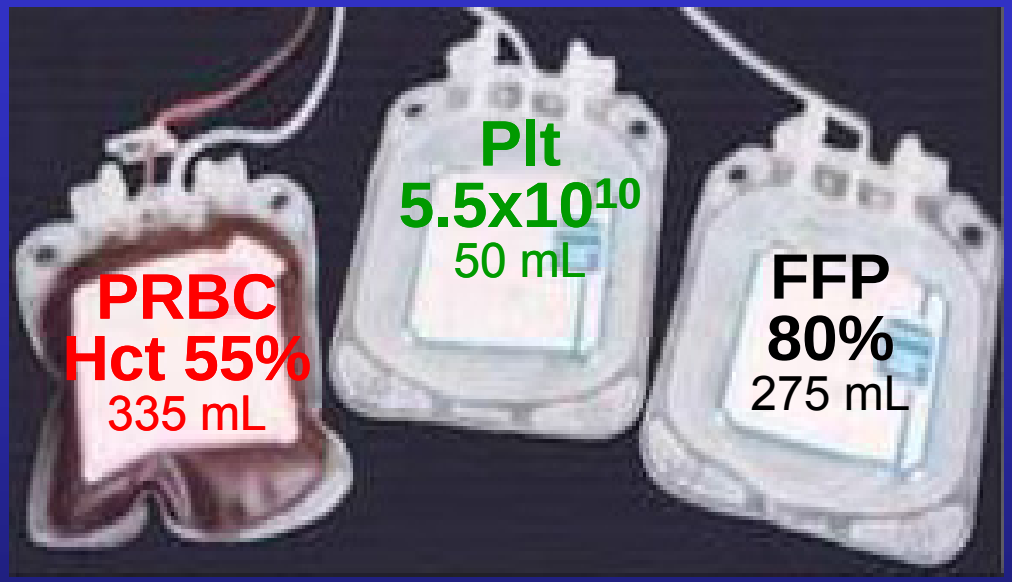


- RBCs stored in 300 mL of EAS-67 in PVC bags at 4°C
- Bags stored upright, not mixed
- Recovery measured by ^{51}Cr single-label technique
- n = 9

“Bloody vicious cycle”

Component Therapy vs What we bleed





So Component Therapy Gives You

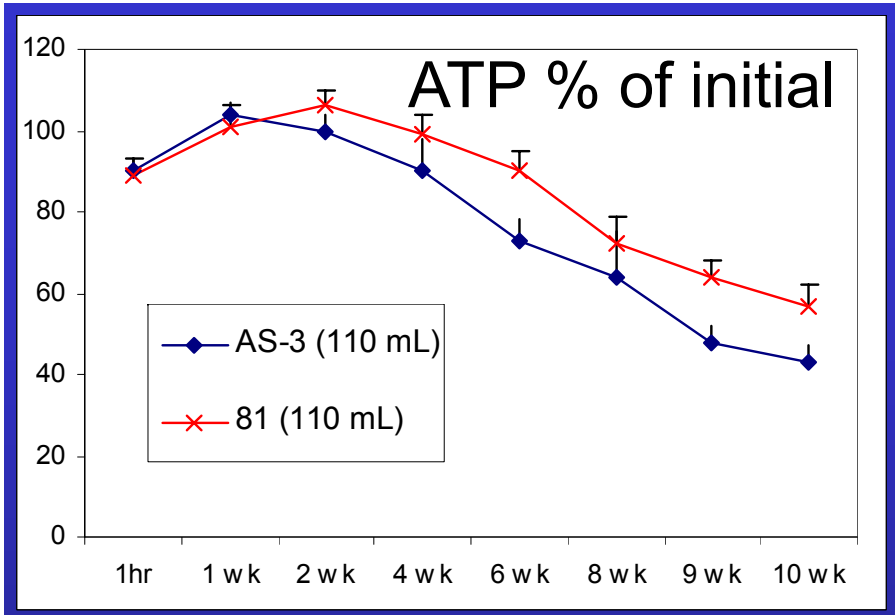
1U PRBC + 1U PLT + 1U FFP

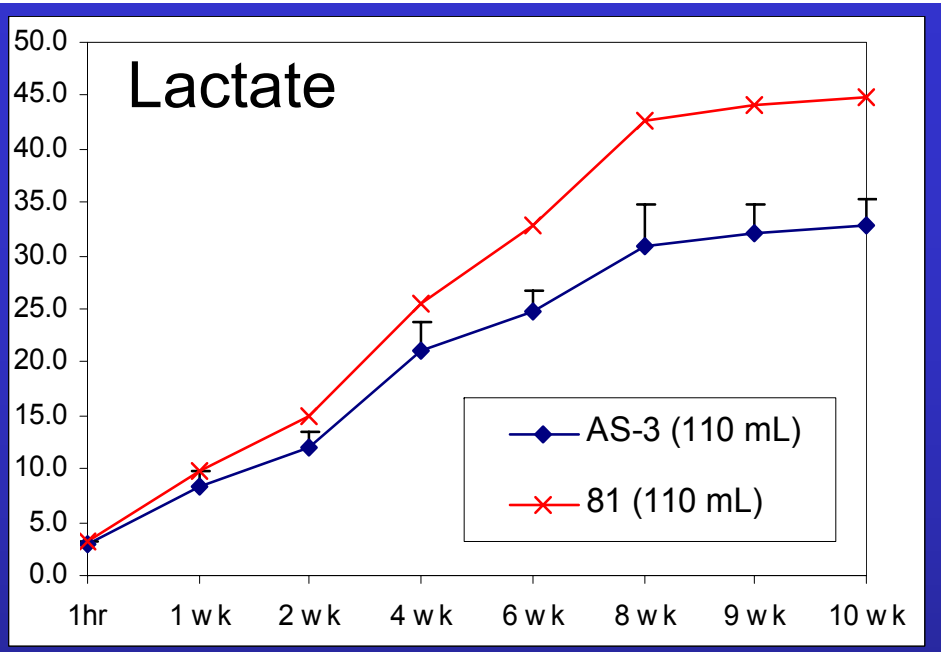
- ***Hct 29%***
- ***Plt 87K***
- ***Coag activity 65%***
- ***950 mg fibrinogen***

