



# Data Mining for Beryllium Toxicity

- *Underutilized Decision Resources* -

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Hello, I'm Tom from Kansas City and  
this is our race (board) track circa 1922



# Pratt & Whitney 'Double Wasp' WWII vintage



# Bannister Federal Complex -KC



## HSWG Objectives – & Mining correlates

- 1) *Accumulating & disseminating* information concerning *Be* process best work practices, as well as, data from health studies concerning hazards associated with *Be*;
- 2) *Obtaining* a better understanding of exposure risks;
- 3) (*Better appreciating*) illnesses ...caused or exacerbated by workplace exposure to *Be*.

# Historical Aspects of Discovery

## *Bioinorganic Chemistry & Immunology*

- Multidisciplinary approach using detailed *bioinorganic chemistry and immunology*;
- *How do those (2) terms define your craft?*
- Hx background of *Be*-exposure & disease, followed by occurrence of *Be* in environment, toxicokinetics, biological effects, *Be* lung disease & other human health effects.

Living on the edge of *Noble*, often *Toxic*, metals

- How should we go about evaluating relatively *rare metals* used as super alloys & in machine tools & catalysts?
- *Tungsten* would be an example – *Be*, too;
- In one sense, *Tungsten* commonly used in surgical implants, in another may cause problems, e.g., 'Green' bullet.

# Controlled medical terminologies (*CMTs*)

Important tools in a variety of medical informatics applications, ranging from patient-record systems to decision-support;

*CMT's* typically organized in semantic network structures consisting of 10s - 100s of thousands - concepts;

Overwhelming size & complexity - serious barrier to their maintenance & utilization;

*Objective-oriented* databases (the *OOHTR*) address problems posed by extensive scope & high complexity;

Objective oriented health care terminology repository.

What is data mining anyway?

*Ontologies* allow unification of language

- Galen,
- Unified Medical Language System (UMLS),
- Medical Entities Dictionary (MED),
- SNOMED-CT, LOINC,
- Foundational Model of Anatomy (FMA),
- Gene Ontology (GO),
- NCI Thesaurus, &
- DOLCE+;
- *Standard vocabulary – 10 CFR is not a std. vocabulary.*

# Powerful toxicants have found their way *Into modern medical practice*

- *Thalidomide* – Original problem birth defects  
– Modern use – cancer treatment;
- *Botulinum toxin* – Original problem serious food poisoning – Modern uses torticollis, spasticity, neck pain, many others;
- *Noble metals* – Somewhat time-reversed experience as implants but lingering concerns regarding toxicity (Tungsten, titanium...);
- *Trojan horse concept.*

# Why *Be*, Why Now?

Highly pure antigens – Possibly improve vaccine safety;

*Adjuvant research* historically lagged behind other Vx research such as antigen discovery;

Very limited number of adjuvants (*Aluminum salts, monophosphoryl lipid A & oil emulsions* currently approved);

*Be* considered an ideal adjuvant, maximizing vaccine immunogenicity s/ compromising tolerability or safety;

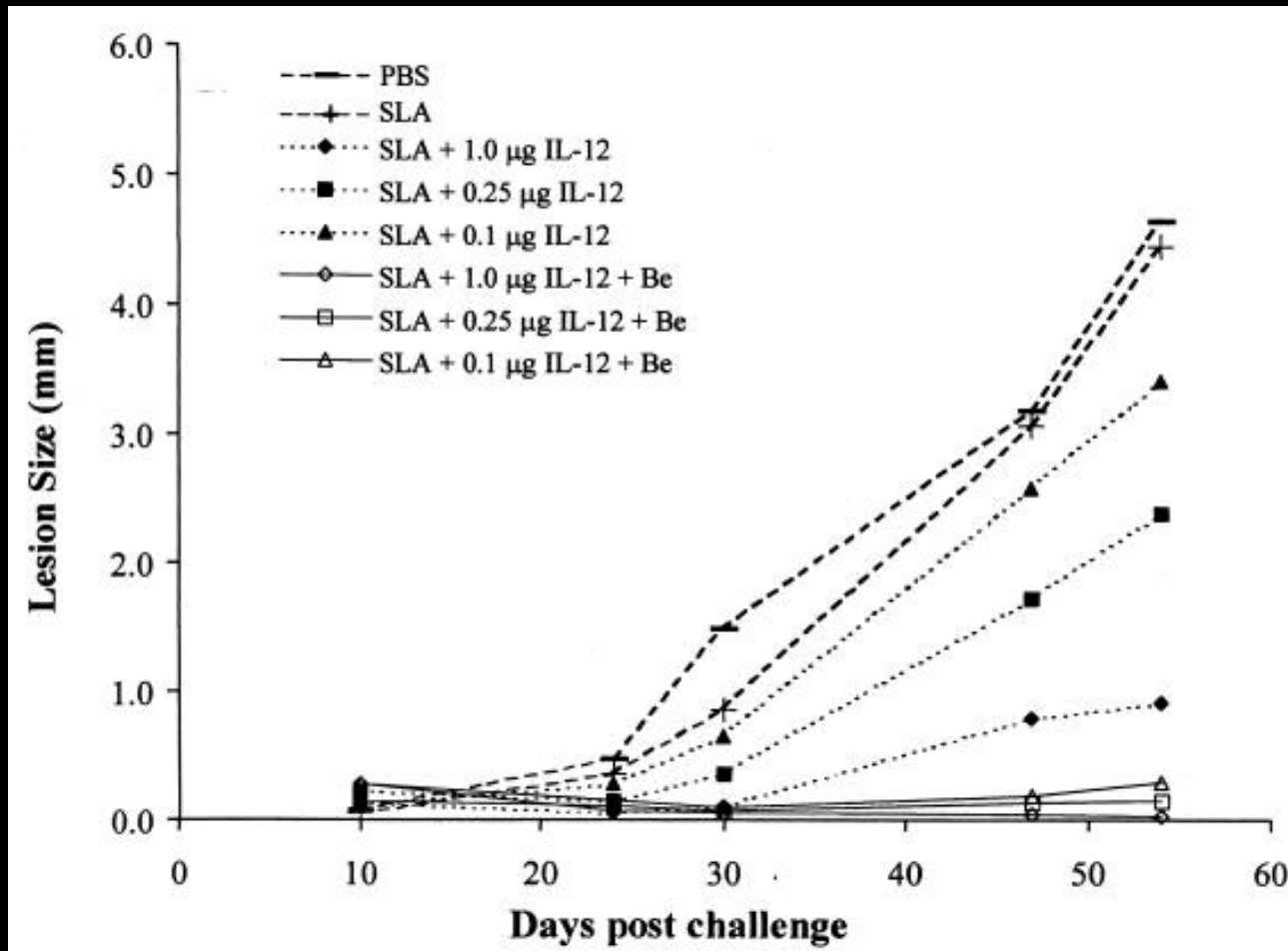
*BeSO<sub>4</sub> is on the radar screen for adjuvant use.*

