

Complex Interactions Between Coagulation,  
Endothelium and Inflammation: A Pyramid  
Towards Outcome In Cardiopulmonary  
Bypass

Bruce D. Spiess, MD

Professor

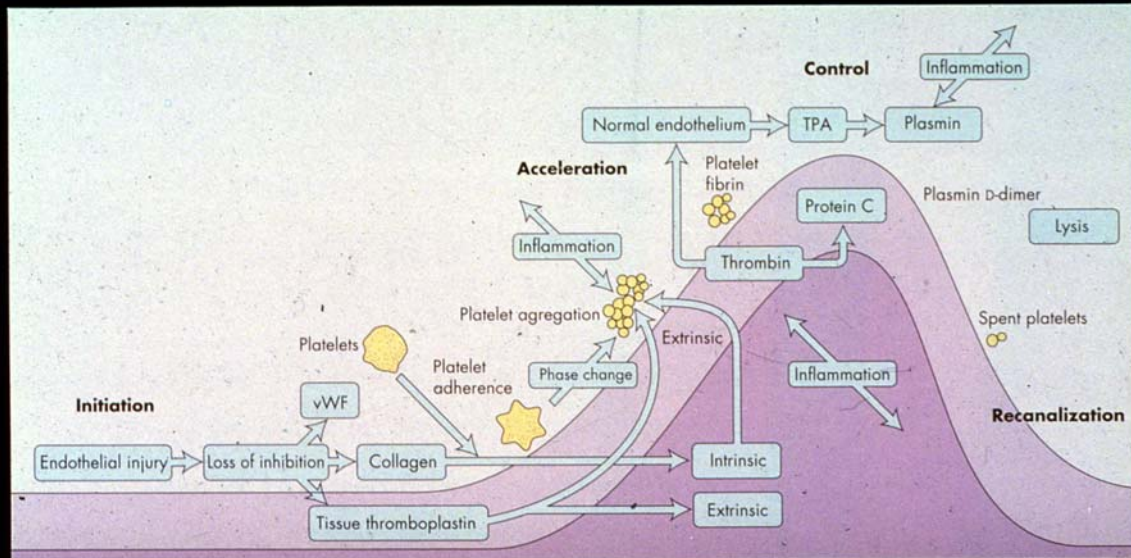
VCU/MCV

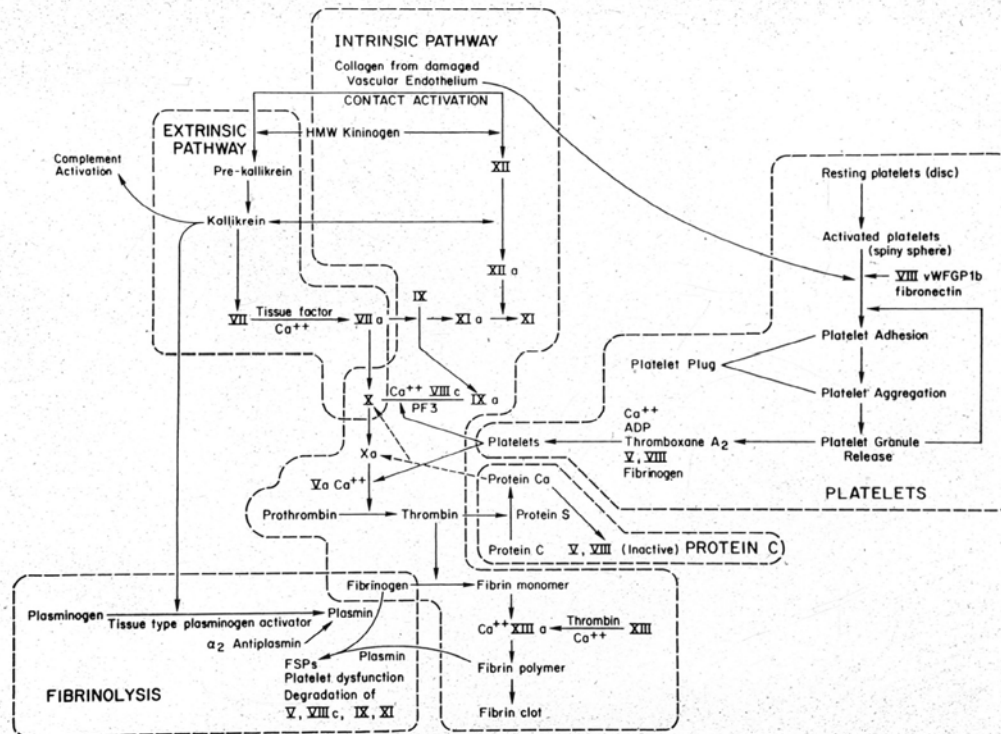
# Coagulopathy of Bypass

- Contact Activation
- Hemodilution
- Fibrinolysis
- Platelet Activation/Dysfunction
- Consumptive Coagulopathy
- Dysfunction from Transfusion
- Genetics
- Drugs

# Interactions

- Coagulation: Proteins and Platelets
- Endothelium: Healthy or Perturbated
- Inflammation: Cellular / Humoral





# The Insult of Cardiopulmonary Bypass

**Contact of Blood with the Foreign Surface of the Bypass Circuit May Activate :**



- **Kinin Generation**
- **Neutrophils**
- **Complement System**
- **Coagulation System & Platelets**
- **Fibrinolytic System**

# Homeostasis/Buffering

- Multiple Arrows=Buffering
- CPB overrides buffering capabilities
- Adverse Outcomes
  - Bleeding
  - MI
  - Stroke
  - Death





# Bypass-Induced Brain Edema



Pre-Op



Post-Op

# Endothelium

- Active Cellular Metabolism- maintenance of Homeostasis
- Heparan/Proteoglycan
- PgE
- NO
- TPA
- Protein C/S

# Endothelium Perturbed

- Ischemia Reperfusion Injury
- NfKB-intracellular upregulation
- Prothrombotic-Tissue Factor
- Loss of inhibition
- Platelet and White cell adhesion/cross talk

