Pharmacokinetics of

PROTEIN DRUGS

PEPTIDE AND PROTEIN DRUGS

In this lecture, the general differences in the kinetic behavior of protein drugs relative to that observed with small molecules is emphasized. The kinetic behavior of antibody drugs is also contrasted to that of other protein drugs.

Definition

Terminology for polypeptide and protein drugs is not well defined, but all contain multiple amino acids that are linked via peptide bonds. They are therefore poly-peptides. Many have used a specific number of amino acids, e.g., 50, as the cut-off for defining when a polypeptide becomes a protein; but there is no “official” definition.

Subsequently, for the purposes of this lecture, “protein” is used as an all-encompassing term for all compounds containing two or more amino acids.

Breadth of Drugs in This Category

It is virtually impossible to summarize succinctly the pharmacokinetic and pharmacodynamic properties of protein drugs for the class as a whole because of the wide range of compounds and activities involved. For our purposes, however, it is useful to divide protein drugs into two groups:

Non-antibody and **Antibody**

Table 1. Examples of Polypeptide and Protein (Non- antibody) Therapeutic Agents[a]

- Wide variety of uses
- Sizes of molecules vary greatly
- Some synthetic, some from recombinant technology
- Some pure, but most are heterogeneous

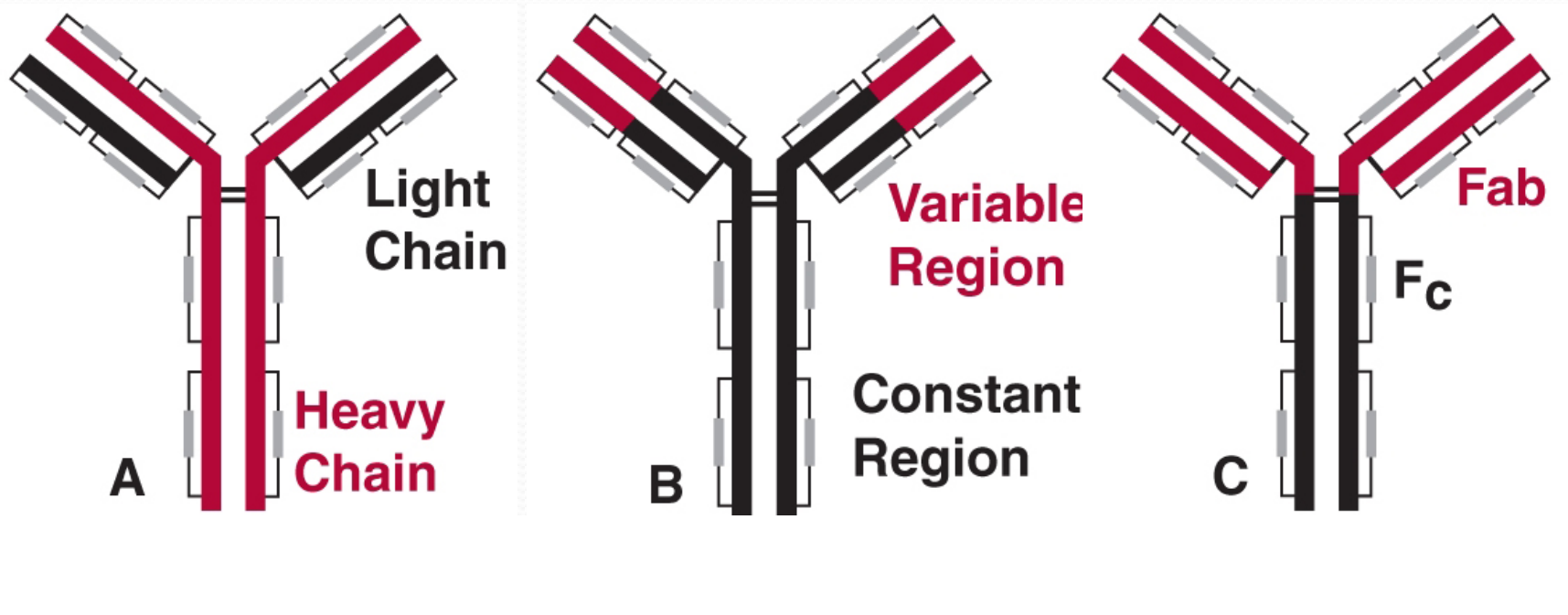
[a]In "Protein Drugs" file accompanying lecture.

Table 2. Examples of Monoclonal Antibodies, Their Therapeutic Use, Half-life, and Route of Administration[a]

- Classified by technology used to produce them
- Variety of uses
- Long half-lives
- Most administered intravenously
- Dosing interval often one week or more

[a]In "Protein Drugs" file accompanying lecture.

Structure


Light
Chain
Heavy
Chain
A
Variable
Region
Constant
Region
B
Fab
Fc
C

Nomenclature

Monoclonal antibodies (mab) are named by the World Health Organization's Non-Proprietary Names and the United States Adopted Names by a common scheme (last modified in 2009). The prefix is variable for specifying the antibody (See Table 3). Every mab has its own prefix.

The stem (or suffix) -**mab** identifies the drug as a **monoclonal antibody**. Substems, identifying the target system and the source of the antibody, in that order, are used (see Table 3).

Table 3. Nomenclature of Monoclonal Antibodies

	Target Substem		Source Substem		
Prefix	Substem	Meaning	Substem	Meaning	Stem
	-anibi-	angiogenesis inhibitor	-a-	rat	
	-b(a)-	bacterium	-e-	hamster	
	-c(i)-	circulatory system	-i-	primate	
	-f(u)-	fungus	-o-	mouse	
	-k(i)-	interleukin	-u-	human	
Variable	-les-	Inflammatory lesions	-xi-	chimeric	-mab
	-l(i)-	Immune system	-zu-	humanized	
	-mul-	Musculoskel-etal system	-xizu-*	Chimeric/hum-anized hybrid	
	-n(e)-*	Nervous system	-axo-	Rat/mouse hybrid	

Table 3. (Cont.)

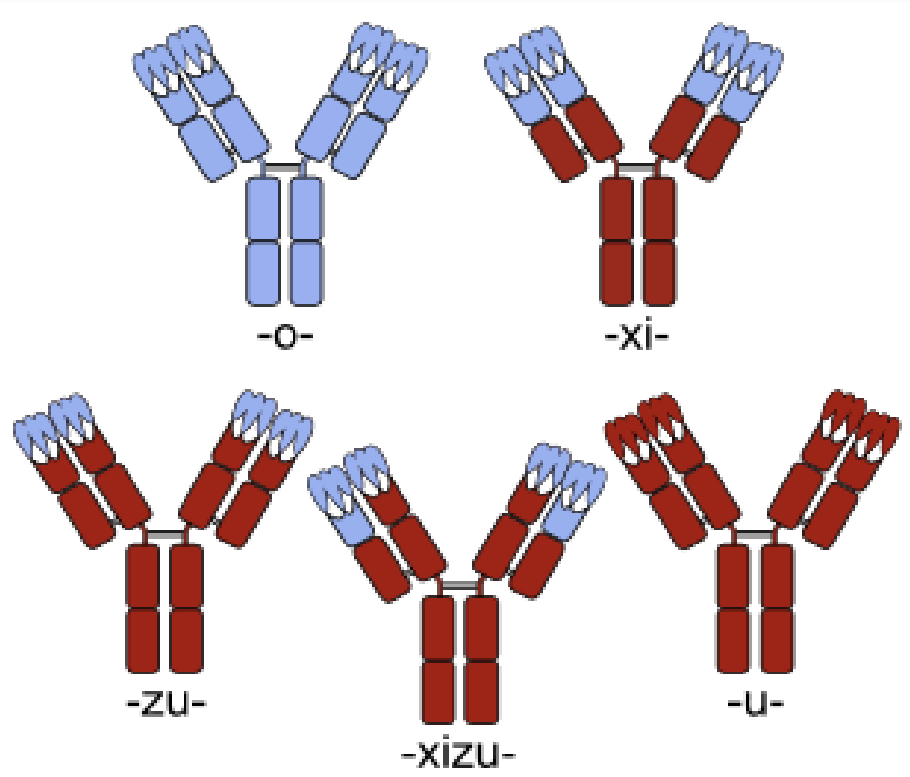
Target Substem			Source Substem		
Prefix	**Substem**	**Meaning**	**Substem**	**Meaning**	**Stem**
	-os-	bone	-a-	rat	
	-toxa-	toxin	-e-	hamster	
	-t(u)-	tumor	-i-	primate	
	-vi(r)-	virus	-o-	mouse	
			-u-	human	
Variable			-xi-	chimeric	-mab
			-zu-	humanized	
			-xizu-*	Chimeric/hum -anized hybrid	
			-axo-	Rat/mouse hybrid	

* Under discussion as of December 2009

Thus, the drug ***retuximab*** is:

1. A monoclonal antibody (retuxi***mab***)
2. Of chimeric (mouse and human) origin (retu***xi***mab)
3. Acting on a tumor (re***tu***ximab**).**

Cetuximab acts on tumors and is of chimeric origin, but it differs from retuximab in its chemical structure, as identified by its prefix (**Ce**- vs. **Re-**).



