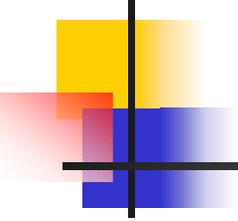


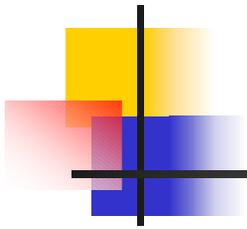
Pharmacokinetic and Pharmacodynamic Considerations in the Development of Macromolecules

Pamela D. Garzone, Ph.D.



OUTLINE OF LECTURE TOPICS

- Immunoassays
- Interspecies Scaling
- Pharmacokinetic Characteristics
 - Scientific Issues
- Pharmacodynamics



REPRESENTATIVE MARKETED MACROMOLECULES

Macromolecule

Trade Name

Erythropoietin

Epogen (Amgen)

Growth Hormone

Nutropin (Genentech)

G-CSF

Neupogen (Amgen)

IL-2

Proleukin (Chiron)

IL-11

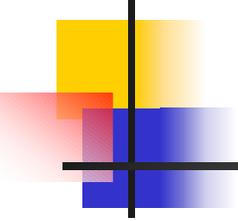
Neumega (GI)

Factor IX

BeneFIX (GI)

rt-PA

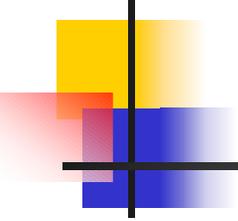
Alteplase (Genentech)



ASSAYS FOR MACROMOLECULES

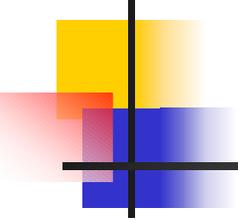
- **Immunoassays**

- ELISA (Enzyme-Linked Immuno-sorbent Assay)
- RIA (Radioimmunoassay)
- IRMA (Immunoradiometric Assay)
- RRA (Radioreceptor Assay)



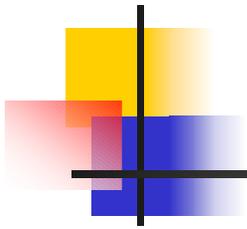
ASSAYS FOR MACROMOLECULES

- Steps in ELISA
 - Adsorb the primary antibody ('capture' the target antigen) to solid phase
 - Wash excess away
 - Apply patient's serum;
 - unbound substances are washed away



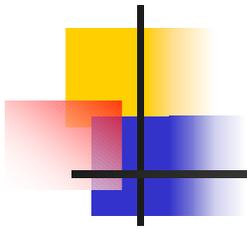
ASSAYS FOR MACROMOLECULES

- Steps in ELISA (continued)
 - Add second enzyme-labeled antibody
 - Add chromogenic substrate; converts (by the enzyme) to brightly colored product
 - Amount of colored products is proportional to targeted products



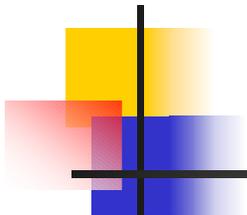
INTERSPECIES SCALING OF MACROMOLECULES

- Factors to Consider
 - Species specificity
 - Glycosylation and sialation
 - Binding proteins
 - Size, shape and charge
 - Relative abundance of tissue receptors



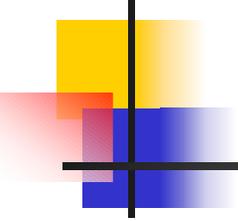
ALLOMETRIC EQUATIONS FOR SOME MACROMOLECULES

Macromolecule	Allometric V_1	Equations CL
Factor IX	$87 W^{1.26}$	$14 W^{0.68}$
Factor VIII	$44 W^{1.04}$	$10 W^{0.69}$
IL-12	$65 W^{0.85}$	$8 W^{0.62}$
GH	$68 W^{0.83}$	$7 W^{0.71}$
rt-PA	$91 W^{0.93}$	$17 W^{0.84}$



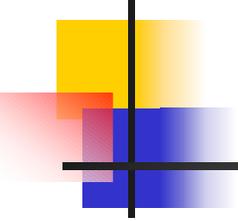
CENTRAL COMPARTMENT VOLUME PREDICTED BY ALLOMETRIC SCALING

Macromolecule	Human Parameter: Predicted (mL)	V₁ Observed (mL)
FIX	18,380	10,150
Factor VIII	3,617	3,030
IL-12	2,406	3,360
GH	2,243	2,432
rt-PA	5,814	4,450



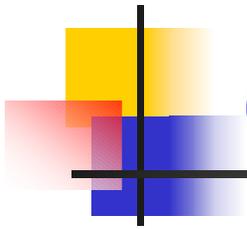
ELIMINATION CLEARANCE PREDICTED BY ALLOMETRIC SCALING

Macromolecule	Human Parameter: Predicted (mL/hr)	Cl Observed (mL/hr)
FIX	248	434
Factor VIII	195	174
IL-12	113	406
GH	148	175
rt-PA	646	620



PHARMACOKINETIC CHARACTERISTIC OF MACROMOLECULES

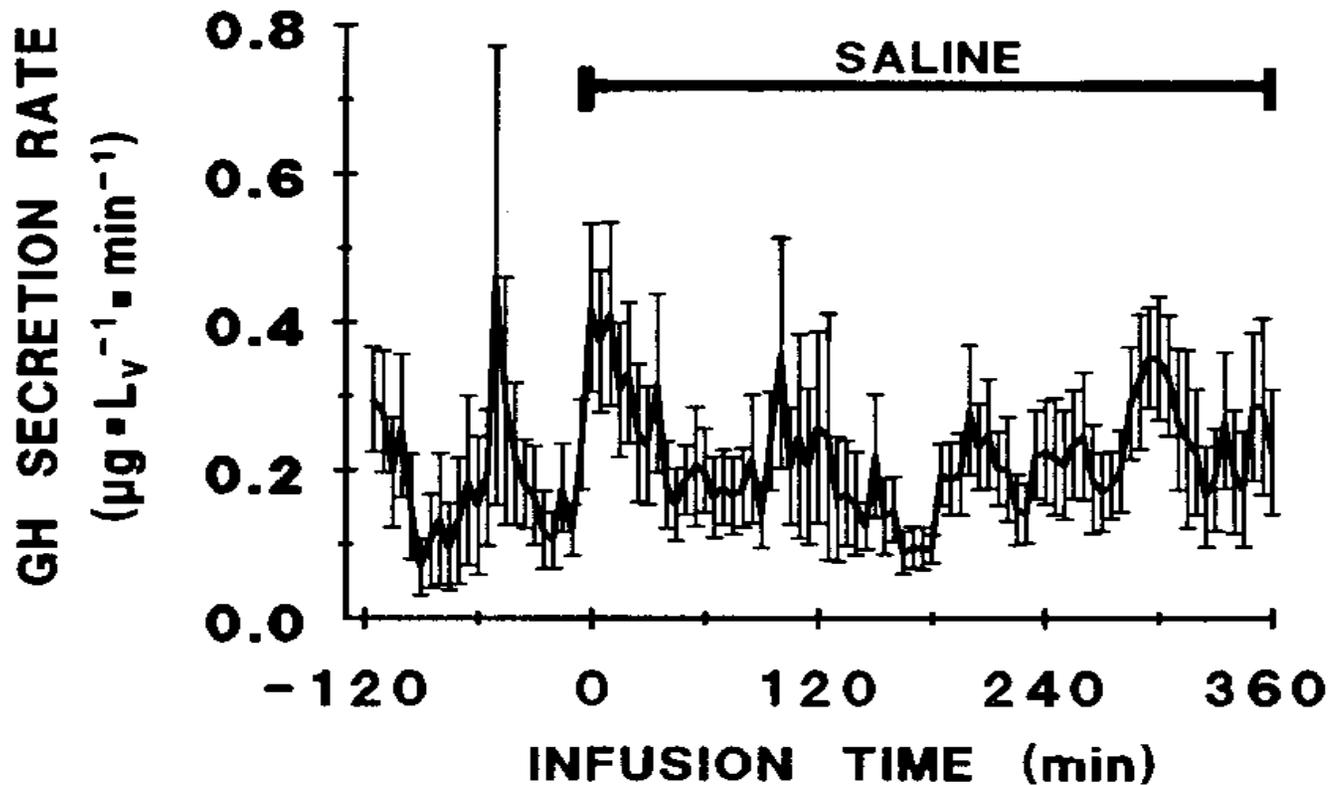
- Endogenous concentrations
- Absorption
- Distribution
- Metabolism
- Elimination