# Potassium

- High Dose Potassium causes endothelial dysfunction for several hours after infusion.
- A probe denudes endothelium
- Ischemia Reperfusion injury

# Heparin

- Universally Utilized in CPB
- More than just an anticoagulant
- May set up a number of the dysfunctions of CPB

# Heparin's Natural Actions

- Present in mast cells-why?
- Not found in plasma or on endothelium.
- Heparan- glycosaminoglycan different from heparin.
- Heparan- bound to the protein surface of endothelium.
- Heparan- scavenged by Platelet Factor-4 (PF4)

# Heparin manufacturing

- Combine 5,000 lbs. intestines, 200 gallons water, 10 gallons chloroform, and 5 gallons toluene. Hold at 90°F for 17 hours.
- Add 30 gallons acetic acid, 35 gallons ammonia, sodium hydroxide to adjust pH, and 235 gallons water. Bring to a boil; then filter.
- Add 200 gallons hot water to filtrate and allow to stand overnight, then skim off the fat.
- Keep pancreatic extract at 100°F for three days, then bring to boil.
- Filter solids and assay for heparin content.



# Little Known Heparin Facts

- Causes Release from Endothelium of Tissue Factor Pathway Inhibitor (TFPI)-cause of post-operative bleeding
- Heparin causes expression of GPIIb/IIIa

# Heparin-Protamine

- Protamine-a bad actor
- More heparin means more protamine
- Heparin-protamine complex
- Margination of platelets-thromboxane release.
- Acute drop of platelet count- perhaps up to 90%
- If you did not have to give heparin you would not have to give protamine!

# TFPI

- Endothelial buffer for thrombin
- Protein manufactured by endothelial cells and secreted into the glycoprotein coating.
- Released by exogenous heparin administration.
- Heparin is utilized in vivo to stimulate TFPI release.

# Heparin and Platelets

- Trace amounts trigger platelet activation
- Expression of PF4
- Expression of Gp Ib
- Expression of Gp IIb/IIIa 1000 receptors to 100,000 per cell
- Schneider et al. *Circulation* 96:2877-2883,1997
- Wahba et al: *Eur J Cardiothorac Surg* 10:768-773,1996.

# HITT

- PF4 antibody
- platelet destruction by IgG
- New tests: ELISA assays (several) and C14 Serotonin releasing assay (Gold standard???)
- 1% of patients for CPB ? Maybe more
- White Clot Syndrome- emboli, renal failure, PE , DIC, Stroke, MI , Death (50%).  
Respect this killer!!

# Antithrombin III

- Circulating Serpin ( Serine Protease Inhibitor)
- Antitrypsin, Anti-Plasmin, Anti-complement, Anti -IXa, AntiXa
- Interaction with Heparin- increases Anti-thrombin activity 1000-100,000 fold
- Heparan Interaction

# AT-III Levels in CPB

- Normal Levels- 80-100% activity
- Mean decrease of 20-35% with routine CPB
- Some patients decrease by 60-70%

# AT-III in CPB

- Hashimoto et al. Heparin and AT III levels during CPB. Correlation with sub-clinical coagulation. Ann Thorac Surg 1994;58:799-805.
- Peds and adult hearts. Some patients had ATIII added back

# AT-III and DIC

- Blanhut B et al. Substitution of AT-III in shock and DIC: A Randomized Study. *Thromb Res* 1985;39:81-89.
- Fourier F et al. Double blind, placebo-controlled trial of AT III concentrate in septic shock with disseminated intravascular coagulation. *Chest* 1993;104:882-888.

# Platelets

- Active Cells ( no DNA)
- GP Ib (VWF site)
- GPIIb/IIIa (fibrinogen, ? Heparin, thrombin)
- Protein Coagulation Interactions

# GPIIb/IIIa

- Most prolific cellular ligand in the body
- 1000 on surface of resting platelet
- 100,000 on surface of activated platelet
- heparin forces expression- why?
- fibrinolysis

# Fibrinolysis

- Universal event in CPB ( how universal)
- Most common cause of bleeding?
- TPA versus PAI-1

# TPA

- Released from endothelium (normal)
- activates platelets
- inhibits platelets (plasmin attacks GPIIb/IIIa), ? Inactivates these sites
- Plasmin- attacks fibrinogen/fibrin
- Must overcome circulating PAI-1
- What causes TPA release-Thrombin?, Cytokines

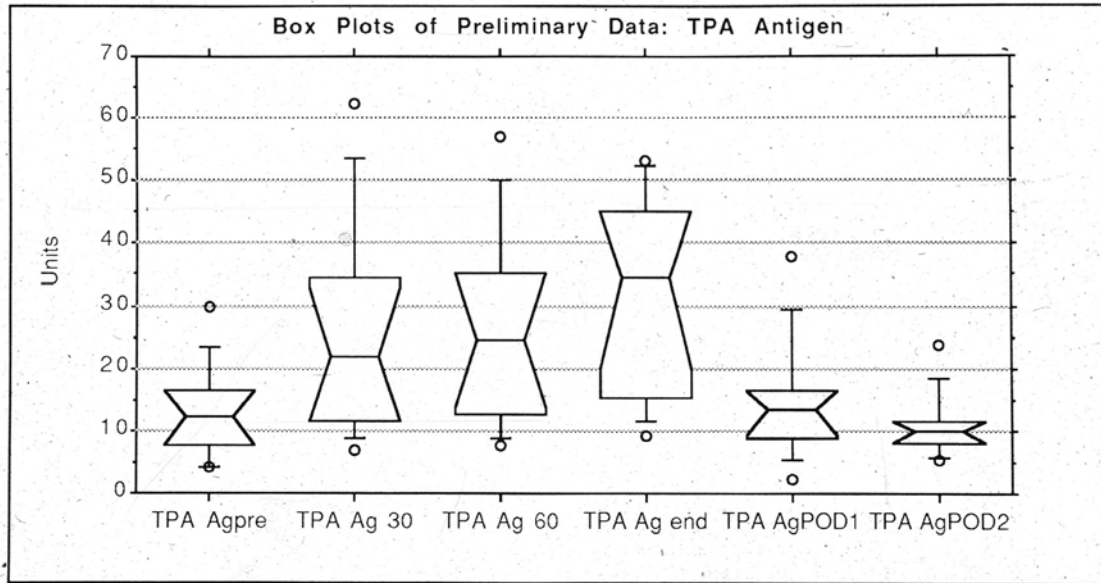
# PAI-1

- Prothrombotic, inhibits TPA
- Manufactured by liver and platelets
- Stimulated by different mechanisms than TPA

