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Title: Biokinetic Models Estimating Rapid Diffusion Using a Priori Knowledge of Flow Rates and Compartment Volumes

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Biokinetic Models Estimating Rapid Diffusion Using a Priori Knowledge of Flow Rates and Compartment Volumes

With a discussion of the possible role of transferrin in Pu metabolism

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Extracellular Fluids

References:

Pierson Jr RN, Price DC, Wang JA, Jain RK. Extracellular water measurements: organ tracer kinetics of bromide and sucrose in rats and man. Am J Physiol Renal Physiol 1978; 235(3):F254-64.

Miller G, Klumpp JA, Melo D, Poudel D. The role of extracellular fluid in biokinetic modeling. Health Phys 2017; 113(6):519-26.

Extracellular Fluids

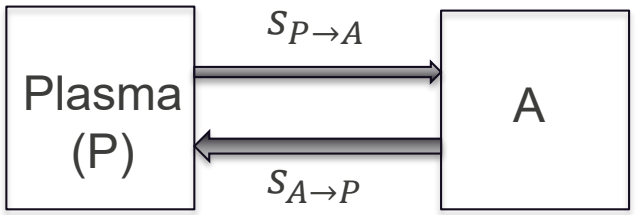
- Intravascular (blood plasma and lymph), interstitial, and transcellular (cerebrospinal fluid and synovial fluid)
- Blood plasma (~4% total body mass in rats) leaks through capillary walls to provide nutrients to cells
- The fluid bathing the cells is called interstitial fluid (~28% rat body mass)
- Only explicitly model blood plasma and interstitial fluid
- Excess interstitial fluid is gradually returned to blood plasma via lymph – this is likely important for plutonium (not explicitly modeled)
- We refer to the interstitial fluid and transcellular fluid in a particular organ as that organ's ECF (i.e., the interstitial fluid plus plasma in the bone is called "bone ECF")

Diffusion Across Vascular Membranes

- Small molecules (e.g., Am-citrates) rapidly diffuse across cellular membranes into interstitial fluids
- Diffusion occurs on a much shorter timescale (i.e., minutes) than most measurements
- Quasi-equilibrium between blood plasma and interstitial fluids in a given organ or tissue is attained when the concentration in blood plasma is equal to the concentration in interstitial fluid
- Diffusion rates obtained from a priori PK information (flow rates and volumes)
- Transport rates into and out of cells (mediated by cellular membranes) determined empirically

$$s_{A\to B}(\min^{-1}) = \frac{F_{A\to B}(ml/\min)}{V_A(ml)}$$

Rapid Diffusion Equations



- Transport rate from plasma to an ECF compartment of an organ, say A ($s_{P\to A}$) , is a function of organ ECF compartment is a function of:
  - -the amount of material in the source compartment (P)
  - -the volume of the source compartment (V_P)
  - -the flow rate of plasma through the organ ($F_{P\to A}$)

- $s_{P\to A}(\mathrm{min}^{-1}) = \frac{F_{P\to A}(\mathrm{ml/min})}{V_P(\mathrm{ml})}$

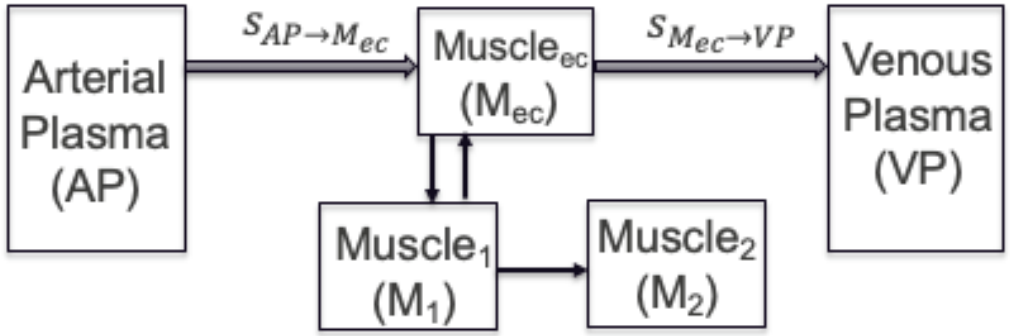
- $\frac{dA}{dt}\left(\frac{\mathrm{Bq}}{\mathrm{min}}\right) = P\ (\mathrm{Bq}) \times\ s_{P\to A}(\mathrm{min}^{-1}) - A(\mathrm{Bq})\ \times s_{A\to P}\,(\mathrm{min}^{-1})$

