PPTA
Plasma Protein Therapeutics Association

Manufacture of Immunoglobulin Therapies

Relationship to Thrombogenicity

Albert Farrugia, VP Global Access, PPTA

Risk Mitigation Strategies to Address Procoagulant Activity in Immune Globulin Products

Universities at Shady Grove Conference Center

Rockville, Maryland

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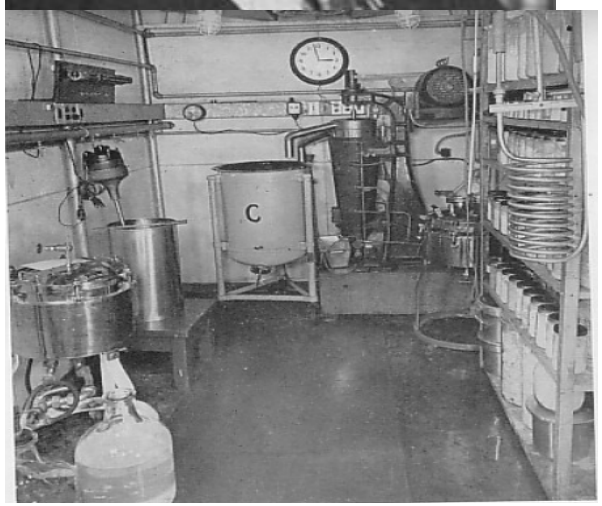
Features of presentation

- Manufacture of IgGs to basic Cohn FII
- IgGs as distinct products
- Thrombogenicity and plasma products – assessment of causality
- Conclusions

Where it all began…..



[Contribution from the Department of Physical Chemistry, Harvard Medical School]

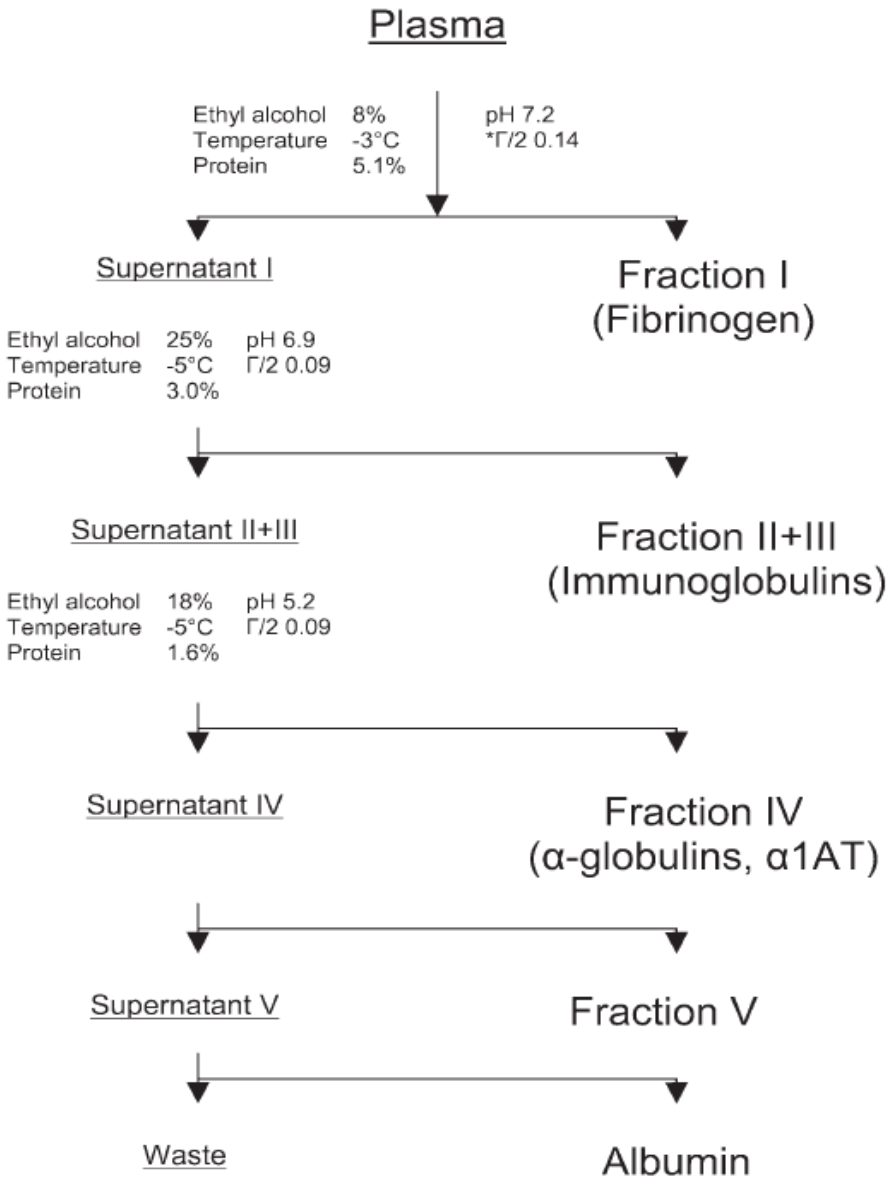
Preparation and Properties of Serum and Plasma Proteins. IV. A System for the Separation into Fractions of the Protein and Lipoprotein Components of Biological Tissues and Fluids[1a,b,c,d]