## *Pulmonary Granulomatosis* or *Vaccine Adjuvant*?

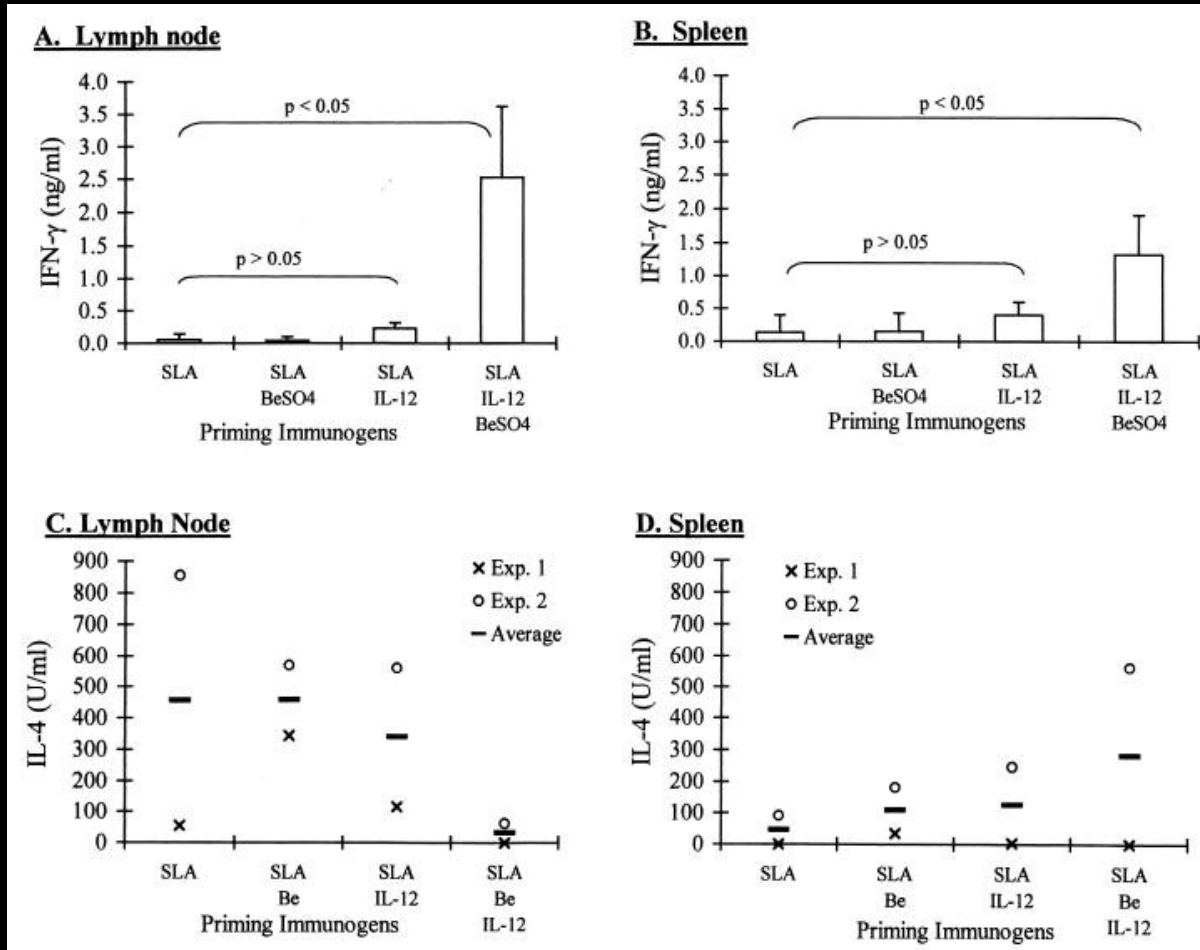
- Be assoc. c/ pulmonary granulomatosis char. by accum. of CD4(+) T cells in lungs & *heightened specific lymphocyte proliferative response* (LPR) to Be c gamma interferon (*IFN-gamma*) release (T-helper1 [Th1] response);
- While an animal model of Be sensitization *NOT* available, *Be exhibits adjuvant effects in animals*;
- Effects of Be on BALB/c mice immunized c soluble leishmanial antigens (SLA) investigated to determine if Be has adjuvant activity for...*IFN-gamma production, an indicator of the Th1 response.*