$$= C_P\left(\frac{\mathrm{Bq}}{\mathrm{ml}}\right) \times F_{P\to A}\left(\frac{\mathrm{ml}}{\mathrm{min}}\right) - C_A\left(\frac{\mathrm{Bq}}{\mathrm{ml}}\right) \times F_{P\to A}\left(\frac{\mathrm{ml}}{\mathrm{min}}\right)$$

- Note that equilibrium is achieved when $C_P = C_A$

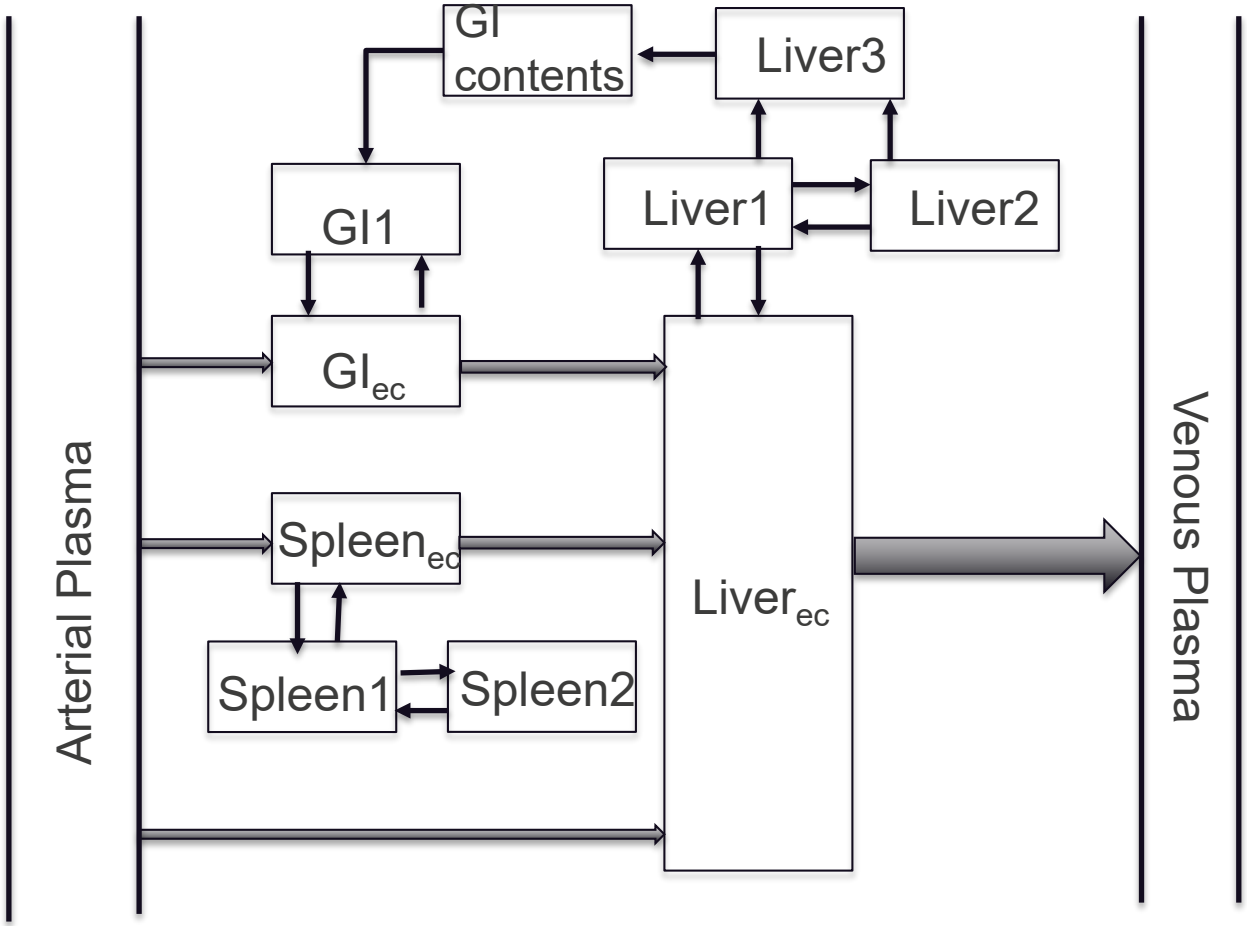
$$s_{A\to B}(\min^{-1}) = \frac{F_{A\to B}(ml/\min)}{V_A(ml)}$$