By E. J. Cohn, L. E. Strong, W. L. Hughes, Jr., D. J. Mulford, J. N. Ashworth, M. Melin and H. L. Taylor[1e]

Edwin Joseph Cohn 1892 – 1953

- Developed protein chemistry, with Edsall, Scatchard….
- Invented plasma fractionation
- Saved thousands of lives
- Never took a patent
- Insisted on right of free publication
- Twice nominated for Nobel Prize

Cohn's Method 6



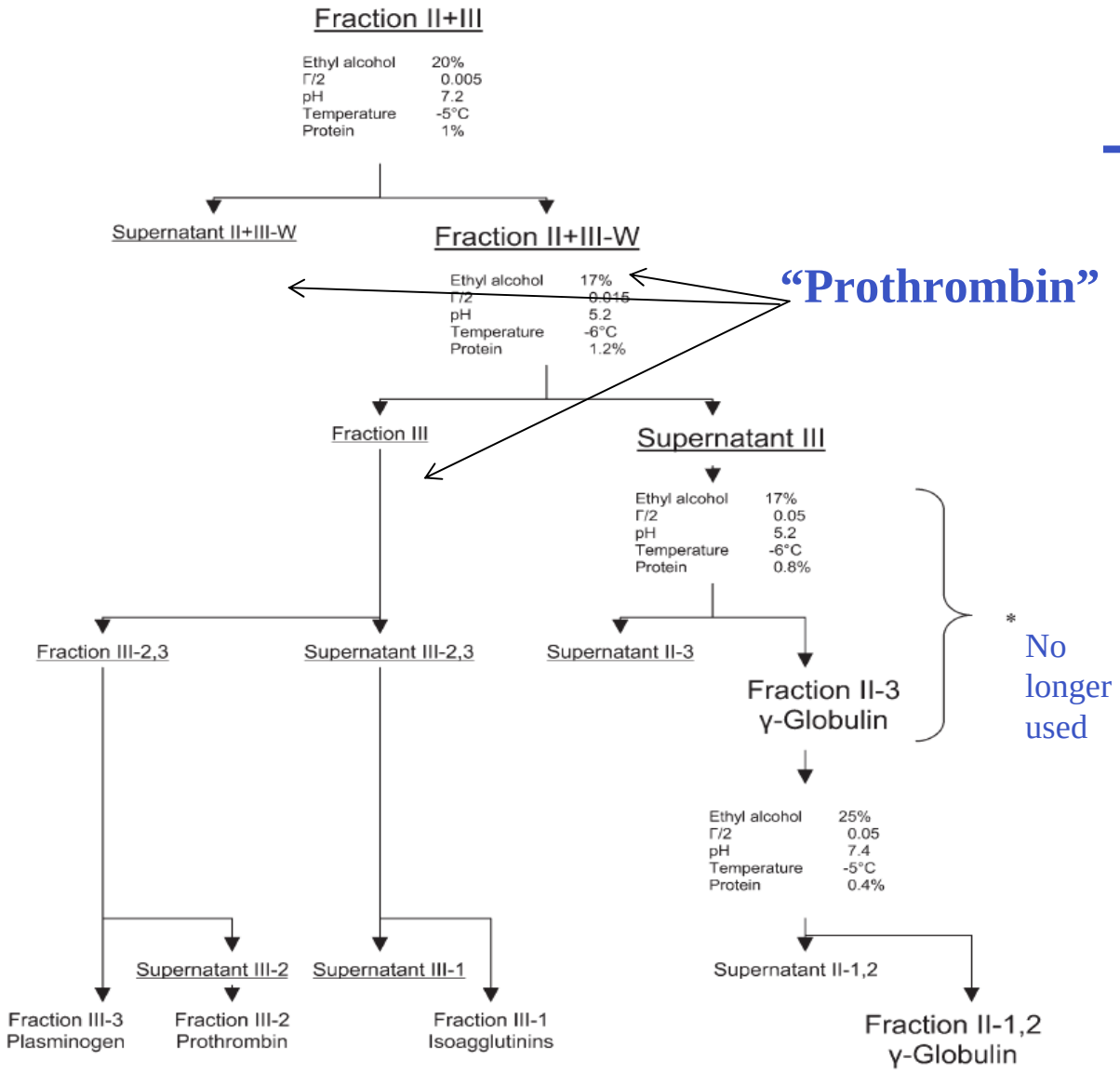
Fibrinogen, AHG, "other factors"