# Lee's work shows *Be*

## *Promotes gamma interferon production*



# Another way of looking at things – Lee 2000



# Complete Freund's Adjuvant (*F/A*)

## *Famous vaccine booster*

In a head-to-head comparison *Be* produces a better interferon response ( $Th_1$  response) than *F/A*;

*Be* >> *F/A* increases levels rabbit anti-nematode Ab;

Metals in general >> attractive given nanotechnology;

Medical equivalent of *nano-* is *micronizing*.

# Let's take a break

- So *what is the deal* c/ molecular biology (*m/b*), forward-thinking experiments & their protocols? Not very *Dr. Welby - Gray's Anatomy* (TV doctor-like) ?
- *What happened to old-line chemistry* or engineering-only approaches – *Why can't they be used?*
- Finally, how long do these stories need to be?
- *What's c/ the abbreviations & 'trade names?'*

## Pointers to understanding 'new' science

### *Picking on McKee's article – MyD88 dependence of Be-induced...*

#### 1) *Most molecular biology stories are long, e.g.,*

- *Release of IL-1 $\alpha$  was **inflammasome** independent and required for beryllium-induced neutrophil recruitment into the lung. Beryllium enhanced classical dendritic cell (cDC) migration from the lung to draining lymph nodes (LNs) in an IL-1R-independent manner, and the accumulation of activated cDCs in the LN was associated with increased priming of CD4(+) **T cells**;*

#### 2) *Almost always a 'ringer,' e.g., manipulate system;*

- *DC migration was reduced in Toll-like receptor 9 knockout (TLR9KO) mice; however, c-DCs in the LNs of TLR9-deficient mice were highly activated, suggesting a **role for more than one innate receptor** in the effects on DCs.*

## Pointers to understanding 'new' science

*Picking on McKee's article – MyD88 dependence of Be-induced...*

### 3) *Lead-ins to m/b reasonably humane –understand every word;*

- Beryllium exposure results in beryllium hypersensitivity in a subset of exposed individuals, leading to granulomatous inflammation and fibrosis in the lung.

### 4) *Deep within their narratives, they struggle c/ same uncertainties;*

- In addition to its antigenic properties, beryllium has potent adjuvant activity that contributes to sensitization via *unknown pathways*.

### 5) *A lot of us give up reading before we get to the important part.*

- Here we show that beryllium induces cellular death and release of interleukin (IL)-1 $\alpha$  and DNA into the lung. Release of IL-1 $\alpha$  was *inflammasome independent* and required for beryllium-induced neutrophil recruitment into the lung. Beryllium enhanced classical dendritic cell (cDC) migration from the lung to draining lymph nodes (LNs) in an IL-1R-independent manner, and the accumulation of *activated cDCs in the LN* was associated with increased priming of CD4(+) T cells.

## MyD88 Dependence of Be-induced *Dendritic Cell Trafficking & CD4<sup>+</sup> T-cell Priming*

- *Be induces cellular death & release of*
- *Interleukin (IL)-1 $\alpha$  & DNA* into lung;
- Be - *Potent adjuvant activity* contributes to sensitization via *unknown* pathways;
- Be-enhanced classical dendritic cell (*cDC*) migration from *lung to* draining lymph nodes (*LNs*) in an IL-1R-independent manner.
-

## *Details within McKee's article...*

DC migration was reduced in Toll-like receptor 9 knockout (TLR9KO) mice; however, c-DCs in the LNs of TLR9-deficient mice were highly activated, suggesting a role for more than one innate receptor in the effects on DCs.

The adjuvant effects of beryllium on CD4(+) T-cell priming were similar in wild-type, IL-1R-, caspase-1-, TLR2-, TLR4-, TLR7-, and TLR9-deficient mice.

In contrast, DC migration, activation, and the adjuvant effects of beryllium were significantly reduced in myeloid differentiation primary response gene 88 knockout (MyD88KO) mice.

Collectively, these data suggest that beryllium exposure results in the release of damage-associated molecular patterns that engage MyD88-dependent receptors to enhance pulmonary DC function.

## *Be* is also a Candidate for *Tumor Suppression*

- *Be* salts (e.g., *Be Sulfate*) produce cytostatic effect in some Ca-cell types;
- Possibly *increase expression* of proliferation *inhibitors*, *reduced expression* of proliferation *promoters*, maybe both – Ying & Yang;
- Role of p53, the tumor-suppressing transcription factor, in mediating Be-induced *cytostasis*?