Brown – human parts

Blue – mouse parts

-o-	mouse	-xizu-	chimeric/humanized
-xi-	chimeric	-u-	human
-zu-	humanized		

Nomenclature of monoclonal antibodies - wikipedia

Antibodies named before the new rules were established in 2009 retain the name given them under the older rules.

For example, **adalimumab**, a human monoclonal antibody targeting the immune system, in the new system would be **adalumab**.

Some antibodies have an additional word indicating that another substance is attached:

Pegol – pegylated to slow degradation or reduce immunogenicity

Vedotin – linked to monomethyl auristatin E, a cytotoxic agent

Pendetide – attachment of a derivative of pentetic acid to chelate a radionuclide

Table 4. FDA-approved Polyclonal Immune Globulins and Antibody Fragments

Crotalidae immune Fab

Digoxin immune globulin

Hepatitis B immune globulin

Intravenous gamma globulin

Lymphocyte antithymocyte immune globulin

Normal immune globulin

Pertussis immune globulin

Rabies immune globulin

Rho(D) immune globulin

Tetanus immune globulin

Vaccinia immune globulin

Varicella zoster immune globulin

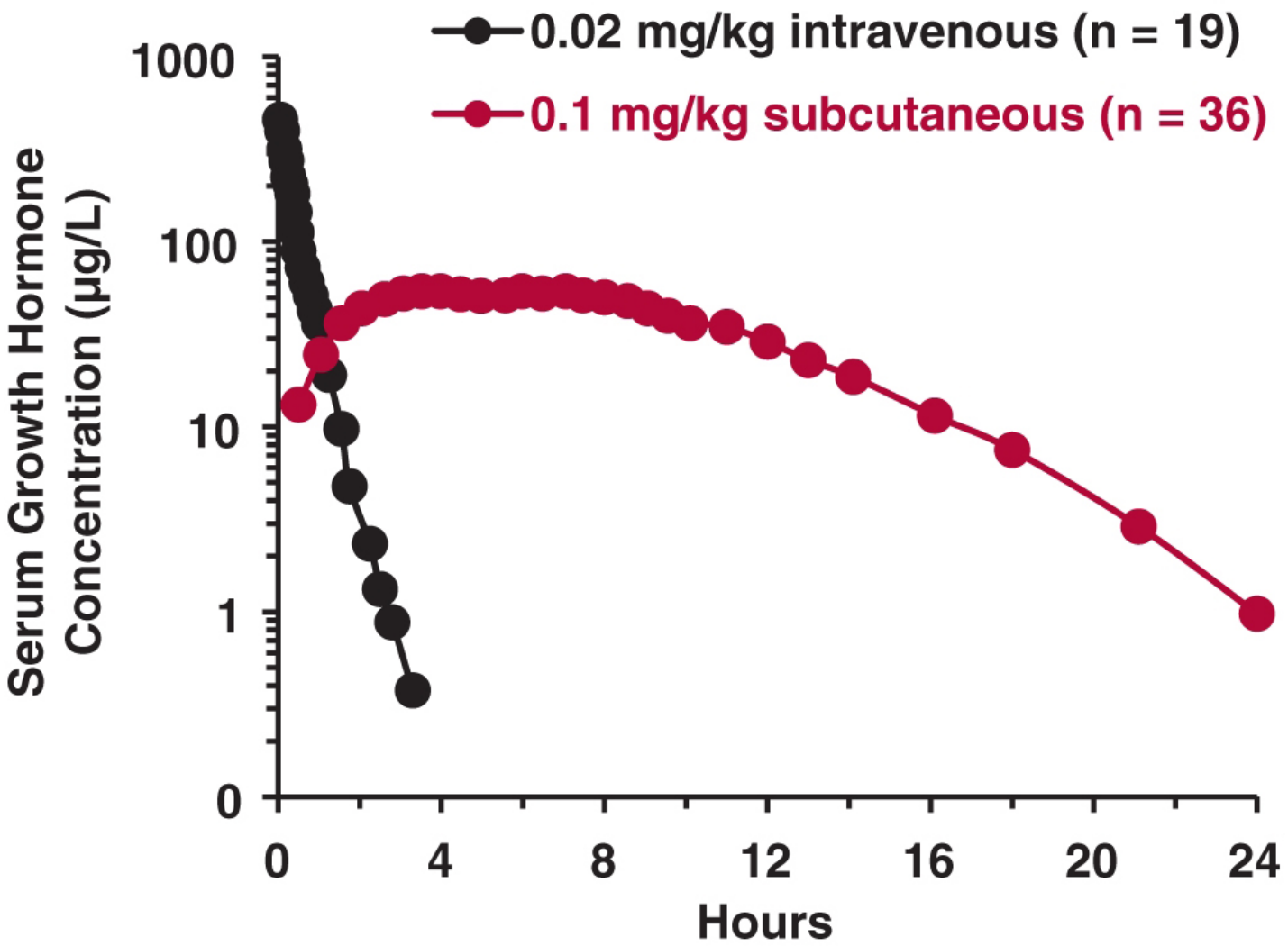
EXTRAVASCULAR ADMINISTRATION

Oral Administration

- Unstable in Gastrointestinal Tract (Foodstuff)
- Extremely Low and Erratic Bioavailability

Other Extravascular Routes

- Subcutaneous
- Intramuscular


0.02 mg/kg intravenous (n = 19)
0.1 mg/kg subcutaneous (n = 36)
Serum Growth Hormone Concentration (µg/L)
1000
100
10
1
0
0
4
8
12
16
20
24
Hours

INTRAVENOUS ADMINISTRATION

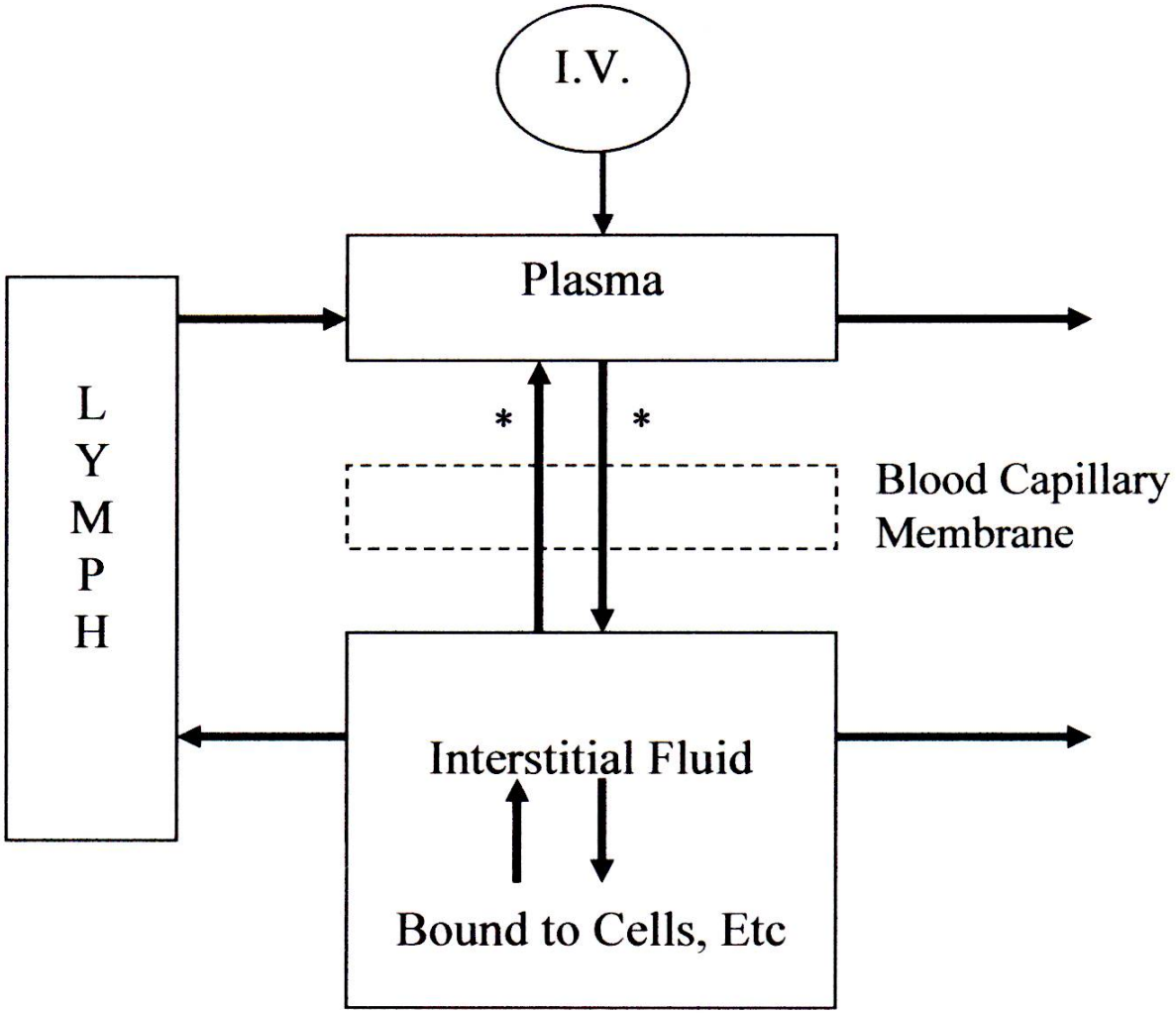
- The most pharmacokinetically reliable mode of administration.
- Less convenient than i.m. or s.c. for both patients and caregivers.
- As the half-life of many **non-antibody** protein drugs is quite short (< 3 hours), infusion is often needed.
- Most **antibodies** have half-lives of 0.3 to 30 days and can be given relatively infrequently (e.g., once weekly or every other week).

