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Aluminium speciation in relation to aluminium bioavailability, metabolism and toxicity

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Abstract

Aluminium toxicity may act in two distinct ways, depending on the level of contamination. Relatively low aluminium levels from environmental origin (mainly from drinking water poor in silica) have been shown to be statistically associated with senile dementias of Alzheimer type (chronic intoxication). In addition, high aluminium therapeutic levels (from phosphate binders, antacids, . . .) can induce different, more rapid, symptoms (acute intoxication). In all cases, aluminium toxicity is largely conditioned by aluminium bioavailability, which in turn hinges upon aluminium coordination chemistry in vivo. The highly polarising power of the Al^{3+} ion dictates its particular affinity for oxygen donors that abound in essential biomolecules and dietary substances. The influence of these substances on aluminium bioavailability, metabolism and toxicity can be assessed through animal models. However, understanding

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the mechanisms through which aluminium–ligand interactions may influence physiological processes on the molecular level requires a knowledge of the speciation of the metal in the main biofluids. Access to this critical information can a priori be gained through direct experimental analysis of relevant biological samples. It is in this way that aluminium protein-bound fractions, involving essentially transferrin, have been identified, but using such a direct approach to analyse the ultrafiltrable pool of the metal is a virtually insurmountable task, hence the necessity to have recourse to computer-aided speciation techniques based on simulation models. Following a previous review published in this journal on nearly the same topic [Coord. Chem. Rev. 149 (1996) 241], this article updates the knowledge available on both biological and chemical fronts. After a review of experimental investigations led on the roles of aluminium–ligand interactions in aluminium bioavailability, metabolism and toxicity, contributions of experimental and computer-aided speciation to the understanding of the relevant processes are then analysed. Significant progress has been made in the diverse aspects of the biological field, in particular, in relation to the role of dietary ligands on aluminium gastrointestinal absorption, excretion and tissue distribution. Also, very intensive research has been pursued on the design of new aluminium sequestering agents to treat acute intoxications. Some progress has also been made on the chemical side relative to computer-aided speciation applications to gastrointestinal and blood plasma conditions. However, the gap is increasing between the large body of observations made by physiologists and toxicologists and the few data painfully obtained by coordination chemists to interpret the relevant phenomena.

Keywords: Aluminium speciation; Aluminium–ligand interactions; Aluminium bioavailability; Aluminium absorption; Aluminium excretion; Aluminium toxicity; Computer simulation models

1. Introduction

The first special volume of this journal devoted to aluminium chemistry (vol. 169, 1996) already contained an exhaustive review by the same author on 'Chemical speciation studies in relation to aluminium metabolism and toxicity' [1]. The present paper, whose title has been proposed by the Guest Editor very close to the former, is meant to cover the same topic. It will therefore mainly consist of an overview of the relevant literature appeared in the meantime. For the information of new readers, however, a brief recall will be made of the basic principles of aluminium chemistry in the updated context of aluminium toxicity.

A number of points regarding the relationship between aluminium chemistry and toxicity are now well understood. In particular, much progress has been made during the last decade on the comprehension of the role of aluminium as a pro-oxidant agent in vivo (see Ref. [2]). Yet, much remains to be done to elicit the various mechanisms through which aluminium can exert its toxic properties. In the first place, most of aluminium metabolic processes, in particular, regarding the access of the metal to the brain, are still unknown. As always when a metal ion is involved in relatively labile coordination equilibria in vivo, global analytical techniques can be used to follow its routes of absorption, retention and excretion. However, these are generally insufficient to unravel the molecular mechanisms that underlie the macroscopic data to which they give access. In particular, they are unable to pinpoint the species which, though occurring at very low concentrations, may trigger aluminium toxic effects. Chemical computer-assisted speciation still represents at the present time the only possibility to bridge this gap. The main objective of this review is therefore to illustrate the effective capacities of this technique in this context, by putting its most recent applications in perspective with parallel advances in research on aluminium bioavailability, metabolism and toxicity.