## Both Sarcoid and Be Disease Defy Treatment

### *How does this inform mechanism of injury?*

Chronic beryllium disease (CBD) characterized by accumulation of macrophages in *Be-specific CD4+ T* cells that proliferate & produce *Th1 cytokines*;

5-Amino salicylic acid (*5-ASA*) currently used to Tx inflam. bowel disease - both anti-oxidant & -inflam actions;

*5-ASA's* ability to << BAL cell *BeLPT* & Be-stimulated BAL cell *TNF- $\alpha$*  levels suggests it impacts Be-specific immune resp.;

Get Roughly a 20% decrease in BeLPT from *5-ASA*;

## Why not try Rx used to treat other *Anti-oxidant & -inflammatory Conditions*

CBD characterized by accumulation of macrophages & *Be*-specific CD4+ T cells that proliferate & produce Th1 cytokines.

5-Amino salicylic acid (*5-ASA*) currently used to tx inflam bowel disease -

17 CBD pts randomized 3:1 to receive *5-ASA* 500-mg capsules or placebo 4/da \* 6 wks P/O;

Primary study endpoints included changes in *Be* lymphocyte proliferation (*BeLPT*);

Secondary endpoints included changes in BAL lavage fluid, cells, serum & blood cell glutathione (GSH) levels, BAL - TNF- $\alpha$  levels, lung function & QOL;

Bottom line: *5-ASA decreased BAL cell BeLPT by 20%*

**Day et al. Lung 2017 Oct 27 – New!**

# Why don't things work '*right*'?

*Microorganisms - a more consistent insult than Be--*

- Designed to *withstand infectious insults 1st*, everything else *2<sup>nd</sup>*;
- Particles often confusing to host defense mechanisms?
- So what is lipopolysaccharide-binding protein (*LBP*)?
- *An endotoxemia* associated protein potentially useful as *inflammatory biomarker* for both infectious \* non-infectious origins in Hepatitis C -infected subjects;
- *LBP* enhances responsiveness of alveolar macrophages to bacterial lipopolysaccharide;
- *What would happen if we supplied it?*

# Riddle of too much – Or too little...

- Virtually every *anti-seizure* drug will also...
  - *Cause seizures* in overdose;
- Virtually every oncologic (*'chemo' Rx*) can also...
  - *Cause cancer*...sometimes even in small doses;
  - Findings draw our attention to *dose-responsing*,
  - But dose-responsing includes *time-element* of pharmacokinetics (PK).