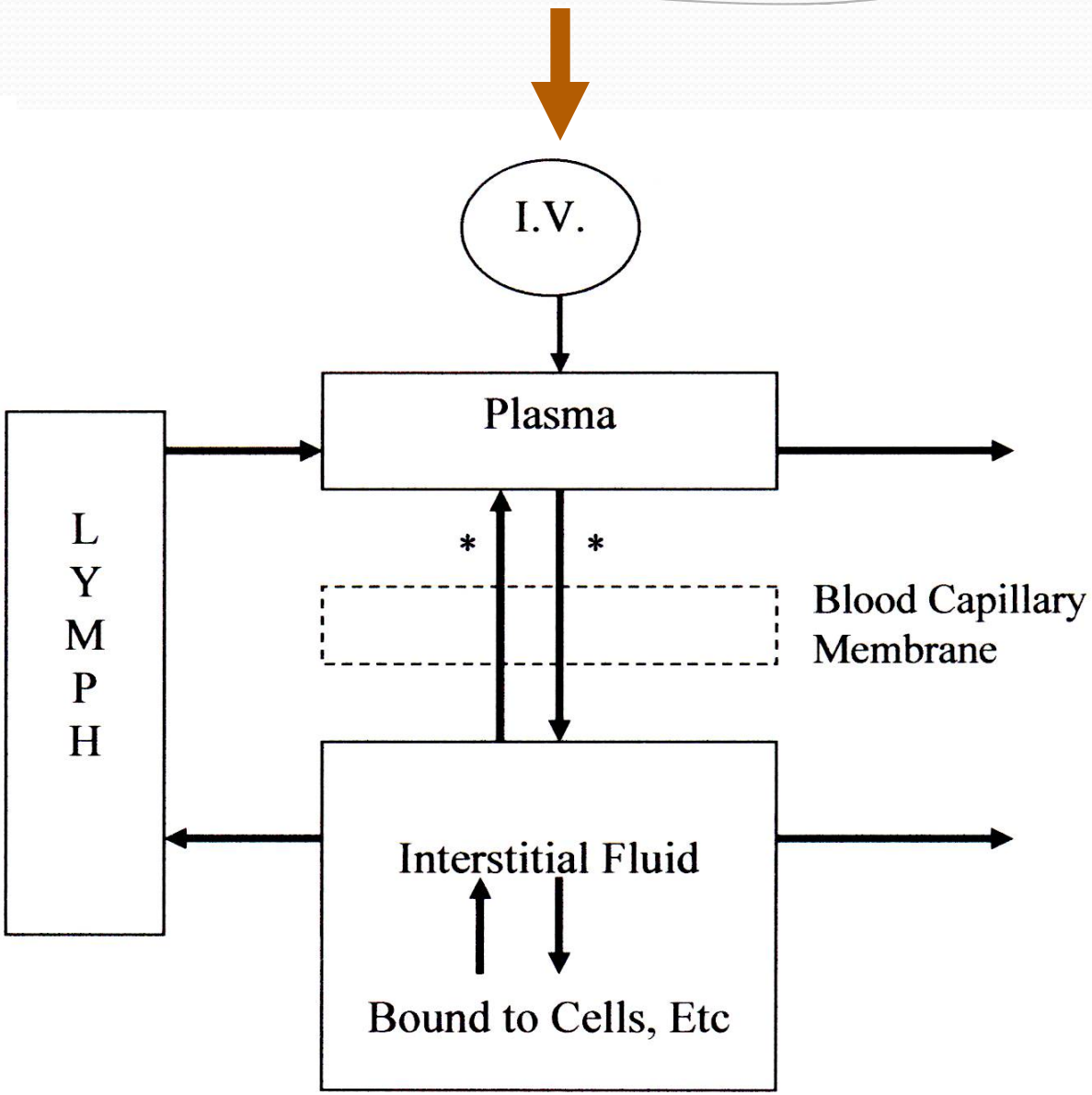
Distribution

- Comparison of Protein Drugs with Conventional Drugs

Table 5. Comparison of the Distribution of Small (M.W. < 1000 g/mol) Conventional Drugs with Large (M.W. > 5,000 to 10,000 g/mol) Protein Drugs

Conventional Drugs	Protein Drugs
• Volume of distribution varies greatly (7 – 40,000 L/70 kg. Drug can distribute to any combination of organs/tissues. Usually extensively distributed to the tissues, unless highly bound to plasma proteins and not bound in tissues.	• Usually limited to an apparent volume between that of plasma (3L/70 kg) and extracellular fluids 16 L/70 kg).
• Readily cross blood capillary membranes	• Cross blood capillary membranes slowly, which may contribute to long terminal half-life of some proteins.


I.V.
Plasma
L
Y
M
P
H
*
*
Blood Capillary
Membrane
Interstitial Fluid
Bound to Cells, Etc


I.V.
Plasma
L
Y
M
P
H
*
*
Blood Capillary
Membrane
Interstitial Fluid
Bound to Cells, Etc

Volumes of Distribution

Table 6. Volumes of Distribution of Selected Non-Antibody Proteins

Proteins[a]	V_1(L/kg)[b]	V_{ss}(L/kg
r-Activated Factor VII	-	0.08
h-Albumin	0.06	0.11
Bivalirudin	-	0.20
Cyclosporine	-	1.2
rh-Factor VIII	-	0.07
rh-Follicle Stimulating Hormone	0.06	0.16
Gonadotropin-Releasing Hormone	-	0.22
r-Hirudin	-	0.20
Human Tumor Necrosis Factor Binding Protein-1	0.06	0.14
rh-Insulin-like Growth Factor	-	0.23
rh-Interleukin-10	-	0.06
rh-Interleukin-2	0.06	0.11
Pegylated-r-Interleukin-2	0.03	0.05
rh-Soluble CD4	0.07	0.10
r-Superoxide Dismutase	-	0.10
Tenecteplase	0.07	0.12

[a]r = recombinant, h = human [b]Initial dilution space [c]Volume at steady stat

Table 7. Representative Non-antibody Protein Drugs that Bind to Other Proteins (carrier proteins) in Plasma

Cyclosporine
Deoxyribonuclease I
Growth Hormone
Insulin-like Growth Factor-I
Insulin-like Growth Factor-II
Interferon
Interleukin-2
Nartogastrim
Nerve Growth Factor
Tissue Plasminogen Activator

Table 8. Volumes of Distribution of Selected Antibodies and Antibody Fragments[a]

Proteins[a]	V_{ss} (L/70 kg)[b]
Adalimumab	4.7 - 6.0
Alefacept	6.6
Alemtuzumab	12.6
Basiliximab	8.6
Bevacizumab	3.0
Cetuximab	3.5 - 5.0
Digoxin immune Fab	6.0
Etanercapt	10.4
Omalizumab	3.6
Trastuzumab	3.0

[a]Extracted from the 2008 PDR. [b]Volume of distribution at steady state.

Tissue Distribution of Antibody Drugs

- Because of their large size, antibodies enter the interstitial space of tissues with great difficulty.
- The tissue interstitial-plasma concentration ratio is low, varying between tissues, a balance between slow transcapillary movement into the interstitial space and loss from it via the lymphatic system.
- Capillary permeability and tissue-plasma concentration ratio, tends to be higher in inflamed tissues.