# Fibrinolysis and CPB

- Chandler, Fitch, Spiess et al. Individual Variations in the Fibrinolytic Response During and After CPB. *Thromb Haemost* 1995;74:1293-1297.
- TPA 250 fold variability
- PAI-1 500 fold variability
- Why-genetics

**Table 3. Predictive Values of Tests**



Hemoglobin (< 12g/dL)

85.7

7.1

18.8

66.7

## Gene Polymorphisms for Plasminogen Activator Inhibitor-1/Tissue Plasminogen Activator and Development of Allograft Coronary Artery Disease

- Benza RL, et al. *Circulation*. 1998;98:2248-2254.
- Recipient genotype for PAI-1 2/2 allele correlates with development of new graft coronary artery disease. It also has to do with the donor genotype.

# Tissue Plasminogen Activator and the Risk of Myocardial Infarction the Rotterdam Study

- Van der Bom, JG et al.  
Circulation.1997;95:2623-2627
- 7983 subjects studied >55 years old (MI=121)
- Genotyped all subjects for TPA ALU Insertion/Deletion
- Homozygous insertion 2.24 times MI rate compared to homozygous for depletion

# The Two Allele Sequences of a Common Polymorphism in the Promoter of the Plasminogen Activator Inhibitor-1 (PAI-1) Gene Respond Differently to Interleukin-1 in HepG@ Cells

- Dawson SJ. Et al. J Biol Chem 1993, 15:10739-10745.
- TNF, interleukin-1 regulates PAI-1 release.
- NFkB regulates mRNA for PAI-1 expression
- certain insertion/depletion alleles ( del allele) have more PAI-1 and more MI

# Gene-Environment Interaction in the Determination of Levels of Haemostatic Variables Involved in Thrombosis and Fibrinolysis

- Humphries SE. Et al. Thrombosis and Haemostasis 1997;78:457-461
- molecular transcription for messages to manufacture beta fibrinogen and PAI-1 are cytokine mediated

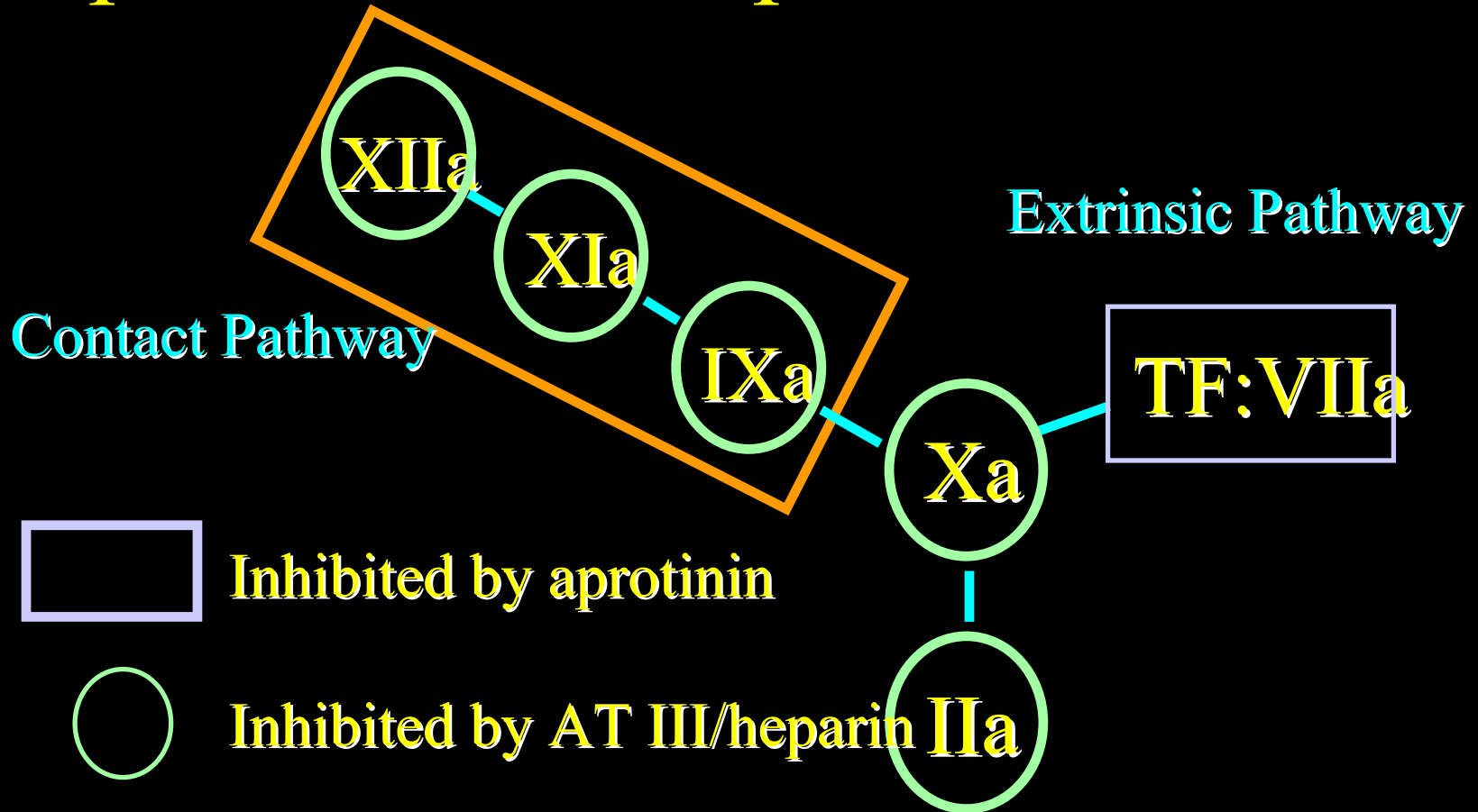
# The Increase of Plasminogen Activator Inhibitor Activity is Associated with graft Occlusion in Patients Undergoing Aorto-Coronary Bypass Surgery