"Prothrombin"

NB

This preceded the discovery and characterization of all the clotting factors and the cascade eg FXI discovered in 1953

Oncley's Method 9 to access IgG



Plasma: fresh or reconstituted dry plasma, ACD – or EDTA-stabilized 5,5% protein.

8–10%* ethanol**
pH 7.2 ± 0.2
– 3° C
5% protein

centrifugation

→ precipitate: *fraction I*, containing fibrinogen (ca. 50% clottable protein) and AHG. "Small pool" process: one unit of fraction I from plasma from only two blood donations. Distributed in lyophilized form.

supernatant
globulins and
albumin

19% ethanol
pH 5.85 ± 0.05
– 5° C
4.5% protein

centrifugation

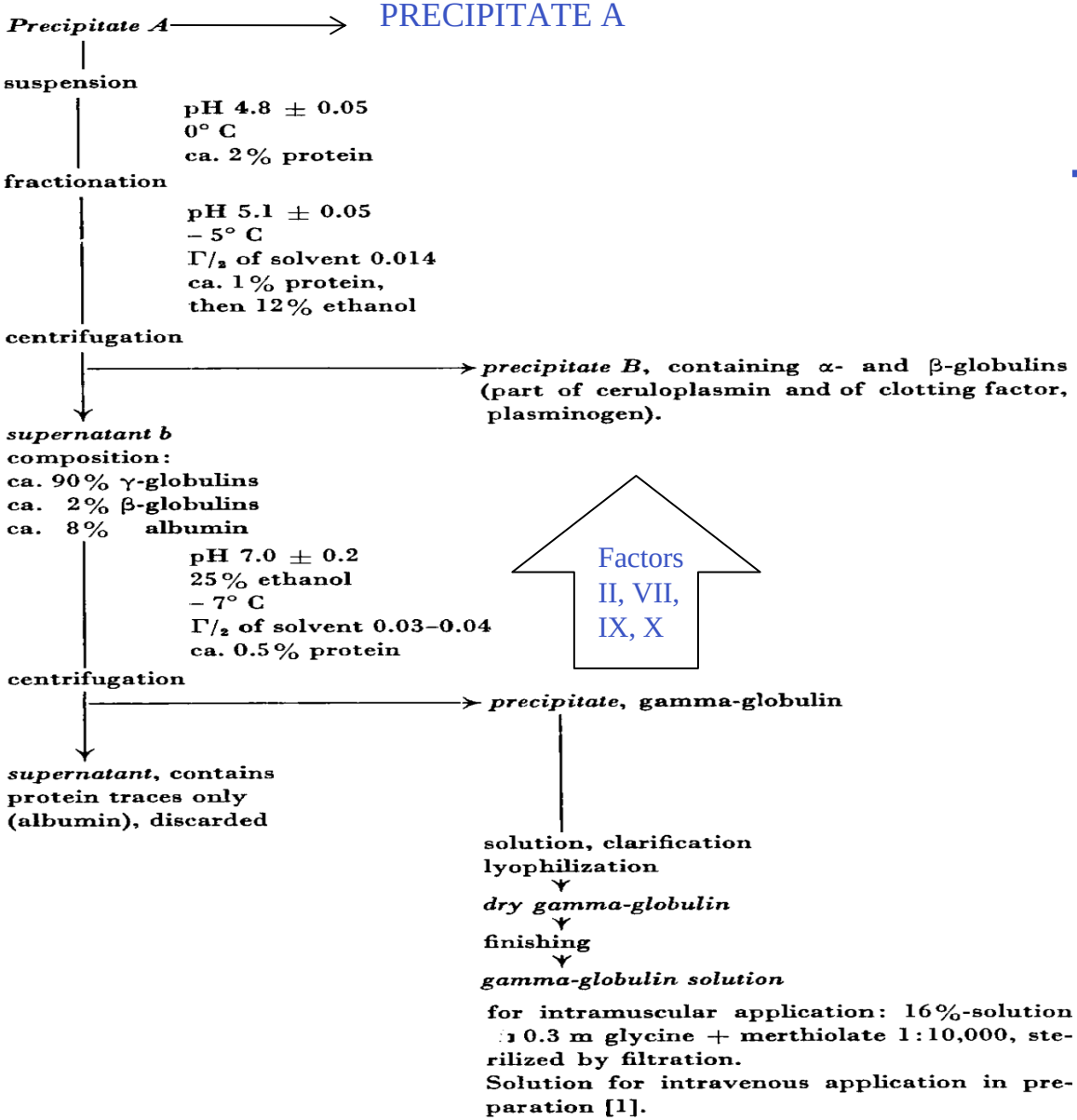
→ PRECIPITATE A

→ *precipitate A*, containing all of the gamma-globulins and part of the α- and β-globulins. (further processing see Fig. 2).

supernatant a

Kistler and Nitschmann's fractionation scheme

Kistler and Nitschmann's fractionation scheme (continued)

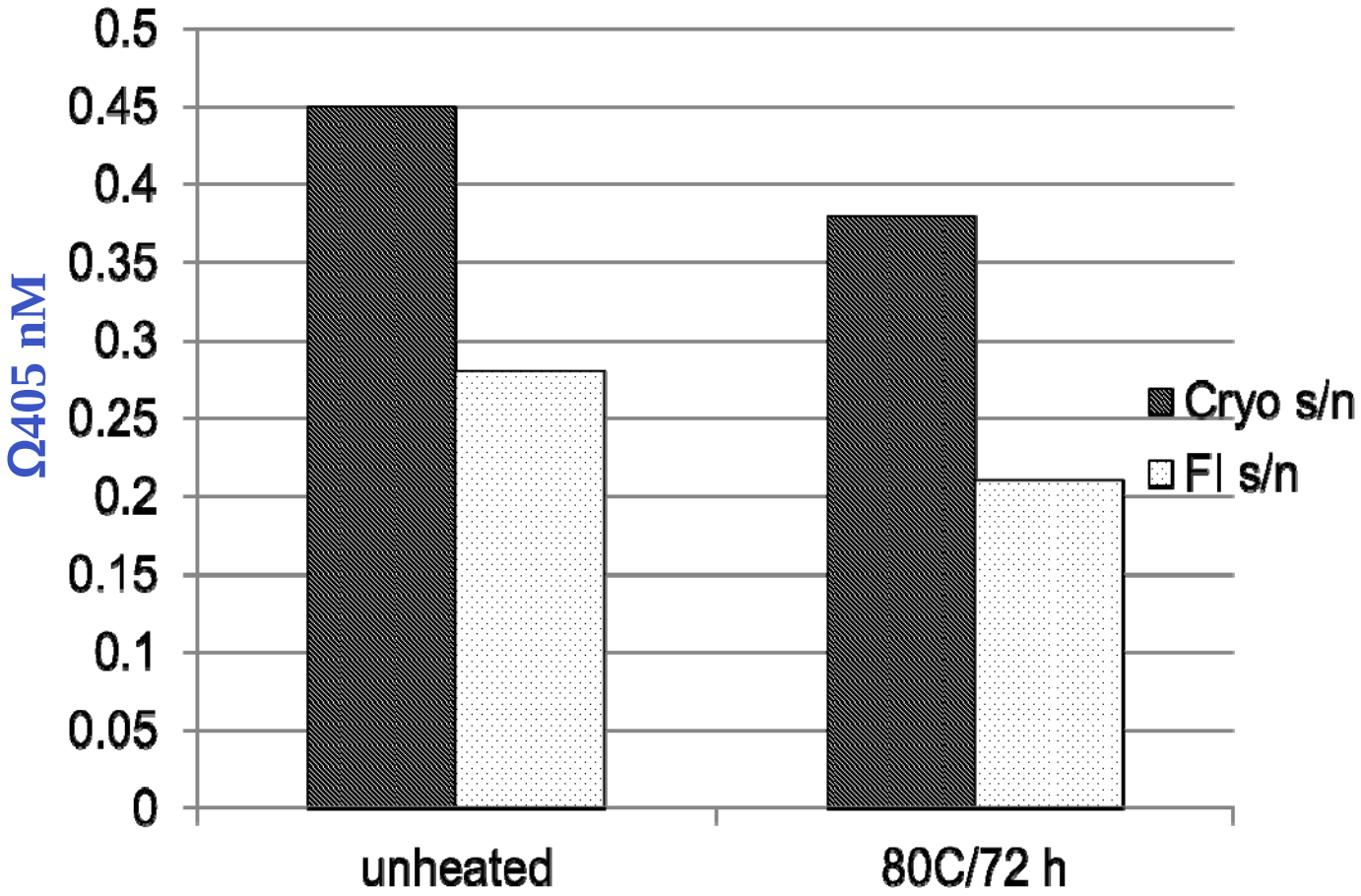


"[T]he method was aimed at obtaining yields as high as possible while achieving purity such as seemed necessary and reasonable."

