Scloersants and Coagulation

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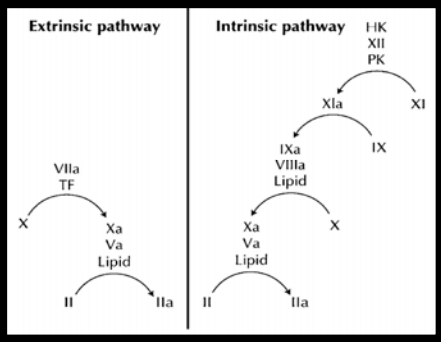
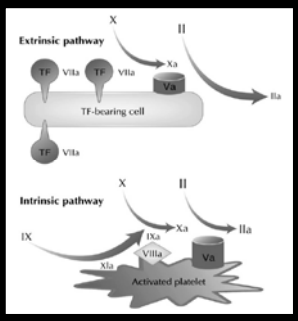
Historical view of coagulation



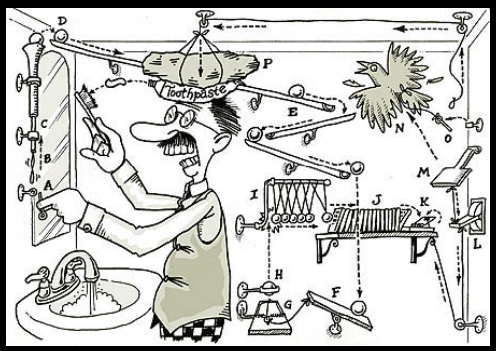
Cell Based Model

- Now recognized that cells do more than just provide a phospholipid surface
- Cells considered to direct haemostasis



Hoffman M and Monroe DM. Hematol Oncol Clin N Am 21 (2007) 1-11.

Coagulation Pathways



Basic haemostasis

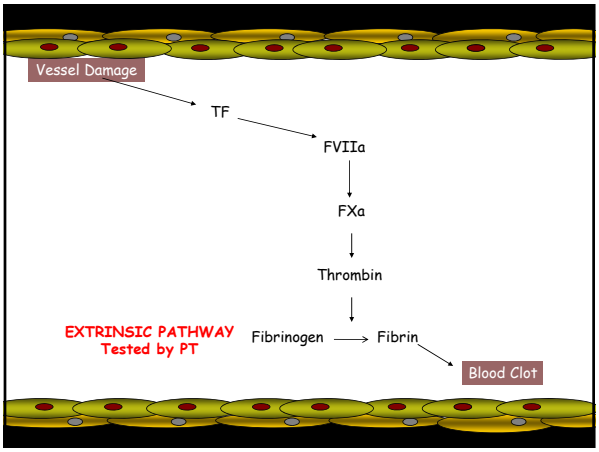
- Haemostasis = balance between 'procoagulant' and 'anticoagulant' forces
- Usually divided into 2 phases:
  - Primary haemostasis = formation of platelet plug
  - Secondary haemostasis = activation of coagulation cascade

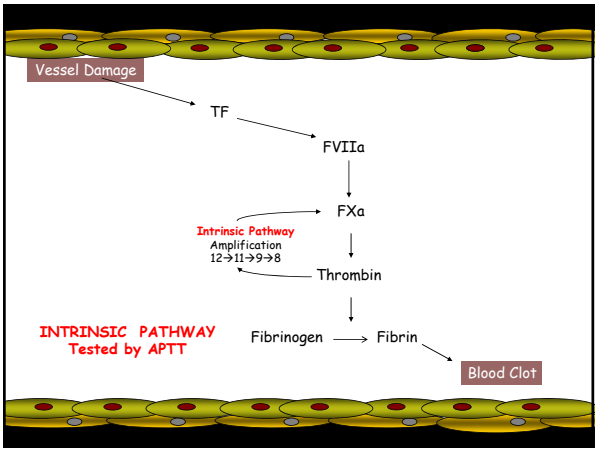
Primary haemostasis

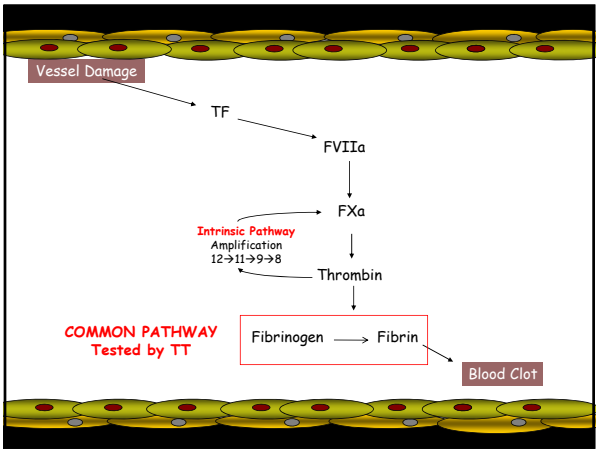
- Platelet plug formation at sites of injury
- Occurs within seconds of injury
- Important in stopping blood loss from capillaries, small arterioles, and venules