2. Aluminium–ligand interactions and aluminium bioavailability and metabolism

2.1. General considerations on the risks bound to aluminium

In spite of its abundance and ubiquity on the Earth's crust, aluminium has been excluded by nature from all life processes probably because of both its poor availability and its unfavourable chemical properties [3]. As 'detrimental' in the sense of the current evolutive classification of elements (see Ref. [1]), aluminium may exert its toxicity within two distinct concentration ranges in vivo, these reflecting two different types of contamination. Whereas low level chronic environmental contamination may result from aluminium dietary intakes exceeding human natural capacities of accommodation, high level acute iatrogenic intoxication may afflict patients treated with high doses of oral aluminium-based drugs (phosphate binders, antacids, antidiarrheics, ...). Taking this distinction into account (see Fig. 1 of Ref. [1]) renders the long lasting debate on the possible implication of aluminium in the genesis of Alzheimer's disease (AD) [4] largely irrelevant. Clearly, there is no comparison between the high aluminium burden rapidly constituted within the body of uremic patients diagnosed with 'dialysis encephalopathy' [5, see also Ref. [6] for more references] and the trace amounts of aluminium accumulated along decades in the brain of AD patients consuming low-silica aluminium-contaminated drinking water ($>100\ \mu g\ l^{-1}$) (see Ref. [7] and references therein). For solution chemists with a knowl-

edge of aluminium coordination complexity, different overall concentrations of the metal in identical locations in vivo are expected to result in different distributions of its possible complexes, hence in different expressions of its toxicity. Therefore, arguing against a possible contribution of aluminium to the genesis of AD on the sole ground that brain tissues characteristic of dialysis encephalopathy and AD display distinct histological features seems excessive, not to say irrelevant. (Also excessive at the very least is the widespread impression that AD is of genetic origin whereas the sporadic form of the disease represents more than 95% of reported cases [8]!) Even though no cause and effect relationship has yet been characterised between aluminium and AD likely to substantiate the repeatedly observed correlation between high aluminium level in drinking water and high AD incidence rate (see Ref. [7] and references therein), there remains the possibility for aluminium to act as an AD inducer of environmental origin [9] like manganese is supposed to do in prion diseases [10,11]. Finally, denying any significance to the above-mentioned correlation on the argument that the aluminium contained in drinking water represents a relatively small fraction of the whole aluminium dietary intake comes down to neglect the notion of bioavailability, which actually determines the extent of aluminium absorption, retention and excretion.

2.2. Aluminium–ligand interactions and aluminium gastrointestinal absorption

Contrary to essential and beneficial metal ions whose utilisation by the body has, in the course of evolution, led to the elaboration of specific processes capable of acquiring necessary amounts from oral intakes and/or excreting excesses within certain limits (see Refs. [12,13]), a detrimental element like aluminium is not expected to benefit from any sophisticated metabolic regulation. As already stressed [1], aluminium absorption should therefore simply depend on the solution chemistry of the Al^{3+} ion in the gastrointestinal fluid. Thus, the only absorption criteria that aluminium shares with other metals are: (i) the solubility of the Al^{3+} ion within the gastrointestinal pH interval, and (ii) its neutralisation by complexation (e.g. by the anion of the ingested salt), the neutral species formed being expected to facilitate the metal diffusion through the intestinal membrane. In addition, the large hydration of the Al^{3+} ion can allow it to cross the intestinal epithelium paracellularly, via solvent drag [14].[1] Finally, aluminium may also share some absorption pathways with essential substances for which it may substitute or with which it may combine (see Ref. [1] for more references).