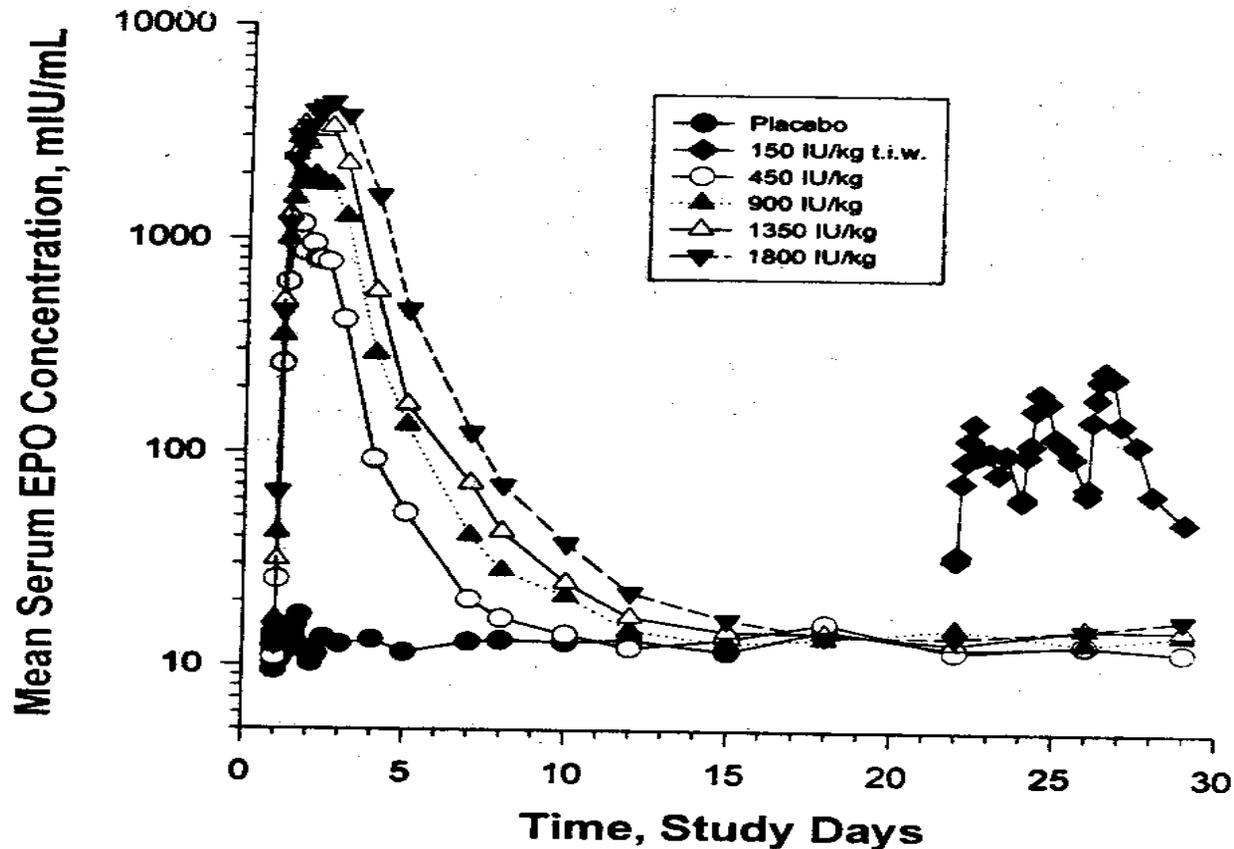
THE PROBLEM OF ENDOGENOUS CONCENTRATIONS OF MACROMOLECULES

- Endogenous concentrations - What do you do with them?
- Two examples
 - Growth Hormone
 - Erythropoietin

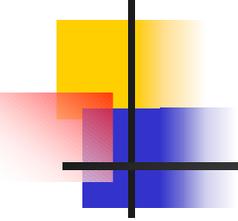
Growth Hormone

$$[F(t) = B + A^{-kT}]$$


ERYTHROPOIETIN KINETICS



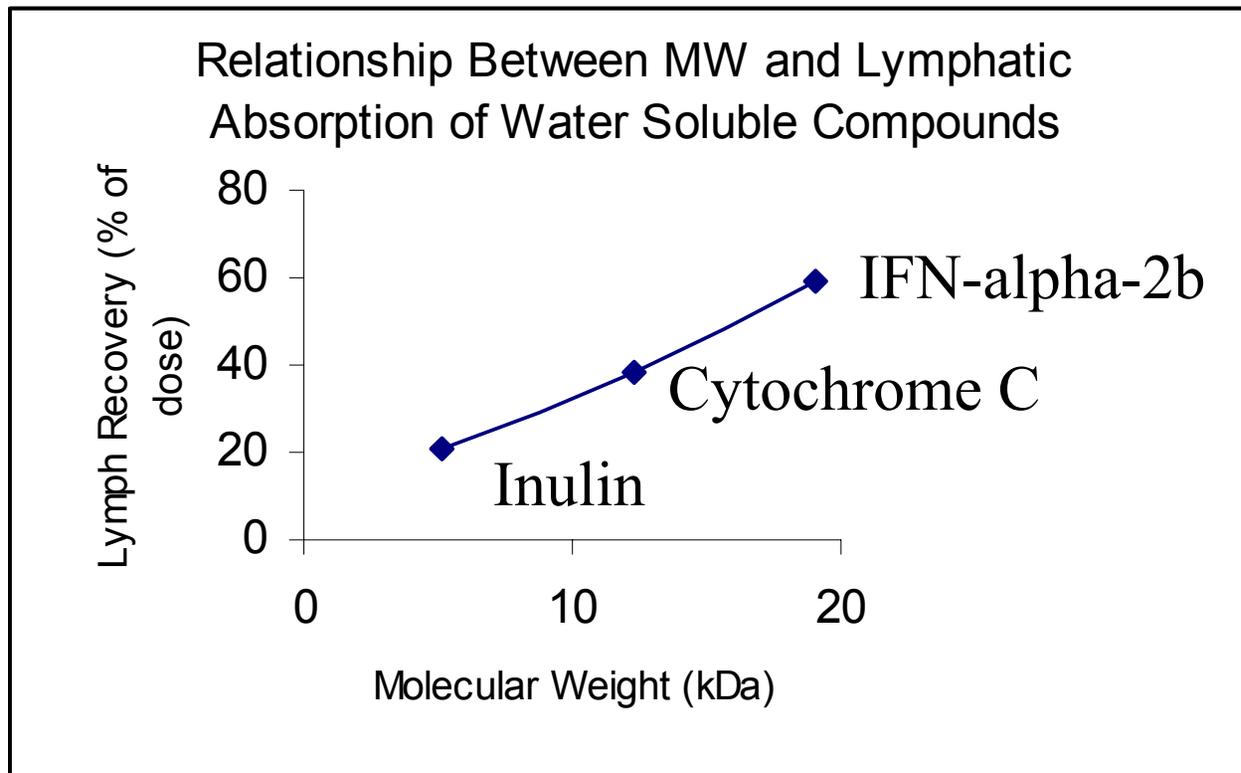
Cheung et al CPT 1998; 64:412-423



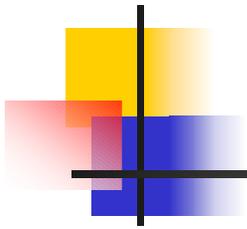
ABSORPTION OF MACROMOLECULES

- Flip-flop model
- Site of administration

RELATIONSHIP BETWEEN MW AND LYMPHATIC ABSORPTION OF WATER SOLUBLE COMPOUNDS



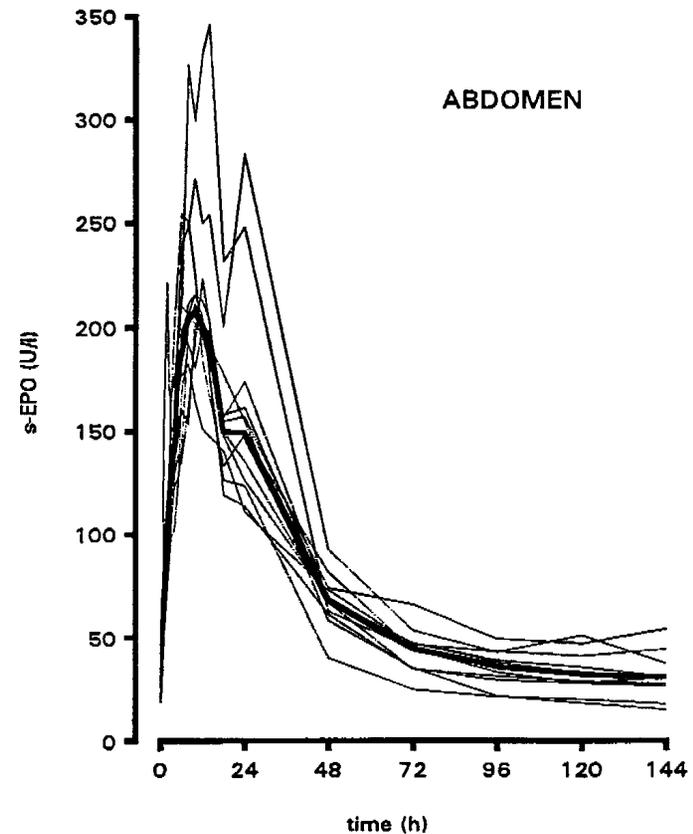
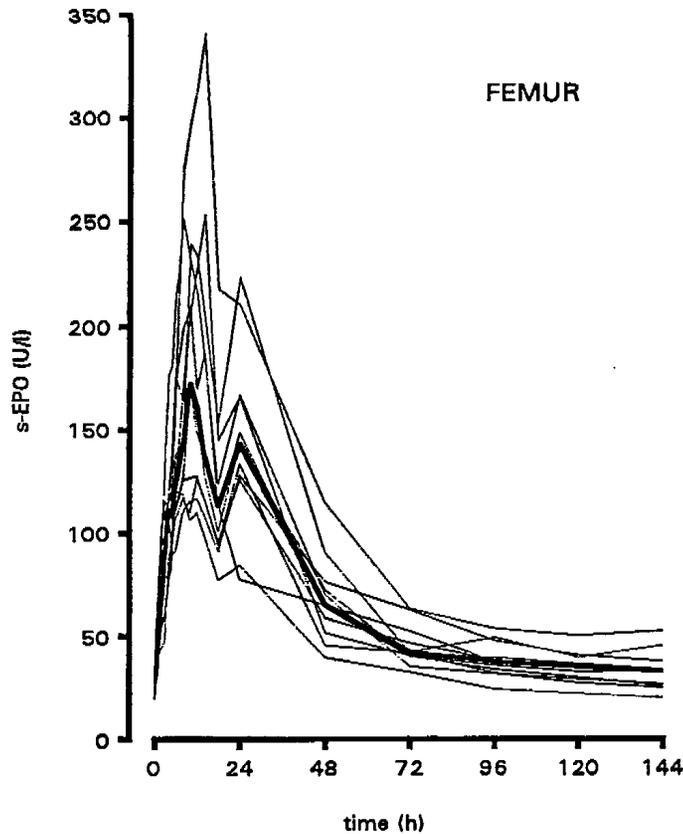
Supersaxo A et al. Pharm Res 1990; 7:167-169