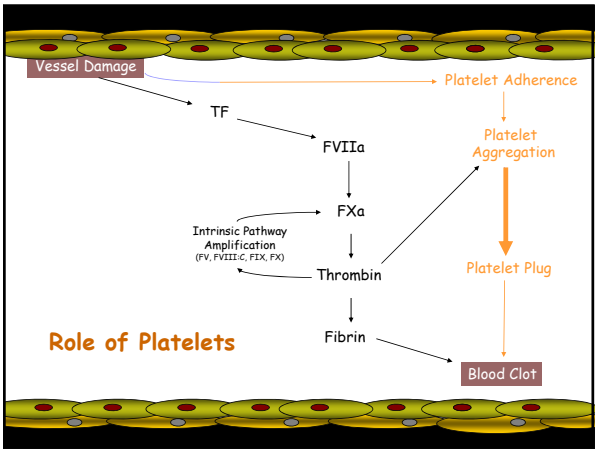
Secondary haemostasis

- Reactions of plasma coagulation system that result in fibrin formation
- Requires several minutes for completion
- Fibrin strands strengthen the primary haemostatic plug
- Important in larger vessels and
- Prevents bleeding hours or days after the injury









Sclerocoagulation

Parsi K, Exner T, Connor DE, Ma DD, Joseph JE
In Vitro Effects of Detergent Sclerosants on Coagulation, Platelets and Microparticles
Eur J Vasc Endovasc Surg. 2007;34:731-40

Sclerocoagulation Studies

- The effects of STS and POL on:
  - Clotting tests
  - Clotting factors
  - Red cell lysis
  - Platelet lysis
  - Platelet derived microparticles

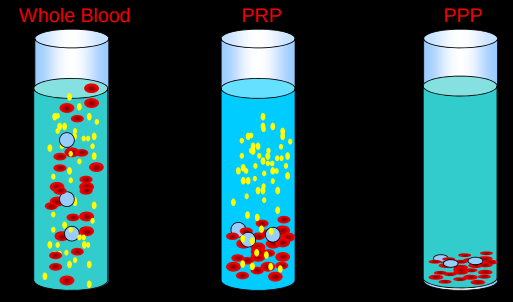
Measurement of

- Clotting times
  - Platelet-rich plasma (PRP)
  - Platelet-poor plasma (PPP)
- Haemolysis
  - Whole blood
  - Washed red cells

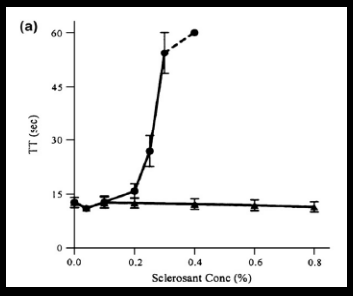
Measurement of

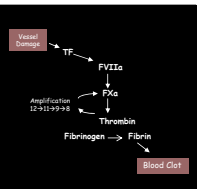
- Clotting factors
  - Factor assayed on pooled normal plasma
    - Activity at 100% initially
    - Mixed with 0.3% STS/POL
    - Duration: 5 and 30 minutes
- Platelet lysis
  - Turbidity measurements
- PMP
  - Flow cytometry

Method



Thrombin Time (TT) PPP

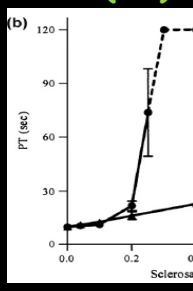


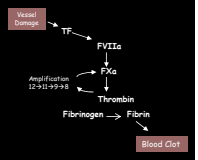


Legend:
- ● - STS
- ▲ - POL

Clotting Test	Detects
Thrombin Time (TT)	Fibrinogen and thrombin

Prothrombine Time (PT) PPP





Legend:
- ● - STS
- ▲ - POL

Clotting Test	Detects
PT	Extrinsic Pathway Fibrinogen and Factors VII, X, V, II

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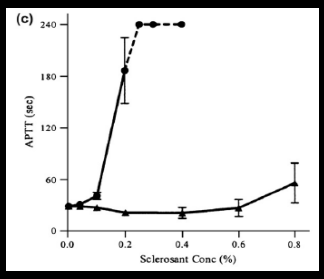
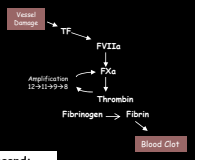
APTT PPP