Elimination

Table 9. Comparison of the Elimination of Non-Antibody and Conventional Drugs

Conventional Drug	Protein Drug
• Renal excretion	• Renal filtration often followed by tubular processing of proteins with molecular size <≈ 30,000 g/mol (metabolism, reabsorption of amino acids)
• Hepatic metabolism	• Cellular processing in hepatic cells and other tissues.
	• Conservation of amino acids

Renal Handling (Processing)

Table 10. Renal Handling of Peptides/Small Proteins (< 30,000 g/mol)

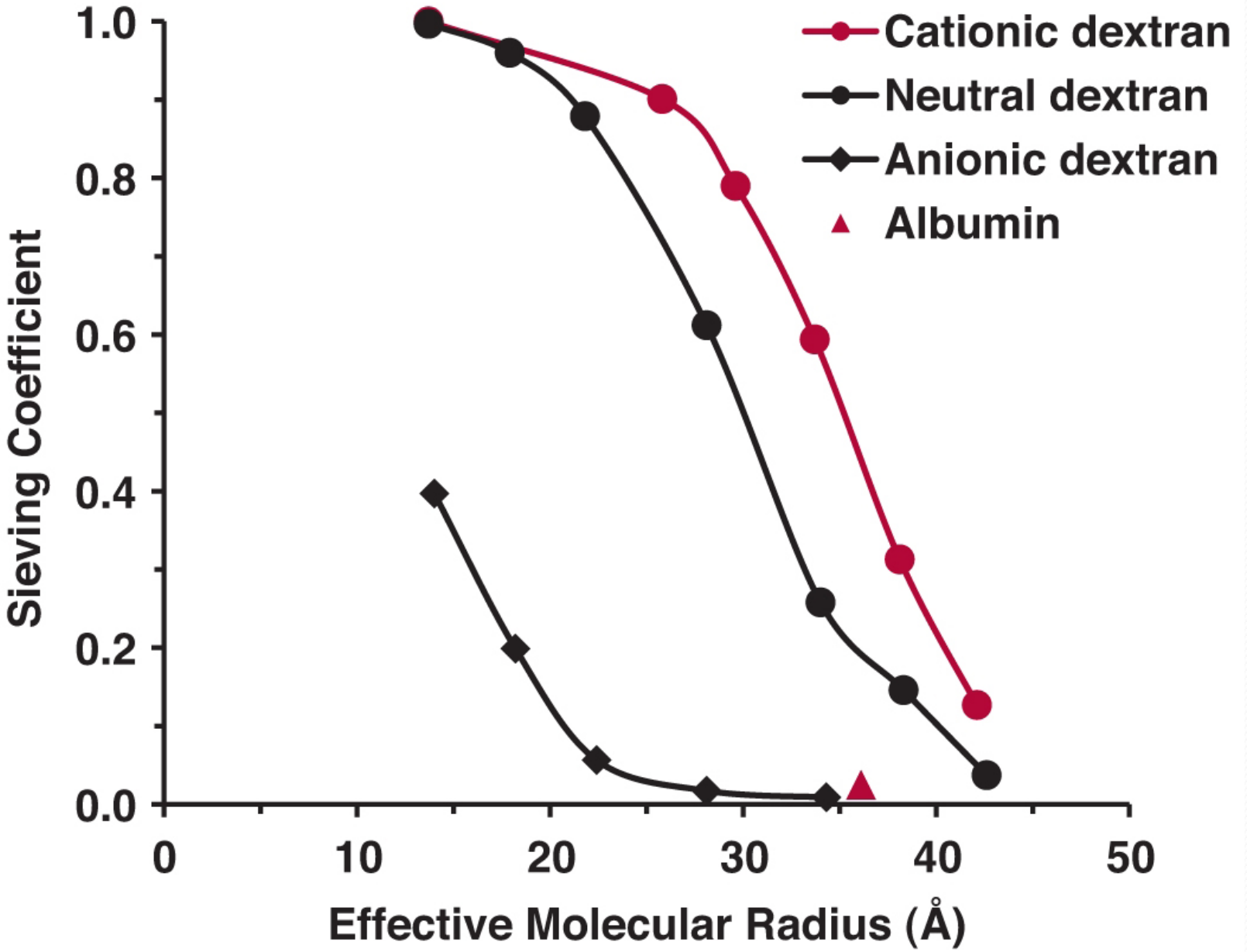
Peptide/Proteins	Location of Hydrolysis	Examples
I. Catabolism after Filtration in the Glomerulus		
Small linear Peptides (< 10 amino acids)	Luminal Membrane of Proximal Tubule	Angiotensin I and II Bradykinin, Luteinizing Hormone Releasing Hormone
Complex Peptides/Proteins	Within Proximal Tubular Cells	Calcitonin, Glucagon Growth hormone, Insulin, Oxytocin, Vasopressin
II. Peritubular Transport into Tubular Cells		
Selected Peptides/Proteins	Within Proximal Tubular Cells	Angiotensin II, Calcitonin Insulin, Interleukin-2, Parathyroid Hormone, Vasopressin

Table 11. Glomerular Sieving Coefficients of Selected Non-Antibody Proteins

Protein	Size (Da)		Glomerular sieving coefficient
Insulin	6,000		0.89
Bovine Parathyroid hormone	9,000		0.69
Lysozyme	14,600		0.75
Myoglobin	16,900		0.75
Growth Hormone	22,000		0.65
Superoxide Dismutase	32,000		0.33[b]
Horseradish	40,000	(anionic)	0.007
Peroxidases		(neutral)	0.06
		(cationic)	0.34
Bence-Jones (λ-L chain)	44,000		0.085
Albumin	69,000		0.001[c]

Factors Determining Glomerular Filtration

- Molecular size
- Charge
- Shape and rigidity
- Polymerization
- Protein binding


Cationic dextran
Neutral dextran
Anionic dextran
Albumin
Sieving Coefficient
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
Effective Molecular Radius (Å)

Metabolism - *Non-Antibody Drugs*

- Carrier-mediated membrane transport.
- Endocytosis/ Phagocytosis.

- Highly dependent on structure (including sugars), charge (density and distribution), size, and hydrophilicity-lipophilicity of compound.

- Liver is a major metabolic organ.
  One exception: For many small poly-peptides the kidney is the major metabolic organ.

Metabolism - *Antibody Drugs*

- Essentially neither excreted nor metabolized in the kidneys, although antibody fragments are filtered and metabolized in kidney.
- Speculated to be metabolized in diverse cells of body, particularly those of the reticuloendothelial system .

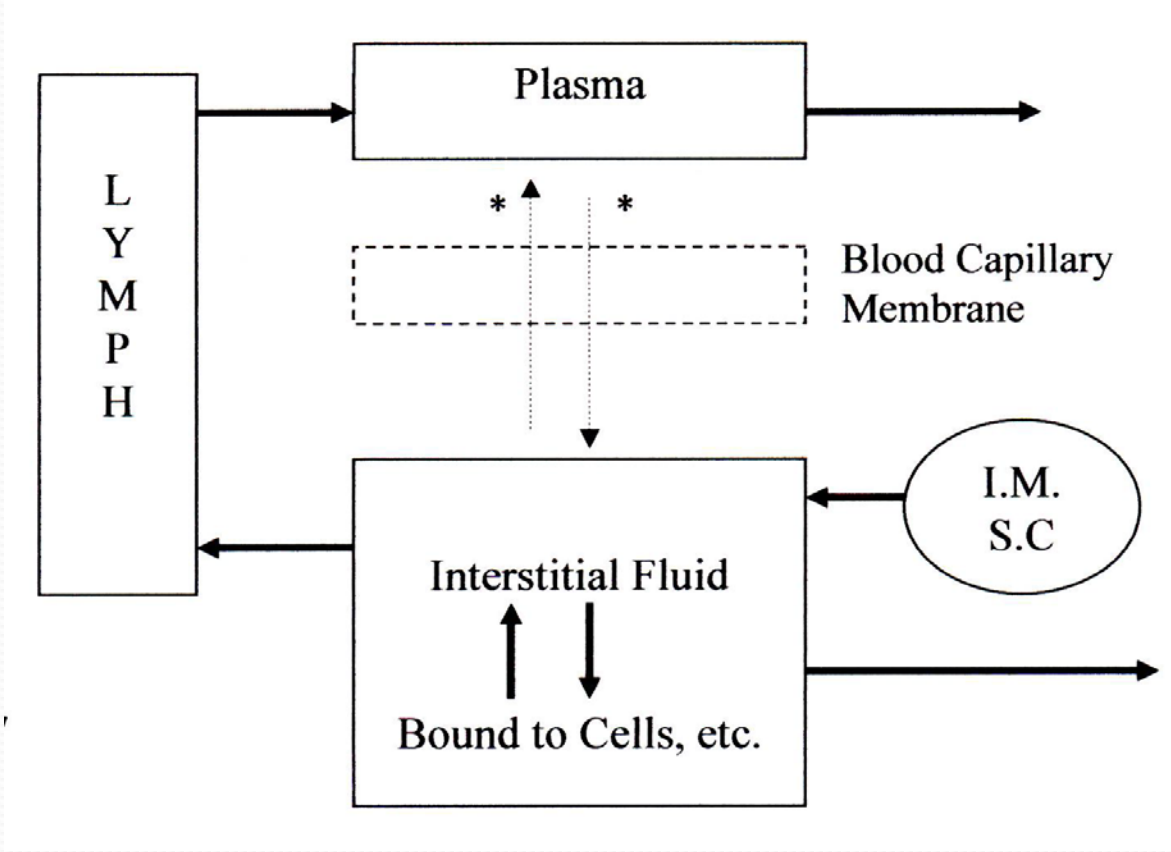
SUBCUTANEOUS AND INTRAMUSCULAR ADMINISTRATIONS