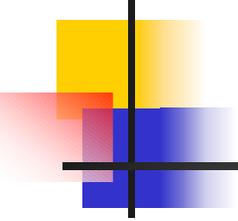


COMPARISON OF ABSORPTION AND ELIMINATION RATE CONSTANTS

Macromolecule	Route of Administration	K_a (hr ⁻¹)	K_e (hr ⁻¹)
GH	SC	0.23 ± 0.04	0.43 ± 0.05
	IV		2.58
IFN- α -2b	SC	0.24	0.13
	IV		0.42
Erythropoietin	SC	0.0403 ± 0.002	0.206 ± 0.004
	IV		0.077

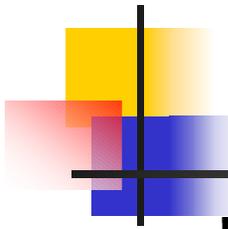
Effects of Site of Injection on EPO Concentration vs Time Profile





DISTRIBUTION OF MACROMOLECULES

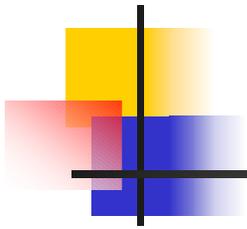
- Volume of Distribution
- Binding Proteins



DISTRIBUTION VOLUMES OF REPRESENTATIVE MACROMOLECULES

Macromolecule	MW (kDa)	V_1 (mL/kg)	V_{ss} (mL/kg)
Inulin	5.2	55	164
Factor IX	57	136*	271*
IL-2	15.5	60	112
IL-12	53	52	59
G-CSF	20	44	60
rt-PA	65	59	106

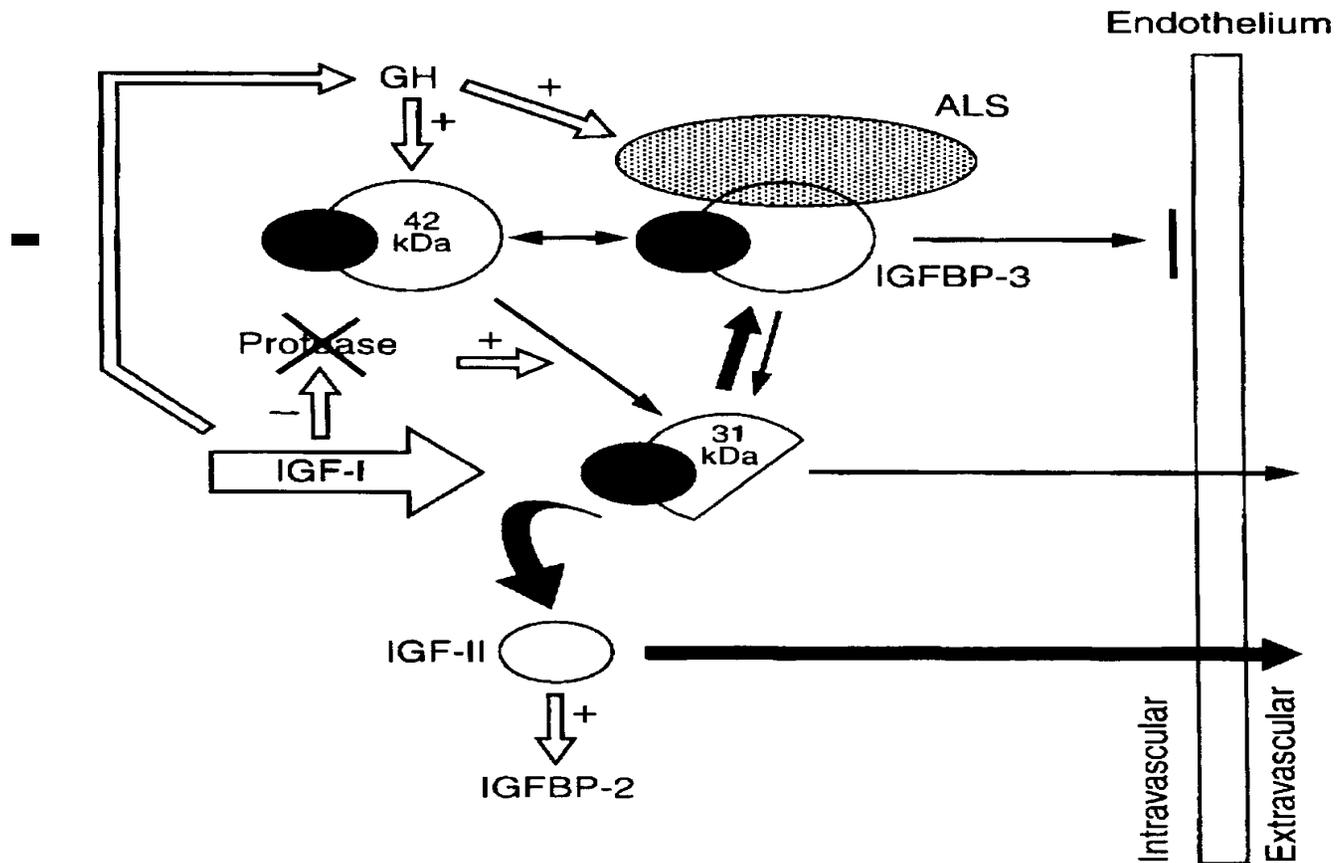
* Calculated from literature

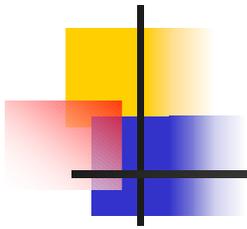


EFFECTS & RELEVANCE OF MACROMOLECULE BINDING TO α_2 -MACROGLOBULIN

Macromolecule	Effect	Relevance
NGF		Assay interference
IL-1	Regulation of proliferation of thymocytes	Regulatory protein
IL-2	Impaired proliferation of T-cells	Inactivation
TGF$_{\beta}$	Growth of kidney fibroblasts	Clearance

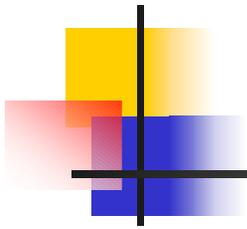
Hypothetical Model of the Effects of IGF-1





