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Biochemistry of the actinides

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Abstract

In this plenary paper the evolution of the trace element content of the human body to its present-day composition, the role that non-essential elements play in human biochemistry, the relatively recently researched roles of actinide complexes present in humans, and means of decontaminating wounds and other biological tissue are considered, and desirable researches and legislation required for the next century are referred to.

1. Introduction

For a human, metal ions found in the body may be roughly classified as being essential, beneficial, neutral or detrimental. However, some metals may move between these categories depending on the chemical species in which they exist and, also, on their concentrations [1].

A metal which is classified as being essential is one without which life is not possible. In practice, this criterion means that, in the presence of a deficiency of the metal, there is an impairment of the biological activity, which is usually reversible on admission of that metal at the correct concentrations and species. A related definition is that of a beneficial metal ion in whose absence normal health is somewhat impaired but, nevertheless, the life of the organism is not absolutely threatened.

Those metal ions which are present in humans at concentrations which have neither beneficial nor detrimental effects on the organism are often classed as neutral. However, from Fig. 1 it may be seen that all metal ions below a threshold of concentration in an organ have no effect upon the state of health of the organism and, therefore, could be said to be neutral [2].

The toxic metal ions include classical polluting elements such as cadmium, lead and mercury but it must be stressed that just as all metals can be below threshold concentration and, therefore, neutral, so too all metals above a certain concentration can be detrimental to the health of the human body. These features are all illustrated in Fig. 1.

In summary, the human body consists of some 20–30 essential and beneficial elements which, not surprisingly, are those elements which were most widespread and

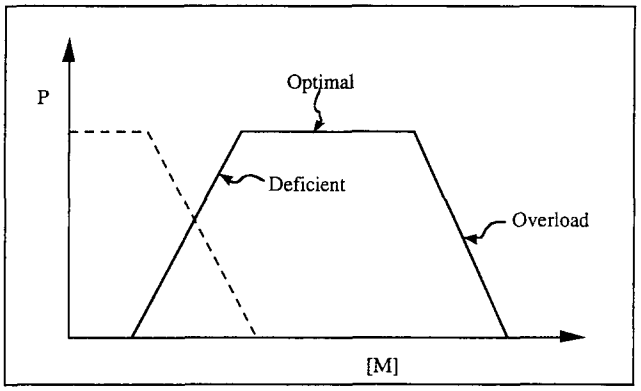

Fig. 1. The physiological response P to the concentration of a metal-containing species: – – –, toxic metals; ——, essential and beneficial metals.

bio-available on the surface of the earth when the evolution of predecessors to humans was initiated millions of years ago (Fig. 2). Our primitive ancestral cells are ostensibly derivatives of those evolving on ancient beaches, washed with sea water and heated by the sun's rays.

Since those times there have been three eras of addition to the elements found in a normal human.

(1) Several thousand years ago, early civilizations discovered outbreaks of elements normally found deep in the mantle of the earth and fashioned them into primitive weapons, jewellery etc. (for example, gold, silver).

(2) The introduction of deep mining for minerals, which was closely coupled with the industrial revolution some two centuries ago, added an additional range of heavy or unusual elements to the ionogram of humans (for example, lead, mercury, platinum).

(3) The manufacture of actinide elements in the early part of this century, colloquially known as the

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H*																He
Li	Be											$B^{\dagger}$	C^{*}	N^{*}	O^{*}	$F^{\dagger}$	Ne
Na^{*}	Mg^{*}											Al	$Si^{\dagger}$	P^{*}	S^{*}	Cl^{*}	Ar
K^{*}	Ca^{*}	Sc	Ti	$V^{\dagger}$	$Cr^{\dagger}$	$Mn^{\dagger}$	$Fe^{\dagger}$	$Co^{\dagger}$	$Ni^{\dagger}$	$Cu^{\dagger}$	$Zn^{\dagger}$	Ga	Ge	As?	$Se^{\dagger}$	Br?	Kr
Rb	Sr	Y	Zr	Nb	$Mo^{\dagger}$	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn?	Sb	Te	$I^{\dagger}$	Xe
Cs	Ba	|	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
		(Lanthanides)															
Fr	Ra	|															
		(Actinides)															

Fig. 2. Broad classification of the elements of the periodic table: *, major essential elements; †, trace elements believed to be essential for plants and/or animals; ?, elements whose biological properties are still not conclusively proven. From Christie and Williams [1].

plutonium era, and the distribution of elements through the biosphere, for example by above-ground nuclear warhead tests which released the order of some 6×10^6 g of plutonium into our atmosphere in the 1960s, have all led to an additional two dozen elements being present in humans over and above the essential and beneficial elements listed. This means, for example, that all animals and humans contain exceedingly low amounts (well below the threshold level for biochemical activity) of plutonium in our lungs and other tissues.