Comparison of Protein Drugs with Conventional Drugs

Table 12. Systemic Absorption of Protein Drugs Compared to Conventional Drugs Following Subcutaneous and Intramuscular Injections

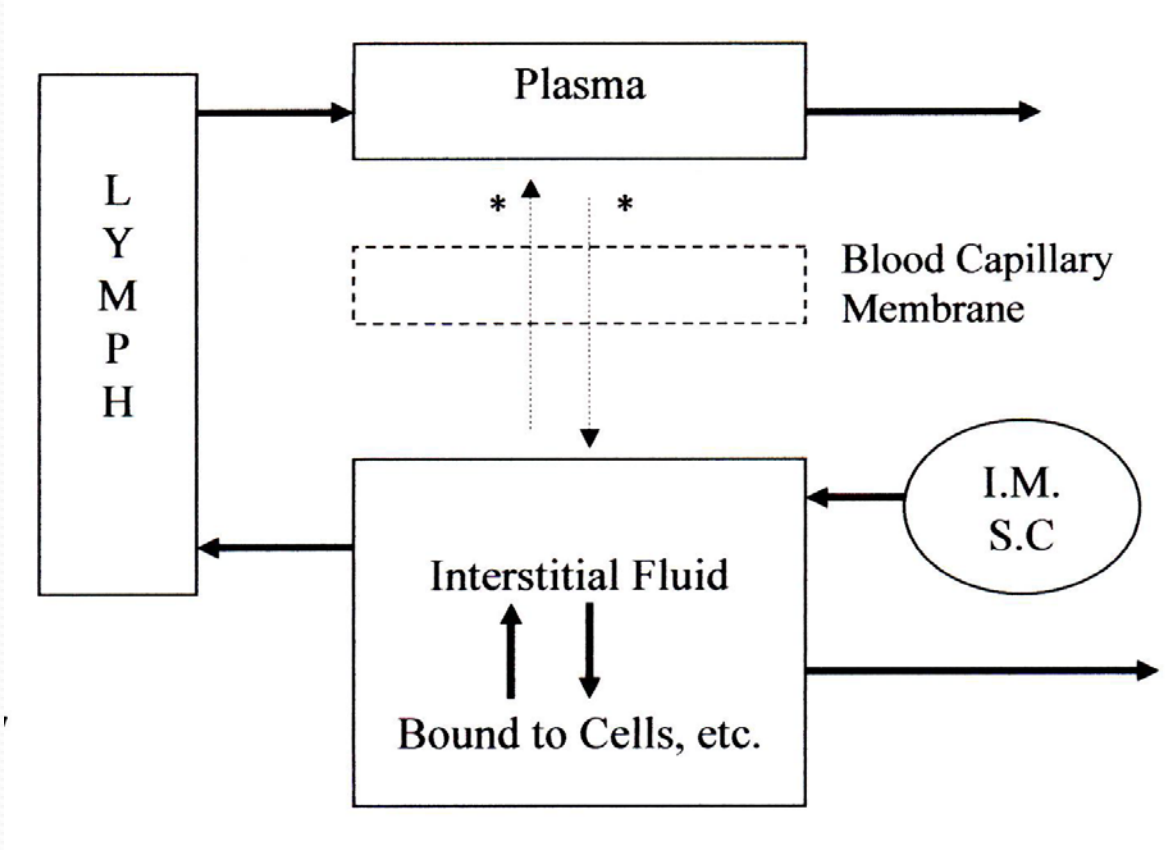
Conventional Drugs	Protein Drugs[a]
• Rapidly enter systemic circulation through blood capillaries (polarity and charge do not matter).	• Larger molecules (> 15,000-20,000 g/mol) primarily reach circulation via lymphatics.
• Systemic absorption usually almost complete (F ≈ 1.0).	• Subject to proteolysis during interstitial and lymphatic transit. First-pass loss is sometimes extensive.

Model for Systemic Absorption and Disposition



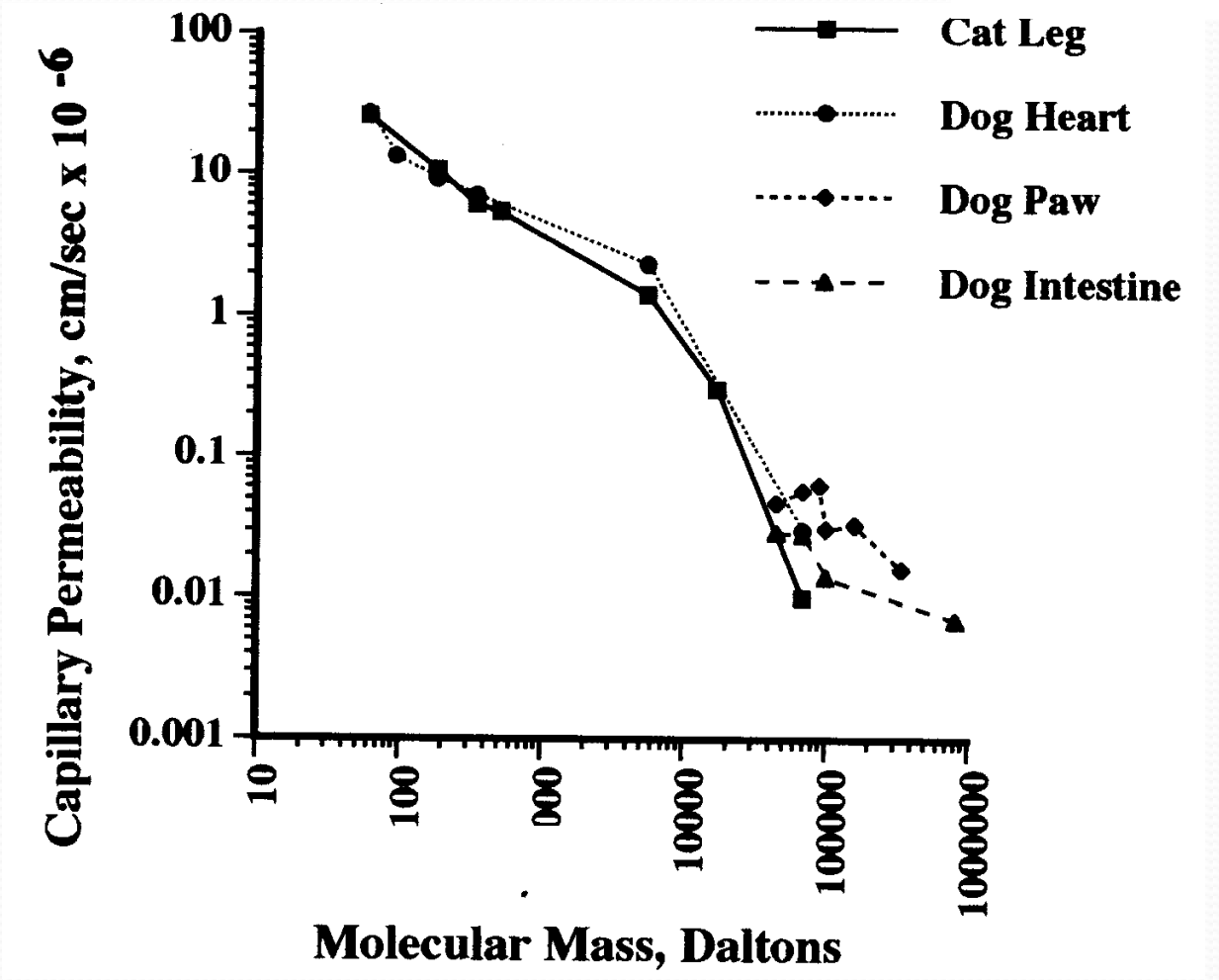
*** The rate of movement across capillary membranes is slow relative to other pathways.**

Model for Systemic Absorption and Disposition



*** The rate of movement across capillary membranes is slow relative to other pathways.**

Capillary Permeability



Extent and Rate of Absorption

Extent

Table 13. Bioavailability of Selected Non-antibody Protein Drugs

Protein	Subcutaneous	Intramuscular
rh-CD4-IgG	-	0.23
rh-Erthropoietin		
(Hemodialysis)	0.14-0.25	-
(Healthy)	0.36	-
(Hemodialysis)	0.23	-
(CAPD)	0.24	-
rh-Follicle Stimulating Hormone	0.74	0.74
rh-Growth Hormone	0.72	-
rh-Granulocyte Macrophage Stimulating Factor	0.31	
rh-Interleukin-2	-	0.37
rh-Interleukin-3	1.00	-
h-Interferon-γ	-	0.55
Lenograstim	0.30	-
Octreotide	1.00	-