2.2.1. Aluminium bioavailability

The average fraction of aluminium absorbed from a given dose is estimated to be very low ($\sim 1\%$ from whole animal techniques) even though it is subject to large variations (0.06–27% in animals and 0.001–24% in humans) depending on the aluminium intake (see Refs. [15,16]). However, the observed decrease in aluminium absorption percentage with increasing metal doses (10- to 100-fold greater from small amounts of environmental aluminium, i.e. ~ 5 mg, than from large doses of therapeutic aluminium, i.e. 1–3 g per day, in humans [15]) is far from cancelling the risk of toxicity for patients on long term aluminium-based treatments, even with normal renal function and within manufacturer's label recommendations [17,18]. The perception of an apparent capacity of adaptation of aluminium absorption to ingested doses largely results from the use of the percentage scale which may, for example, convey the wrong impression that 4% of 125 mg represent less metal than 26% of 5 mg [19]. The use of the percentage scale to evaluate aluminium absorption is also misleading in that it may suggest that a regulatory mechanism is at the origin of the above apparent compensation, whereas this effect simply results from the influence of gastrointestinal Al^{3+}–ligand interactions on aluminium bioavailability (to be seen later).

Even the generally low capacity of aluminium to be absorbed under normal conditions of life is insufficient to ensure a complete safety in the long term. Although labile aluminium present in drinking water is rapidly complexed (and often precipitated) by food constituents as water enters the gastrointestinal tract, resulting in a very low concentration of available Al^{3+} ion [16,20], any saturation of the aluminium-binding capacity of feed components is expected to allow the Al^{3+} ion in excess to remain fully bioavailable [21]. This might bear some consequences for the genesis of AD (see Ref. [7]), especially as trace amounts of ^{26}Al from a single exposure have been shown to directly enter the brain tissue of rats and as it has been calculated that the amount of aluminium taken into the human brain from drinking water over 7–8 decades is almost equal to the amount of aluminium measurable in the mature brain [22]. Also of interest in this respect is the recent discovery that only the organic monomeric fraction of the aluminium present in drinking water is associated with AD [23].

Finally, the extent of aluminium absorption may be subjected to variations due to disease. In particular, it has been shown to be significantly increased in patients with AD [24,25] as well as Down syndrome (DS) [26].

[1] It is noteworthy that the term 'passive diffusion' is often used by physiologists to designate both the transcellular diffusion of a neutral metal complex and the paracellular diffusion of the hydrated form of the metal ion involved.

2.2.2. Site of aluminium absorption

Based on compared solubilities of aluminium trihydroxide and monophosphate salts within the gastrointestinal pH range, aluminium absorption was initially suggested to 'largely (occur) in the acidic milieu of the proximal duodenum or stomach and minimally, if at all, in the rest of the gastrointestinal tract' [27]. Aluminium was believed to be gradually solubilised in the chlorhydric stomach after ingestion, being then (re-)precipitated and made unavailable on entering the milder conditions of the duodenum (see Ref. [1] for more references). It was later realised [28] that like essential metals also precipitating at neutral pH which were absorbed through their interactions with ligands present in the intestinal fluid, aluminium could also be bound to such substances and prevented from precipitating during transit.

More than secretory ligands [29], exogenous ligands were effectively found to be determining in extending the fraction of intestine over which aluminium can be absorbed (see Ref. [1] for references). All segments of the small intestine (duodenum, jejunum, ileum) have in fact a potential for aluminium absorption [30], depending on prevailing conditions. In patients on aluminium hydroxide therapy, for example, the acidity of the upper part of the gastrointestinal tract is certainly the key factor for aluminium absorption as releasing a larger fraction of soluble Al^{3+} ions [27]. In the presence of citrate, on the contrary, the role of the ligand becomes predominant and serum aluminium peaks 4 h of administration, suggesting small bowel absorption [31]. Thus, even though proximal small intestine seems to be the most favourable site for aluminium absorption, physiological conditions ultimately determine the site actually used [32]. More recently, it has been confirmed that despite the potential for hydroxypolymerisation of aluminium at intestinal pH, the small bowel and colon absorbed aluminium passively and paracellularly but the stomach did not, the predominantly proximal absorption of aluminium observed in vivo being thought to reflect the proximal absorption of dietary constituents (e.g. citrate) that enhance mucosal permeation of the metal [33].