Legend:
- ● - STS
- ▲ - POL

Clotting Test	Detects
APTT	Intrinsic Pathway- Fibrinogen, factors XII, XI, PKK, HMWK, IX, VIII, X, V, II

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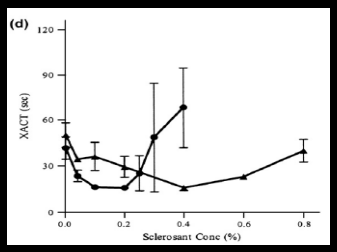
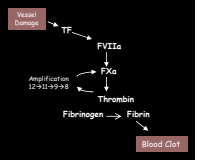
XACT PRP





Legend:
- ● - STS
- ▲ - POL

Clotting Test	Detects
XACT	Procoagulant phospholipid, factors V and II

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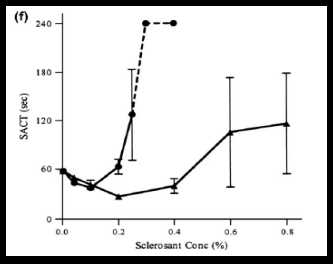
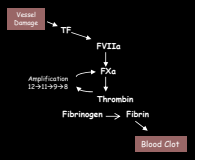
SACT PRP





Legend:
- ● - STS
- ▲ - POL

Clotting Test	Detects
SACT	Surface activated clotting time Procoagulant phospholipid

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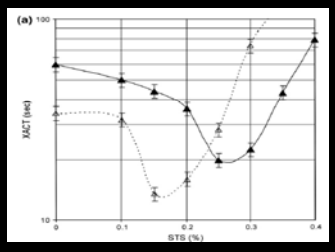
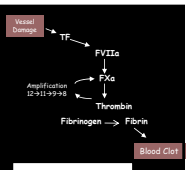
STS Effect on XACT





Legend:
- △ - STS initially
- ▲ - STS after 2.5h incubation

Clotting Test	Detects
XACT	Procoagulant phospholipid, factors V and II

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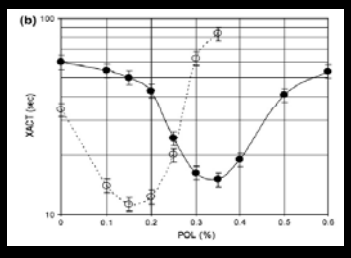
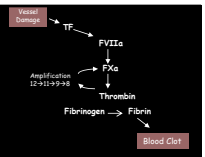
POL Effect on XACT





Legend:
- ● - POL initially
- ○ - POL after 2.5h incubation

Clotting Test	Detects
XACT	Procoagulant phospholipid, factors V and II

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Effect on Clotting Factors

Parsi K, Exner T, Connor DE, Ma DD, Joseph JE
In Vitro Effects of Detergent Sclerosants on Coagulation, Platelets and Microparticles
Eur J Vasc Endovasc Surg. 2007;34:731-40

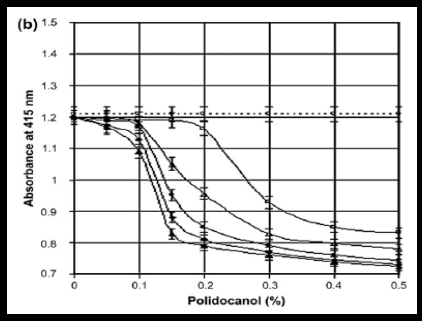
Effect of Sclerosants on Clotting Factors

FACTOR	PNP with 0.3% POL		PNP with 0.3% STS	
	T = 5 min	T = 30 min	T = 5 min	T = 30 min
II	102%	97%	96%	92%
V	70	59	54	7
X	76	75	90	20
VII	82	79	72	5
VIII	117	106	97	61
IX	111	119	88	31
XI	152	126	90	42
XII	135	135	105	107