- Rifon J. et al. Brit J Haem 1997;99:262-267.
- 23% patients had graft occlusion
- Baseline resting PAI-1 activity was almost double the group without graft occlusion
- Low TPA activity in 45% with graft occlusion 8% in those without

## Plasminogen Activator Inhibitor-1 Promoter 4G/5G Genotype and Plasma Levels in Relation to a History of Myocardial Infarction in Patients Characterized by Coronary Angiography

- Ossei-Gerning N et al. *Arter thromb Vasc Biol* 1997;17:33-37.
- 4G/4G genotype has much higher levels of PAI-1
- Odds ratio for MI 2.0 with this genotype

# Aprotinin and Heparin Inhibition



# Aprotinin

## A Serine Protease Inhibitor

Binds with the human serine proteases:

**Trypsin**

**Plasmin**

**Plasma kallikrein**

**Tissue kallikrein**

**Elastase**

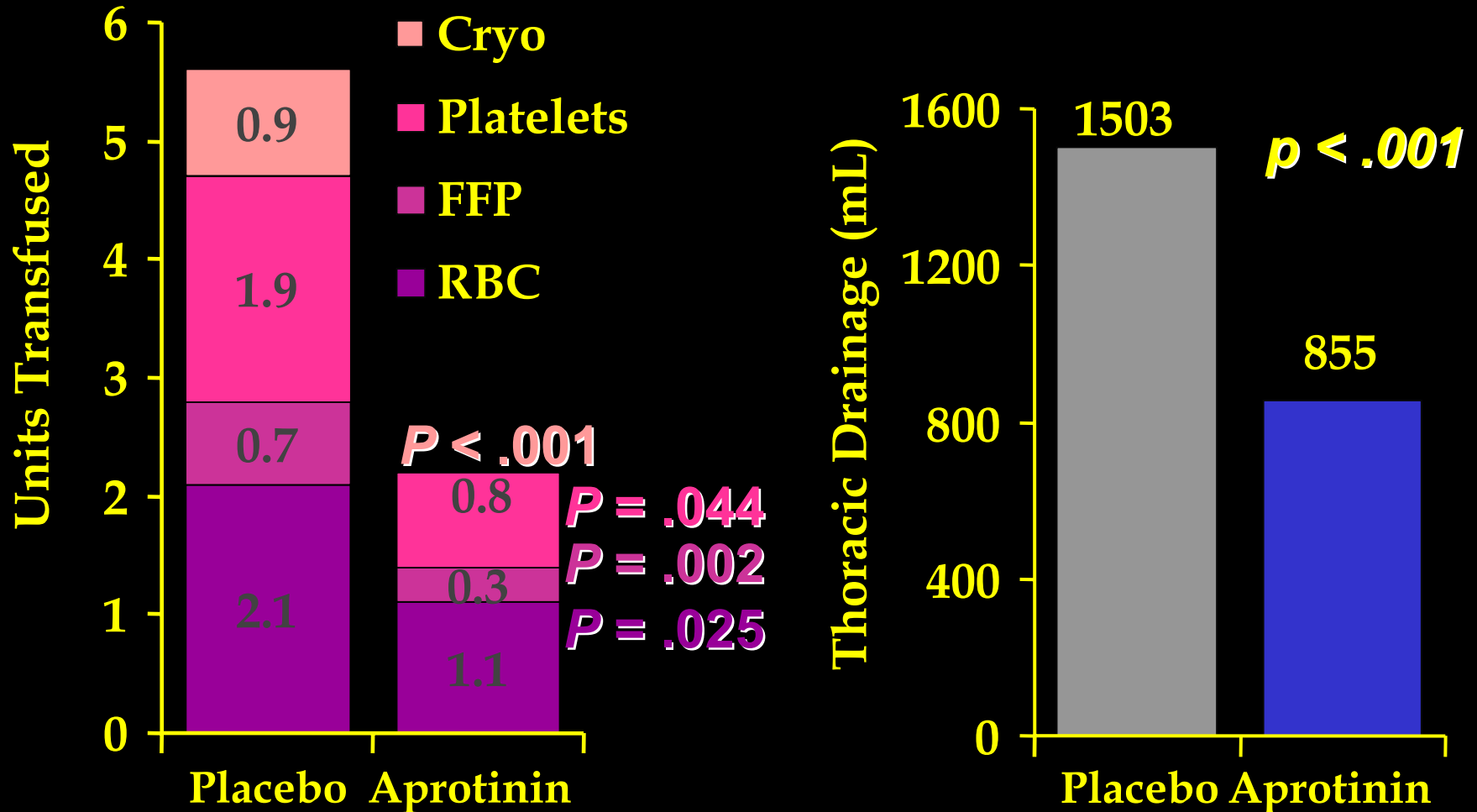
**Urokinase**

**Thrombin**

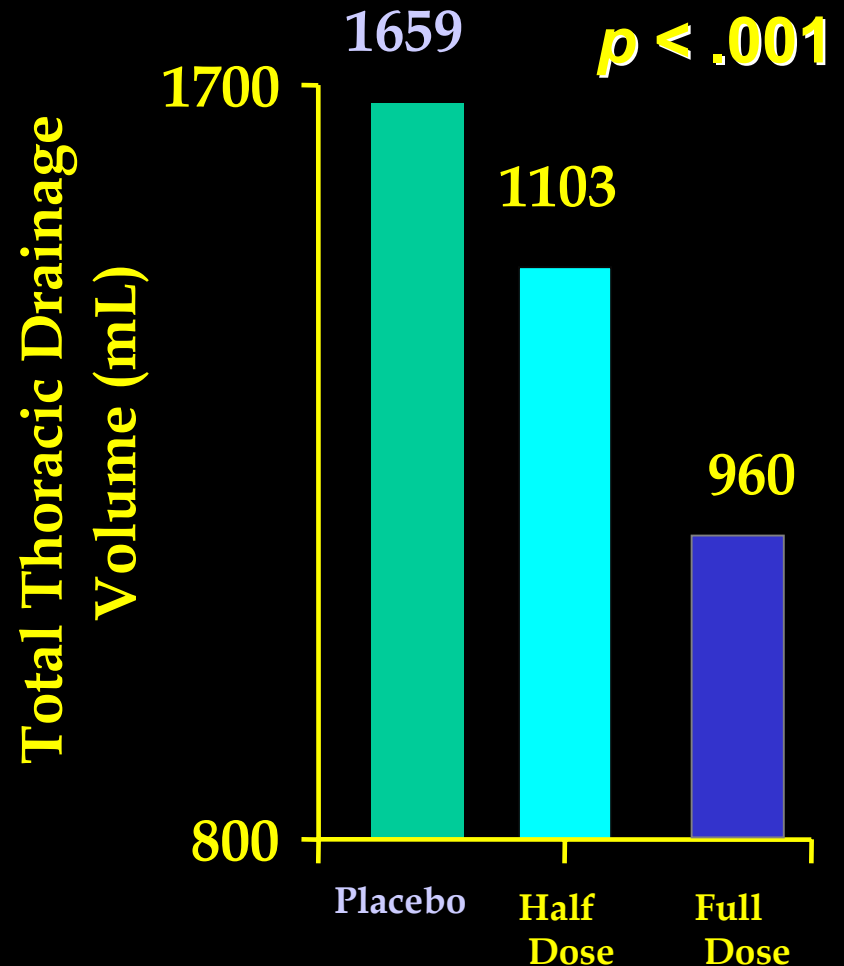
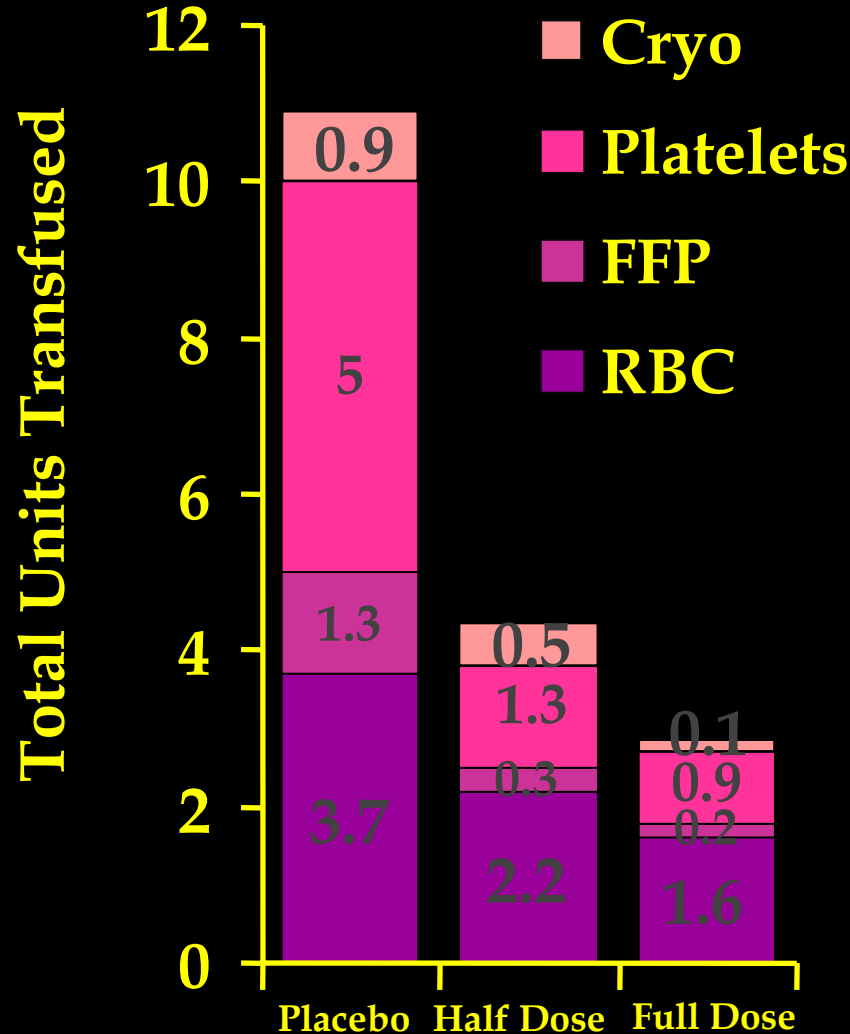
decreasing  
affinity