2.2.3. Mechanisms of aluminium absorption

Kinetically, chemical substances may be transported in vivo through passive transfer (simple diffusion or filtration) or specialised transport (active transport, facilitated diffusion or pinocytosis). Physiologically, intestinal absorption may be transcellular (through the brush-border—mucosal—membrane, the cytoplasm, and the basolateral—serosal—membrane of the enterocyte, successively) or extracellular. Transcellular routes, respectively, include lipid and aqueous routes for non-ionic and water-soluble solutes, and a carrier route for hydrophilic substances too large for the aqueous route. They may involve passive, facilitated and active processes. The main extracellular route is the paracellular route via the 'leaky' junctions between cells which, though often classified as passive diffusion, rather consists of a solvent drag phenomenon (see Ref. [1] for more references).[1]

Regarding aluminium, the notion is widely accepted of its large uptake by the gut resulting in little absorption, adsorption of Al^{3+} ions onto the insoluble mucus and precipitation of their salts combining to limit metal net absorption. Aluminium absorption is therefore generally perceived as a biphasic process involving first a fast mucosal uptake, then a slow release of the metal into the blood [1]. The comprehension of this process is obscured by two factors: the chemical form under which aluminium is ingested is rarely that under which it is eventually absorbed, and concentration dependence may be confused with species dependence. According to van der Voet [32], each aluminium species most probably has its own absorption mechanism (see Ref. [1]), hence the importance of computer-aided speciation to analyse the problem on the molecular level.

Detrimental metals may share absorption pathways with their essential chemical neighbours. Possible aluminium interactions with calcium and iron have been the most extensively investigated in this respect (see Ref. [1]). However, the conclusions reached about these were questioned on the argument that the small size of the Al^{3+} ion would not favour substitution for the larger Ca^{2+} and Fe^{2+} ions. In fact, the Al^{3+} ion bears a greater resemblance with Mg^{2+}, which the indication of a similar solvent drag mechanism for Al^{3+} [33,34] and Mg^{2+} [35] seems to confirm (see more references in Ref. [1]). This mode of absorption was again recently confirmed in rats, with the parallel characterisation of a transcellular and saturable route, the aluminium level in blood being not modified with increased aluminium in the intestinal lumen [36].

2.2.4. Effects of dietary components

The co-ingestion of aluminium with ligands may reciprocally influence the gastrointestinal absorption of both. Among dietary ligands demonstrated to enhance aluminium absorption, citrate has been the most often cited (see references in Ref. [1]; see also, more recently, Refs. [36–41]). As already pointed out [1], the citrate effect was long thought to stem from the formation of the neutral [Al–Cta] species within the $1 < pH < 4$ range. It was then demonstrated by Alfrey's group [34] that citrate mainly acts in the proximal bowel by complexing free endogenous Ca^{2+} ions that line the basolateral membranes of intestinal cells, easing the opening of their 'leaky' junctions, hence the paracellular absorption of Al^{3+} ions by solvent drag (see above).

Other dietary ligands including ascorbate, gluconate, lactate, malate, oxalate, succinate and tartrate can also

facilitate aluminium absorption, as was demonstrated in animal studies (see Ref. [1] for references). The capacity of these ligands to elevate the pH of precipitation of the Al^{3+} ion from near 4.5 to above 8 in vitro [42] at least partially substantiates this effect. In addition to these compounds, fluoride [43], glutamate [36], and also gallate, chlorogenate, caffeate and protocatechuate [41], all of which are largely present in food and beverages, have recently been shown to enhance aluminium gastrointestinal absorption in rats.