•ARMAND & HESS, TRANSFUSION MED. REV., 2003

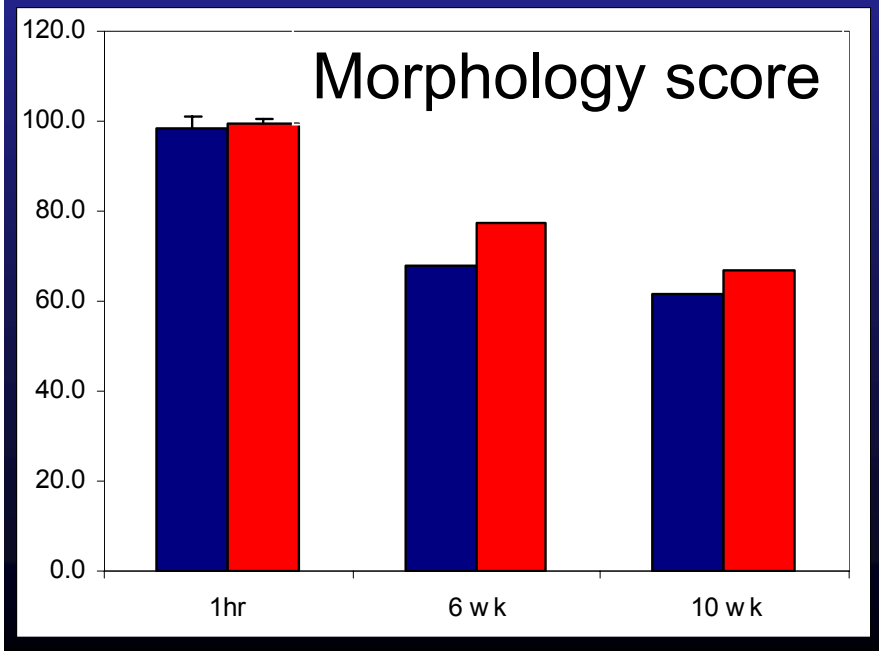
Formulation of AS-7

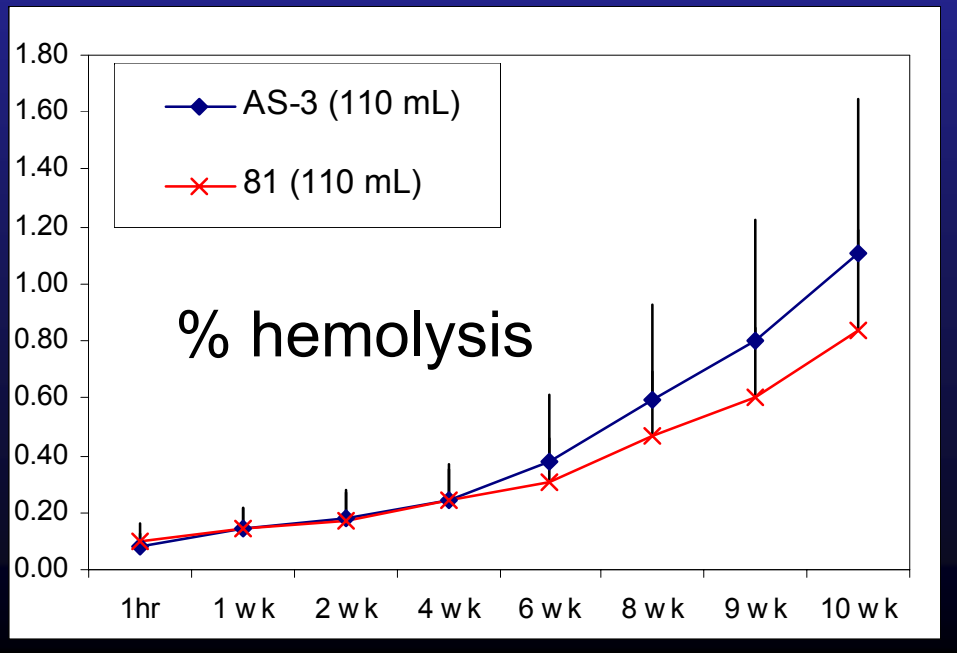
	AS-7
Adenine	2
Dextrose	80
Mannitol	55
Na_2HPO_4	12
$NaHCO_3$	26
mOsm, kg/L	244
mOsm supernatant @ 1h	270
pH (22ºC)	8.5