Adding Complexity…



- $s_{AP\to M_{ec}}(\min^{-1}) = \frac{F_{AP\to M_{ec}}(\mathrm{ml/min})}{V_{AP}(\mathrm{ml})}$
- $s_{M_{ec}\to VP}(\min^{-1}) = \frac{F_{M_{ec}\to VP}(\mathrm{ml/min})}{V_{M_{ec}}(\mathrm{ml})}$
- $\frac{dM_{ec}}{dt} = C_{AP} \times F_{AP\to M_{ec}} - C_{M_{ec}} \times F_{M_{ec}\to VP}$ + (much slower exchange with M1)
- Quasi-equilibrium is achieved when $C_{AP} = C_{M_{ec}} = C_{VP}$

Subsystems Linked by Plasma Flow



Pharmacokinetic Parameters for Rats

- We call transfer rates obtained in this way the “pharmacokinetic front end”

Organ	Vol (ml)	ecVol (ml)	Flow (ml/min)
Plasma	13.4	13.4	
RBC	10.05		
Muscle	137.35	15.779	5.03
Skin	63.148	31.222	3.35
Adipose	23.45	2.982	3.3
Connective tissue	17.42	12.194	0.985
Skeleton	16.75	7.035	0.948
Transcellular water	14.07	13.4	0.796
Liver	13.065	2.01	10.8
Marrow	4.02	2.211	0.227
Small intestine	3.786	2.077	4.44
Other organs	3.786	1.139	0.214
Gonads	3.35	1.039	0.19
Kidney	2.446	0.938	8.38
Large intestine	2.211	0.67	2.593
Brain	1.675	0.201	1.36
Stomach	1.575	0.871	1.847
Lung	1.34	0.603	44.93
Spleen	1.005	0.027	0.42
Heart	0.67	0.369	0.72
Thyroid	0.335	0.067	0.24
Total	334.9	108.2	90.8

Organ volumes for 335g rat obtained from columns 1 and 3 of Table 2 of Pierson *et al.* (1978). The ECF volume and the vascular flow rates to and from the organ are also given.

Application to an Americium Rat Model

Reference:

Miller G, Klumpp JA, Poudel D, Weber W, Guilmette RA, Swanson J, Melo DR. Americium systemic biokinetic model for rats. Radiation research. 2019 Jul;192(1):75-91.