This paper is predominantly targeted at discussing the problems which arise when elements exceed their usual concentrations and which then upset the regular biochemistry associated with healthy existence. The word "usual" is used to reflect both the higher than necessary levels of, for example, iron which accompany the medical condition of siderosis or, alternatively, the higher than threshold levels of lead or plutonium associated with plumbism or with actinide contamination.

A long history of research has been associated with therapies being designed to decontaminate "the human body" by lowering excesses of essential elements such as lead, copper, and potassium, normally present therein and this applied knowledge can now be used to design approaches for the decorporation of radionuclides arising from an incident or industrial accident. This is the main topic of this paper.

2. The toxicity of metal ions

Most of the interesting biological reactions involving metals centre around low molecular mass (LMM) chemical forms of these metals and their ability to stimulate or challenge the normal biochemistry prevailing.

The disruption caused by so-called toxic metal ions present depends on the abundance of the metal ion, its biochemical availability for uptake into cells, and the reactivity of the metal ion complex having entered into such cells. The human body is some 70% water by weight and contains of the order of 10^{15} cells, each of which enclose several thousand biochemical reactions. Thus, the toxicity of metal ions is usually bound up with the aqueous chemistry of LMM metal ions and their complexes.

Such metal ions are usually in a state of labile equilibrium between three different types of complexes *in vivo* and, in addition, there is usually a fourth state in which they can exist but which is relatively inert in terms of the ability to yield up the metal ion at short notice. This is depicted in the scheme below:

inert metal complex	labile protein complex	$\rightleftharpoons$	LMM metal complex	$\rightleftharpoons$	aquated metal ion

Such a four-cornered relationship may be applied to all the essential metals present *in vivo* as well as to toxic metal ions introduced from outside into the human body. Thus, the normal, naturally occurring, biochemistry involving such metal ions can be seriously challenged by competition from LMM complexes of a toxic metal ion. Eventually, this metal carries through to the inert fraction present *in vivo* and thus can be found deposited in bones or tied up as a firmly bound metal–protein complex. Concerning the three labile fractions of high molecular mass, LMM and aquated metal ions, it is possible to procure metal from the circulating protein fraction by administering pharmaceutical agents which a chemist would call "chelating drugs". Such drugs progressively move the metal into the LMM fraction, which is then available for excretion through the kidneys (in the event of its being a charged LMM fraction) or, alternatively, for excretion through the bile duct and through the skin if it is present as net-neutral metal species.

The challenge of identifying the vulnerable essential metal ion bioinorganic chemistry which will be challenged by an intruding toxic metal ion is best summarized in terms of the hard and soft acids and bases approach which has been well described in the literature [1].

The specific toxicity of a metal compound depends on the total concentration, the particular chemical species in which the metal exists, the route of administration of the offending metal compound, and its

chemical "personality" in terms of the lability of its metal complexes between different chelating ligands and different oxidation states.

The field of studying different forms and concentrations in which metals exist is known as chemical speciation (see later) [3]. From a knowledge of the chemical speciation occurring in the biological fluid concerned and from the route of exposure and the initial physical–chemical form, it is now possible to derive therapies in order to arrest further damage or to decontaminate completely the organ concerned.

3. Routes of exposure and forms of contamination [4]

Many metal ions enter into an organism in a form, such as a solid, which is not a real threat because it is not bio-available. Other metal ion species are bio-available or, through a process of labile equilibrium, become bio-available when they enter into the lungs or into the small intestine wherein they equilibrate with other LMM complexes.