A first attempt at a 110 mL additive solution





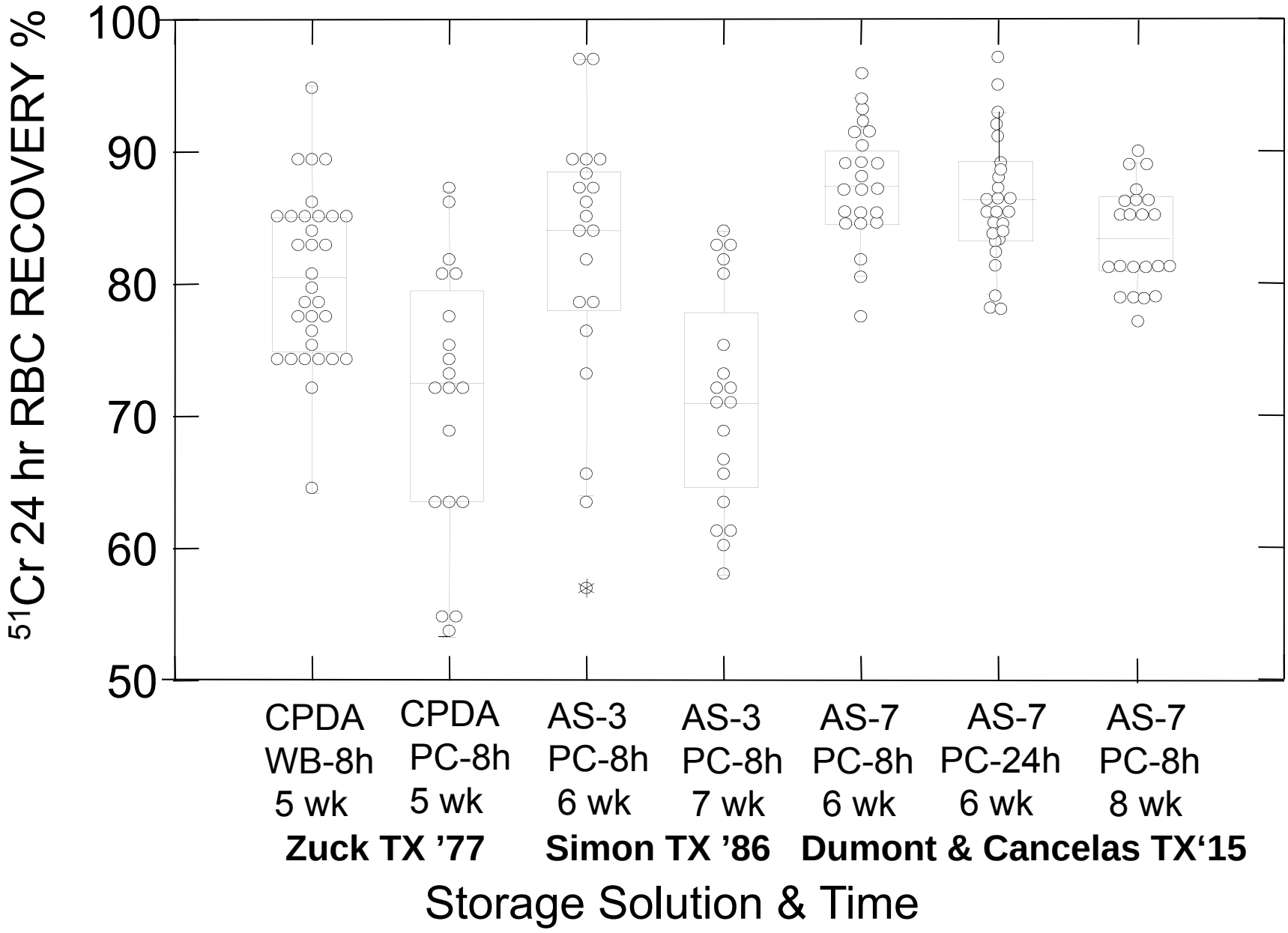
Comparing AS-7 at 8 weeks to AS-1 at 6 weeks of storage