# Aprotinin Use in Primary CABG



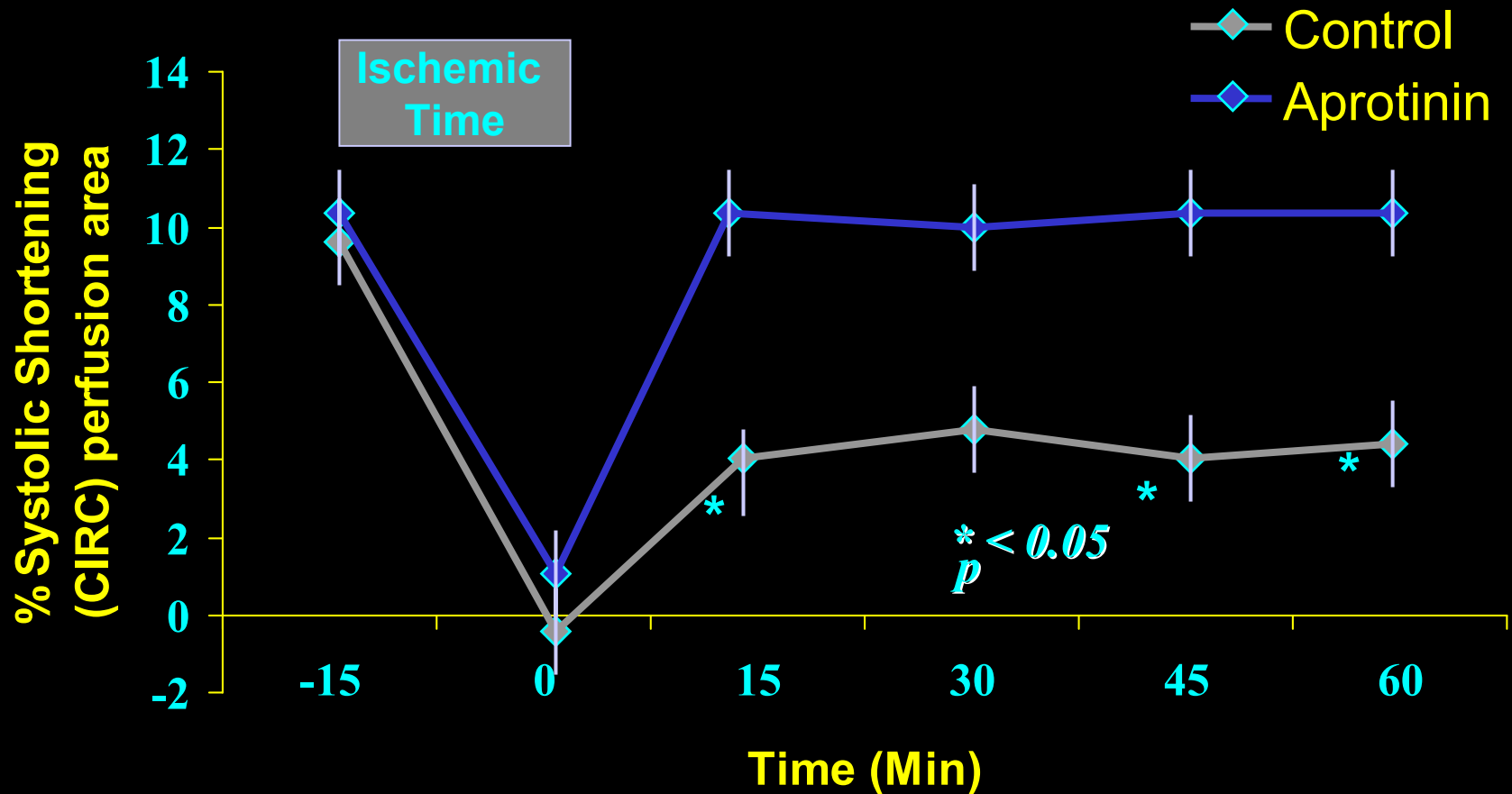
# Aprotinin Use in Repeat CABG



# Aprotinin Pretreatment Diminishes Postischemic Myocardial Contractile Dysfunction in Dogs

- Tuman K. et al. *Anesth Analg*, 1999;89:1096-1100
- Circumflex snare for 15 minutes, hemodynamics and dp/dt, end systolic myocardial length, % systolic shortening etc
- Aprotinin animals had complete return of normal fiber shortening in 15 minutes

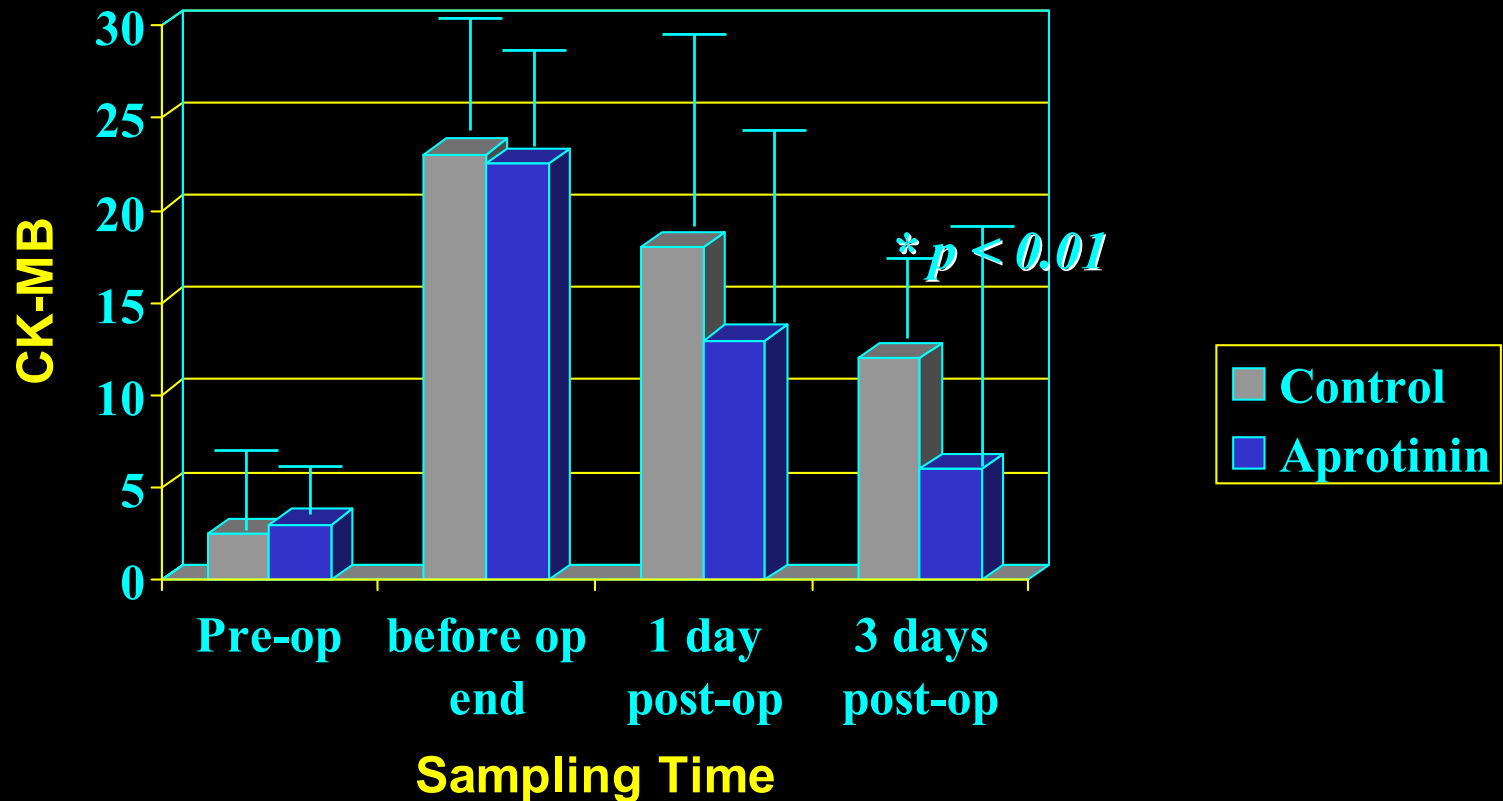
# Aprotinin Effect on Systolic Function in Ischemic Hearts