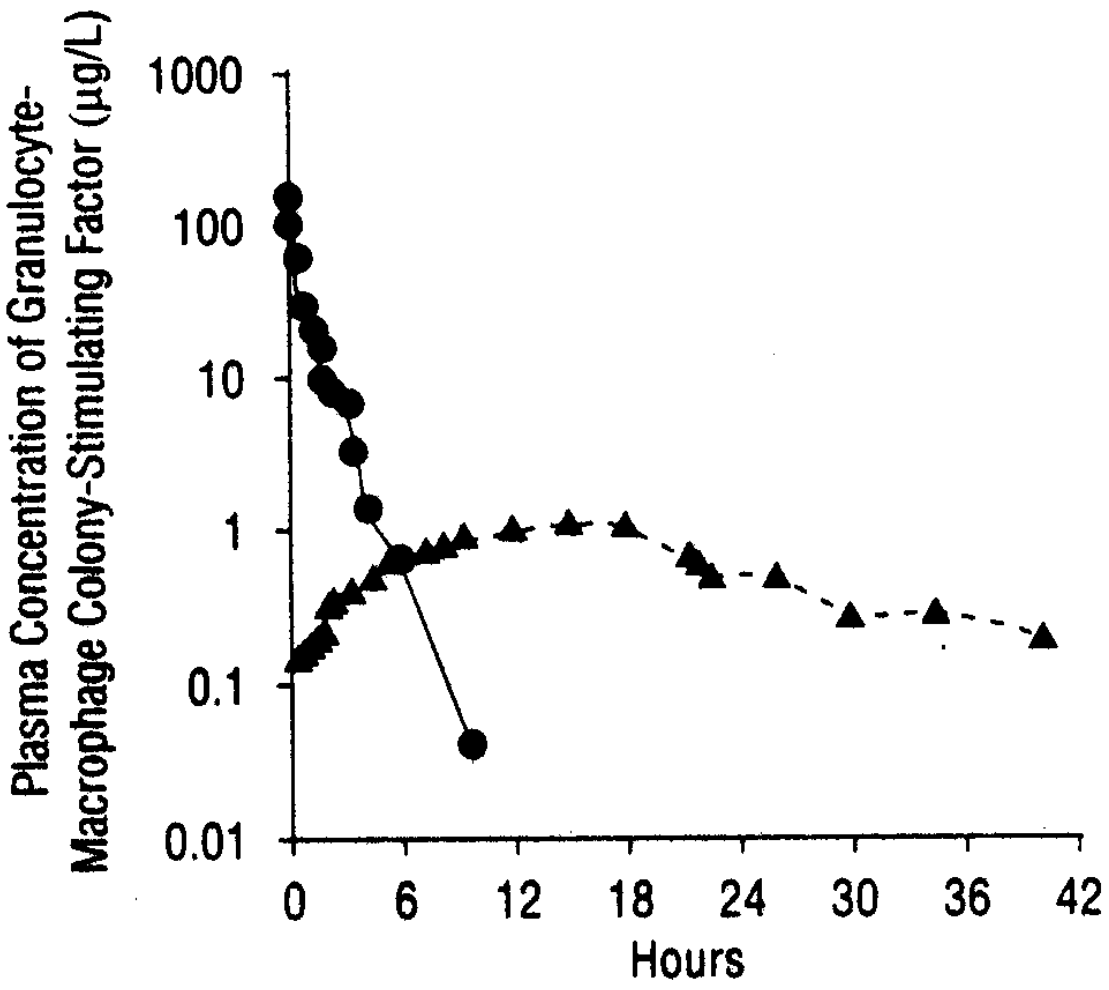
Table 14. Bioavailability of Selected Antibody Drugs[a,b]

	Route of Administration	
	Subcutaneous	Intramuscular
Adalimumab	0.64	-
Alefacept	-	0.80
Efalizumab	0.50	-
Omalizumab	0.62	-

a Most antibody products are administered intravenously.

b Degradation at injection site and during passage through lymphatics.

Rate of Absorption - *Non-Antibody Drugs*



Antibody Drugs

After subcutaneous or intramuscular administration, the peak time is typically about 4 to 8 days for antibodies.

Table 15. Bioavailability of Selected Monoclonal Antibody Drugs after Subcutaneous and Intramuscular Administrations of a Single Dose

Antibody	Weight (kg/mol)	Bioavailability	Route of Administration	Peak Time (Days)	Terminal Half-life (Days)
Adalimumab	148	0.64	s.c.	5.5	30
Alefacept	91.4	0.80	i.m. and i.v.	3.2[†]	11
Efalizumab	150	0.50	s.c.	-	17
Omalizumab	149	0.62	s.c.	7.5	26
Palivizumab	148	-	i.m.	2.0	20 (Pediatric)

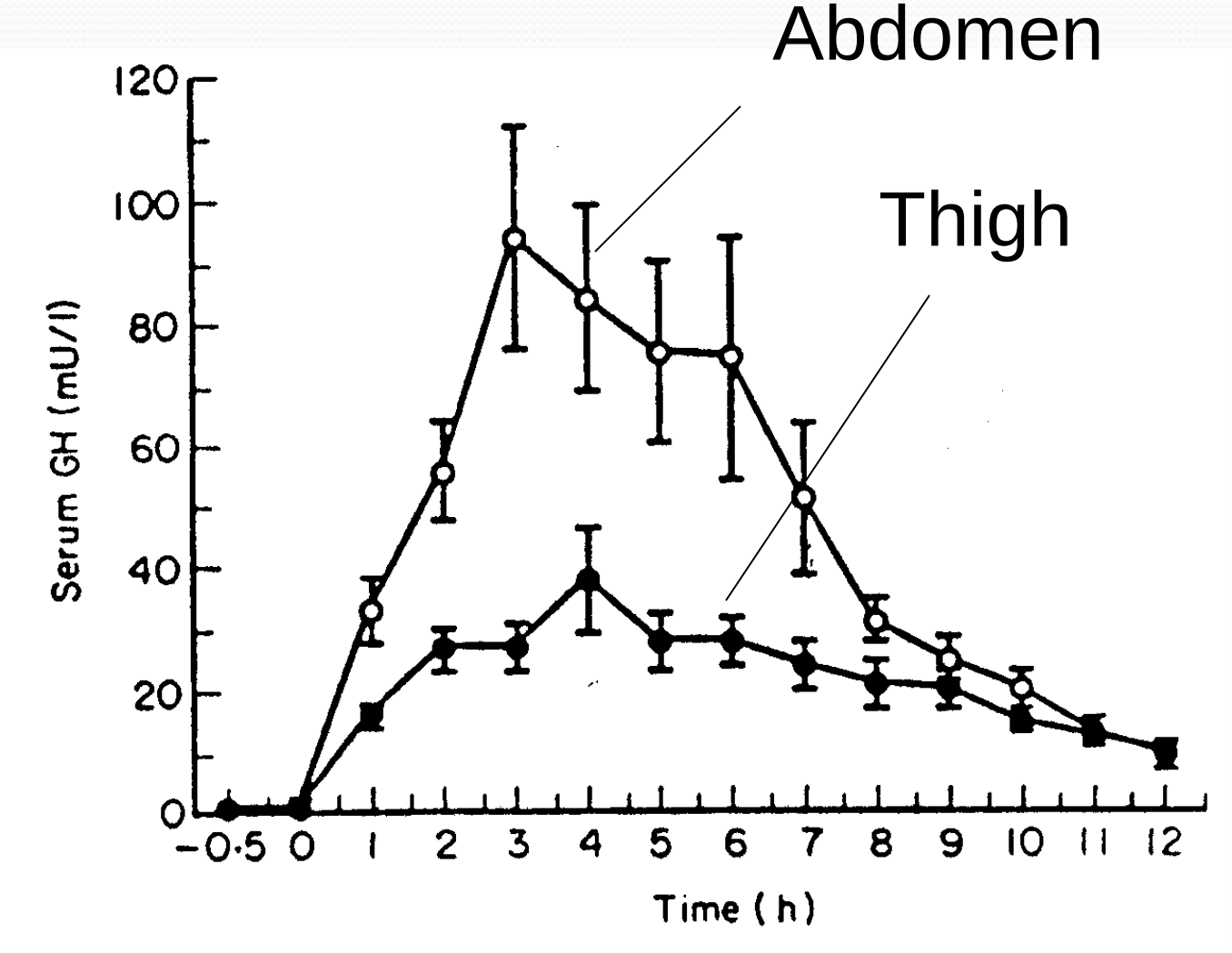
*From: 2008 Physicians' Desk Reference. Montvale, NJ: PDR; 2008.

[†]From: Sweetser MT, Woodworth J, Swan S, Ticho B. Results of a randomized open-label crossover study of the bioequivalence of subcutaneous versus intramuscular administration of alefacept. Dermatol Online J 2006;30:12(3):1.

