

Trace Elements & Toxic Metals

Clinical Biochemistry Department
City Hospital



Lecture outline

- Which elements?
- Essential elements: Cu, Zn, Se
- Iatrogenic elements: Al, Mn
- Elemental poisoning: Pb
- Elemental analysis: atomic spectroscopy

ESSENTIAL METALS

Na	Cu	Co
K	Zn	Cr
Ca	Se	Mn
Mg		Mo
Fe		

METALS USED IN THERAPY

Ag	Ba	Hg
Al	Gd	Cr
As	I	Co
Au		Ti
B		Mo
Bi		V
Li		
Pt		

INDUSTRIAL & 'TOXIC' METALS

Pb
Cd
Ni
As
Hg
Tl
Sb

Which elements? – the historical repertoire

- *Essential*: Cu, Zn, Se (serum); Cu (urine)
 - *'Toxic'*:
Al (plasma, water) – renal dialysis patients
Pb, Cd, Cr, As, Hg, Ni (blood, urine):
Industrial monitoring / occupational exposure
Asian & Far Eastern traditional medicines
Pica – primarily paediatric
- Acute poisoning from accidental or deliberate ingestion of metals solutions or salts is uncommon

Which elements? – the more recent

- Cr, Co, Ni (blood, serum, urine, hip joint fluid)
- B (blood, plasma, brain, urine) – cancer chemotherapy
- Mn (blood) – overload during PN
- Pt (serum) – cancer chemotherapy
- Li (post mortem blood) – H.M. Coroner
- Li – blood spot analysis
- Br (serum) – therapeutic monitoring
- Ga, In, Te (urine) – occupational monitoring
- Tl (blood, urine, liver, faeces) – Iraqi poisoning episode
- Sb (blood) – treatment of Leishmaniasis
- I (serum) – betadine overdose
- Mo (serum, urine) – concern over dental implant.

Copper Function

- Catalytic co-factor & structural component for metalloproteins (~ 24 enzymes):
Cytochrome-c oxidase
Ferroxidases (in caeruloplasmin)
Cu-Zn superoxide dismutase
Amine oxidases
Tyrosinase (catechol oxidase)
- Post-absorption transport attached to albumin (not caeruloplasmin)

Copper investigation

Disease: inherited disorder of Cu-transporting P-type ATPase

Menkes: gene expressed in all tissues except liver
defective intestinal absorption of Cu
deficiency of numerous Cu-containing enzymes

Wilson's gene expression liver, kidney to lesser extent
impairs : biliary excretion of Cu
incorporation of Cu into caeruloplasmin

Nutrition: malnutrition, malabsorption, burns
medical diets, parenteral nutrition
excess Zn intake

Assessing copper status

Serum/plasma [Cu]: neonates – 4 months: 1.6 – 7.9 µmol/L
4 – 6 months: 4.7 – 17 µmol/L
7 – 12 months: 7.9 – 20 µmol/L
children > 6/12 & adults: 11 – 25 µmol/L
pregnancy > 15/40: 25 – 39 µmol/L

Influenced by: steroid hormones (inc HRT)
acute phase response

Complemented by: caeruloplasmin
24 hour urine Cu for Wilson's Disease
(not affected by IUD's)

Alternatives: Liver biopsy; 'free' copper

Zinc Function

- Catalytic co-factor & structural component for metalloproteins (~ 200 enzymes):

Alkaline phosphatase
Alcohol dehydrogenase
RNA polymerase
Carbonic anhydrase

- Zinc finger proteins:
DNA transcription
Hormone receptors

Zinc - clinical relevance

1. *Acrodermatitis enteropathica*: (Moynihan; 1974)
Rare, autosomal, recessive inherited disease of Zn absorption.

Identification of SLC39A4, a gene involved in AC
Kury S et al. *Nat Gen* 2002; Jun 17

A novel member of a zinc transporter family is defective in AC
Wang K et al. *Am J hum Genet* 2002; 71(1):66-73

2. *Most common circumstances:*

Malnourishment; malabsorption; burns.
Special 'medical' diets; enteral & parenteral feeding.