# Look at Lithium & Beryllium

## – *What is similar, what is different?*

|                                       |  |                                        |  |                                       |  |                                         |  |                                            |  |                                        |  |                                         |  |                                        |  |                                      |  |                                         |  |                                          |  |                                         |  |                                        |  |                                       |  |                                        |  |                                        |  |                                          |  |                                         |  |                                        |  |
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| hydrogen<br>1<br><b>H</b><br>1.0079   |  |                                        |  |                                       |  |                                         |  |                                            |  |                                        |  |                                         |  |                                        |  |                                      |  | helium<br>2<br><b>He</b><br>4.0026      |  |                                          |  |                                         |  |                                        |  |                                       |  |                                        |  |                                        |  |                                          |  |                                         |  |                                        |  |
| lithium<br>3<br><b>Li</b><br>6.941    |  | beryllium<br>4<br><b>Be</b><br>9.0122  |  |                                       |  |                                         |  |                                            |  |                                        |  |                                         |  |                                        |  |                                      |  |                                         |  | boron<br>5<br><b>B</b><br>10.811         |  | carbon<br>6<br><b>C</b><br>12.011       |  | nitrogen<br>7<br><b>N</b><br>14.007    |  | oxygen<br>8<br><b>O</b><br>15.999     |  | fluorine<br>9<br><b>F</b><br>18.998    |  | neon<br>10<br><b>Ne</b><br>20.180      |  |                                          |  |                                         |  |                                        |  |
| sodium<br>11<br><b>Na</b><br>22.990   |  | magnesium<br>12<br><b>Mg</b><br>24.305 |  |                                       |  |                                         |  |                                            |  |                                        |  |                                         |  |                                        |  |                                      |  |                                         |  | aluminium<br>13<br><b>Al</b><br>26.982   |  | silicon<br>14<br><b>Si</b><br>28.086    |  | phosphorus<br>15<br><b>P</b><br>30.974 |  | sulfur<br>16<br><b>S</b><br>32.065    |  | chlorine<br>17<br><b>Cl</b><br>35.453  |  | argon<br>18<br><b>Ar</b><br>39.948     |  |                                          |  |                                         |  |                                        |  |
| potassium<br>19<br><b>K</b><br>39.098 |  | calcium<br>20<br><b>Ca</b><br>40.078   |  | scandium<br>21<br><b>Sc</b><br>44.956 |  | titanium<br>22<br><b>Ti</b><br>47.867   |  | vanadium<br>23<br><b>V</b><br>50.942       |  | chromium<br>24<br><b>Cr</b><br>51.996  |  | manganese<br>25<br><b>Mn</b><br>54.938  |  | iron<br>26<br><b>Fe</b><br>55.845      |  | cobalt<br>27<br><b>Co</b><br>58.933  |  | nickel<br>28<br><b>Ni</b><br>58.693     |  | copper<br>29<br><b>Cu</b><br>63.546      |  | zinc<br>30<br><b>Zn</b><br>65.39        |  | gallium<br>31<br><b>Ga</b><br>69.723   |  | germanium<br>32<br><b>Ge</b><br>72.61 |  | arsenic<br>33<br><b>As</b><br>74.922   |  | selenium<br>34<br><b>Se</b><br>78.96   |  | bromine<br>35<br><b>Br</b><br>79.904     |  | krypton<br>36<br><b>Kr</b><br>83.80     |  |                                        |  |
| rubidium<br>37<br><b>Rb</b><br>85.468 |  | strontium<br>38<br><b>Sr</b><br>87.62  |  | yttrium<br>39<br><b>Y</b><br>88.906   |  | zirconium<br>40<br><b>Zr</b><br>91.224  |  | niobium<br>41<br><b>Nb</b><br>92.906       |  | molybdenum<br>42<br><b>Mo</b><br>95.94 |  | technetium<br>43<br><b>Tc</b><br>[98]   |  | ruthenium<br>44<br><b>Ru</b><br>101.07 |  | rhodium<br>45<br><b>Rh</b><br>102.91 |  | palladium<br>46<br><b>Pd</b><br>106.42  |  | silver<br>47<br><b>Ag</b><br>107.87      |  | cadmium<br>48<br><b>Cd</b><br>112.41    |  | indium<br>49<br><b>In</b><br>114.82    |  | tin<br>50<br><b>Sn</b><br>118.71      |  | antimony<br>51<br><b>Sb</b><br>121.76  |  | tellurium<br>52<br><b>Te</b><br>127.60 |  | iodine<br>53<br><b>I</b><br>126.90       |  | xenon<br>54<br><b>Xe</b><br>131.29      |  |                                        |  |
| caesium<br>55<br><b>Cs</b><br>132.91  |  | barium<br>56<br><b>Ba</b><br>137.33    |  | 57-70<br>*                            |  | lutetium<br>71<br><b>Lu</b><br>174.97   |  | hafnium<br>72<br><b>Hf</b><br>178.49       |  | tantalum<br>73<br><b>Ta</b><br>180.95  |  | tungsten<br>74<br><b>W</b><br>183.84    |  | rhenium<br>75<br><b>Re</b><br>186.21   |  | osmium<br>76<br><b>Os</b><br>190.23  |  | iridium<br>77<br><b>Ir</b><br>192.22    |  | platinum<br>78<br><b>Pt</b><br>195.08    |  | gold<br>79<br><b>Au</b><br>196.97       |  | mercury<br>80<br><b>Hg</b><br>200.59   |  | thallium<br>81<br><b>Tl</b><br>204.38 |  | lead<br>82<br><b>Pb</b><br>207.2       |  | bismuth<br>83<br><b>Bi</b><br>208.98   |  | polonium<br>84<br><b>Po</b><br>[209]     |  | astatine<br>85<br><b>At</b><br>[210]    |  | radon<br>86<br><b>Rn</b><br>[222]      |  |
| francium<br>87<br><b>Fr</b><br>[223]  |  | radium<br>88<br><b>Ra</b><br>[226]     |  | 89-102<br>* *                         |  | lawrencium<br>103<br><b>Lr</b><br>[262] |  | rutherfordium<br>104<br><b>Rf</b><br>[261] |  | dubnium<br>105<br><b>Db</b><br>[262]   |  | seaborgium<br>106<br><b>Sg</b><br>[266] |  | bohrium<br>107<br><b>Bh</b><br>[264]   |  | hassium<br>108<br><b>Hs</b><br>[269] |  | meitnerium<br>109<br><b>Mt</b><br>[268] |  | ununnilium<br>110<br><b>Uun</b><br>[271] |  | unununium<br>111<br><b>Uuu</b><br>[272] |  | ununbium<br>112<br><b>Uub</b><br>[277] |  | nihonium<br>113<br><b>Nh</b><br>[284] |  | flerovium<br>114<br><b>Fl</b><br>[289] |  | moscovium<br>115<br><b>Mc</b><br>[288] |  | livermorium<br>116<br><b>Lv</b><br>[293] |  | tennessine<br>117<br><b>Ts</b><br>[294] |  | oganesson<br>118<br><b>Og</b><br>[294] |  |

\* Lanthanide series

|                                        |                                     |                                           |                                        |                                        |                                       |                                       |                                         |                                      |                                         |                                      |                                     |                                      |                                        |
|----------------------------------------|-------------------------------------|-------------------------------------------|----------------------------------------|----------------------------------------|---------------------------------------|---------------------------------------|-----------------------------------------|--------------------------------------|-----------------------------------------|--------------------------------------|-------------------------------------|--------------------------------------|----------------------------------------|
| lanthanum<br>57<br><b>La</b><br>138.91 | cerium<br>58<br><b>Ce</b><br>140.12 | praseodymium<br>59<br><b>Pr</b><br>140.91 | neodymium<br>60<br><b>Nd</b><br>144.24 | promethium<br>61<br><b>Pm</b><br>[145] | samarium<br>62<br><b>Sm</b><br>150.36 | europium<br>63<br><b>Eu</b><br>151.96 | gadolinium<br>64<br><b>Gd</b><br>157.25 | terbium<br>65<br><b>Tb</b><br>158.93 | dysprosium<br>66<br><b>Dy</b><br>162.50 | holmium<br>67<br><b>Ho</b><br>164.93 | erbium<br>68<br><b>Er</b><br>167.26 | thulium<br>69<br><b>Tm</b><br>168.93 | ytterbium<br>70<br><b>Yb</b><br>173.04 |
|----------------------------------------|-------------------------------------|-------------------------------------------|----------------------------------------|----------------------------------------|---------------------------------------|---------------------------------------|-----------------------------------------|--------------------------------------|-----------------------------------------|--------------------------------------|-------------------------------------|--------------------------------------|----------------------------------------|