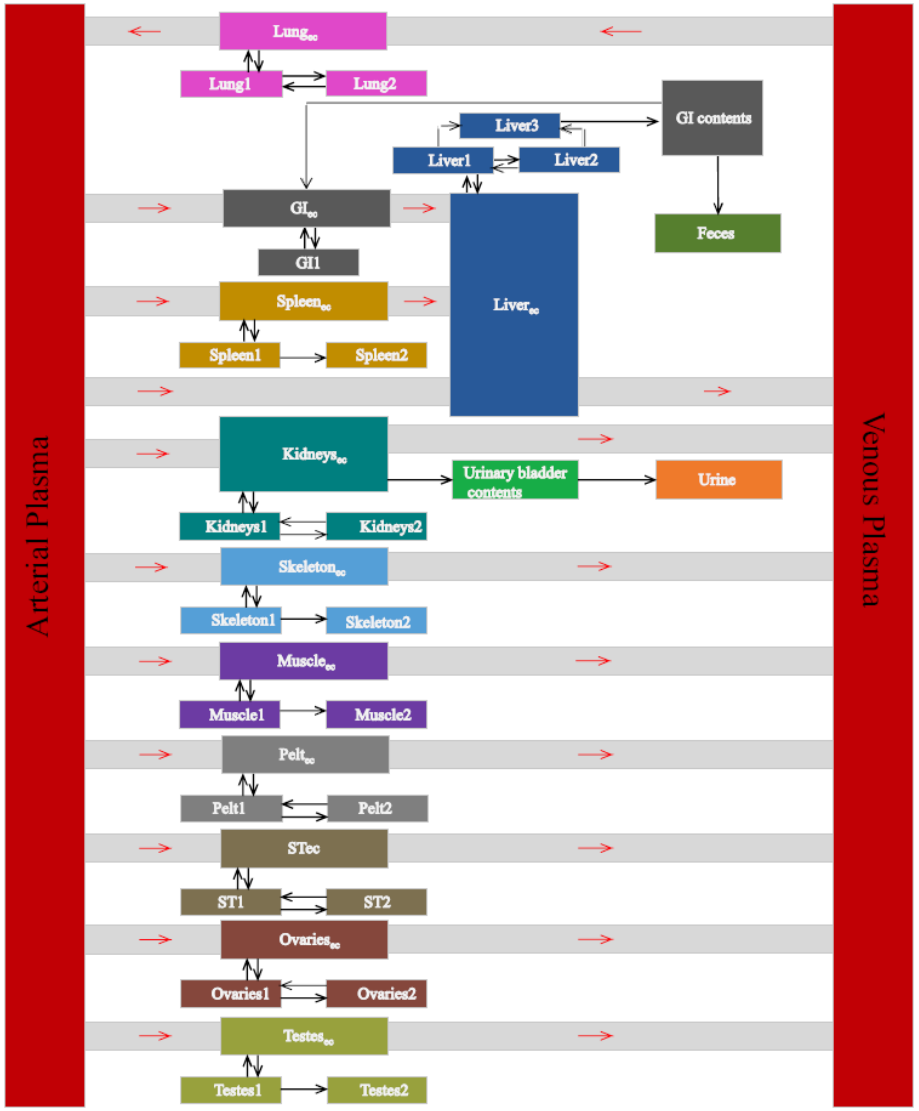
Application to an Americium Rat Model

- Diffusion rates in rats initially determined with bromide
- In theory it should be material-agnostic, since it depends on passive transport and diffusion. But is it?
- We developed an Am biokinetic model which incorporates front-end transfer rates for all available organs and tissues
- We are free to optimize transfer rates from ECF into cells, and within the cellular components of an organ, but the front-end transfer rates (from blood plasma to ECF) are determined a priori and fixed

Rat Data

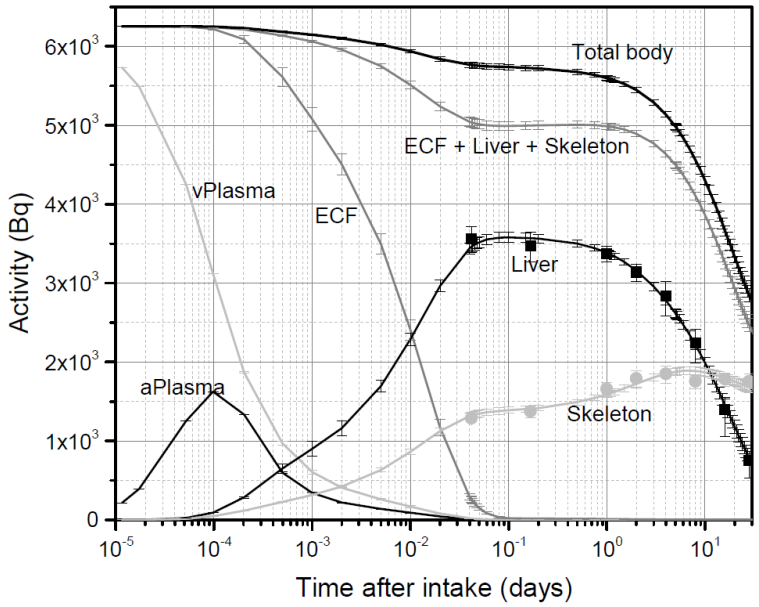
- We obtained a large dataset from LRRI – 25 male and 23 female F344 rats
- Animals were injected with Am-citrate, sacrificed at 1 hour, 4 hours, 1 day, 2 days, 4 days, 8 days, 16 days, and 28 days post exposure
- Urine and feces were collected. At sacrifice, blood, liver, spleen, kidneys, lungs, GI tract, muscle, ovaries, testes, bone, pelt, and soft tissue remains, plus urine and feces, were collected for Am radioactivity analysis (14 measurements x 8 time points = 112 measurements)
- Note that the measured compartments consisted of extracellular and intracellular americium
- Early sacrifice times were sufficient to test the assumption of rapid diffusion