# Aprotinin but not Tranexamic Acid Inhibits Cytokine-Induced Nitric Oxide Synthase Expression

- Hill G and Robbins RA, *Anesth Analg* 1997;84:1198-202

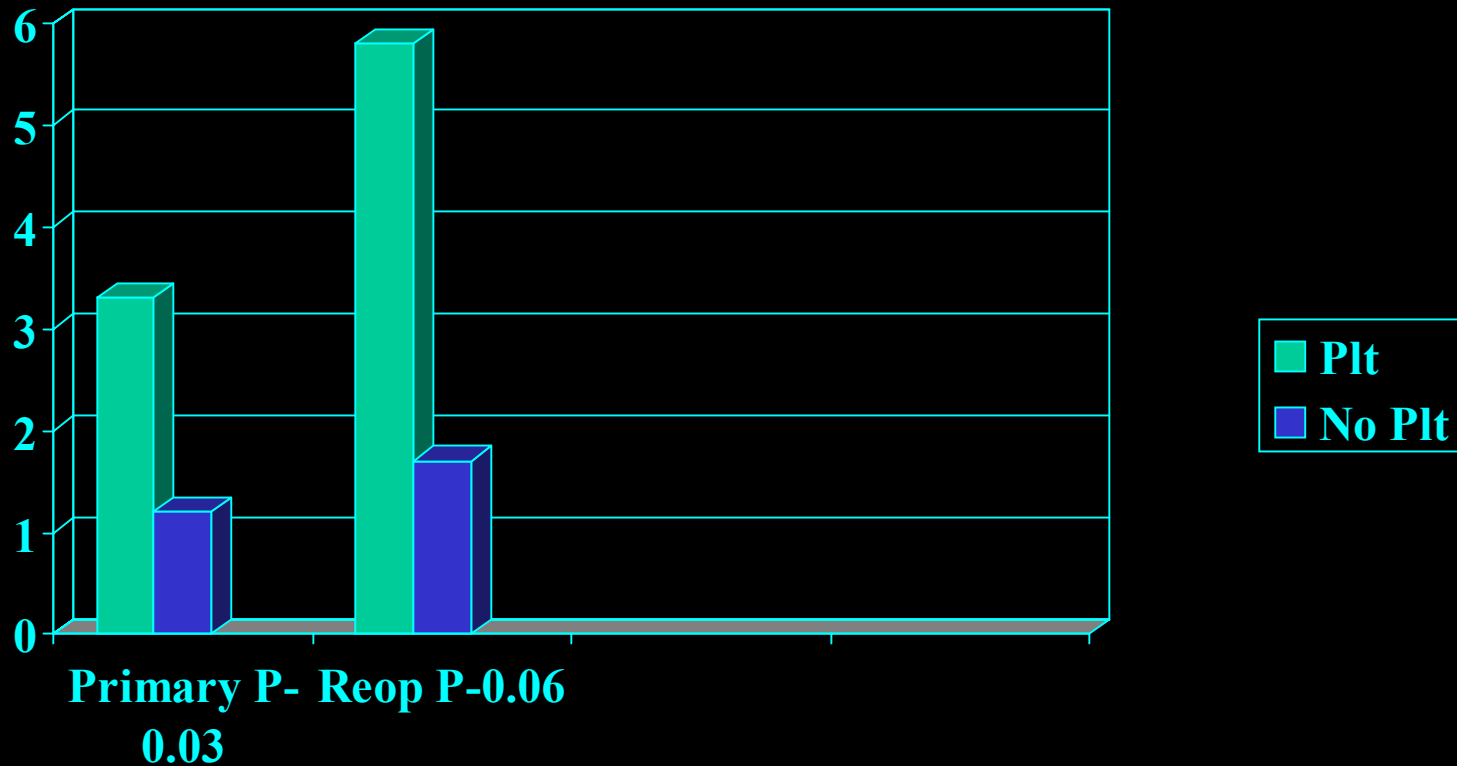
# High-Dose Aprotinin Effect on CK-MB Postoperatively