- AS-1 at 6 weeks (n=10) Transfusion 40:1011

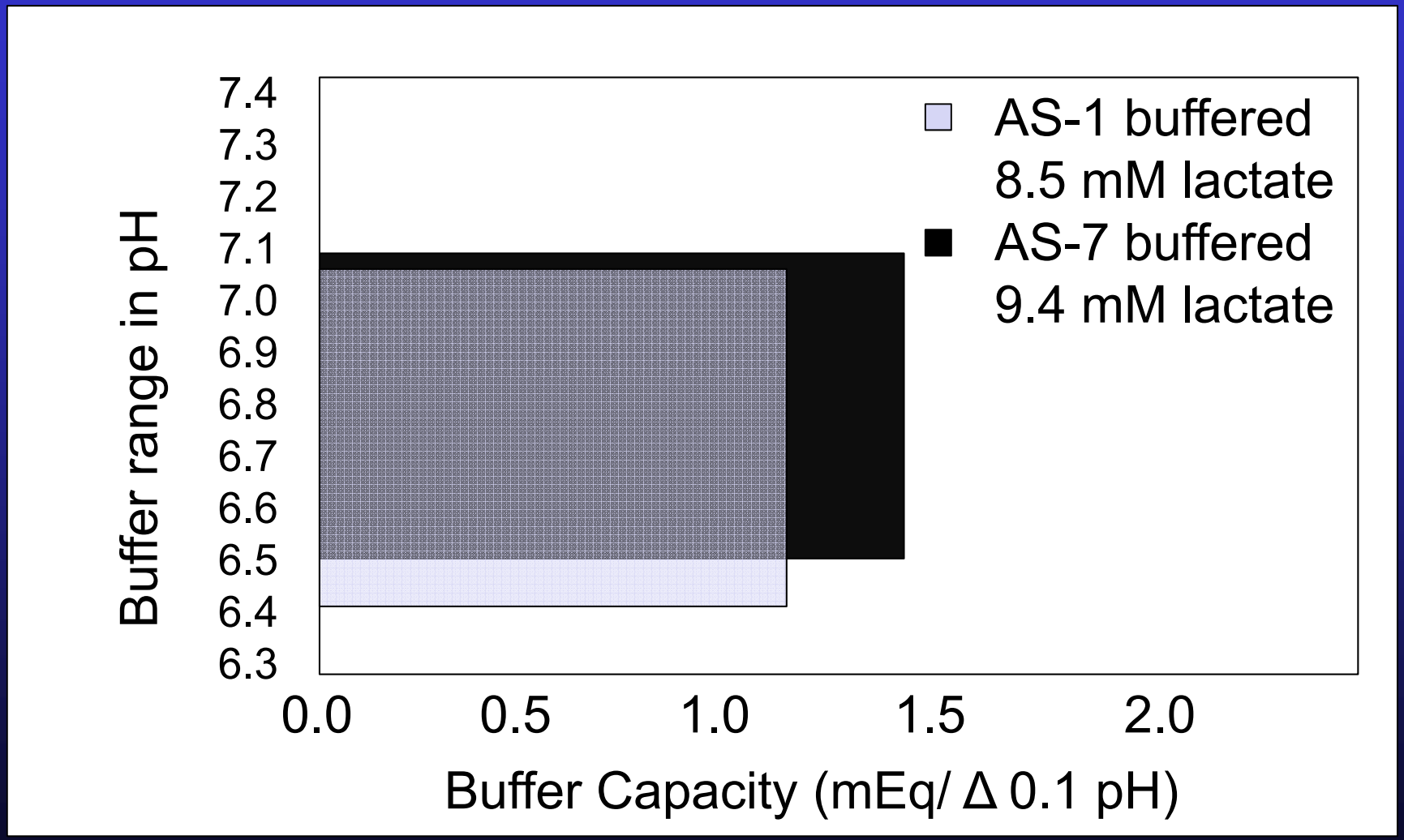
- Recovery 84 ± 8 %
- Hemolysis 0.63 ± .39%
- ATP 2.86 ± .45 µM/gHb
- ATP 64 ± 8%
- Morphology 71 ± 7%
- Vesicle protein 32 ± 7

- AS-7 at 8 weeks (n=6) Transfusion 45:50

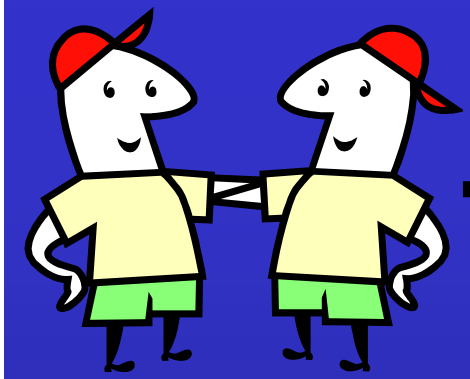
- Recovery 87 ± 2 %
- Hemolysis 0.38 ± .23%
- ATP 2.93 ± .32 µM/gHb
- ATP 64 ± 6%
- Morphology 77 ± 11%
- Vesicle protein 12 ± 6


^{51}Cr 24 hr RBC RECOVERY %
100
90
80
70
60
50
CPDA WB-8h 5 wk
CPDA PC-8h 5 wk
AS-3 PC-8h 6 wk
AS-3 PC-8h 7 wk
AS-7 PC-8h 6 wk
AS-7 PC-24h 6 wk
AS-7 PC-8h 8 wk
Zuck TX '77
Simon TX '86
Dumont & Cancelas TX'15
Storage Solution & Time

Improving buffer range and capacity with a phosphate / bicarbonate buffer system



Buffer utilization with 6 weeks of RBC storage at 4°C



13 Mono- and 5 di-zygotic twin pairs

UNIVERSITY of IOWA HOSPITALS & CLINICS

DeGowin Blood Center

University of Iowa Health Care

Processing

Draw sample

[Hemoglobin]