\*\* Actinide series

|                                      |                                      |                                           |                                     |                                       |                                       |                                       |                                    |                                       |                                         |                                         |                                      |                                          |                                       |
|--------------------------------------|--------------------------------------|-------------------------------------------|-------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|------------------------------------|---------------------------------------|-----------------------------------------|-----------------------------------------|--------------------------------------|------------------------------------------|---------------------------------------|
| actinium<br>89<br><b>Ac</b><br>[227] | thorium<br>90<br><b>Th</b><br>232.04 | protactinium<br>91<br><b>Pa</b><br>231.04 | uranium<br>92<br><b>U</b><br>238.03 | neptunium<br>93<br><b>Np</b><br>[237] | plutonium<br>94<br><b>Pu</b><br>[244] | americium<br>95<br><b>Am</b><br>[243] | curium<br>96<br><b>Cm</b><br>[247] | berkelium<br>97<br><b>Bk</b><br>[247] | californium<br>98<br><b>Cf</b><br>[251] | einsteinium<br>99<br><b>Es</b><br>[252] | fermium<br>100<br><b>Fm</b><br>[257] | mendelevium<br>101<br><b>Md</b><br>[258] | nobelium<br>102<br><b>No</b><br>[259] |
|--------------------------------------|--------------------------------------|-------------------------------------------|-------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|------------------------------------|---------------------------------------|-----------------------------------------|-----------------------------------------|--------------------------------------|------------------------------------------|---------------------------------------|

Well known Rx - *Li* - Unknown Rx – *Be*

*Li* as a *bipolar (BiP) Rx* - Only discovered because of erroneous theory of urea (that had to be solubilized c/ Li salts) as cause of bipolar disorder;

- *Mode of action still unknown.*

Strontium (*Sr* - At. No. 38) similar chemical properties to Ca –  
Sr<sup>89</sup> Beta emitter

- *Bimodally - Cancer ca but also Ca-pain relieving?*

Beryllium is definitely *NOT* an essential element but it may be useful in ways we presently do not anticipate.

*BeSO<sub>4</sub> inhibits endogenous GSK-3β*

*Key regulator in signaling networks - Cell prolif., metab. & develop.*

BeSO<sub>4</sub> inhibits endogenous Glycogen Synthase Kinase 3β (*GSK-3β*) in cultured human cells;

- Exposure to *10 μM* Be<sup>+2</sup> produced decr. in GSK-3β *kinase* activity compared to *10 mM Li<sup>+</sup>* (NB: Be<sup>+2</sup> Very dilute in trial);
- Indicates *Be ~1000-X >> potent* than a classical inhibitor;

Significant dose-dependent reduction in specific activity of *GSK-3β immuno-ppt.* from cells prev. tx c/ either agent.

- *Lithium inhibited GSK-3β kinase activity directly.*

Details not completely explainable but show...

*Be has important role in cell signalling.*

# Discussion of Early Interventions

## *Liposaccharide Binding Protein (LBP) is Protective*

Be oxide-induced *oxidative lung injury* interrupted by protective effects of *LBP*;

Animals exposed to *BeO* by 1-time intra-trach. injection;

Lung content of oxidative markers (*HIF-1, VEGF & HO-1*) measured;

Heme oxygenase-1 (*HO1*) makes *carbon monoxide*;

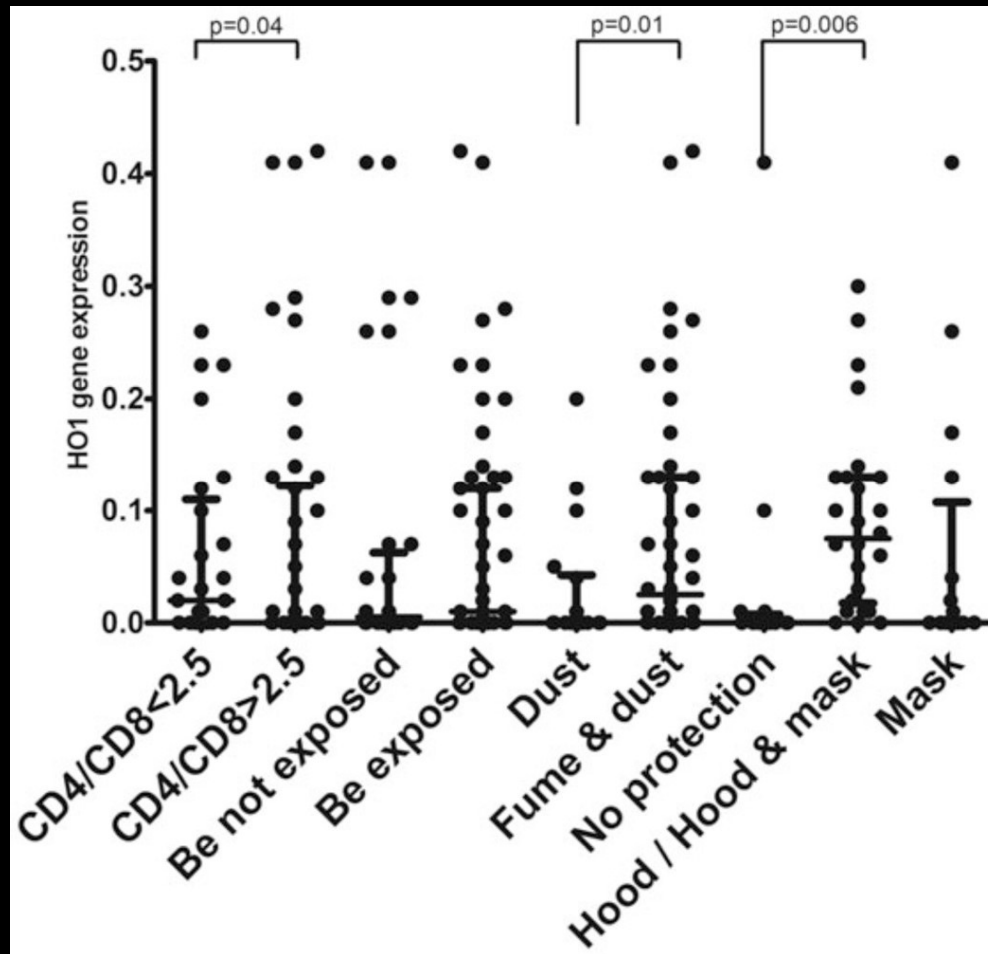
*Angiogenic factors* - VEGF & stromal cell–der. factor-1 induce *HO1*;