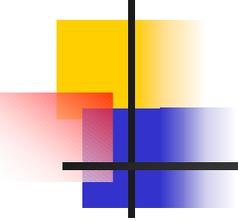
METABOLIC EFFECTS OF MACROMOLECULES

- Effects on P450s



EFFECTS OF MACROMOLECULES ON DIFFERENT CYP ENZYMES

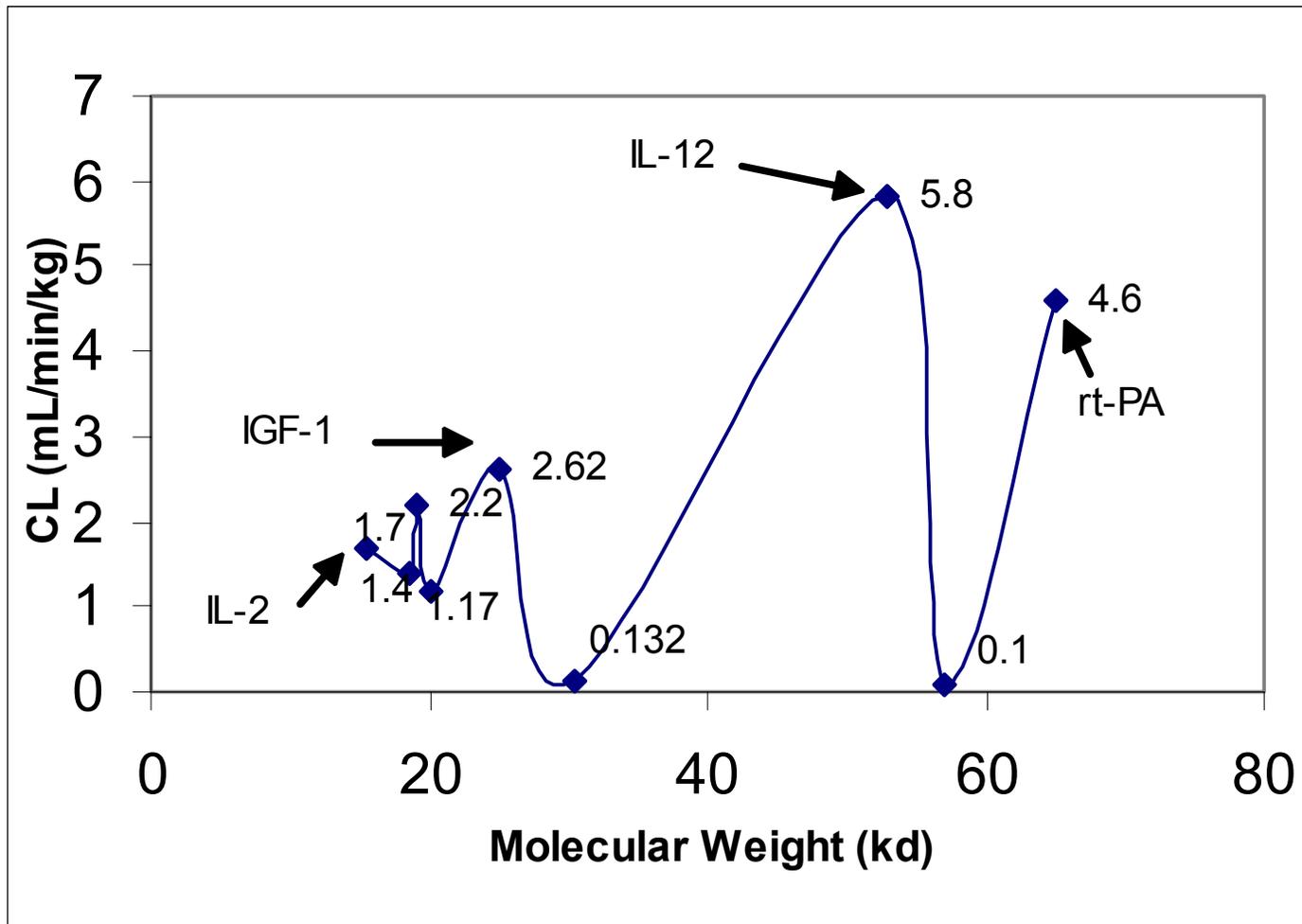
Macromolecule	Isoenzyme	Effects
IFN- γ	CYP2C11	Decreased mRNA and enzyme levels
IL-1	CYP2C11	Decreased mRNA and enzyme levels
	CYP 2D	Decreased mRNA and enzyme levels
IL-2	CYP2D1	Increased mRNA and enzyme levels
IL-6	CYP2C11	Decreased mRNA and enzyme levels
TNF	CYP2C11	Decreased enzyme levels

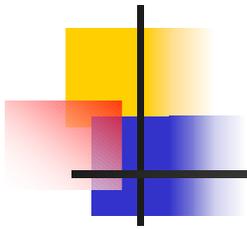


EXCRETION OF MACROMOLECULES

- Contributions of kidney and liver
- CHO vs E. Coli produced
- Receptor mediated clearance