CBC

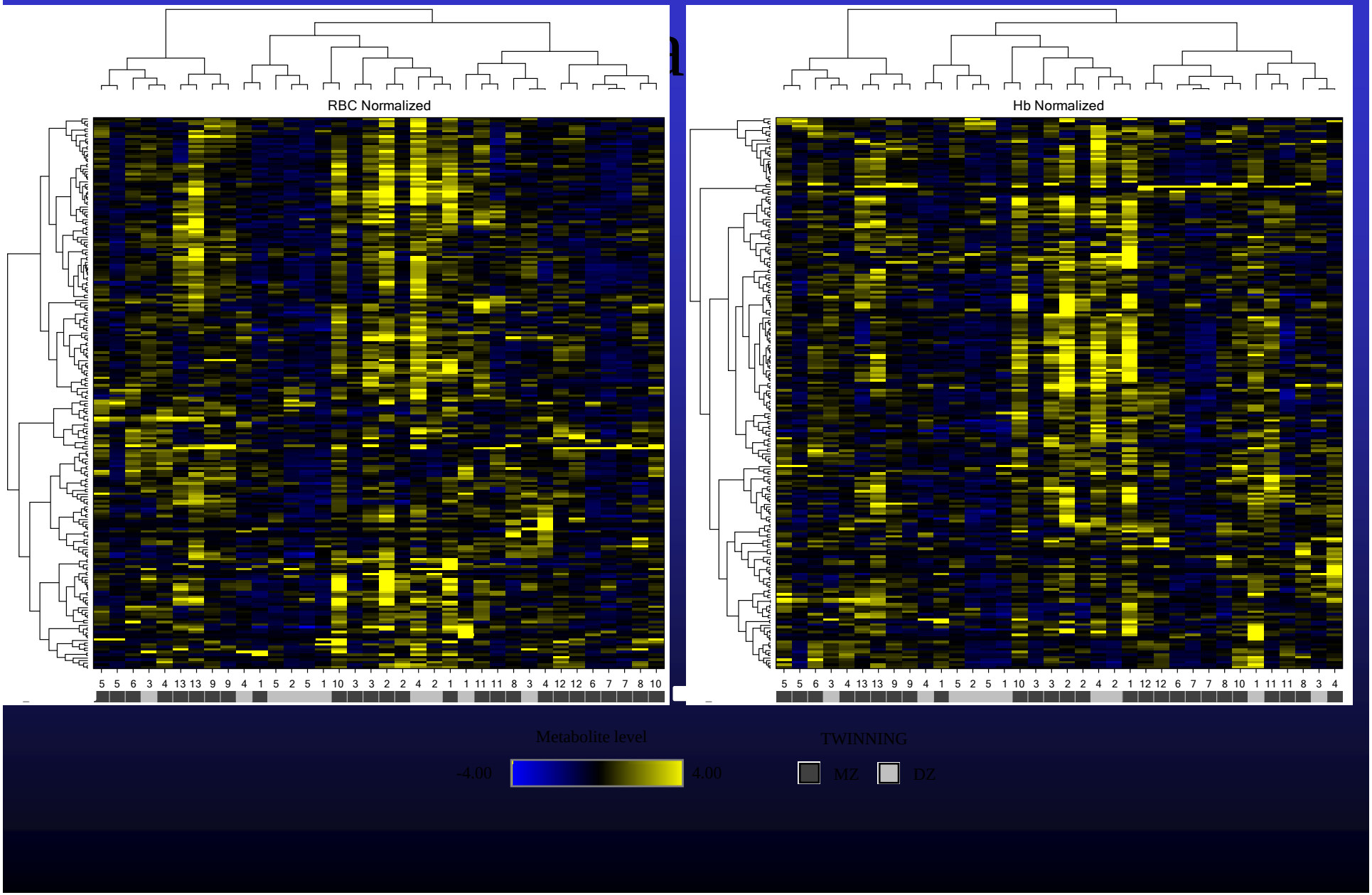
[ATP]

[GSH]

[metabolites]

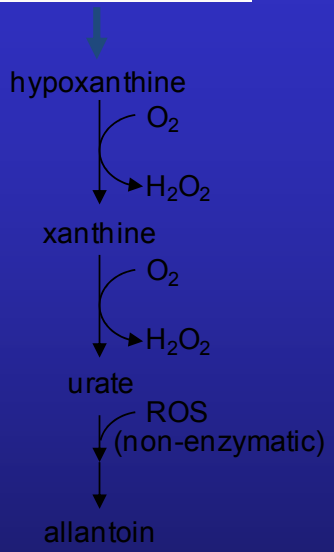
Process

Day: 0,7,14, 28,42, 56

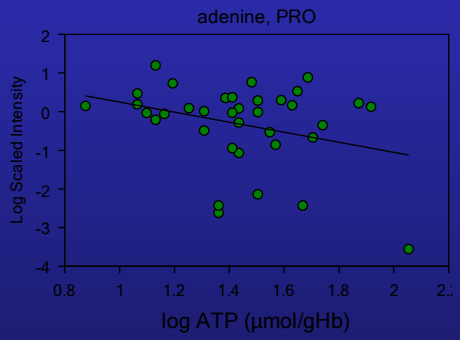

RBC Normalized
Hb Normalized
5 5 6 3 4 13 13 9 9 4 1 5 2 5 1 10 3 3 2 2 4 2 1 1 11 11 8 3 4 12 12 6 7 7 8 10
Metabolite level
-4.00
4.00
TWINNING
MZ
DZ