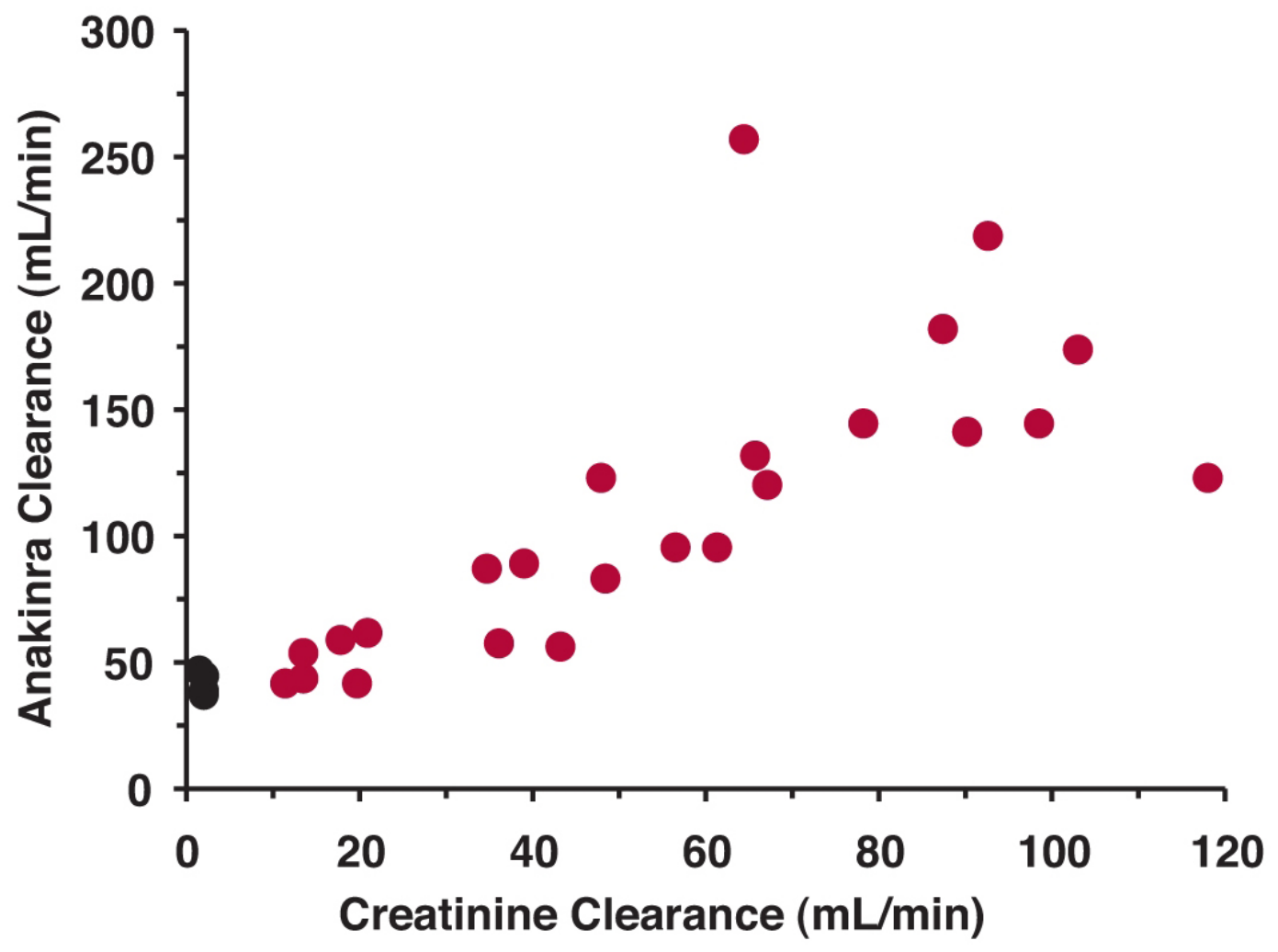
Table 16. Selected Factors Affecting Absorption

• Molecular Size	• Exercise and Rubbing
• Site of Injection	• Blood Flow at Injection Site
• Temperature	• Depth of Injection

Injection site



CONCURRENT RENAL DISEASE



Hirudin

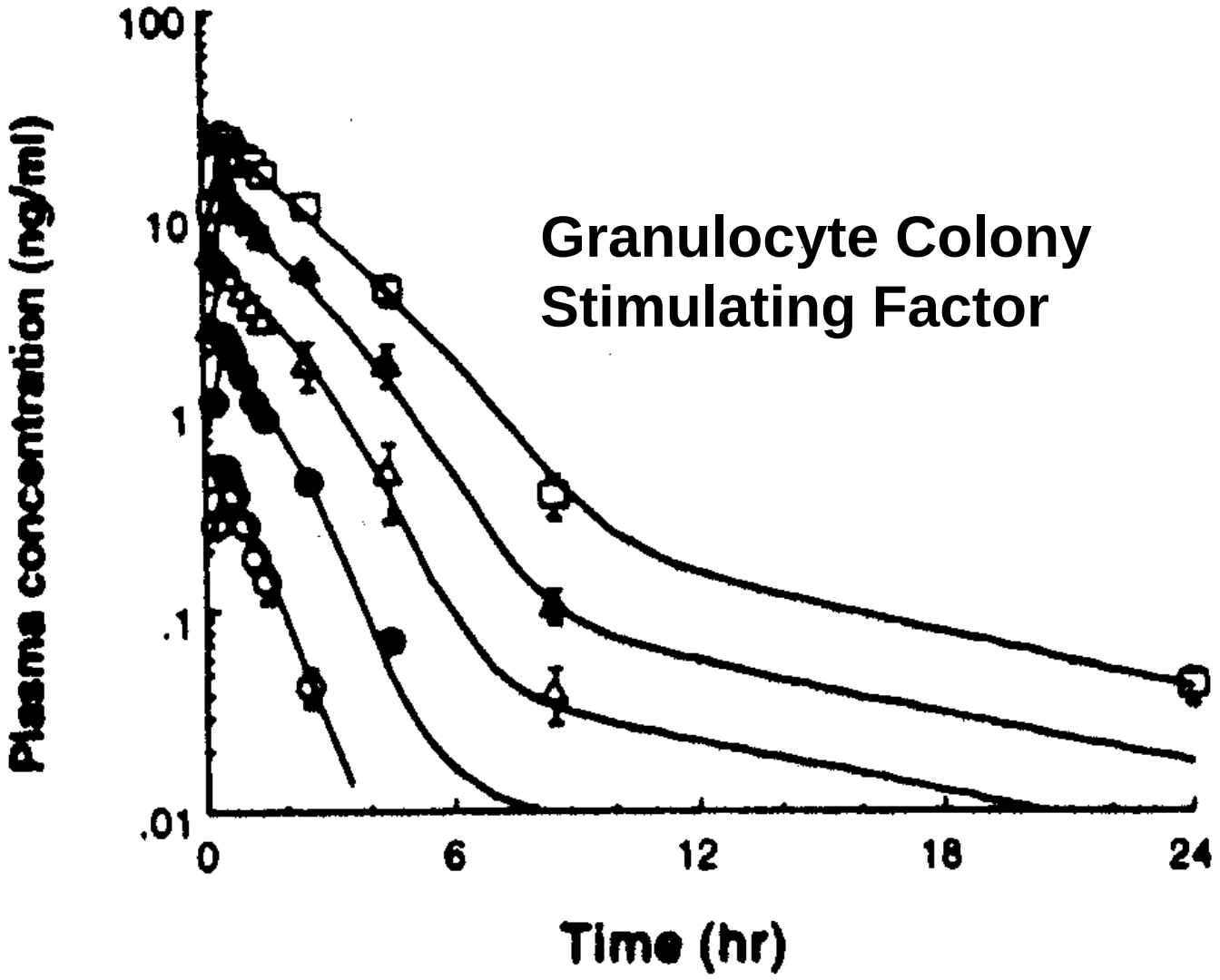
Table 15. Half-life and Fraction Excreted Unchanged of Hirudin in Healthy Volunteers, and in Patients: (1) with Pre-terminal Renal Insufficiency, (2) on Chronic Dialysis or (3) Having Undergone Bilateral Nephrectomy.[a]

	Creatinine Clearance (mL/min)	Half-life (hours)	Percent Excreted Unchanged
Healthy Volunteers (Previous studies)	> 60	0.9 ± 0.2[b] 1.7 ± 1.5[c]	38 ± 10
Pre-terminal Renal Insufficiency (N = 4)	14 ± 5	24 ± 11	39 ± 8
Chronically Dialyzed (N = 3)	< 10	33 ± 7	-
-			
Bilateral Nephrectomy (N = 2)	-	168 and 316	-

NONLINEARITIES

Target Mediated Drug Disposition

- Seen in plasma because V_{ss} is small and at nonsaturating (low) doses much of the drug is bound to high-affinity, low-capacity target (site of action).
- Seen with some small molecular weight drugs too (e.g., bosentan).
- Hence need to include fate of bound complex in PK/PD modelling.


Granulocyte Colony Stimulating Factor
Plasma concentration (ng/ml)
100
10
1
.1
.01
0
6
12
18
24
Time (hr)

Antibodies and Antibody Fragments

Saturable binding to target antigen molecules and to cell surfaces (the mechanism involved in their catabolism) often result in nonlinear kinetic behavior.