RELATIONSHIP BETWEEN MOLECULAR WEIGHT AND ELIMINATION CLEARANCE





CELL SURFACE RECEPTORS FOR CLEARANCE OF CARBOHYDRATES & MONOSACCHARIDES

Specificity

Cell Type

Gal/Gal/NAc

Liver parenchymal cells

Gal/GalNAc

**Liver Kupffer and
endothelial cells
Peritoneal macrophages**

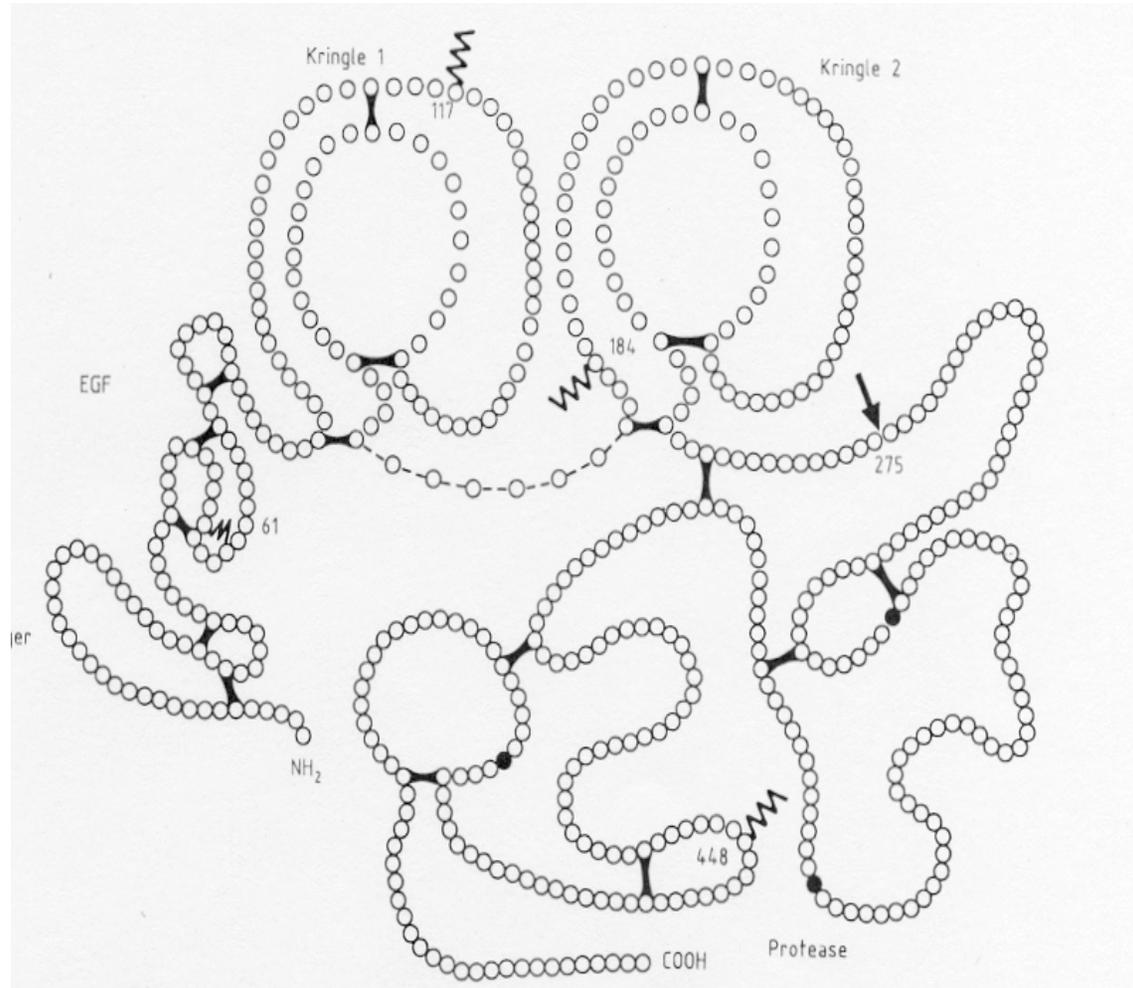
Man/GlcNAc

**Liver Kupffer and
endothelial cells
Peritoneal macrophages**

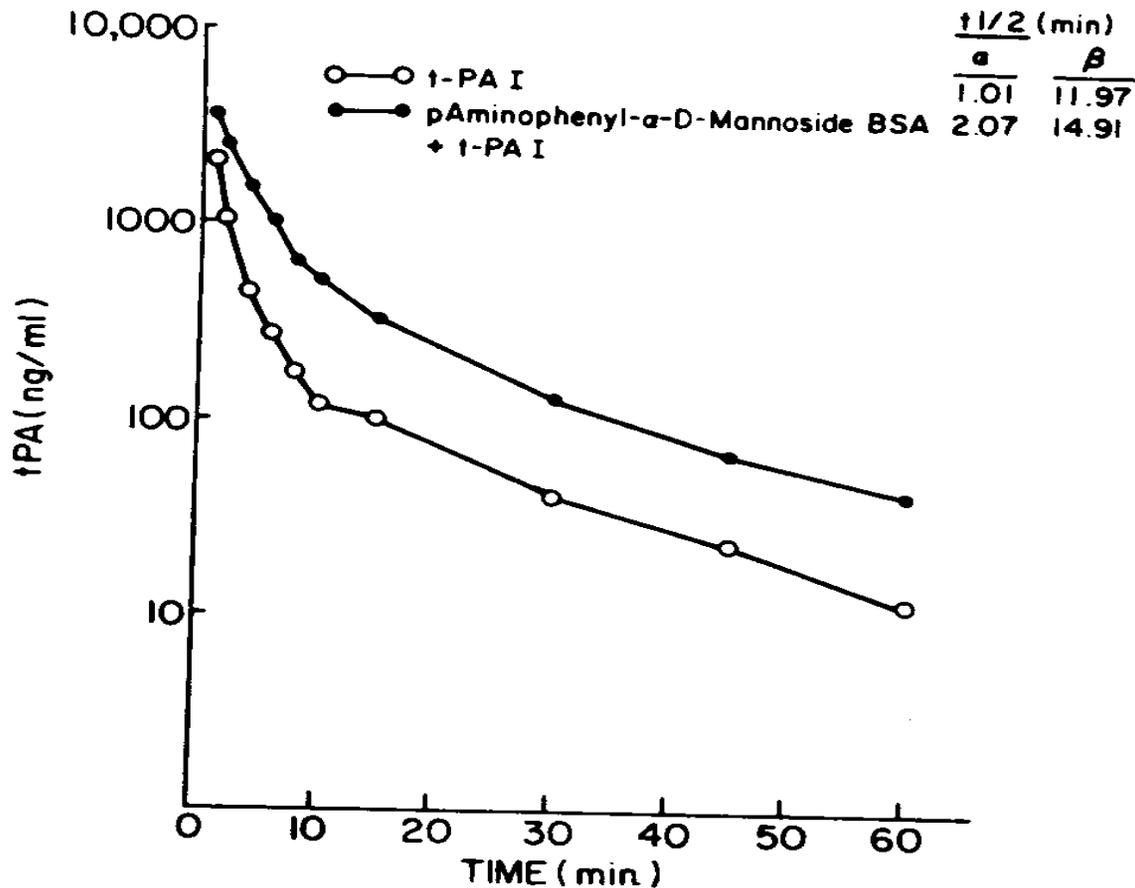
Fuc

Liver Kupffer cells

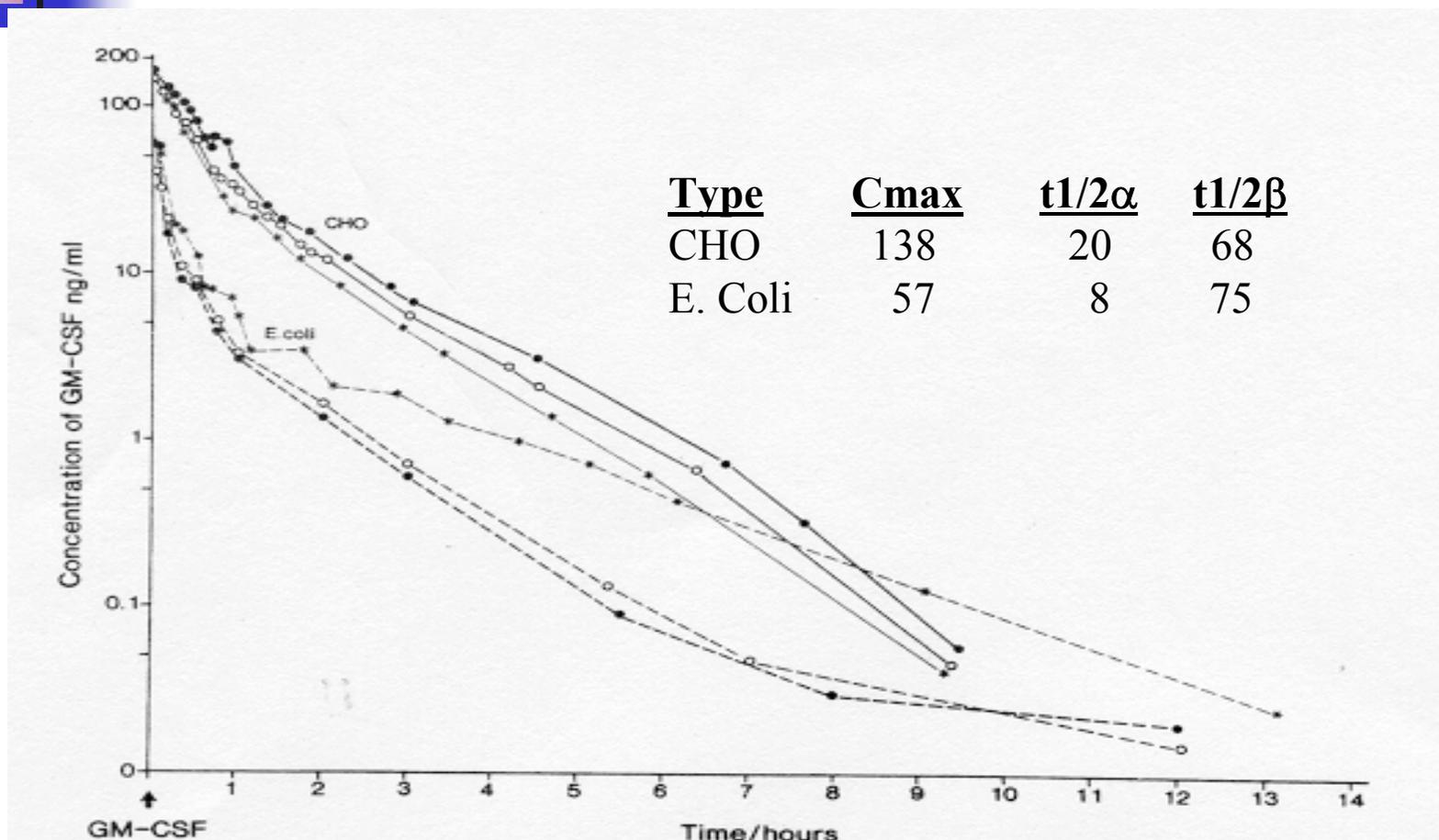
STRUCTURE OF rt-PA



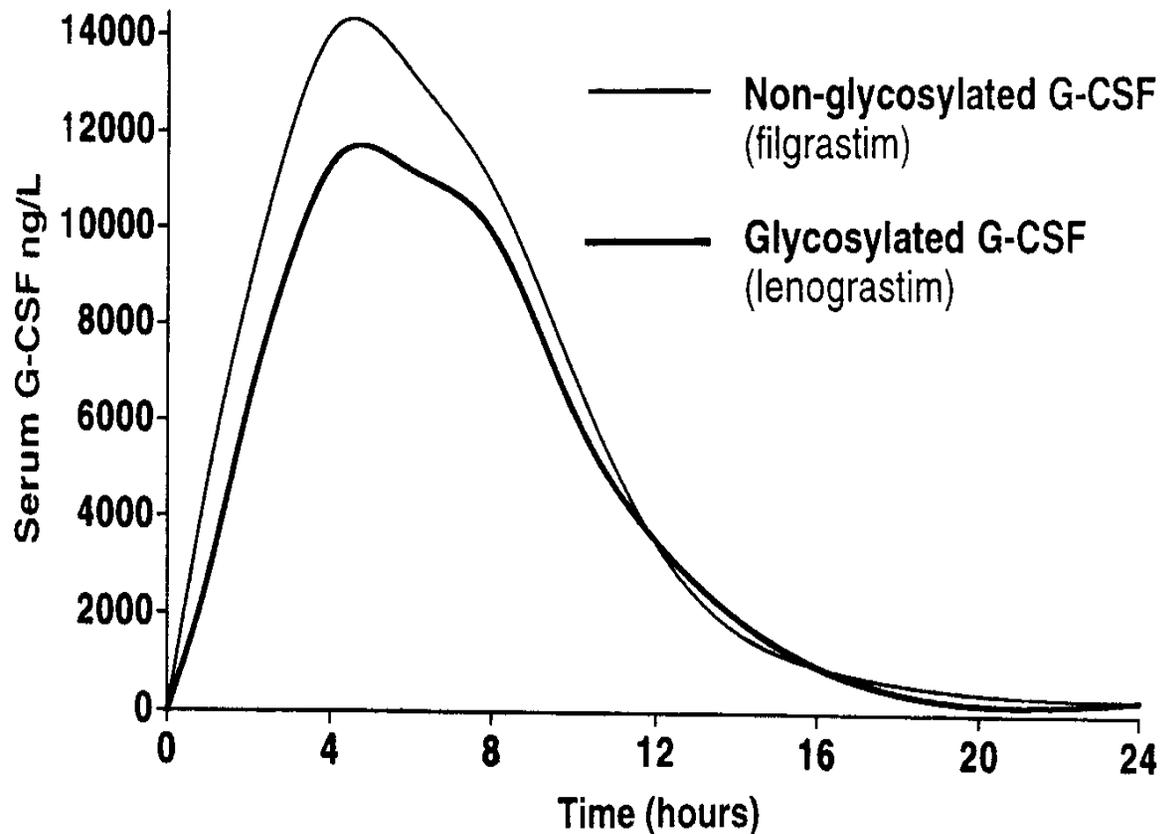
CLEARANCE OF DIFFERENT FORMS OF rt-PA IN RABBITS