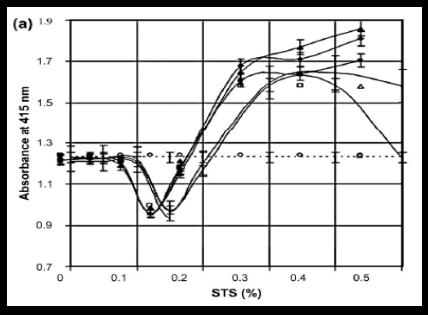
Effect on Platelets

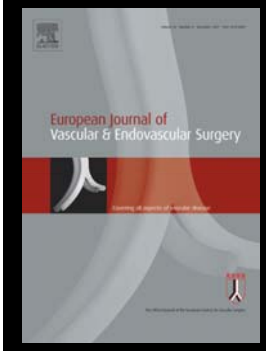
Parsi K, Exner T, Connor DE, Ma DD, Joseph JE
In Vitro Effects of Detergent Sclerosants on Coagulation, Platelets and Microparticles
Eur J Vasc Endovasc Surg. 2007;34:731-40

POL Effect of on Platelets (PRP)



STS Effect on Platelets (PRP)

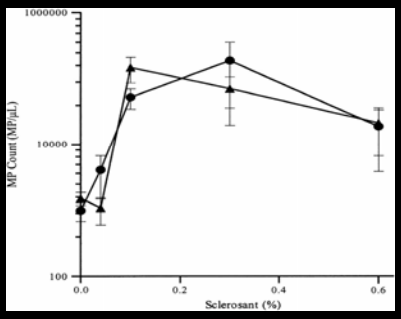




Effect on Platelet Derived Microparticles

Parsi K, Exner T, Connor DE, Ma DD, Joseph JE
In Vitro Effects of Detergent Sclerosants on Coagulation, Platelets and Microparticles
Eur J Vasc Endovasc Surg. 2007;34:731-40

Effect on PLT Microparticles



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Summary

- High Concentration (>0.6%)
  - STS prolongs all clotting times
  - Possess anticoagulant properties (STS > POL)
  - STS significantly destroyed FV and FVII
  - Lyse PLT
  - Lyse PMP
- Low Concentrations (STS 0.1-0.3%)
  - Shortened XACT and SACT
  - Procoagulant properties (POL > STS)
  - Activated PLT
  - Released PDMP

Normal Thrombus Generation

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Interaction of STS with the coagulation system

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Scleroantithrombotic

Parsi K, Exner T, Low J, Ma DD, Joseph JE
In Vitro Effects of Detergent Sclerosants on Antithrombotic Mechanisms
Eur J Vasc Endovasc Surg. 2009;38:220-228

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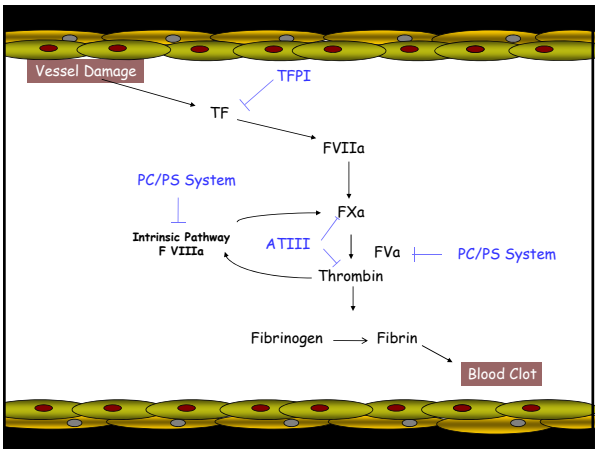
Antithrombotic Mechanisms

- Help to limit coagulation where it is not required
- Inactivate specific clotting factors such as:
  - Antithrombin (AT) – FXa and FII
  - Proteins C and S (PC, PS) – FVa and FVIII
  - Tissue factor pathway inhibitor (TFPI) - TF

Antithrombotic Mechanisms

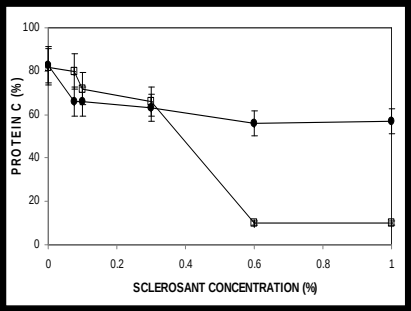
- Therapeutic anticoagulant drugs act via similar mechanisms
  - Heparin binds to AT
  - Increases its ability to inactivate factors X, thrombin
  - For heparin to work you need AT