"Slight deviations in ionic strength will influence yield and purity of the gamma-globulin remarkably. A rigorous standardisation of the processing is therefore indispensable."

Clues from the past – Role of F I

Thrombin generation in PCCs from different starting materials



- FI removal decreases *in vitro* thrombogenicity
- Does this have implications for Cohn fractionation ie co-precipitation of FI+II+III ?

Farrugia et al Vox Sang **1989;57:4-9**

Clues from the past – Purification of FXI

- FXI shares isoelectric properties and molecular weight characteristics with IgG
- Therefore, it is not surprising that it co-purifies with IgG in preliminary steps (JBC 252, 6432-6437, 1977)
- FXI is also absorbed from cryo s/n with heparin agarose, co-purifying with ATIII (Bolton-Maggs T&H 1992, 67 (3) 314-319)

Cohn Fractionation and coagulation factors

- Coagulation factors with the exception of fibrinogen and FVIII (?FV) partition to FII + III
- Fractionation of FII + III results in factors being precipitated into FIII away from FII
- Nevertheless, some factor content in FII must be expected
- Further removal of factors must rely on the finishing operations used to purify Ig from FII
  - IEC
  - Diafiltration
  - VI
  - Etc – Company presentations

An anonymous product

Pediatr Blood Cancer

BRIEF REPORT
Cerebral Sinus Thrombosis Following IV Immunoglobulin Therapy of Immune Thrombocytopenia Purpura

Arwa Z. Al-Riyami, MD,[1] James Lee, MD,[2] Mary Connolly, MD, BCh,[3] and Evan Shereck, MD[4]*

We present a pediatric patient treated with high dose intravenous immunoglobulin (IVIG) for acute immune thrombocytopenic purpura (ITP), who developed cerebral sinus thrombosis in the absence of any identifiable hypercoagulable state. This report describes the successful management of this rare complication in this challenging setting. This report shows IVIG induced cerebral sinus thrombosis in ITP. Pediatr Blood Cancer © 2011 Wiley-Liss, Inc.

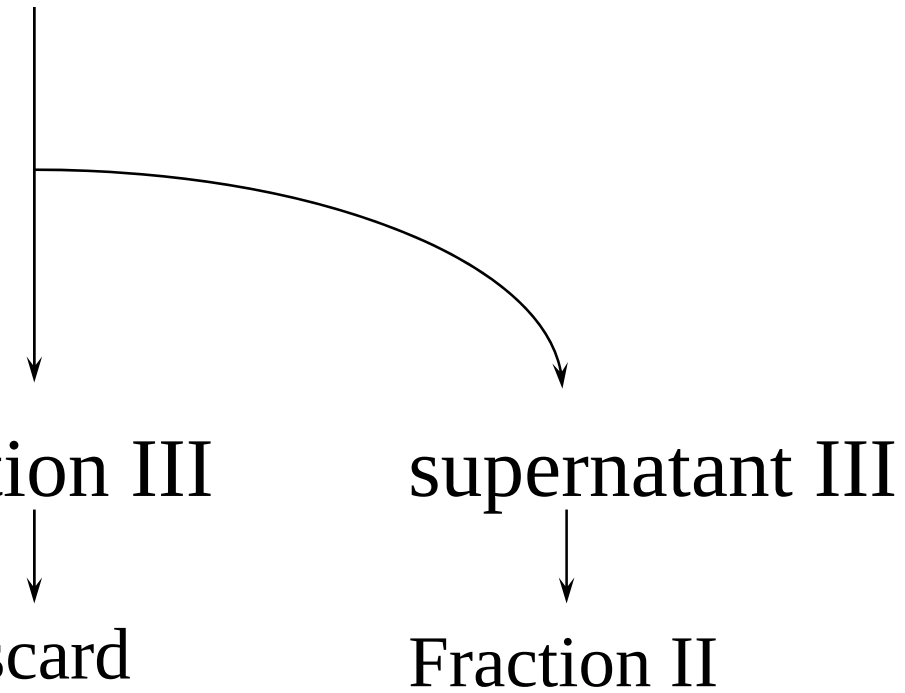
Key words: anticoagulation therapy; cerebral sinus thrombosis; immune thrombocytopenic purpura; intravenous immunoglobulin