SERUM CONCENTRATION-TIME PROFILES FOR CHO VS. E. Coli PRODUCED GM-CSF

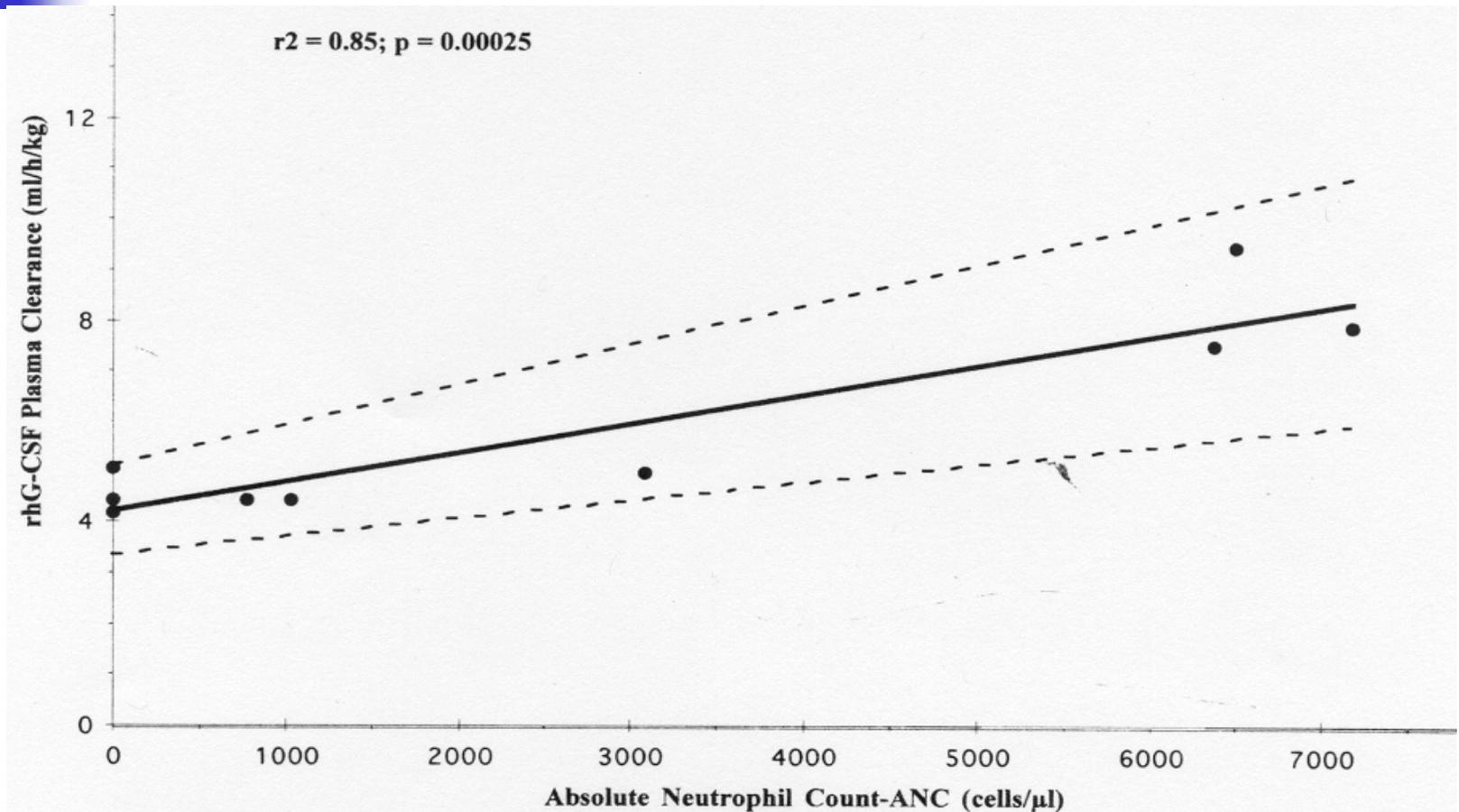


SERUM CONCENTRATION-TIME PROFILES FOR NON-GLYCOSYLATED VS. GLYCOSYLATED G-CSF

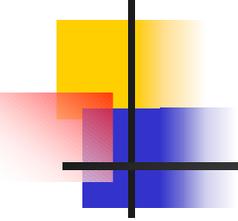


Watts et al. Br J Haematol 1997; 98:474-479

RELATIONSHIP BETWEEN G-CSF CLEARANCE AND ABSOLUTE NEUTROPHIL COUNT

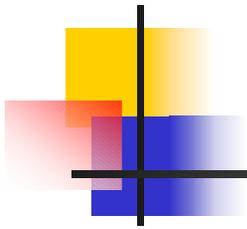


Ericson SG et al. *Exper Hematol* 1997; 25:1313-1325



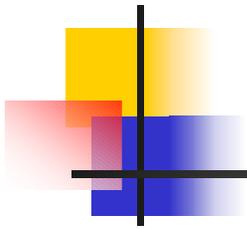
CHARACTERISTICS THAT AFFECT THE PHARMACOKINETICS OF MACROMOLECULES

- Physical characteristics
- Post-translational modification
- Binding
- Route of administration
- Duration of administration
- Frequency of administration



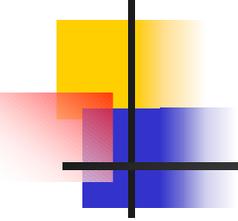
PATIENT CHARACTERISTICS THAT AFFECT PHARMACOKINETICS OF MACROMOLECULES

- Age
- Gender
- Disease
- Concurrent drugs



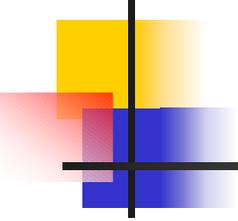
GROWTH HORMONE

- Reverses effects of aging
 - Restores IGF-1 levels to that of young adult
 - Increases lean body mass and decreases adipose tissue mass
 - Increases skin thickness
 - Increases vertebral bone density



GROWTH HORMONE (cont.)

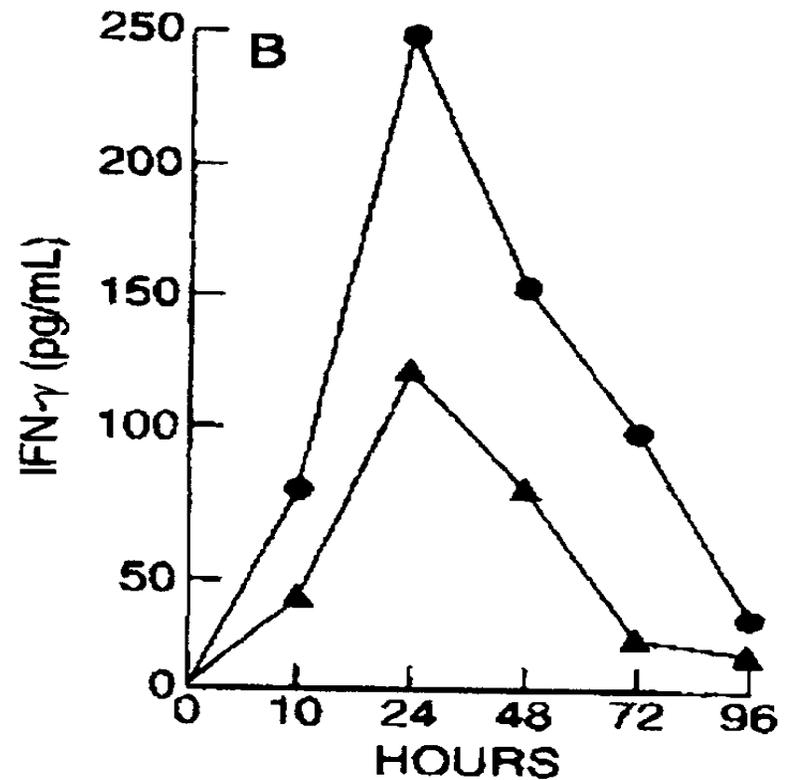
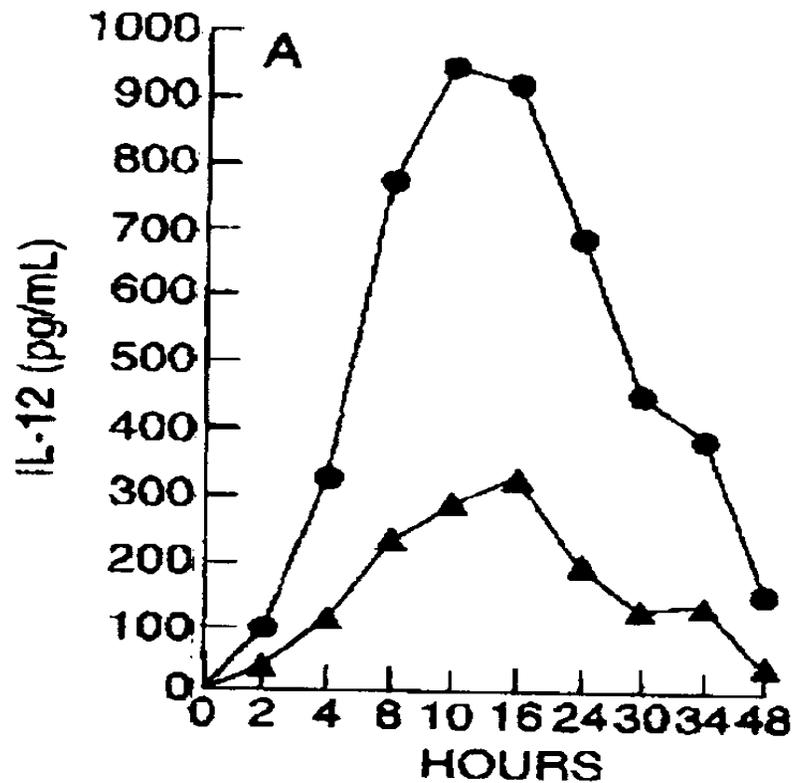
- Daily rhGH dose/kg required to normalize IGF-1 response in GH deficient women is higher than in men
 - Estrogen replacement also significantly increase rhGH dose requirement