Methods

- Freeze dried samples spiked with POL and STS
- PC and AT determined using chromogenic assays
- Free PS determined by immuno-turbidimetric method

Effect on Protein C (PC)



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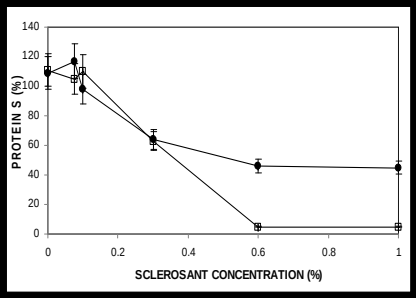
Effect on Protein S (PS)



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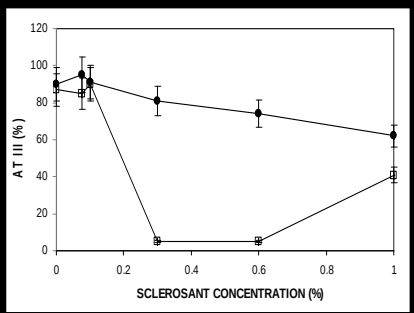
Effect on Antithrombin (AT)



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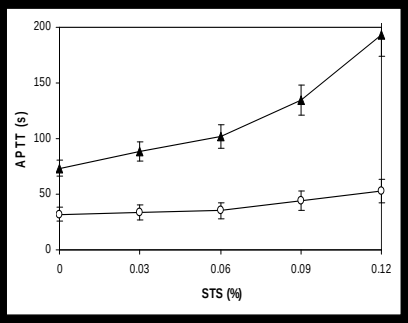
Apparent rise in AT

- Compared samples containing 1.5% STS
  - In bovine serum albumin (BSA) VS hydrolysed gelatine (no plasma)
  - BSA fully neutralises STS
- Sample containing BSA displayed no AT
- Sample containing hydrolysed gelatin produced 46% AT activity
- Rise in AT activity due to the direct effect of STS on thrombin used in this assay

Effect on APC induced anticoagulation

- Normal plasma
- POL and STS
- Tested with and without APC present in $CaCl_2$
- APTT measured

STS Effect on APC



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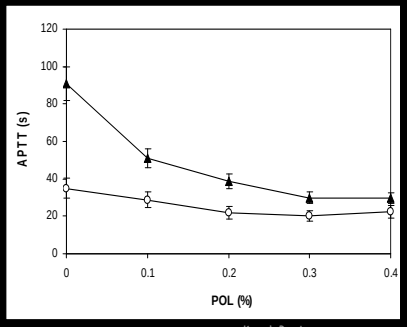
POL Effect on APC



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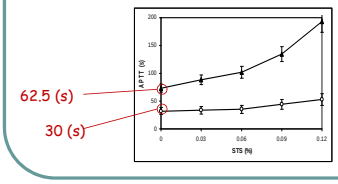
Activated PC resistance (APCR)

APC Ratio = clotting time with APC/ clotting time without APC



Activated PC resistance (APCR)

APC Ratio > 2 Normal

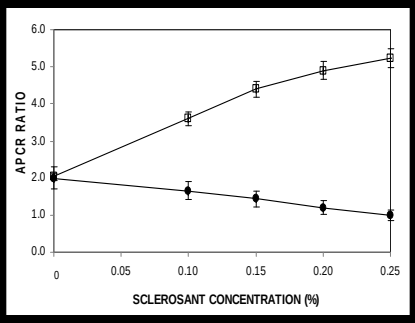
APC Ratio < 1.8 indicates abnormality

- lack of sensitivity of FV to APC or APC Resistance
- APCR
  - FV Leiden mutation or
  - other factors that reduce the sensitivity of FV to APC eg liver disease or OCP

Activated PC resistance (APCR)

- Normal and APC resistant plasma containing 0.15%-0.25% sclerosants were mixed with APC
- dRVVT-LR based assay was used
- Ratios of clotting times with APC to those without APC were derived:

Effect on APC Resistance



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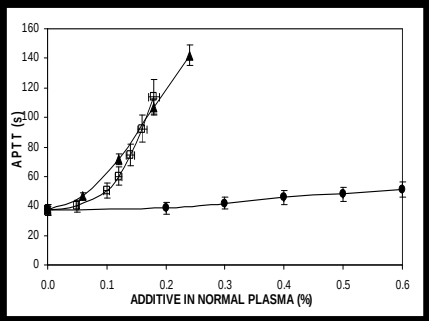
Comparison with heparin