Ligands may also prevent aluminium absorption. In addition to phosphate whose well-known capacity to precipitate aluminium in the form of the [$AlPO_4$] salt is used in oral aluminium-containing drugs to control phosphatemia of uremic patients, another ligand commonly found in food and beverages (particularly in water), silica, can also reduce aluminium absorption. Birchall even attributed the essentiality of silicon to its capacity to limit aluminium access to the body (see Ref. [1]), which seems to be confirmed by recent results on the protective role of silica with respect to the possible induction of AD by aluminium in drinking water [7,44]. The effect of silica was recently investigated on rats by two different groups. In the first study using ^{26}Al measurements, silicon administered at concentrations up to 14 mg l^{-1} in drinking water failed to depress physiological intestinal aluminium absorption under basal conditions or after stimulation by citrate [38]. In the second, which used ICP spectrometry aluminium determinations, higher doses of silicon (up to 118 mg l^{-1}) also administered in drinking water were shown to effectively prevent aluminium gastrointestinal absorption [45].[2] It has been more recently reported from an investigation on healthy volunteers that the oligomeric, high-aluminium-affinity form of soluble silica reduced aluminium availability from the gastrointestinal tract, but that monomeric silica had no effect [46].

Finally, metal ions also naturally occur in aliments and beverages. The effects of Na^+ and Ca^{2+}, alone and in combination, on the intestinal absorption of Al^{3+} have been recently investigated on rats. Two one-sided negative interactions have been characterised between aluminium and sodium ions (Al^{3+} being able to displace Na^+, but not the contrary) and between calcium and aluminium ions (Ca^{2+} being able to replace Al^{3+}, but not the contrary) during the process of mucosal uptake. Both interactions are maintained on the systemic level. It has been suggested from this that the Al^{3+} ion might try to mimick Ca^{2+} in its Na^+-dependent passage [47].

[2] Interestingly, concentrations of silica considered to reduce significantly the risk of dementia and AD for populations consuming drinking water with high aluminium levels (> 0.1 mg l^{-1}) was defined as ≥ 11.25 mg l^{-1} [7].

2.3. Aluminium–ligand interactions and aluminium excretion

The kidney appears to be the major route for the elimination of systemic aluminium [48,49]. Under ordinary conditions in healthy people, it is thought to be able to excrete all of the absorbed aluminium. However, in patients fed by total parenteral nutrition receiving aluminium-contaminated solutions, its capacity is exceeded even in those with normal renal function, and part of the infused metal is retained [48,50]. To which extent the normal kidney can excrete the aluminium absorbed by patients treated with large oral loads is difficult to quantify. Aluminium renal excretion can be inefficient [51] for a number of reasons. One of these is protein-binding, which limits aluminium ultrafilterability, even though the fraction of metal that is protein-bound decreases with increasing plasma aluminium concentration, and at very high plasma aluminium levels, the ultrafilterability of the metal is very low ($\sim 1\%$) as a consequence of formation of colloid [51].

In addition to proteins, small ligands capable of significantly binding aluminium in plasma are also expected to interact with the renal excretion of the metal. In particular, citrate, isocitrate, ascorbate, lactate, malate and tartrate have been shown to enhance aluminium urinary excretion (see Ref. [1] and references therein). A large consensus was reached on citrate, whose influence was attributed to its capacity to enlarge the ultrafiltrable fraction of aluminium in serum. In contrast, other organic acids would combine urinary excretion—also via the low-molecular-mass (l.m.m.) ultrafiltrable pool of the metal—and biliary excretion—through its high-molecular-mass (h.m.m.) fraction. The above consensus about citrate was questioned on the ground that aluminium excretion rose more slowly than plasma citrate and that plasma aluminium fractions did not significantly changed with citrate infusion, suggesting that the increased excretion of aluminium was independent of the aluminium filtered load and might be the result of changes in the handling of the metal within the kidney [52]. A possible alternative explanation may be that the significant changes that are visible in urine may result from insignificant changes affecting the stationary states pertaining to plasma, the additional l.m.m. aluminium mobilised by citrate being excreted as soon as formed [1].