Generally speaking, the main routes of entry into humans are (a) pulmonary exposure through inhalation, (b) ingestion through the oral route with subsequent absorption via the gastrointestinal tract, (c) transdermal adsorption and (d) uptake via cuts, wounds, abrasions, burns, and other injuries. The offending metal ion which is now present at its primary deposition site may well re-speciate by equilibrating with other ligands and metal ions present and, thence, be transported via the blood stream to a secondary deposition site such as the liver, surfaces of bone, or other vital organs. Much information is available in the literature concerning the speed of migration from primary to secondary sites. Similarly, there have been many comparisons of the toxicity of the most well-known actinide element, plutonium, being administered to humans via various routes and compared with other well-known toxins [5]. Table 1 compares the toxicity of a range of toxins with that of plutonium and is based on that of Taylor [6].

Generally, processes of detoxifying humans are far more efficient when the offending metal ion is intercepted *en route* from primary to secondary sites rather than having to back-extract the metal from the secondary site and to mobilize and liberate it into the blood stream before it is passed on to the classical routes of excretion.

It is a sad reflection on the co-called technological society in which we live that legislative limits in terms of toxic metals are usually laid down as total amounts permitted in the whole body or in the diet rather than being based on the exact chemical species of these metals, the route of administration, and the target organ. A related problem is that often the legislative top limit of a permitted metal ion is sometimes below that which is achievable in analytical laboratories on a routine analysis basis. For example, the permitted level of aluminium for drinking water in the EC is the order of 200 ppb. Precious few routine analytical laboratories functioning in an atmosphere in which aluminium is ubiquitous can consistently and reliably analyse water samples at that low level.

4. Chemical speciation

No organic chemist would think of expressing the analytical levels of carbon present in an ethanolic solution in water, in a calcium carbonate solution, or in a potassium cyanide solution solely in terms of the total carbon presence. Rather, the toxicity and mood-changing aspects of these three aforementioned compounds of carbon are highly dependent on the chemical speciation. Similarly, food, beverages, and contaminants are often assessed in an analogous manner in terms of their total concentrations present. Such concentrations do not reflect the chemical species and the bio-availability of the metal which, in turn, can vary markedly

TABLE 1. Overview of the relative toxicities of plutonium to humans from three common sources: wounds (injection), breathing, and the food routes

	Route of intake	Lethal dose (μg)	Time to death
Reactor plutonium	Injection	140	More than 15 years
Snake venoms	Injection	5–100	Hours to days
Reactor plutonium	Inhalation	300–12000	More than 15 years
Nerve gas	Inhalation	1000	Hours
Reactor plutonium	Ingestion	10^6	More than 15 years
Anthrax	Ingestion	0.1	Hours to days

From Taylor [6]. Reactor plutonium is the most toxic form.

as concentration, pH, temperature etc. change. Similarly, oxidation states can determine whether a metal is toxic or non-toxic. Table 2 illustrates some of the paradoxes involved [7].

Chemical speciation is neither a theory nor a technique but, more specifically, it is a new means of considering the underlying chemistry which occurs in the biological system [3]. Sadly, many of the chemical species to which we are referring occur at levels at the limits of analytical chemistry or even below the levels at which analytical chemistry can determine reliable data. Thus, computer simulation of the chemical speciation prevailing needs to be applied in order to extrapolate chemical speciation studies down to representative biological concentrations. Chemical speciation using a computer begins where analytical chemistry gives way to much of nature's biochemistry.

Chemical speciation defines the composition of each of the species in which a metal exists in an actual sample, and this includes knowledge of the ratios of ligands to metal ions, the oxidation state of all the metal ions present, and the concentrations of each chemical species of the metal under consideration. Nowadays, modern computers can use extensive databases (typically of up to 10 000 LMM complexes participating in blood plasma).

Much of chemical decontamination of a polluted patient involves using a drug to liberate the metal from within the tissues (for example, D-penicillamine is used to liberate zinc, lead and copper therefrom because it forms net-neutral complexes) and thence swapping the chelating agent for another chelating drug such as ethylenediaminetetraacetate (EDTA) which is used to turn the aforementioned metal ions into net-charged species, which are then excreted through the renal route. The above process, which is known as synergistic chelation therapy and, indeed, the whole field of chemical speciation via computer modelling and painstaking laboratory experiments to validate the models, has been well reviewed in the literature [3]. The chemical speciation modelling approach has been applied to a large number of biological fluids, such as blood, saliva, intestinal juice, urine, cerebrospinal fluid, and also to a wide range of materials which interact with human bodies, such as the solid and liquid ingredients involved in foods, milks (both human and bovine) and also beers and other drinks.