*BeO* can cause abn. expression related genes of lung tissue (rats);

*LBP* has protective effects on BeO caused lung injury.

# Oxidation steps are critical to the damage from Beryllium

*Exciting to see how even a biological indicator  
(gene expression) changes c PPE!*



Normally think of exercise as an oxidative stressor,

*Watch what happens c Be as opposed to Pb*

Mechanisms & consequences of exercise-induced *oxidative stress*;

Reactive oxygen species (*ROS*) generated in the mitochondria of contracting skeletal myocytes;

Diffusion across myocyte membrane allows *ROS* transport to neighboring muscle tissue & to other body regions;

Intense exercise can induce oxidative damage within myocytes, BUT magnitudes of moderate-intensity exercise-assoc. increase in ROS ~ modest.

~2-fold increase in intra- & extra-cellular *ROS* conc. during exercise.

# American College of Sports Medicine defines *'Exercise is Medicine'*

*Cd, W, Te, Be & Pb* ~ non-essential metals

*Pervasive in industrial environments;*

Training periods compared to non-training control- *Suggests increased losses through sweat & urine;*

Concentrations expressed in micrograms of metal,  
*e.g., Be, basically per ml (mg of creatinine);*

Be (*0.54 mcg* training vs. *0.07 mcg* non-training,  $P \leq 0.001$  ~ per gm creatinine);

Pb (*0.94 mcg* training vs. *2.1 mcg* non-training  $P \leq 0.001$  ~ per gm creatinine).

*Pb accumulates– but Be is lost through increased exercise.*

# Metallurgists Not Only Ones Using *Be*

*Molecular biology labs very interested, too*

## *Beryllium & LPT*

- ~ *Dozen* Medline entries.

## *Beryllium & ATP & ATPase & Mg*

- ~ *400* Medline entries

Interest in *Be* involves manipulating *kinases* (*phosphate*) relative to very central cellular energy process;

*Be* typically used c/ *Al* salts, including *fluoride* (as the anion analog to *phosphate*);

*'BeFx'* ~ 'bad chemistry' but refers to *beryllium fluoride*.

# Molly Thomas original paper in 1966

## The Inhibition of Phosphoglucomutase by Beryllium

By W. N. ALDRIDGE AND MOLLY THOMAS  
Medical Research Council Toxicology Research Unit,  
Woodmansterne Road, Carshalton, Surrey

(Received 1 July 1965)

1. The inhibition of phosphoglucomutase by beryllium has been examined. 2. Inhibition by beryllium does not occur unless a complex-forming agent such as cysteine or imidazole is present. It is therefore similar to activation by magnesium. 3. The inhibition is progressive and the rate follows first-order kinetics, which may be defined by a bimolecular rate constant. 4. In the presence of magnesium the rate of inhibition is less. By using a fixed time for inhibition competition between beryllium and magnesium may be demonstrated. After inhibition has taken place, the addition of magnesium does not reverse it.

It has previously been reported that beryllium inhibits phosphoglucomutase (D-glucose 1,6-diphosphate-D-glucose 1-phosphate phosphotransferase, EC 2.7.5.1) and that this inhibition is less in the presence of magnesium (Cochran, Zerivie & DuBois, 1951). The inhibitory activity of beryllium against this enzyme has been confirmed (Thomas & Aldridge, 1966) and the present paper describes some novel features of the kinetics of the inhibitory process. A preliminary account of this work has been published (Aldridge & Thomas, 1964).

### MATERIALS AND METHODS

*Special chemicals.* The following chemicals were obtained from the sources indicated: dipotassium gluco-1-phosphate, cysteine hydrochloride and  $\text{BeSO}_4 \cdot 4\text{H}_2\text{O}$  from British Drug Houses Ltd., Poole, Dorset; imidazole from Koch-Light Laboratories Ltd., Colnbrook, Bucks.; crystalline rabbit-muscle phosphoglucomutase from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany.