Model Structure



Am Model fit to Data

- The data were consistent with the assumption of rapid diffusion
- The model was able to represent a rich dataset of measurements
- This model can be used as the base model for a subsequent analysis of decorporation with DTPA
- Can we do the same thing with Pu?



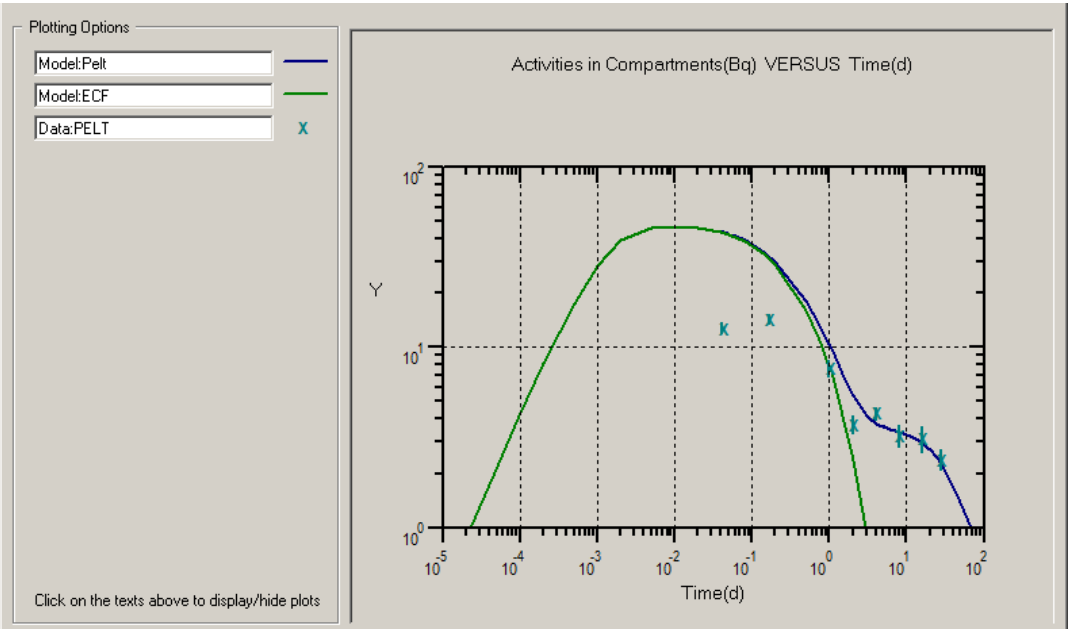
Application to a Plutonium Rat Model

References:
Durbin PW, Horovitz MW, Close ER. Plutonium deposition kinetics in the rat. Blood. 1972 Jun 1;60(52):48.

Miller G, Klumpp JA, Poudel D, Dumit S, et al. Unpublished work.

Front-End Parameters for Pu

- We obtained a similarly rich dataset for plutonium in rats
- Attempted to fit that data using the same front-end parameters
- Rapid dilution into organ ECF predicts minimum activities in organs and tissues at early times – this prediction is inconsistent with measurements of Pelt