Example: Aflibercept (EYLEA)

A protein comprised of segments of two VEGF (vascular endothelial growth factor, MW ~ 40,000 g/mol) receptors fused in the constant region (Fc) of human IgG1. The drug forms complexes with VEGF, decreasing the angiogenesis produced by VEGF, and is therefore called **VEGF Trap** (MW ~ 110,000 g/mol). Currently approved for treating macular degeneration and in clinical trials for cancer treatment.

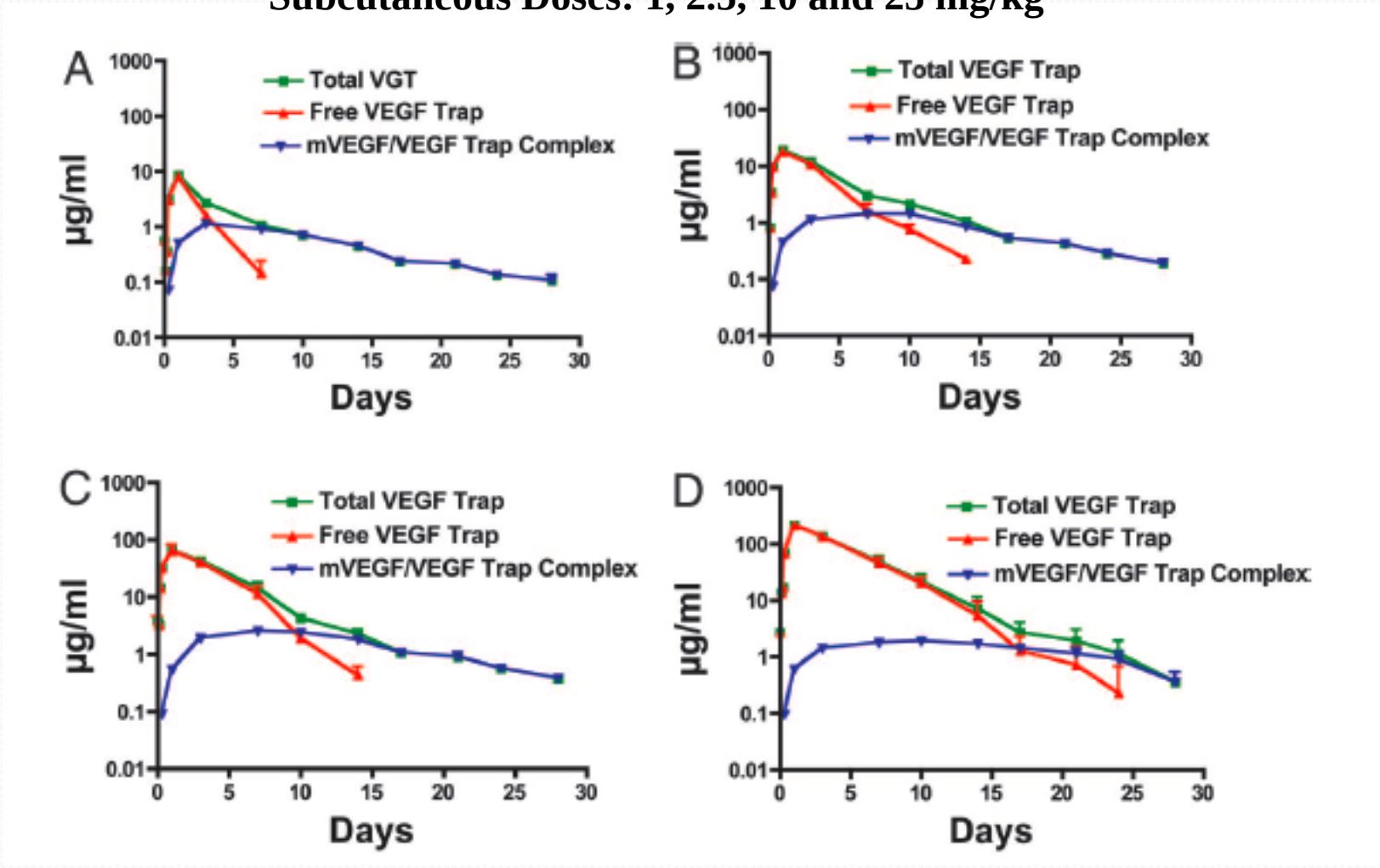
(Turnover time in minutes)

VEGF Trap + VEGF = Complex

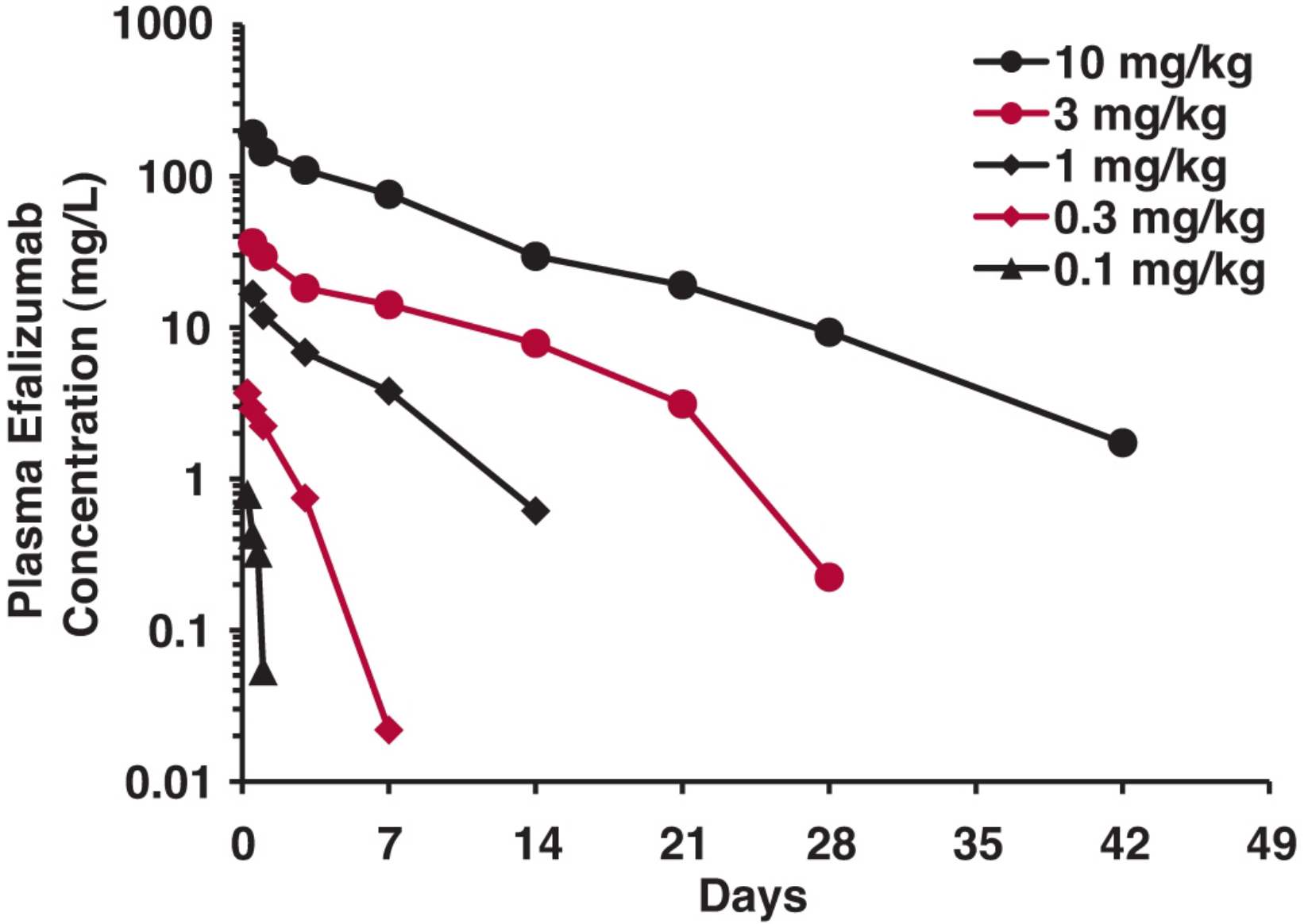
“Free” “Bound”

VEGF Trap – total, free and bound

Subcutaneous Doses: 1, 2.5, 10 and 25 mg/kg



Rudge et al. PNAS 2007


Plasma Efalizumab Concentration (mg/L)
1000
100
10
1
0.1
0.01
0
7
14
21
28
35
42
49
Days
10 mg/kg
3 mg/kg
1 mg/kg
0.3 mg/kg
0.1 mg/kg

In general, non-linear kinetic behavior tends to be the rule, rather than the exception, for both non-antibody and antibody drugs. Their kinetic behaviors are often reported as linear when data are acquired within a narrow range of therapeutic doses. Half-lives and clearances are given for most of them, but care must be taken in using these values.