More recently, the more effective excretion of aluminium intravenously administered to conscious rats as citrate than sulphate [53] or chloride [54] was once again attributed to the much greater filterability of aluminium administered as citrate [53,54]. It was suggested that 'following aluminium sulphate administration, the filtered aluminium (might) be an aluminium citrate form (...) then reabsorbed in the same way as aluminium administered as citrate' [53]. Another possibility may be

that infused aluminium citrate is readily excretable whereas (free) aluminium administered as sulphate or chloride may first be taken up by transferrin and rendered less easily excretable. Interestingly, aluminium concentrations were significantly higher in the livers of rats receiving the chloride [54]. As an illustration of the relative excretability of aluminium injected intravenously, a ^{26}Al investigation on humans revealed that $27 \pm 7\%$ of the administered metal were retained in the body for 5 days, faecal excretion being negligible ($\sim 1\%$) [55]. (The negligibility of aluminium elimination through the liver was confirmed independently [56].)

The effect of silicon on aluminium urinary excretion was investigated in patients following renal transplantation [57]. Unlike aluminium, serum silicon progressively decreased with improving renal function and was significantly positively correlated with serum aluminium, but not with silicon excretion. Aluminium urinary excretion peaked between 4 and 8 days post-transplantation and was highly significantly positively correlated with urine silicon. It was suggested that the two elements might be cleared by the kidney through a common mechanism or as a chemical species, possibly as hydroxy-aluminosilicate [57]. The role of silicic acid in the renal excretion of aluminium in healthy individuals was also analysed [58]. The majority ($\sim 56 \pm 8\%$) of the 600 μmoles of silicon (given with 2.67 μmoles of aluminium in 1.13 l of beer) was excreted within 8 h, concomitant with a significant increase in aluminium excretion. Aluminium excretion reached a maximum and then declined, consistent with depletion of aluminium body stores [58]. This was separately confirmed using the ^{26}Al isotope [58].

2.4. Aluminium–ligand interactions and aluminium distribution in vivo

The fraction of aluminium that reaches the systemic circulation and is not excreted in the urine rapidly accumulates into tissues where it is strongly bound. Thus, plasma aluminium only reflects recent exposure and is a poor index of total body aluminium burden [59]. For example, the uptake of ^{26}Al into bone was shown to occur within ~ 1 h of oral administration in rats and remained irreversible for the 30 days of the experiment at least [39]. Once again, aluminium–ligand interactions are expected to play a determining role in the processes involved (see Ref. [1] and references therein). In the above study, for example, even though the time necessary for aluminium accumulation in bone was found to be the same with or without citrate, the extent of aluminium accumulation was increased by a factor of ~ 5 in the presence of citrate [39]. Citrate also led to a two-fold increase in aluminium retention in liver and brain [39]. These results confirmed previous observations relative to: (i) brain tissue/blood aluminium ratios that were found superior for aluminium citrate than for lactate in rats and rabbits [60]; (ii) 1.5-fold greater aluminium body loads in rats fed aluminium hydroxide with citrate compared to aluminium hydroxide alone [61]; and (iii) induction of aluminium deposition in rat brain by citrate-maltol causing the same effect in serum and bone [62].

3. Aluminium–ligand interactions and aluminium toxicity

3.1. Aluminium–ligand interactions and neurodegenerative disorders

The main concern about aluminium toxicity refers to its implication in various neurodegenerative processes, potentially AD [4] (see Section 2.1). A general review on the toxicology of aluminium in the brain has recently been published by Yokel [63].

3.1.1. Aluminium and its access to brain

The first step in the uptake of metal ions into brain is the passage across the blood–brain barrier (BBB). The BBB is formed at both the brain capillary endothelium and choroid plexus epithelium by a single layer of cells joined by tight junctions. In contrast to the loose junctions of the intestinal epithelium (see Section 2.2), these junctions closely link adjacent cell membranes, thereby sealing off the paracellular cleft to aqueous diffusion. To cross the barrier, most solutes must therefore: (i) dissolve in and diffuse across the lipid endothelial cell membrane by passive diffusion—which largely depends on the lipophilicity of the substance—or (ii) be transported across by specific carrier, channel, or receptor mechanisms [64]—e.g. glucose. The barrier contains ion transporters (e.g. Na^+–K^+ ATPase, Ca^{2+} ATPase) and channels that aid in the secretion of the cerebrospinal fluid (CSF) and contribute to the regulation of the ionic homeostasis of brain extracellular fluid [65]. Formed at the choroid plexus epithelium, the CSF cushions the brain and acts like a 'neural lymph' as there is free exchange of solutes between CSF and brain interstitial fluid. Some solutes gain access to brain indirectly from the choroid plexus via the CSF pathway, others from the small 'circumventricular organs' that lack a BBB, or via retrograde transport from areas outside the barrier [64].