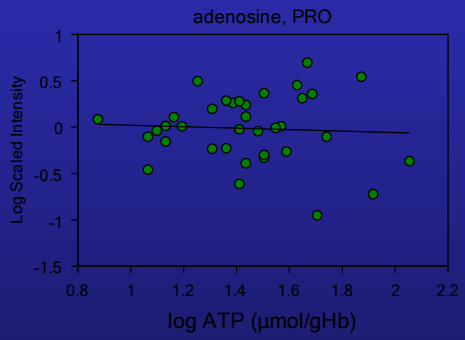
Purine nucleotide degradation

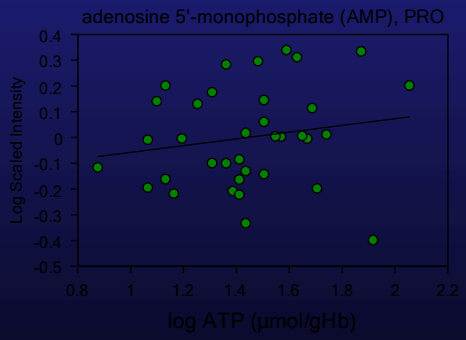
purine nucleotides

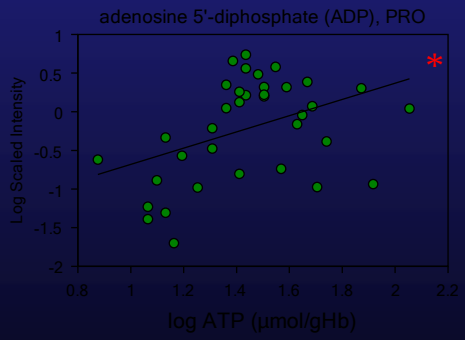


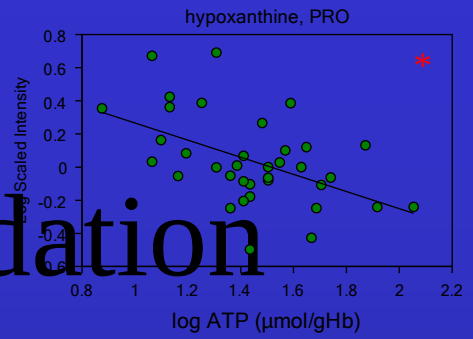
Biochemical Name	Heritability		
	WB	RBC	PRO
adenine	34.2	35.4	31.3
adenosine	13.4	-1.1	44.1
adenosine 5'-monophosphate (AMP)	66.8	45.1	-83.1
adenosine 5'-diphosphate (ADP)	79	78.3	73.8
hypoxanthine	64.9	66.1	17.2
xanthine	-11.4	-0.1	-39.4
urate	5.5	8.6	-25.9
allantoin	2.2	-25.8	46.2