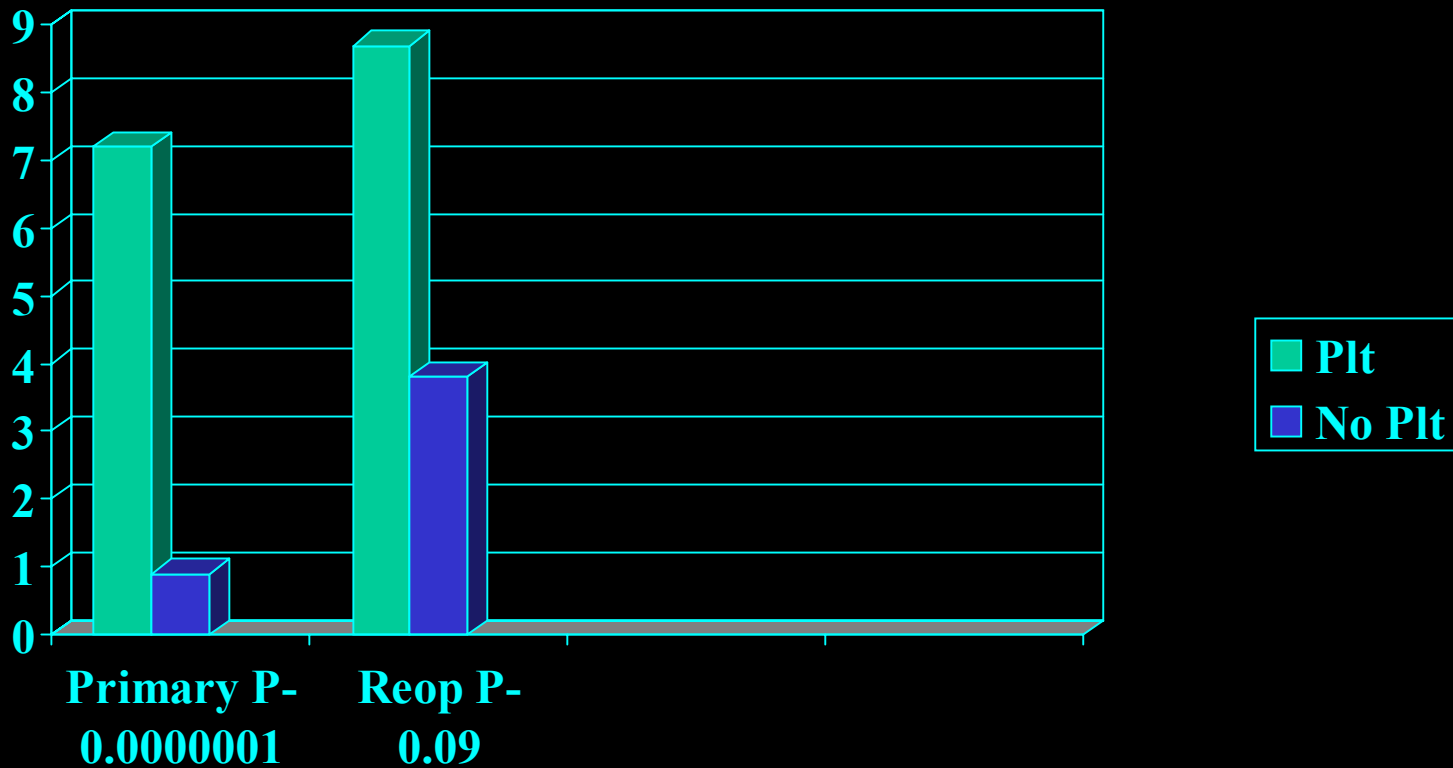
# Aprotinin Database Findings

- Royston, Dietrich, Fitch, Levy, Spiess and the Coagulation CPB Think Tank
- Stroke over 2 fold increase without A.
- Stroke 5.8 fold increase if platelets transfused
- Respiratory complications
- Infections

# Stroke In Primary and Reops



# Death in Primary and Reop



# Effects of Platelet Storage

- 1.) Storage Defect- 60% activity?
- 2.) Platelet White Cell Conjugates
- 3.) Bacterial Contamination- LPS
- 4.) Procoagulant Platelets

# Allogeneic Blood-An Inflammatory Soup

- Activated white cells
- Complement C5a-very large concentration dependent on age
- Cytokines -I16, I18
- Platelets- infected units, LPS, TNF-a

# Cytokine Generation in Stored Platelet Concentrates

- Stack G., Snyder EL. Transfusion 1994;34:20-5
- Does white cell reduction change this, cost to do this, effect on outcome?

# Activated VIIa-Why might it be usefull

- 20% of Heart Surgery Patients have “Excessive Bleeding”
- Tissue Factor is thought to drive the coagulopathy of bypass, not contact activation- cause , ischemia reperfusion injury

# TFPI

- Another Point of View on the Mechanism of Thrombin Generation During Cardiopulmonary Bypass: Role of Tissue Factor Pathway Inhibitor.
- Kojima et al JCTVA 15:60-64,2001
- TFPI does not stop thrombin generation but TFPI is a factor that we do not test for and cannot predict.
- What does it tell us about what heparin does to endothelium?

# Platelet Receptors and CPB

- PAR-1 Receptor- Thrombin transmembrane receptor down regulated by CPB? Partially preserved by aprotinin
- Gp1b receptor degraded by CPB, partially preserved by aprotinin
- ?? GPIIb/IIIa
- Where does VIIa work?

# VIIa and Bypass Rescue for Excess Bleeding

- Hendricks et al. An effective treatment of severe intractable bleeding after valve repair by one single dose of activated factor VII. *Anesth Analg* 2001;7:321-326
- Dietrich *Anesth Analg* 2002;94:1369-1370
- Hendricks *Anesth Analg* 2002;94:1370-1371.
- Sheth et al. Heart transplant in a factor VIII-deficient patient with a high titre inhibitor: perioperative management using high-dose continuous infusion factor VIII and recombinant factor VIIa. *Haemophilia* 2001;7:227-232

# Conclusions

- CPB Coagulopathy Complex
- High Degree of Variability: Multiple Polymorphisms of Population
- Monitoring Incomplete: Seat of Pants
- No Universal Therapy
- Aprotinin- Very Effective
- Platelet/Blood Transfusion Worsens Outcome
- Nova VIIa- Promising Need more research