*Determination of phosphoglucomutase.* The method used was essentially that of Najjar (1948). The final composition of the medium was: cysteine (25 mM),  $\text{MgSO}_4$  (1.5 mM), glucose 1-phosphate (1–1.8 mM), pH 7.4; the total volume was 1.6–2.0 ml. The progress of the reaction was followed at 30° by the determination of the acid-labile phosphorus (3 min. at 100° in  $\text{s-H}_2\text{SO}_4$ ) by the method of Fiske & Subbarow (1925). The reaction was zero-order until 75% of the substrate had been converted. For some experiments, and always for those experiments where the enzyme was incubated in the absence of cysteine (and was therefore largely unbuffered), a final concentration of 4.2 mM- $\text{KHCO}_3$  was added as 0.4 ml. of 21 mM- $\text{KHCO}_3$  saturated with  $\text{CO}_2 + \text{N}_2$  (5:95). Such an addition does not influence the inhibition by beryllium in the presence of cysteine. The time allowed for activation of the enzyme by cysteine and magnesium was never less than 1 min. In the experiments described below the enzyme was often preincubated with

$\text{MgSO}_4$  or cysteine alone with or without  $\text{BeSO}_4$ . In these cases a further 1 min. with both  $\text{MgSO}_4$  and cysteine present was always allowed before the substrate was added. The concentrations of  $\text{BeSO}_4$  stated were always those during the preincubation, which was at 30° except where otherwise stated.

Most of the experiments were carried out with a partially purified phosphoglucomutase from rabbit muscle prepared as described by Thomas & Aldridge (1966). The response of this preparation to cysteine and  $\text{BeSO}_4$  was identical with that of the crystalline preparation.

### RESULTS

*Complex-forming agents and inhibition by beryllium.* Phosphoglucomutase requires magnesium, a complex-forming agent and glucose 1,6-diphosphate as coenzyme for maximal activity (Najjar, 1948; Robinson & Najjar, 1961; Sutherland, 1949; Cori, Colowick & Cori, 1938; Leloir, Trucco, Cardini, Paladini & Caputto, 1948; Cardini, Paladini, Caputto, Leloir & Trucco, 1949; Milstein, 1961b). Since the enzyme as normally isolated is in the phosphoryl-enzyme form (Jagannathan & Luck, 1949; Najjar & Pullman, 1954), no glucose 1,6-diphosphate, other than that in the glucose 1-phosphate, has been added. For most of the experiments described below cysteine has been used as the complex-forming agent because with our enzyme preparation this has given a higher activity than imidazole. In the absence of magnesium or cysteine or both the enzyme has no activity. Although Robinson & Najjar (1961) demonstrated that the activation of phosphoglucomutase by imidazole and magnesium was a time-dependent process and for full activation several minutes were required, we have been unable to demonstrate such a time-dependent activation with cysteine

~51 years later

*1966 Be findings from Dr. Molly Thomas (RIP)*

- Inhibition of *phosphoglucomutase* by *Be*;
- No inhibition by *Be* s/ 'complexers' such as *cysteine or imidazole* (activation similar to *Mg*);
- Progressive inhibition - Rate ~1<sup>st</sup>-order;
- Damp out inhibition c/ presence of *Mg*;
- Competition is set up between *Be & Mg*;
- When enzyme inhibition complete,
  - *addn. of Mg won't reverse it.*

## Way forward in light of the...*HSWG Objectives*

Controlled medical terminologies & use of data mining

*Important to Accumulating & Disseminating Information;*

The '*Why Be, Why Be Now*' not a trivial question for *2018*;

Not unlike thalidomide & BoTox, *Be* joins other high toxicity entries to become useful in ways we can't imagine;

Studies of *Be* as a vaccine adjuvant & as a Cancer (*oncologic*) - New insights into systems biology.

*Better Appreciate (-ing) Illnesses.*

## HSWG Objectives

*Obtaining a better understanding of exposure risks*

Perceived difficulty physical scientists & engineers often have c/  
language of molecular biology can be *overcome in a few steps*;

Advanced knowledge of a disease requires a *carefully  
understood model*, *Be* is not an exception.

## Primary, Secondary & Tertiary Prevention (from Dr. John Last)

*Is biochemical knowledge a form of primary prevention?*

- *Primary prevention* applies to the prevention of the *initial event* (accident or disease beginning);
- Prevention often at the *secondary* level, involving a range of strategies from product design to drug treatment;
- Last originally defined *tertiary prevention* as
- ‘measures to reduce long-term impairment & disabilities, minimize suffering and to promote adjustment to irremediable conditions;’
- *Dr. Miller’s & my talks bookend most of what goes on...*

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--*Voice of Hound, a dog & the book's main character...*

When I travel, it looks to a human as if I am just nosing about, checking out all kinds of things, the occasional tasty rabbit or ground squirrel, but my path is more aligned with continuously updating my surroundings.

My ancestral leanings are to regard the land adjoining the Blue River, with its mouth just east of the Cliffs, as tracts of forests, wet meadows and even a brief prairie.

First, to get out of Kansas City Northeast I have to traverse an almost completely industrialized corridor devoid of natural vegetation from Independence Avenue down to roughly 17<sup>th</sup> street along the river...

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