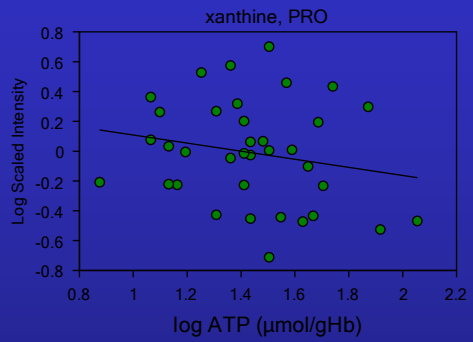


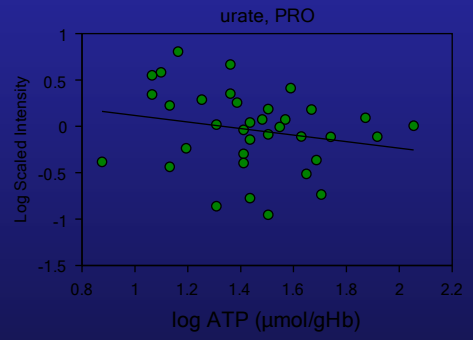


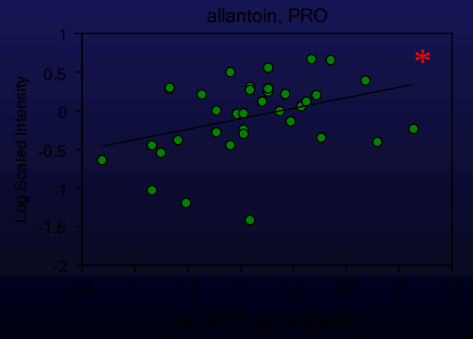






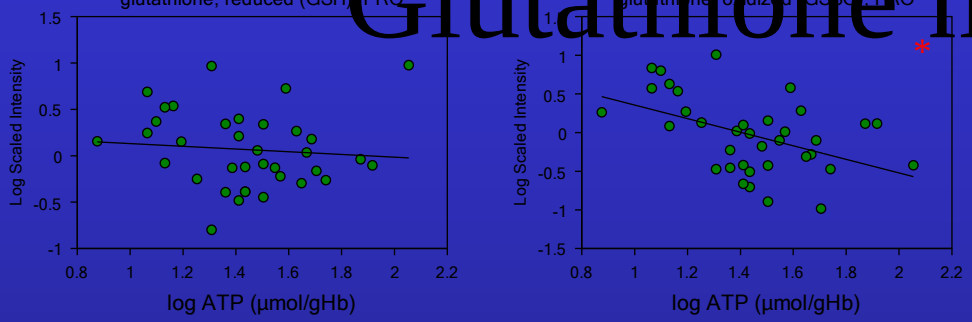


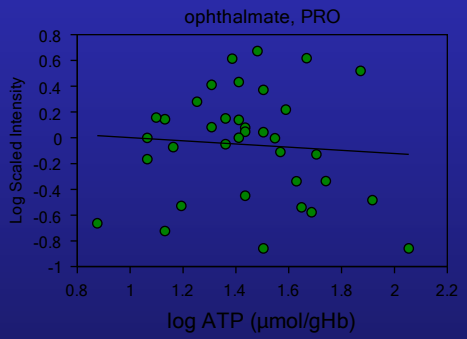


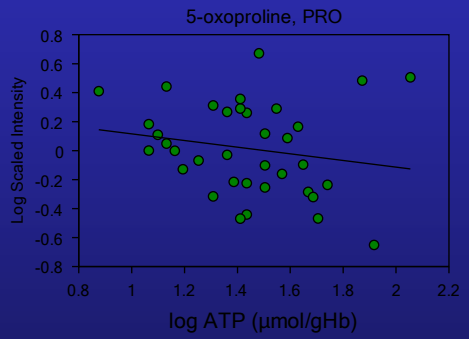


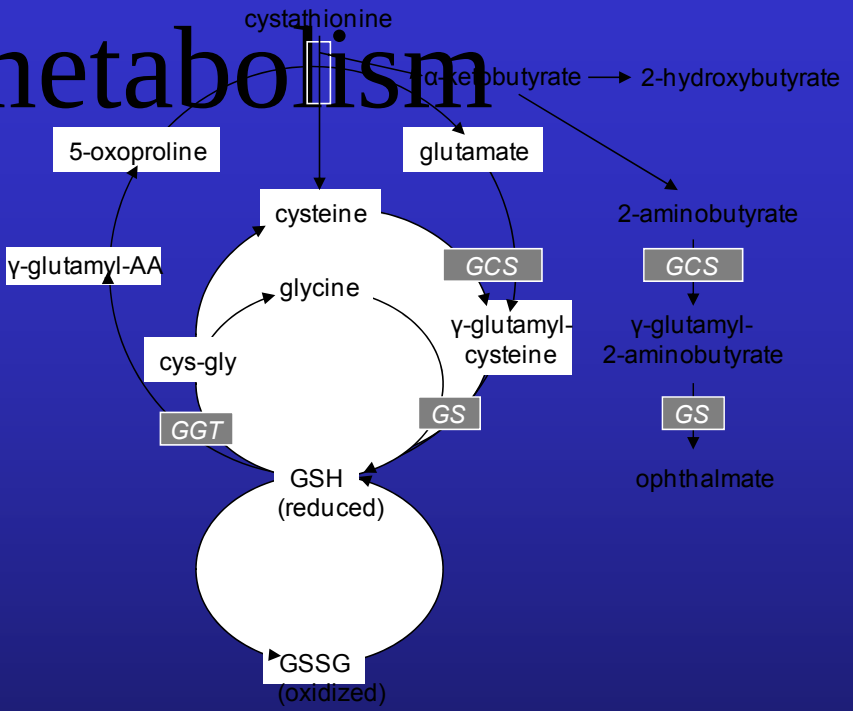
* *denotes correlation with p<0.05*

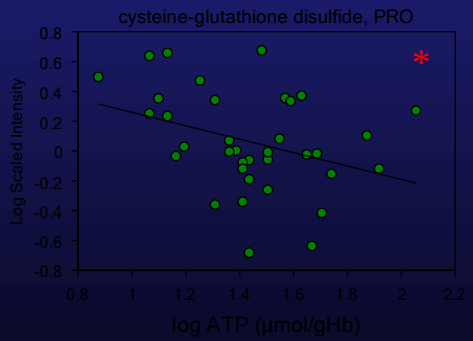
Glutathione metabolism











Biochemical Name	Heritability		
	Hb	RBC	PRO
glutathione, reduced (GSH)	**41.3**	**44.3**	19
5-oxoproline	**57.5**	**47.8**	23.4
glutathione, oxidized (GSSG)	**38.2**	34.9	32.9
cysteine-glutathione disulfide	**62.1**	**66.8**	12.2
ophthalmate	-28	-59.3	3.8

* *denotes correlation with p<0.05*

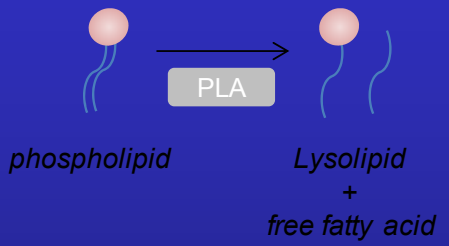
Phospholipids

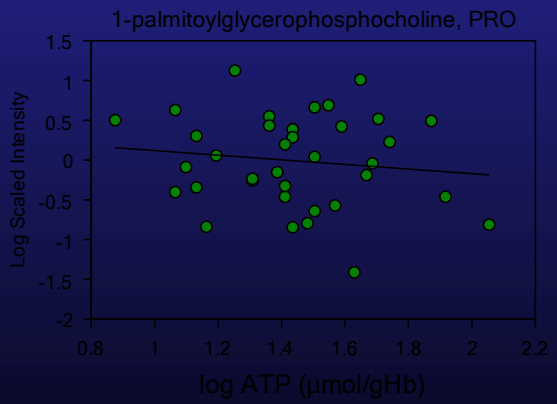
Choline-conjugated membrane glycerophospholipids:
- Most prevalent
- Highest fraction in outer leaflet

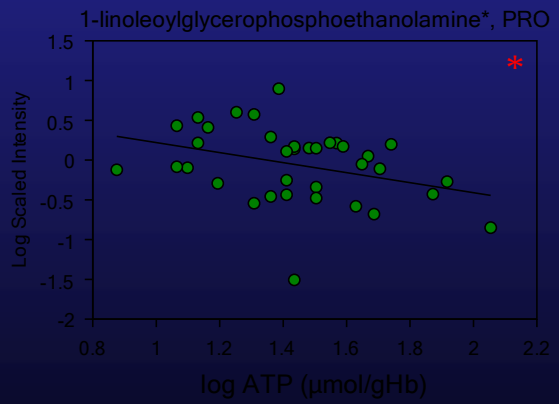
Ethanolamine-conjugated membrane glycerophospholipids:
- Highest fraction in inner leaflet

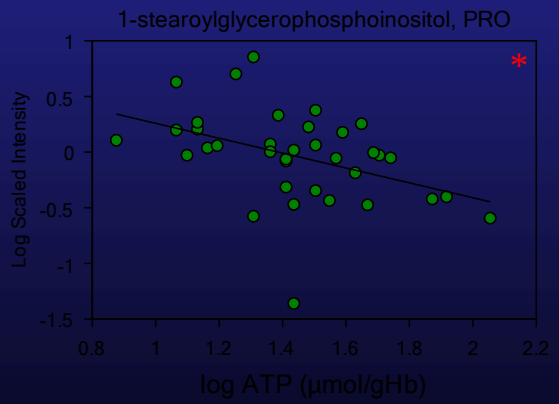
Inositol-conjugated membrane glycerophospholipids:
- Highest fraction in inner leaflet
- Key reservoir for arachidonate

Serine-conjugated membrane glycerophospholipids:
- Highest fraction in inner leaflet
- Presence on outer leaflet in apoptosis