- Sclerosants at various dilutions were added to normal plasma +/- heparin
- APTT was measured to compare the anticoagulant activity of heparin against activity of sclerosant

Comparison with heparin



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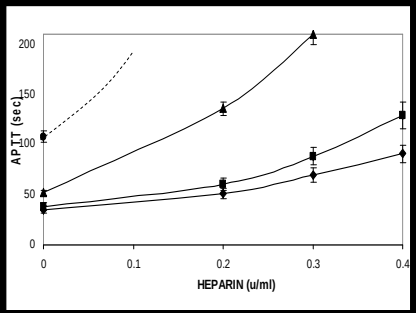
Interation with Heparin

- Sclerosants added to normal plasma with and without heparin present
- APTT measured

STS
Interaction with heparin



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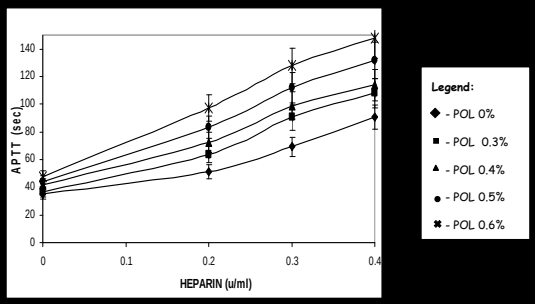
POL
Interaction with heparin



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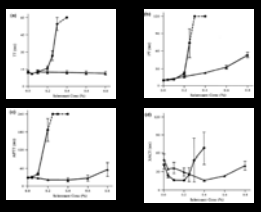
Summary STS High Cn

- **Anti-thrombotic**
  - Destroys factors V and VII
  - Potentiates the effect of APC
  - Mimicks Anti-FII and anti-FXa effect of AT
  - Directly destroys clotting factors
- Pro-thrombotic
  - Destroys PC, PS and AT

Net effect

ANTI-THROMBOTIC

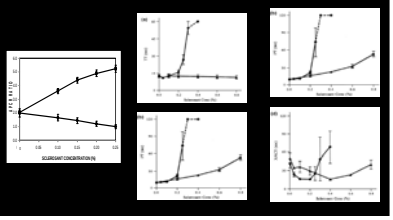


Summary POL HIGH Cn

- POL by contrast:
  - Does not demonstrate an inhibitory effect on APC
  - Increases APC resistance
  - Does not inhibit clotting factors *in vitro*

Net effect

Neutral
? =/- Pro-thrombotic

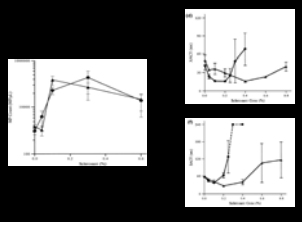


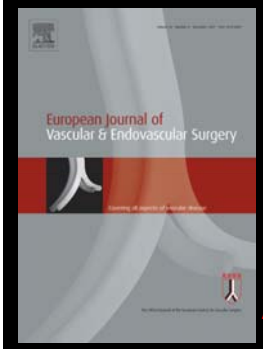
Summary STS and POL Low Cn

- **Anti-thrombotic**
  - nil
- Pro-thrombotic
  - Activate PLT
  - Release PMP
  - Shortens XACT, SACT

Net effect

PRO-THROMBOTIC





Scleroneutralization

Parsi K, Exner T, Ma DD, Connor DE, Joseph JE, Herbert A
The Lytic Effects of Detergent Sclerosants on Erythrocytes, Platelets, Endothelial Cells and Microparticles are Attenuated by Albumin and other Plasma Components *in Vitro*
Endovasc Surg. 2008;36:216-223

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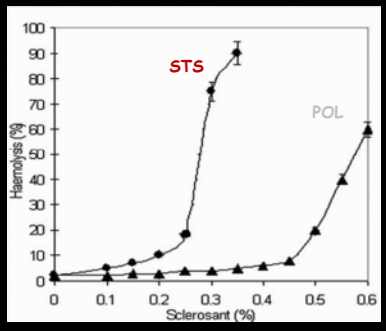
Objectives

- To investigate the *in vitro* effects and interactions of STS and POL on
  - RBC
  - Platelets
  - PMPs
  - Endothelial cells
  - Protective effects of plasma components including albumin