Given its slow transfer, aluminium—like iron—is thought to be taken up into brain in part by a transferrin-dependent mechanism, but l.m.m. complexes may play an important role [64]. Although it does not disrupt the integrity of membranes, aluminium may directly alter the BBB function. It is known to bind to BBB endothelial cells and to be deposited around blood vessels in patients with dementia. It can also increase the passage of many compounds thought to cross the BBB

by transmembrane diffusion, and can selectively affect saturable transport systems [1].

Brain cells possess a specific high-affinity receptor for transferrin that is independent of the metal being transported. This transferrin–transferrin receptor system is postulated to be the route whereby iron accedes to the brain from the general circulation. It has been demonstrated [66] that a metal ion other than iron is capable not only of binding to transferrin but also of utilising this interaction to gain access to cells in the brain via the above system [1]. Thus, aluminium may be capable of interfering with normal cellular iron homeostasis and disrupt iron-dependent cellular processes in the CNS. (Interestingly, ferritin from AD brains has a six-fold higher aluminium content than normal age-matched controls [1].) It is unlikely, however, that transferrin-receptor mediated endocytosis is the sole mechanism of aluminium entry into the brain [67]. On the other hand, evidence has been provided of an energy-dependent transport of aluminium out of brain extracellular fluid into blood or brain cells [67], this process being later shown to be proton-dependent [68].

Aluminium incorporation into neurones and astrocytes is ligand dependent. The uptake of aluminium into both neuronal and astroglial cells has recently been examined in parallel to aluminium speciation [69]. The relative accumulation of aluminium maltolate, lactate, chloride and fluoride was investigated and correlated with cell viability and intracellular distribution. Significant differences in aluminium incorporation and toxicity were observed in both neuronal and glia cells with the largest effects exhibited by the maltol species. This was accompanied by a nuclear accumulation in the neuronal cell line but a perinuclear, vesicular distribution in astrocytes that partially co-localised with cathepsin D, a lysosomal marker [69]. Thus, aluminium–ligand interactions are likely to modulate aluminium toxic effects in the brain.

3.1.2. Aluminium and Alzheimer's disease

Senile plaques (SPs) and neurofibrillary tangles (NFTs) are the main neuropathological hallmarks of Alzheimer's disease—together with dystrophic neurites and synaptic loss more recently [70]. Both structures may contain a fibrous complex known as amyloid, whose main component is a 40–43 residue peptide known as amyloid-β peptide (Aβ). Structurally filamentous, SPs comprise both neuronal and non-neuronal elements. Neuronal components consist of degenerative and regenerative neurites. Non-neuronal components consist of amyloid protein and extracellular deposits such as aluminosilicates [1]. Neurofibrillary tangles are primarily located intracellularly, consisting of paired helical filaments (PHFs) in the neuronal axon. As they persist in the brain even after the neurone has died, extra-neuronal NFTs can be seen as 'ghosts' of original neurones in severely affected areas of the AD brain. The main component of PHFs is a protein normally associated with microtubules and rapid axonal transport, τ. Abnormal τ was first thought to be hyperphosphorylated (see Ref. [1]), but a study on τ phosphorylation in fresh brain biopsy material has since shown that this degree of phosphorylation is normal, and the paired helical filament τ that comprises the NFTs just does not dephosphorylate after death as rapidly as that in normal brain [71]. Intracellular NFTs do not contain demonstrable amyloid, but amyloid is deposited onto extracellular NFTs, which do not contain τ protein (any longer—τ being presumably stripped away during neurone degeneration). Age-stratified studies have shown that Aβ amyloid deposition precedes NFT formation by about two decades [72].