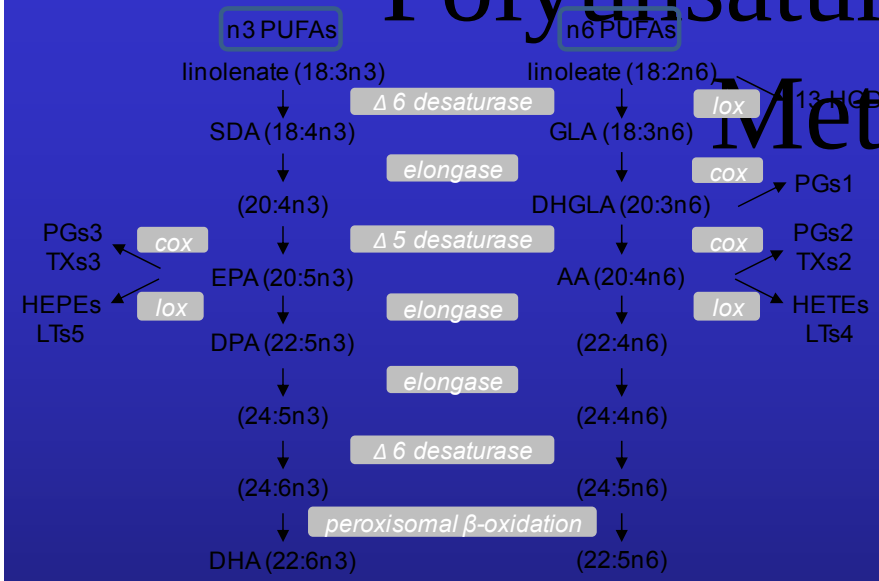


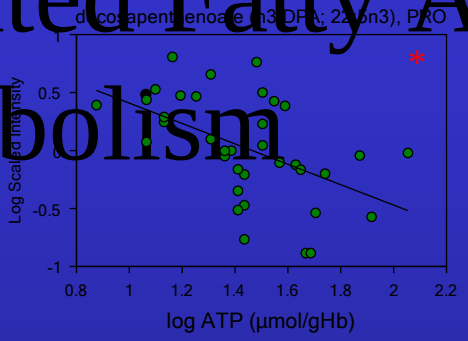


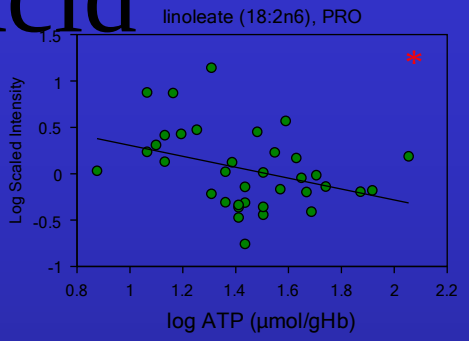


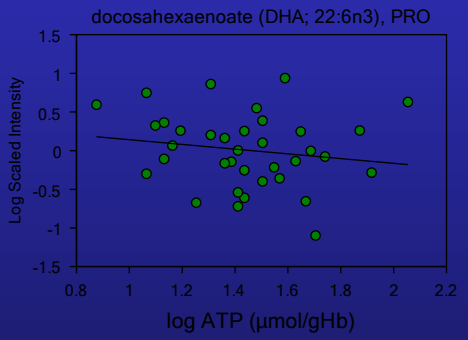
* *denotes correlation with p<0.05*

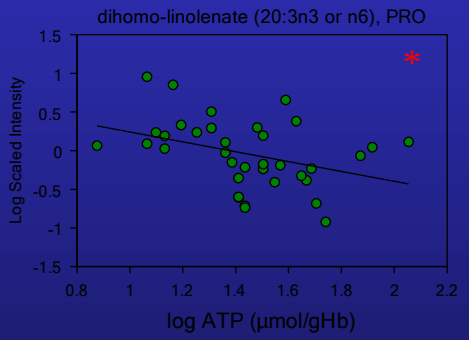
Polyunsaturated Fatty Acid Metabolism

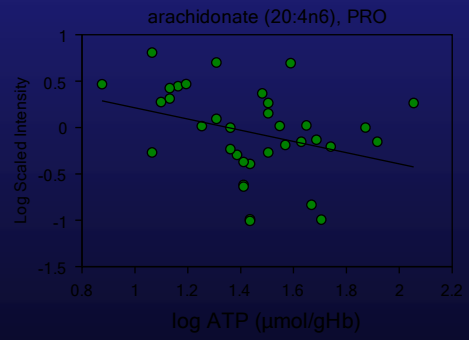












Biochemical Name	Heritability		
	Hb	RBC	PRO
linoleate (18:2n6)	51.6	45.3	25.5
dihomo-linolenate (20:3n3 or n6)	60.4	56.3	44.5
arachidonate (20:4n6)	54.5	56.5	22.1
docosapentaenoate (n3 DPA; 22:5n3)	50.5	51	27.7
docosahexaenoate (DHA; 22:6n3)	-7.9	-12.3	-46.5

* *denotes correlation with p<0.05*

Benefits of Better Storage

- Longer storage
  - Reduction of outdating
  - Ease seasonal shortages
  - Aid autologous blood programs
- Improved quality of RBCs
  - More viable cells reduce requirements
  - Reduction of RBC breakdown products such as procoagulant microvesicles
  - Faster recovery of 2,3-DPG
  - Decrease incidence of transfusion-related acute lung injury (TRALI/ARDS)?


Thank you
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