- BUT – Are we justified in considering all Ig products as being the same?
- Igs are biologics where the product characteristics are dependent on the process of manufacture
- Individual processes vary, in ways which can affect the product

Manufacturing: “Minor” changes with “major” impact

Fraction II + IIIw precipitation

pH 5.4 - stable
pH 5.1 - fragmentation



CBER studies – Courtesy Dr D Scott

Manufacturing: “Minor” changes with “major” impact

			Stability
Plasminogen	pH 5.1-5.2 →	supernatant (Fraction II)	-
Plasminogen	pH 5.4 →	precipitate (Fraction III)	+

CBER studies – Courtesy Dr D Scott

Manufacturing: “Minor” changes with “major” impact

- PKA Incident
  - During Fraction II + III precipitation more filter aid was added and the stirring time was lengthened.
  - As a result there was increased activation of pre-kallikrein activator (PKA)
  - Elevated PKA in IGIV was associated with increased adverse events: hypotension, chest tightness and wheezing.

CBER studies – Courtesy Dr D Scott

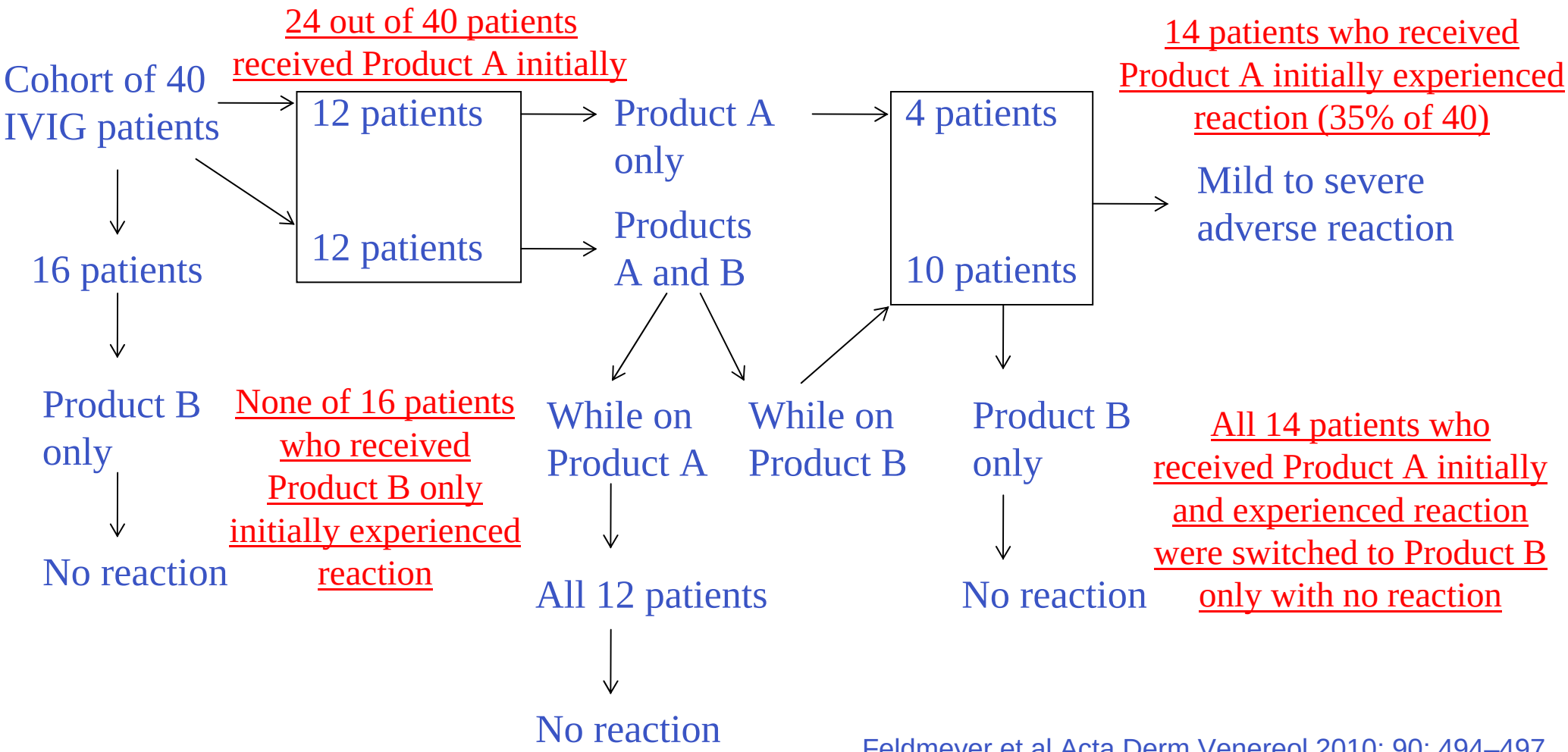
Features of Intravenous Immunoglobulin Products