TABLE 2. Examples of elements having ambivalent biological effects dependent on their chemical speciation

Parent element	Predominantly beneficial species	Potentially toxic species
As	As(V) compounds	As(III) compounds
Ba	Chloride	Nitrate
C	Widespread in biochemistry	Cyanide ion
Cr	Cr(III) compounds	Cr(VI) compounds
Cu	Carbonate	Chloride

From Williams [7].

5. The biochemistry of the actinides

Much has been written during the last two decades concerning the forms in which actinides exist, having entered into the human body by routes described earlier [5]. Essentially, the species prevailing are dictated by the facts that highly charged cations, such as those formed by the actinides, avidly seek out oxygen donor groups on biochemicals, typically carboxylates etc. Secondly, the metal ions themselves are exceedingly prone to hydrolysis at pH values experienced *in vivo* and, in the absence of competition from other ligands, they will complex with water, rapidly hydrolyse and form polymeric hydroxy solids, which are often difficult to dismantle reversibly.

Much of the overall biochemistry of the actinides resembles that of other highly charged cations found in the human body. For example, plutonium in the +4 oxidation state displays many biochemical parallels to that of the ferric ion present in normal biochemistry. Similarly, non-essential element aluminium in humans forms polymers similar to the hydroxy polymers of actinides just described.

Paradoxically, although many contaminating metal ions, and the ions of aluminium, and even iron, can be safely deposited in tissues such as bones, it is highly undesirable for radiotoxic metals such as ^{239}Pu to be incorporated therein, since the α radiation emitted close to the bone marrow will have serious effects.

Although considerably more challenging to normal biochemistry in terms of their radiotoxicities, the keenness to polymerize and the insolubility brought about by hydrolysis often gives the actinides a bio-availability which is exceedingly low. Typically, only 0.1% of plutonium present in the gastrointestinal tract is considered to be bio-available by the International Committee on Radiological Protection. This is reflected in the table of comparative toxicities shown earlier. This sparse bioavailability is essentially that of an LMM complex of the actinide not being able to penetrate from extracellular fluid through the cell membrane (which has been crudely likened to a lipid protein layer of organic solvent) into the intracellular fluid of the cell concerned.

As there are no known essential biochemical functions for any of the actinides, the existence of so-called active transport mechanisms for the uptake of these metals across the intestinal mucosa and throughout the rest of biochemistry is not under serious consideration. The ions, however, may occasionally be taken up by the

intake mechanism for iron but, by and large, the main route of bio-availability is that of the passive diffusion of net-neutral LMM complexes of the actinide across cell boundaries and in the general direction of down a concentration gradient. Thus, the gradient may be adjusted to move the metal ion into tissue or, alternatively, the chemical speciation gradient may be so arranged to move the metal ion out of a tissue during decorporation therapy, and by avoiding precipitation and particulate matter and the complications of redoxing as much as possible.

A lengthy chapter concerning the biochemistry of the actinides appears in a handbook on the physics and chemistry of the lanthanides soon to be published [5]. Each actinide element has a distinct biochemical response which is dependent on the ligands to which it is complexed *in vivo* and it is important to realize that this behaviour, reactivity, threat, toxicity etc. can completely change when the metal ion redoxes or changes its ligands which bind it intermittently. The review describes each of the actinides present in blood plasma and the forms in which they have been identified, the challenges that such actinide complexes pose to naturally occurring iron biochemistry, the metalloproteins which are typically found complexed in equilibrium with LMM fractions of the actinides, and the forms in which such agents occur within cells and bones.

6. Interactions with chelating drugs *in vivo*

Being such highly charged metal ions, it is easy to understand that they are firmly bonded to oxygen donor groups from naturally occurring biochemicals. Thus, the main thrust of decontamination therapy involves using polydentate (in order to acquire the maximum benefit from the chelation effect), multiple carboxylate or multiple phenolate groups to wrap totally all coordination positions of the metal ion in electron donor pairs without causing precipitation or polymerization. This requires additional charge groups on the ligand in order to maintain water solubility for such large metal complexes. The net overall charge on such complexes needs to be arranged to be net-neutral for membrane permeability or, indeed, net-charged (either positive or negative) for excretion through the kidneys to follow the lines dictated by synergistic chelation therapy mentioned earlier.