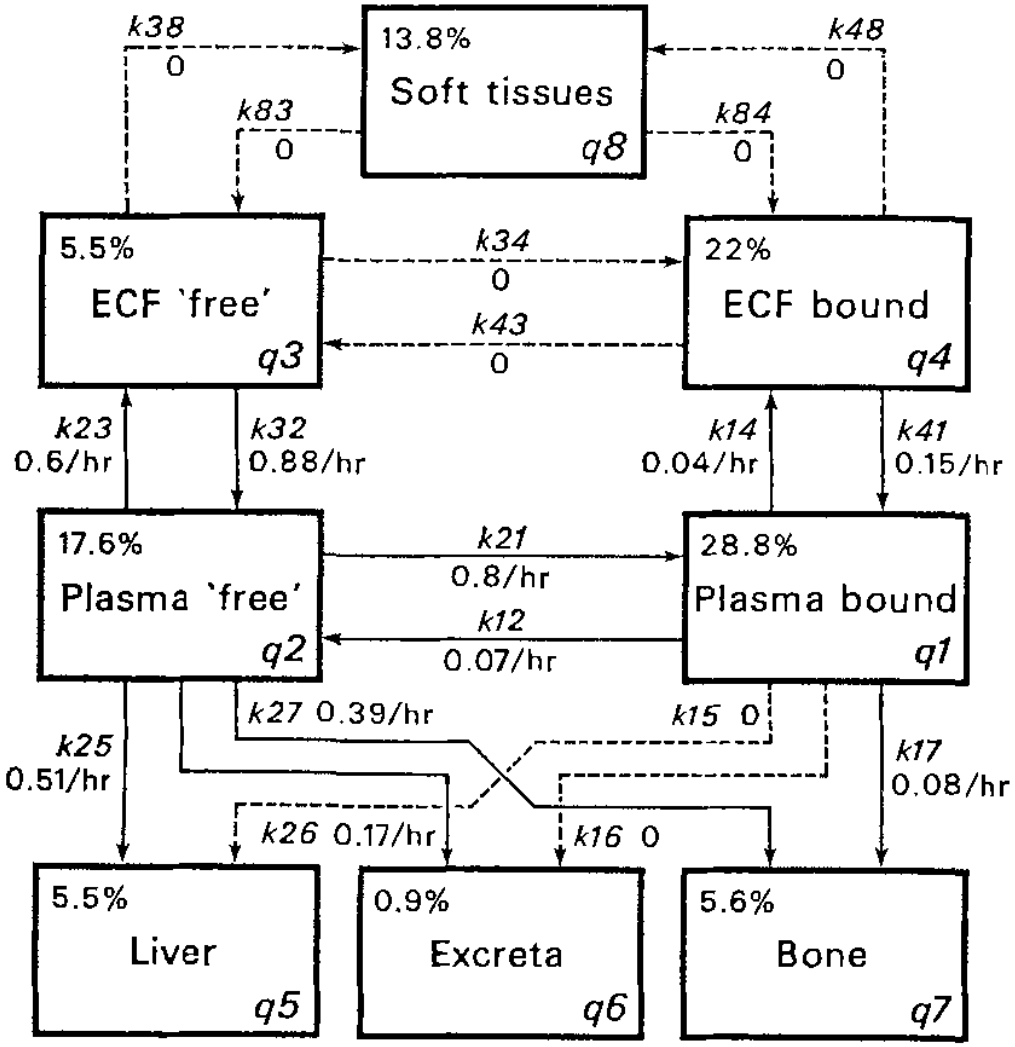
Pu Data Inconsistent with Rapid Diffusion Across Vascular Membranes – Should we be surprised?

- Rapid diffusion across cell membranes only occurs for small molecules
- But it has long been known that Pu rapidly forms complexes with transferrin (Tf) – a protein which brings iron into cells
- In 1972, Durbin showed that protein-bound Pu (hereafter, “bound Pu”) forms very rapidly
- 95% of circulating Pu is protein-bound 7 days after injection

Early observations about bound Pu (Durbin 1972)

- Durbin found that unbound (“free”) Pu in blood plasma binds to Tf at approximately the same rate that it diffuses into the ECF
- Slow diffusion into ECF, more rapid return back to blood plasma (likely via the lymph)
- Slow dissociation back to unbound Pu, likely occurring at surface of reticulocytes (immature red blood cells)
- Bound Pu has no cellular uptake except by bone (possibly due to high abundance of erythroblasts in bone marrow)

The Durbin Model of Bound and Unbound Plutonium



The Role of Transferrin in Metabolism of Iron and Pu

References:
Jensen MP, Gorman-Lewis D, Aryal B, Paunesku T, Vogt S, Rickert PG, Seifert S, Lai B, Woloschak GE, Soderholm L. An iron-dependent and transferrin-mediated cellular uptake pathway for plutonium. Nature chemical biology. 2011 Aug;7(8):560.