Concerns for Patients with Certain Risk Factors

Risk Factor	Sodium Content	Sugar Content	Osmolality and Osmolarity	pH	IgA	Volume Load
Renal dysfunction	✓	✓	✓			✓
Heart disease	✓		✓			✓
Diabetes mellitus, prediabetes		✓				
Elderly	✓	✓	✓			✓
Neonatal, pediatric	✓		✓		✓	✓
Thromboembolic risk	✓		✓			✓
IgA deficiency				✓		

Siegel Pharmacotherapy 2005;25(11 Pt 2):78S–84S

Not All Intravenous Immunoglobulin Preparations are Equally Well Tolerated



Feldmeyer et al Acta Derm Venereol 2010; 90: 494–497

All IG products are different

IVIG in Kawasaki Disease

Factors	Product A	Product B	Product C	Product D	*P*
Fever after IVIG (days)	1 (0-11)	1 (0.5-6)	1 (0.5 -20)	1 (0.5-8)	0.69
No responsiveness	10 (11%)	2 (1%)	12 (13%)	5 (7%)	0.001
Coronary artery abnormality - acute	5 (5%)	11 (6%)	7 (8%)	6 (8%)	0.86
Coronary artery abnormality - convalescence	4 (4%)	3 (2%)	9 (10%)	1 (1%)	0.01
Giant coronary aneurysm	0 (0%)	0 (0%)	3 (3%)	0 (0%)	0.03

Thrombogenicity and causality
An old story

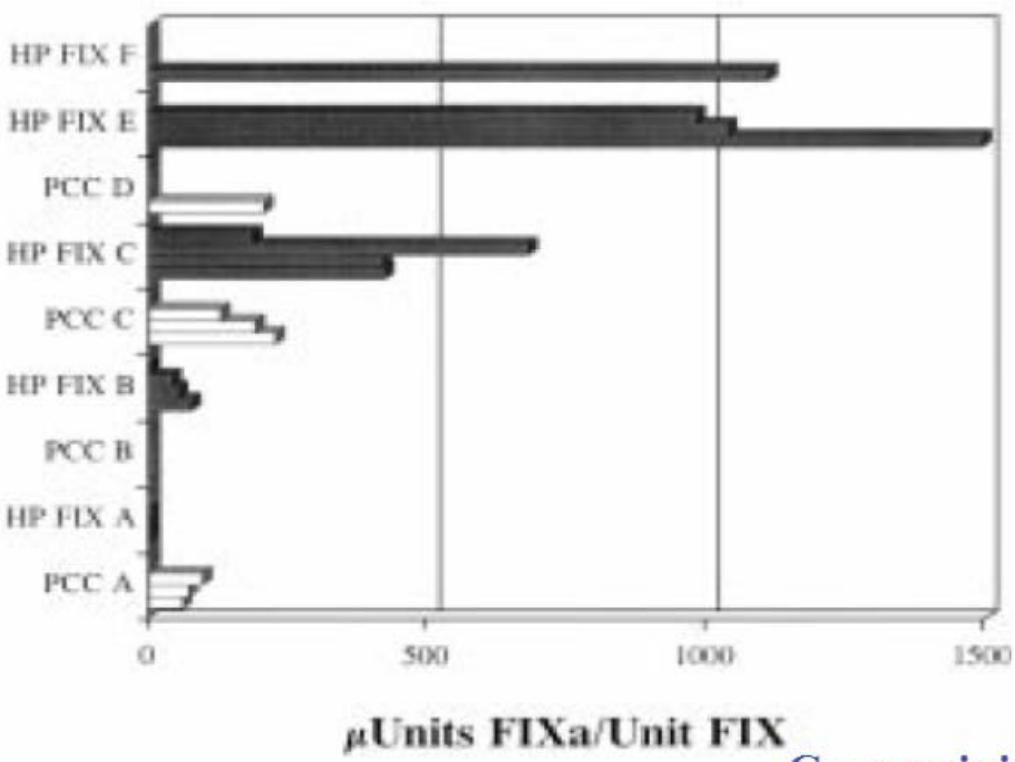
- Class effects are difficult to ascribe to products which are so different
- Some attention must be given to Virchow's triad
    a. Changes in the vessel wall
    b. Changes in the blood flow
    c. Changes in the blood constituents
- So far, attention has focused on c
- This is not justified if the "Pre-Octagam" literature is scrutinized
    a. Patient characteristics – age, mobility, other risk factors
    b. Viscosity changes, infusion rates