Haemolysis

- Sclerosants were added to
  - washed red cells
  - plasma containing sedimented blood cells
- Absorbance measured at 520nm, peak absorption for free Hb released from lysed cells

Haemolysis



NP with 50% packed red cells

● STS
▲ POL

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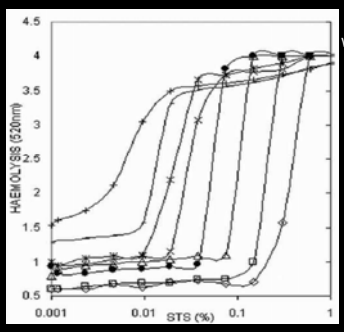
Sclerosants Neutralized by Albumin

- Mixture of Bovine Serum Albumin (BSA) and sclerosants added to washed red cells
- Absorbance measured at 405nm

STS is Neutralized by Albumin

Less albumin
More lysis

More albumin
More cells



Washed red cells

◇ BSA 8%
□ BSA 4%
△ BSA 2%
● BSA 1%
× BSA 0.5%
⋆ BSA 0.25%
- BSA 0.125%
+ BSA 0%

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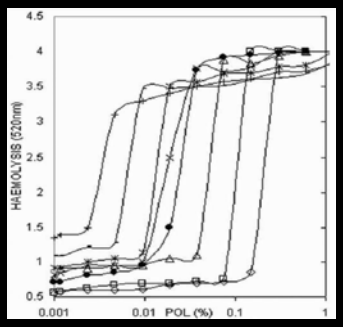
POL is Neutralized by Albumin

Less albumin
More lysis

More albumin
More cells



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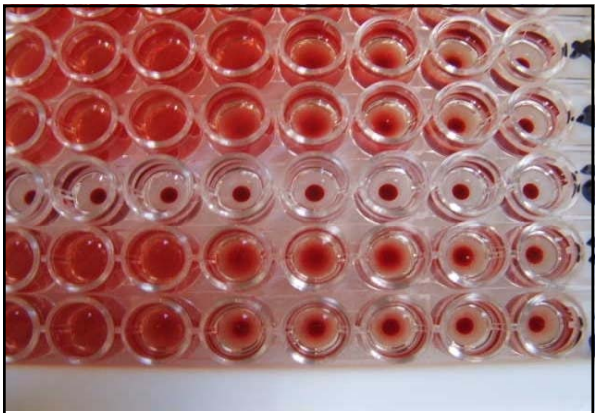


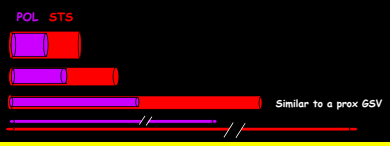
Summary

- Haemolysis in plasma was caused by
  - STS > 0.25%
  - POL > 0.45%
- Albumin neutralized haemolysis induced by the sclerosants
- Sclerosants had a similar lytic effect on platelets at high concentrations also inhibited by albumin

Take home message!

1ml of 3% STS will be neutralized by 5mls of blood
1ml of 3% POL will be neutralized by 2.5ml of blood

Diameter mm	Length cm
14	3.2
8	10
5	25.5
2	159



- Veins fill up from tributaries, deep veins and other veins, which would make these calculation meaningless!!
- Why foam is more potent than liquid !

POL is inhibited more than STS!

POL
was more active than STS in
both saline and 4% BSA

50% Heamolysis	STS (%)	POL (%)
In saline alone	0.006 (1x)	0.0035 (1x)
In saline + 4% BSA	0.2 (33.3x)	0.1 (28.6x)
In whole blood	0.3 (50x)	0.57 (163x)