Wound healing; loss of taste

Assessing zinc status

Serum/plasma : < 7 $\mu\text{mol/L}$? deficiency
7 – 11 $\mu\text{mol/L}$? Not significant
11 – 24 $\mu\text{mol/L}$ normal for all ages
> 24 $\mu\text{mol/L}$? supplementation

Influenced by: diurnal variation, fasting
pregnancy, malignancy, renal failure
low albumin, acute phase response

Complemented by: CRP, alkaline phosphatase

Alternatives: leucocyte zinc; serum 'free' zinc

Biological role of selenium

- Structural component of two amino acids:
Selenocysteine (animals)
Selenomethionine (plants)
- In animals (inc humans) all forms of ingested selenium can only be utilised following reduction to Se^- and metabolic incorporation into selenocysteine

Human selenoproteins

- Glutathione peroxidase : cytosolic, RBC
(GSH-Px) plasma
gastro-intestinal
phospholipidhydroperoxide
- Iodothyronine 5'-deiodinase
- Thioredoxin reductases

- Selenoprotein P
- Selenoprotein N

Role of selenium in human health

- Deficiency linked to:
Heart disease
Cancer
Infertility
Just about everything!
- Recently, excessive supplementation linked to:
Increased incidence of Prostate cancer
Increased incidence of Type 2 diabetes

Serum Selenium Reference Range (U.K.)

- < 18 months: 0.4 – 0.7 $\mu\text{mol/L}$
- 18 months - 4 years: 0.6 - 1.1 $\mu\text{mol/L}$
- 5 - 16 years: 0.7 – 1.5 $\mu\text{mol/L}$
- Adults (> 16 years): 0.9 – 1.7 $\mu\text{mol/L}$
- ? Definition of reference range:
Empirical data for 'normal healthy' population *or*
Values related to functioning of biochemical expression

ALUMINIUM TOXICITY
IN CHRONIC RENAL FAILURE

- Consequences:
 - Dementia
 - Osteomalacia
 - Anaemia
- Sources of aluminium:
 - Dialysis fluid & tap water
 - Phosphate binders

'NORMAL' PLASMA [Al]- $\mu\text{g/L}$: 1964-1985

- 1964 400 \pm 277 Arch Env H; 8: 52 - 57
- 1970 200 - 300 Lancet; ii: 494 - 496
- 1977 6.0 \pm 3.0 N Eng J Med; 296: 1389 - 1390
- 1979 140 \pm 60 N Eng J Med; 301: 728 - 729
- 1985 17.3 \pm 6.1 Analyst; 110: 19 - 20
- 1985 5 - 8 Clin Chim Acta; 147: 247 - 254
- (After Taylor et al, 1986)

PLASMA [Al] REFERENCE VALUES FOR
DIALYSIS PATIENTS (C.E.C)

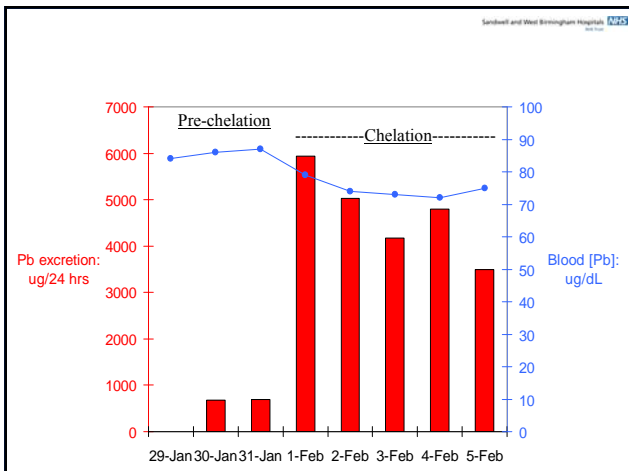
- < 10 $\mu\text{g/L}$ Normal (no history of C.R.F.)
- < 60 $\mu\text{g/L}$ Desirable in C.R.F. patients
- > 60 $\mu\text{g/L}$ Excessive accumulation
- > 100 $\mu\text{g/L}$ Cause for concern; high risk for children
- > 200 $\mu\text{g/L}$ Urgent action required; high risk for all
- C.E.C Upper limit for dialysis fluid: 30 $\mu\text{g/L}$