The example of the thrombogenicity of PCCs in hemophilia B

- PCCs (FII, (VII), IX & X) were the first treatment modality for hemophilia B
- Thrombogenicity was a well known AE following their administration
- Several tests – NAPTT, TGT_{50} – were introduced to attempt release of non-thrombogenic product
- The tests detected activated factors, but the problem persisted
- Ultimately, understanding a different mechanism – zymogen overload – ameliorated the problem

Put not your trust in tests – first understand the problem

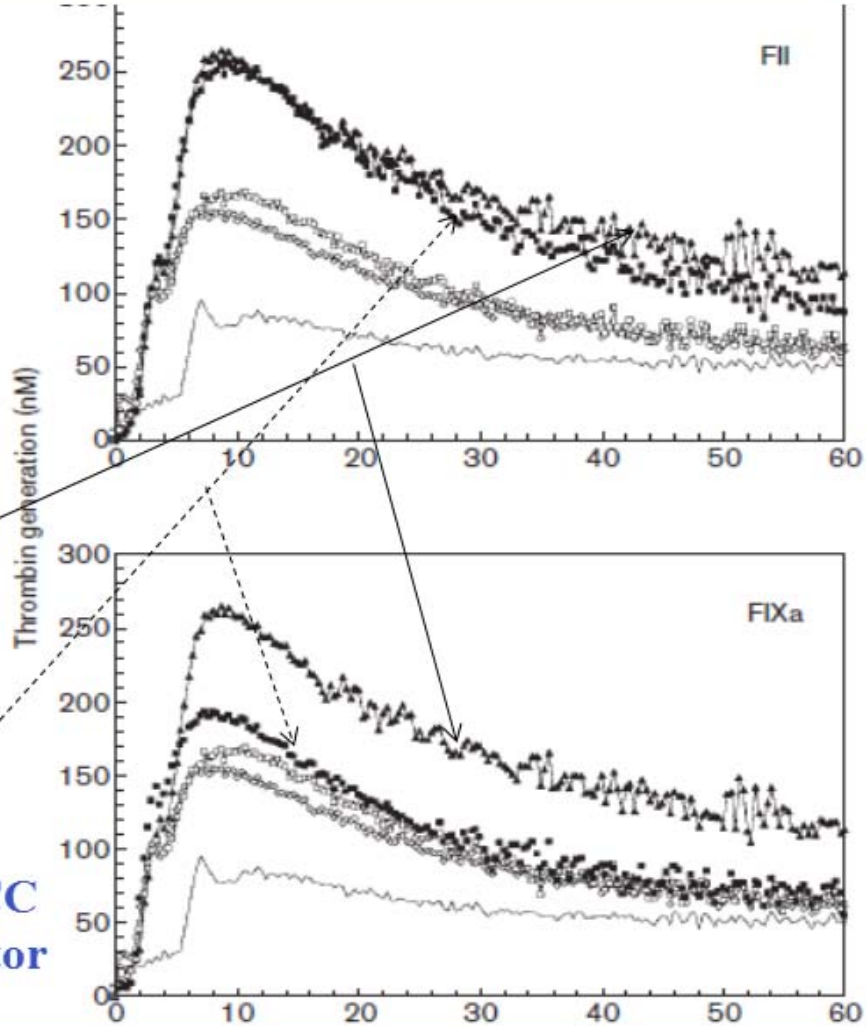
Causality in PCC thrombogenicity
PEI 1998-2004



- **HP FIX (non thrombogenic) has higher FIXa than PCC**
- **High FII levels are causal in thrombogenicity**

Coumarinized Plasma + thrombogenic PCC

Coumarinized Plasma + non-thrombogenic PCC + added coag factor



Kusch et al Thromb Haemost 1998; 79: 778–83
Dusel et al Blood Coagul Fibrinolysis 2004; 15:405–411

Conclusions (1)

- The pro-coagulant globulins of plasma co-fractionate into FII + III in Cohn fractionation, and then partition into FIII upon further Ig purification
- FXI in particular has physico-chemical properties which make it co-fractionate with Ig in preliminary steps
- The minute FXI levels measured in most products show that subsequent steps effectively clear FXI from Ig
- FI may contribute to *in vitro* thrombogenicity

Conclusions (2)

- Ig products have different safety and efficacy profiles which make causal linkage to particular effects difficult
- In particular, strict alignment to product composition ignores other factors arising from Virchow's Triad of thrombotic risk
- Previous experience with the thrombotic risk of PCC should prove cautionary in ascribing TEEs from Ig solely to activated factors

Thank you