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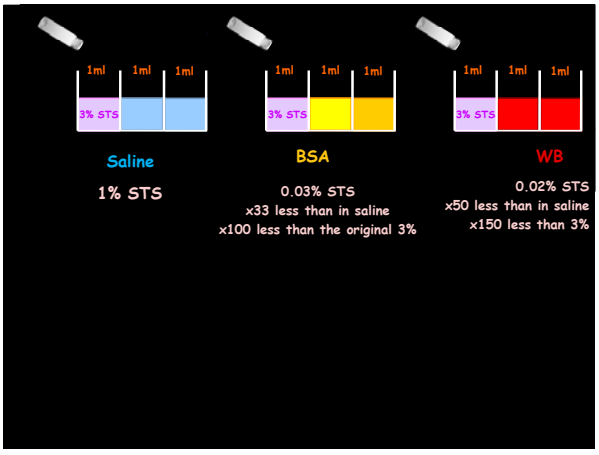
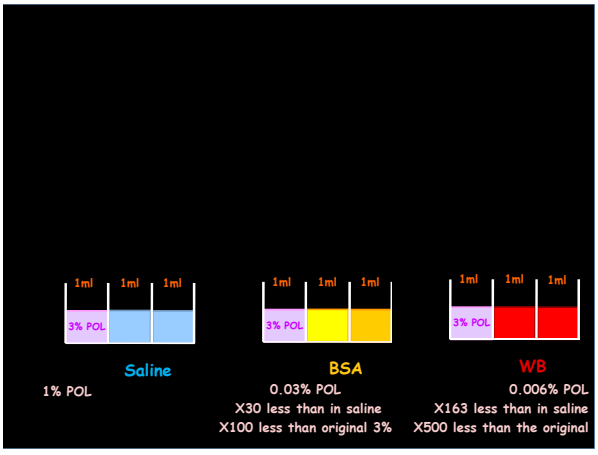
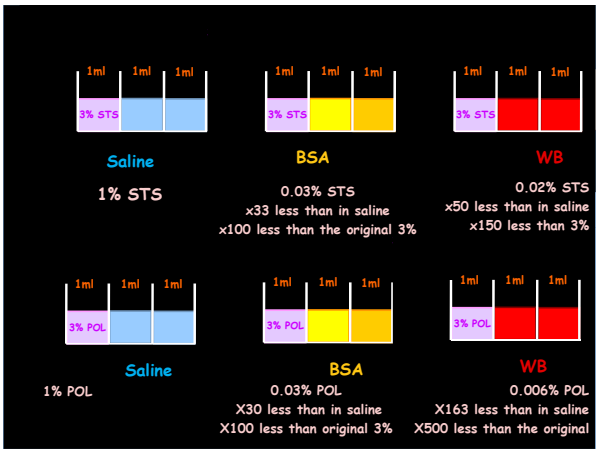


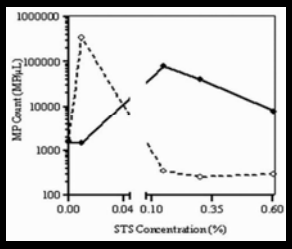




Platelet microparticle (PMP)

- PMP formation was assessed in response to sclerosants
  - In PRP
  - Washed platelet samples
- Flow cytometry performed using Annexin V and CD41a as markers

Microparticle count



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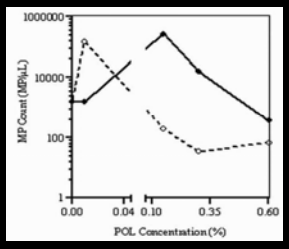
Microparticle count



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Results

- In PRP, STS and POL concentration of 0.2% induced PMP
- In washed platelets, STS and POL concentration of 0.01% induced PMP

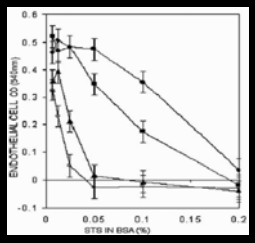
Endothelial cell lysis

- HUVECs were cultured to confluence
- Mixtures of sclerosants and BSA were added to HUVECs
- Lysis was determined by absorbance at 540nm

Endothelial cell-lysis

More albumin
More cells

Less albumin
Cell lysis



Endothelial cells

- BSA 1.5%
- BSA 0.5%
- BSA 0.17%
- BSA 0%

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Results

- POL had less lytic effects on endothelial cells
- Both plasma and BSA reduced the lytic effect of STS on the endothelial cells

Conclusion and Summary

- Sclerosants at therapeutic concentrations lyse BLOOD CELLS and ENDOTHELIAL CELLS
- Both sclerosants induce release of PMP
- Albumin significantly inhibits both sclerosants
- POL is more potent than STS if in the absence of plasma components

Conclusion and Summary

- The LESS ALBUMIN present the LESS SCLEROSANT CONCENTRATION
- Post-sclerotherapy DVT is possibly rare due to neutralization of sclerosants by albumin in deep veins

"You are completely free to carry out whatever research you want, so long as you come to these conclusions."

Interaction of detergent sclerosants with the coagulation system: an update

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