Whereas nature has evolved exceedingly metal ion specific ligand complexing systems for those metals which are essential to life processes, laboratory-made chemicals at best can only achieve selectivity for the offending metal ion being removed. Thus, all chelation therapy involves some side effects usually implicating the removal of metal ions thought to be essential for the biological process. The quantification of such side effects (by computer simulation and then by monitoring fluids excreted), the recognition of clinically known side effects (such as the lack of taste acuity and tinnitus associated with EDTA removing essential zinc) and the choice of bio-available form of the element for topping-up therapy all constitute a sophisticated and advanced field of specialized medicine. Advanced texts are now appearing specifying the regimens to be used for removing different actinides which have entered the human body via different routes and as a wide range of species [8].

One of the most widespread ligands available to clinicians treating contaminated workers in the nuclear industry is that of diethylenetriaminepentaacetate (DTPA). The agent administered as an acid or a sodium salt may be expected to cause the co-excretion of calcium or of zinc and so the topping-up therapy is somewhat achieved by administering the agent as the calcium or zinc salt ($CaNa_3DTPA$ or $ZnNa_3DTPA$). Many of these agents are difficult to administer because they are so richly endowed with charge groups which are mandatory for complexing with the highly charged actinide cation and for keeping the resultant complex in solution. Thus, aerosols which project them into the lungs by inhalation are high on the agenda for the first line of defence against contamination by an actinide. There is a well-established case history in the literature of a worker contaminated with ^{241}Am to have been treated with over half a kilogram of DTPA administered intravenously over a considerable period of time and without any ill effects attributable to the therapy.

7. Wound decontamination [9]

Most contamination incidents in the nuclear industry involve scuffs or grazes or the epidermis being penetrated by sharp objects such as needles or broken glass. The localization of the actinide thus introduced, its removal which must precede dressing and closure of the wound and the eventual healing of the wound and associated monitoring of residual activity *in vivo* all constitute a skilled profession which touches on many important disciplines.

The field of wound management in terms of cleaning out of contaminants, the administration of essential nutrients, and the control of bacteria within the wound leading to a healthy and timely healing process is now a well-developed specialization in a few surgical research centres around the world. Chemical speciation models have been constructed concerning wound fluid throughout the healing process and the influence of decontaminating agents and of radiation within the healing wound is currently being researched.

Changed attitudes to wound dressing are desirable by, for example, considering that often the rate-determining step in healing a straightforward wound is that of removing the excreted biochemicals and debris from the wound by applying dressings which force this material back into the venous system for whole body excretion through the normal routes. Clearly, this is highly undesirable if the debris contains radioactive fission products or actinides. Similarly, the removal of such actinides using chelating drugs may require topping up therapy directly onto the open wound rather than systematically through the oral route. Alginate-based dressings are currently being researched in this respect.

8. Future research needs

The field of researching the chemical speciation of the actinides in the bio-fluids encountered in the human body is probably at the limit of intellectual, chemical analytical, and computational simulation modelling capabilities. There is clearly a need for more chemical speciation research to validate models being used to guide clinical decisions.

Much work on optimizing decontamination therapies of sites such as wounds can be done on animal carcasses from slaughter houses. Similarly, more needs to be done in terms of establishing internationally accepted treatment regimens applicable in the nuclear industry throughout the world.

Legislation is now being established and enacted both in terms of the chemical speciation as well as in terms of the total amounts of contaminating elements in food and in our environment. Nuclear plant and reprocessing operators would do well to be ahead of the legislation in enhancing their knowledge of chemical speciation lest administrators choose targets which are unachievable or unmonitorable using current state-of-the-art equipment.

Legislative levels, means of treating the injured, operator exposure limits, action levels for decontamination, must all be subject to international discussion and agreements because, as the Chernobyl incident has aptly demonstrated, the plume from such incidents does not stop at international boundaries but, rather, is a challenge to persons not directly the responsibility of the government hosting the nuclear facility.

All the areas touched on in this review would benefit from the top scientists in many countries collectively tackling these fascinating challenges.

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