Manganese

- Enzyme component (xanthine oxidase, Mn S.O.D.)
- Estimated Safe & Adequate Daily Intake: (USNRC)
 - Infants: 0.3 - 1.0 mg
 - Children: 1 - 3 mg
 - Adults: 2 - 5 mg
- Nervous system disorders & ? cholestatic disease related to Mn supplementation in patients on long-term PN.
- Paediatric Mn PN recommendations:
 - 1994: 55 µg/Kg/day (550 µg for 10 Kg child)
 - 1996: 1 µg/Kg/day (10 µg for 10 Kg child)
- ? Remove supplemented Mn altogether



Lead ingot 'prescribed' for treatment of stammer.
('Suck for 3 hours daily')



PAEDIATRIC Pb POISONING FROM PICA (female; 2.5 yrs)

Date	Pb-µg/dL	Hb-g/dL	ZPP- µg/gHb	Pb- µg/g Hb
norm	<10	11.5 - 13.5	<3.2	< 0.7
9/9/98	48	8.5	42	5.65
9/2/99	60	7.9	61	7.59
11/5/99	27	7.4	58	3.65
20/7/99	26	10.9	13	2.39
12/10/99	20	11.3	8	1.77

☠ Contamination

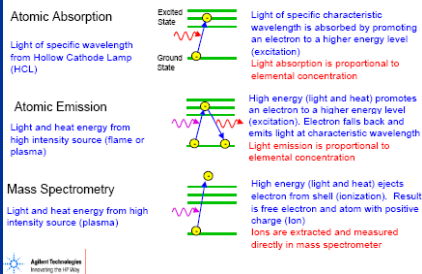
This is a major consideration in trace element investigation, particularly for

- Al – glass, everything!
- Zn – gel tubes, O-rings, glove-powder
- Cr, Co, Mn – stainless steel, needles, tubes

ATOMIC SPECTROSCOPY DEFINITIONS

- *Atomic emission:*
The process whereby an atom excited by thermal, chemical or electrical energy *emits* light of a characteristic wavelength during its return to a lower energy level.
- *Atomic absorption:*
The process that occurs when a ground-state atom *absorbs* energy in the form of light of a specific wavelength and is elevated to an excited state.
- *Ionisation:*
The process by which one or more electrons are *removed* from an atom yielding positively-charged ions.

Atomic Spectrometry



Analytical Instrumentation

- Up to 2003: Flame AA (serum Cu, Zn; urine iron)
CVG-AA (blood, urine Hg)
Furnace AA (everything else)
- April 2003: ICP-MS acquired (7500c)
- April 2009: 7500cx acquired
- April 2010: Mass Hunter software for both instruments

All analyses now by ICP-MS

Inductively-Coupled Plasma

A plasma is a volume of partially ionised gas (Ar) coupled to a high frequency electromagnetic radiation.

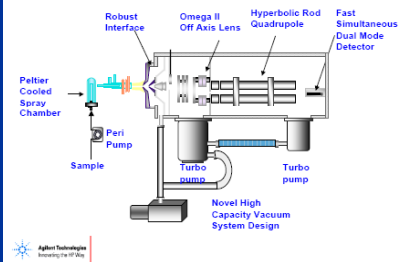
The plasma is initiated by **seeding** from a high voltage spark. Electrons interact with the magnetic field, gain energy and ionise the flowing argon stream by collisional excitation. Power applied via the induction coil allows the plasma to become self-supporting.

Inductively-Coupled Plasma - 2

Very high temperatures:

6000°K -----10,000°K	
atomic emission	ionisation
ICP-AES	ICP-MS
sensitivity – medium to high	sensitivity – very high
specificity – high	specificity – very high
multi-element	multi-element
	isotopic analysis

Schematic Diagram of Agilent 7500a



ICP-MS: an isotopic technique

- Al: 27 (100%)
- As: 75 (100%)
- Cu: 63 (69%), 65 (31%)
- Zn: 64 (49%), 66 (28%), 67 (4%), 68 (19%), 70 (<1%)
- Se: 74 (<1%), 76 (9%), 77 (8%), 78 (24%), 80 (50%), 82 (9%)
- Pb: 204 (1%), 206 (24%), 207 (22%), 208 (52%)