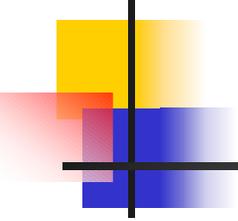
PHARMACODYNAMICS OF MACROMOLECULES

- Important considerations
 - Regimen dependency
 - Endpoints
 - Models

REGIMEN DEPENDENCY OF IL-12 PHARMACOKINETICS AND IFN- γ STIMULATION



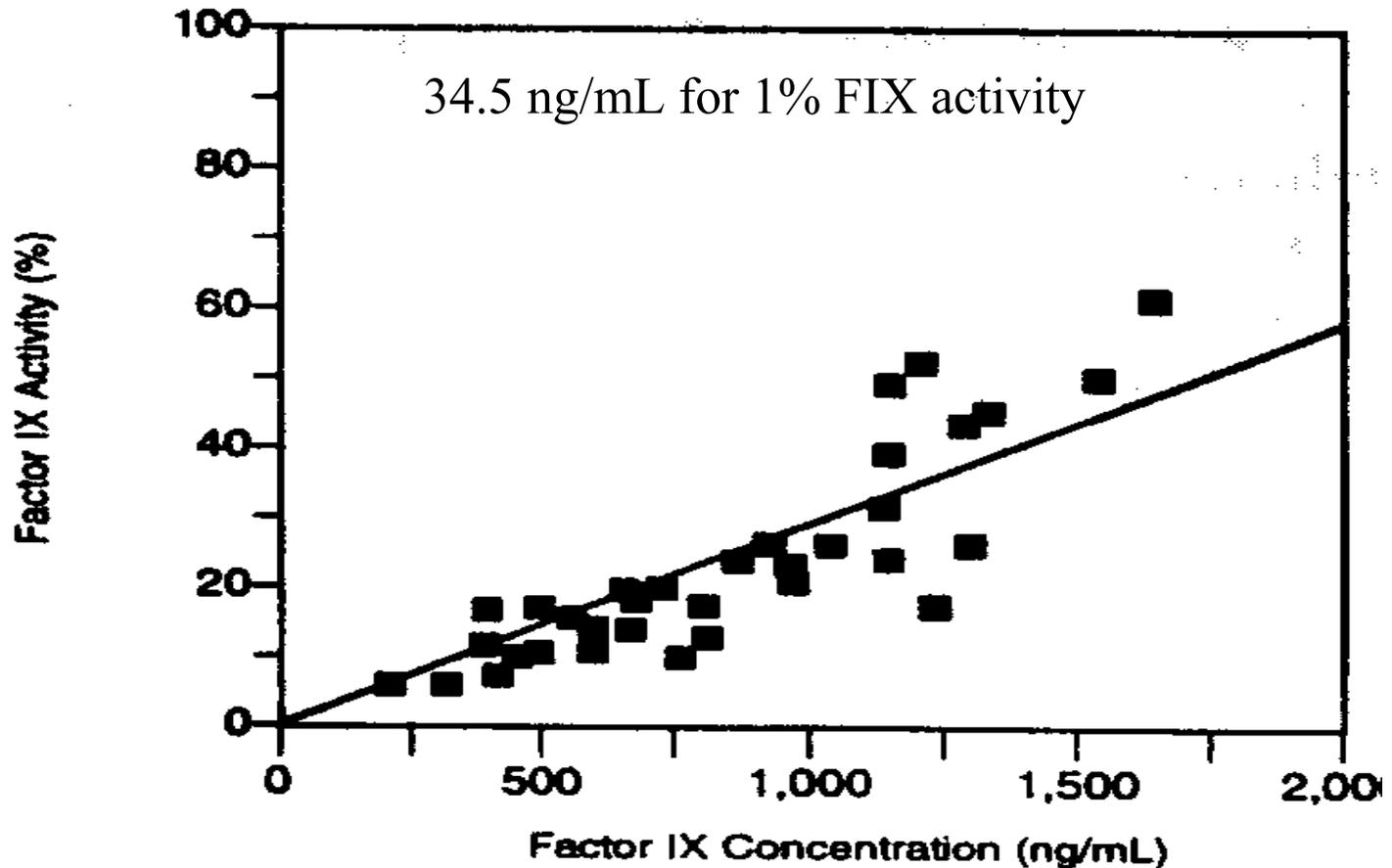
Motzer RJ et al. Clin Cancer Res 1998;4:1183-1191