The role of transferrin in iron metabolism

- Transferrin (Tf) is a protein which brings iron into cells
- Tf binds Fe^{3+} (and Pu^{4+}) at pH 7.4 (normal blood pH)
- Tf takes iron to spleen, liver, or skin – (Muscle uses lots of iron) – but most (~80%) goes to bone marrow
- Tf lives 100 – 120 days. At the end of its life it gets eaten by macrophages in spleen
- Iron gets passed along to new Tf in spleen

The role of transferrin in iron metabolism

- Tf has two lobes (C and N), each of which binds an iron atom
- When both lobes close around an iron, Tf can be recognized by the transferrin receptor protein (TR) on the cellular surface
- Other configurations (such as only one iron atom) cannot be recognized by TR
- Fe-Tf dissociated in bone marrow by erythroblasts (immature red blood cells) – a similar pathway may be the reason for elevated bone uptake of Pu

Iron Homeostasis

- The body needs lots of iron, is entirely dependent on replenishment by diet, but can store iron in cells
- Excess iron gets stored in pools of ferritin called hemosiderin, which is found in cells throughout the body (but especially in the gut and liver)
- Ferritin-bound iron can escape cells via ferroportin (‘iron door’)
- If the body wants less iron, the liver creates a hormone called hepcidin which ‘locks’ the door (deactivates ferroportin); iron gets trapped in duodenal cells (in ferritin)
- Duodenal cells eventually desquamate and iron is excreted via feces. (Same can happen with iron in skin)
- If body wants more iron, it can produce less hepcidin
- When red blood cells are formed they release a hormone telling liver to make less hepcidin

Transferrin to cell cycle

- Transferrin receptor (TR) is on both sides of a cell membrane
- When it binds to Fe-Tf, an endosome (bubble) forms around the whole thing and enters the cell
- H^+ gets pumped into endosome, drops pH to around 5
- At pH 5, Tf releases iron (Tf-Pu dissociates at this Ph as well)
- Fe^{3+} can't escape endosome – a protein is needed to convert it to Fe^{2+}
- Fe^{2+} gets into the cell via Divalent Metal Transporter 1 (DMT-1); DMT-1 also responsible for iron absorption in gut

Transferrin to cell cycle

- Endosome merges back into cell membrane. Tf and TR are returned to blood
- At this point iron gets bound to ferritin or, if cell wants to use the iron for itself, it gets taken into mitochondria
- If the cell is an erythroblast (baby red blood cell), mitochondria incorporate iron into heme (at which point it becomes hemoglobin).
- Erythroblasts eject mitochondria before becoming mature red blood cells. Mitochondria are then eaten by macrophages
- Most iron in body is recycled from red blood cells by macrophages in spleen

The role of transferrin in Pu metabolism

- Early research suggested Pu-Tf complexes are not taken into cells – Pu is not found in red blood cells
- However, one isomer of monoplutonium-monoiron-Tf is active for cellular uptake – Pu_CFe_NTf.
- The other configurations – Pu_2Tf and Fe_CPu_NTf – only bind TR with much lower affinity (effectively zero under physiological conditions)
- Jensen et al found that Tf transports iron into animal adrenal cells (motivated by high adrenal uptake in humans)
- Pu is not found in erythrocytes (mature red blood cells) – why not? Maybe Pu cannot escape the endosome, or maybe it is ejected by mitocondria
- Other pathways for unbound Pu uptake probability still exist, as they do for iron

Summary

- Small molecules rapidly diffuse across vascular membranes
- These “front end” transfer rates are obtained using PK information
- Front end rates should be material-agnostic, provided the molecules are small
- These front end rates were consistent with a large americium rat experiment
- The same front end rates are not consistent with Pu, much of which must not diffuse rapidly across vascular membranes
- The difference in behavior is likely due to binding of Pu with transferrin

Thank you!

Questions?

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