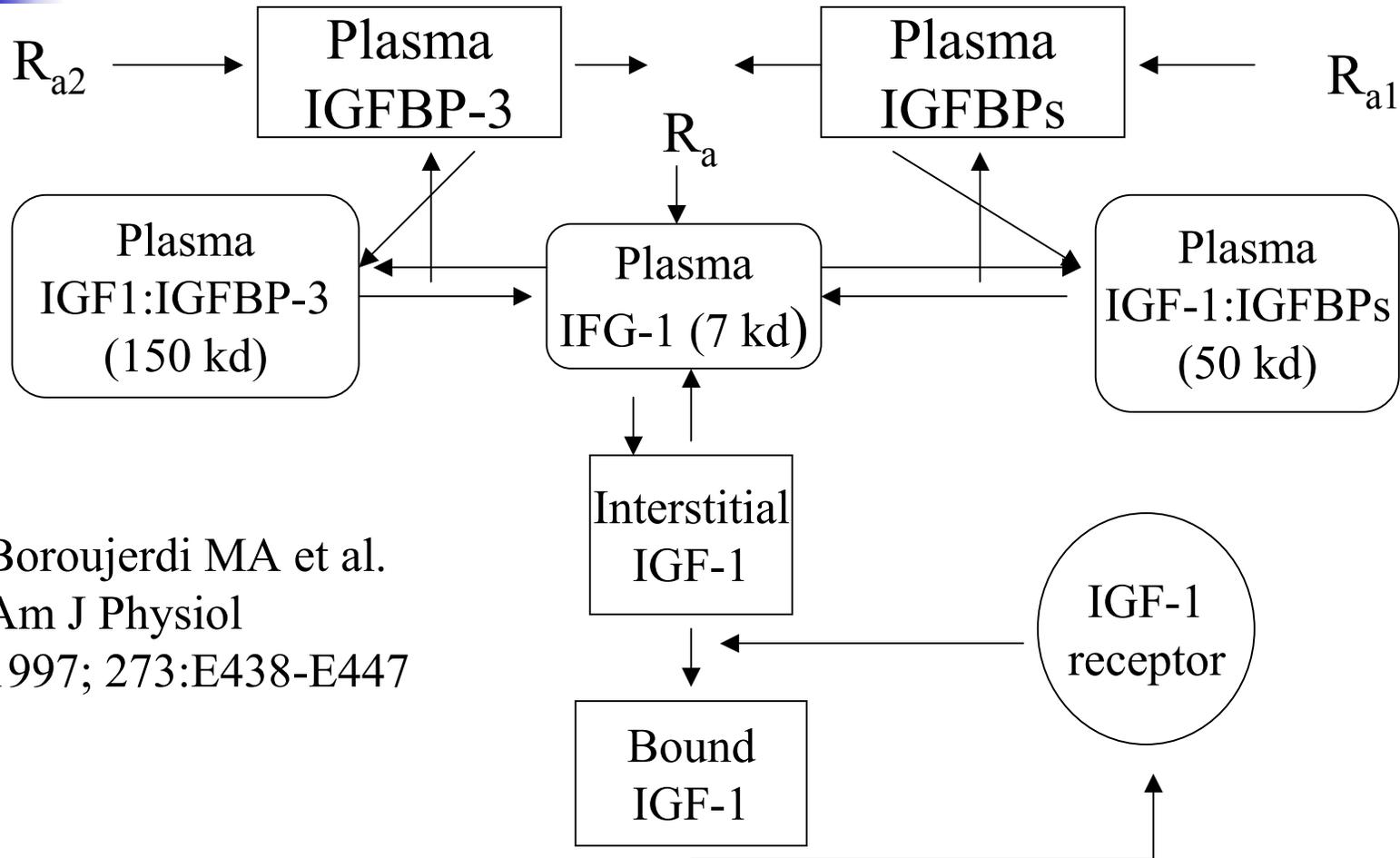
PHARMACODYNAMIC ENDPOINTS

- Easy - replacement proteins
 - rFIX
- Difficult- cascade of events
 - IGF-1

RELATIONSHIP BETWEEN rFIX CONCENTRATION AND ACTIVITY



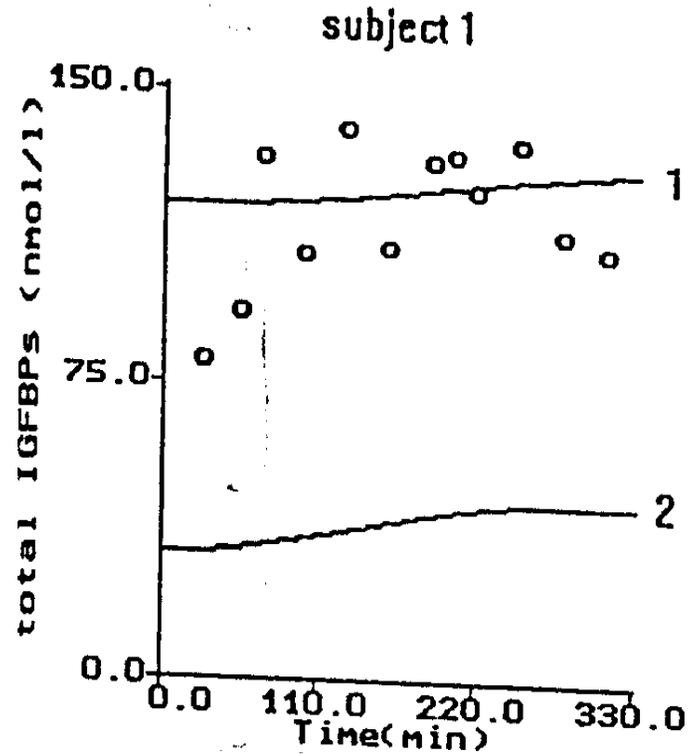
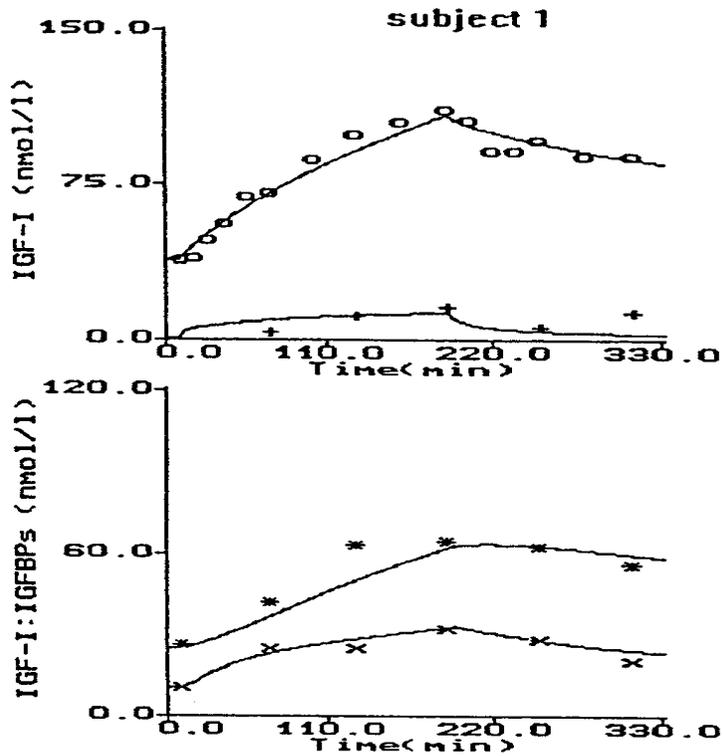
THEORETICAL MODEL OF IGF-1 PHARMACOKINETICS



Boroujerdi MA et al.
Am J Physiol
1997; 273:E438-E447

IGF-1 & IGFBP₃ PHARMACOKINETICS

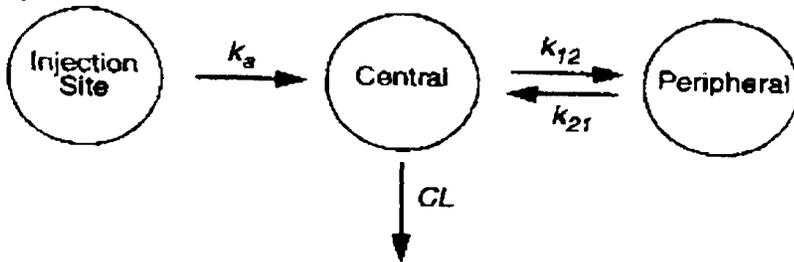
IGF-I PHARMACOKINETICS



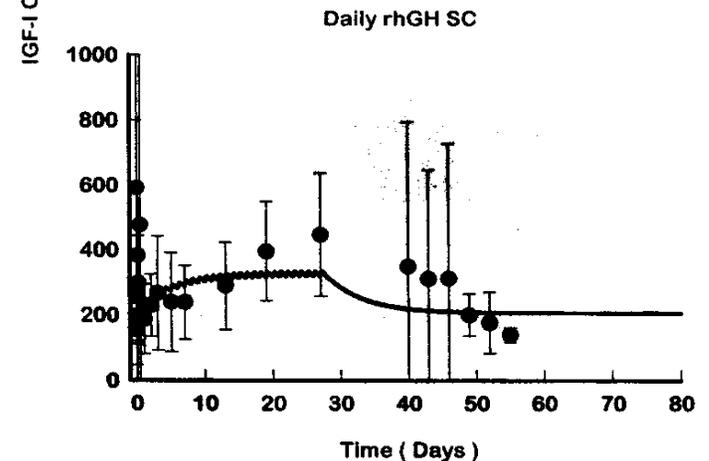
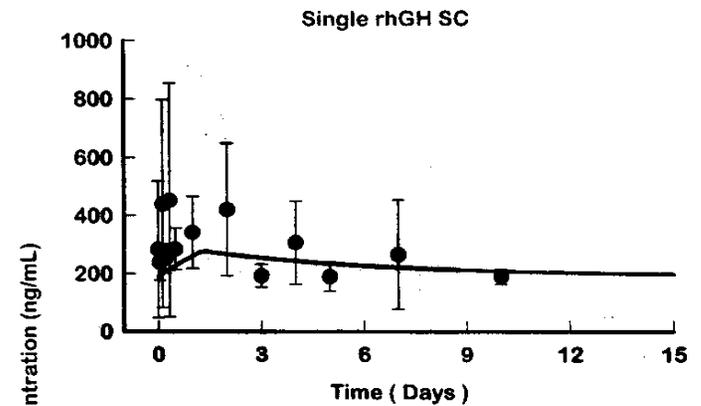
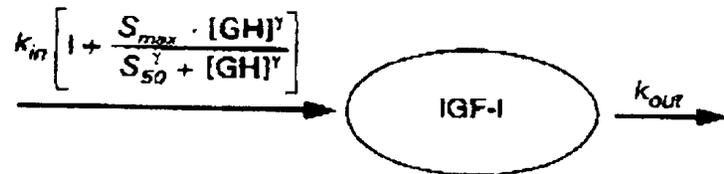
PK-PD MODEL OF rhGH WITH MEASURED VS. PREDICTED [IGF-1] AFTER SINGLE AND DAILY SC rhGH INJECTIONS

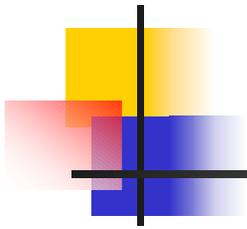
Model of rhGH Pharmacokinetics

SC Injection



Indirect Response Model of IGF-I Induction by rhGH

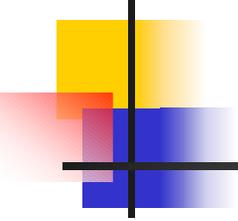




Pharmacokinetic and Pharmacodynamic Considerations in the Development of Macromolecules

■ Summary

- Use scientific judgement and good sense in the interpretation of PK/PD results with macromolecules
- Application of PK principles that have been developed work with macromolecules
- Difficult to select the most appropriate pharmacodynamic endpoint



Pharmacokinetic and Pharmacodynamic Considerations in the Development of Macromolecules

- Acknowledgements
 - Genetic Institute
 - PK/PD Sciences
 - Dr. Joyce Mordenti
 - Dr. Art Atkinson