The first clue in favour of a role for aluminium in the genesis of AD was the finding of deposits of the metal in the brain of AD patients, located within SPs and NFT-containing neurones characteristic of the disease (see Ref. [1] for references). Evidence for selective aluminium accumulation in NFTs of NFT-bearing neurones was obtained in AD subjects [73], and AD-like changes in τ protein processing were found in the brains of renal dialysis patients following prolonged exposure to aluminium [74]. (Aluminium would bind to PHFτ, inducing PHFτ aggregation and proteolysis retardation [75].) Most of former results on this topic were controversial because of possible tissue contamination and practical analytical difficulties (see Ref. [4] for a review). The emergence of new techniques is expected to resolve the controversy. For example, aluminium detected in SPs and NFTs has been shown to be contained in lipofuscin granules[3] with silicon, probably as aluminosilicate [76,77].[3] High levels of aluminium and iron were detected in the nuclei of NFT-free and NFT-containing neurones by laser microprobe mass analysis, suggesting the existence of an association between the deposition of aluminium and iron, and NFT formation [78]. In contrast, a more recent study by nuclear microscopy did not detect aluminium in pyramidal neurones from the brain tissue of AD patients, though significant increases in iron, phosphorus and sulphur concentrations were found with respect to neurones from age-matched controls [79].

Another important argument for the implication of aluminium in AD is the capacity of the metal to cause

[3] Lipofuscin granules are a degenerated form of lysosomes, whose physiological function is to degrade unnecessary substances in the cell. Inorganic materials, which cannot be degraded, accumulate in lysosomes and gradually lead to lysosome dysfunction, catabolic enzymes leaking and hence brain cell degeneration [76].

[4] Aβ aggregation was also induced through free radical generation involving metal-induced oxidation of histidine, tyrosine and methionine residues (see Refs. [80,121]).

the Aβ aggregation that is at the origin of SP formation [80]. Not only was it shown in vitro that aluminium could directly bind the peptide, thereby inducing the random coil to β-sheet conformational change that is required to initiate its aggregation process [81–83], but also that silicate could reverse both binding and conformation [84,85].[4] In this connection, Aβ secondary structure was reported to be stabilised by aluminium and D-Asp substitutions [86]. Circular dichroism spectra of the D-Asp substituted analogues of amyloid peptides, $A\beta_{6-25}$ and $A\beta_{1-40}$, showed a distinct blue-shift on aluminium complexation, suggesting that the aluminium interaction with D-Asp induces the peptide backbone to increase its antiparallel β-sheet character [86]. In contrast to most biological metal ions, aluminium also promoted aggregation of amyloid precursor protein in a dose-dependent manner in vitro, this effect being prevented or reversed by EDTA [87]. Long-term exposure of cultured cortical neurons to aluminium promoted Aβ and enhanced its neurotoxicity [88]. Aluminium interaction with $A\beta_{31-35}$ in trifluoroethanol also induced a gradual shift of the conformational equilibrium toward β-sheet structure, but reflected no specific binding site for the Al^{3+} ion [89]. Regarding the above argument of direct aluminium–Aβ interactions, it can be rightly opposed, however, that whereas Aβ was effectively precipitated by zinc in *physiological concentrations* [90], it was *not* by aluminium [91].

In contrast with Aβ, native conformation was shown to be preserved in aluminium-induced τ aggregation despite NMR evidenced aluminium binding [92]. Compared to other proteins, τ displayed only an average sensitivity towards aluminium-induced aggregation, but this sensitivity increased by 3.5-fold on phosphorylation [93]. Aluminium-induced τ aggregates dissolved in the presence of EDTA whereas aluminium-induced phosphorylated τ aggregates remained insoluble and insensitive to proteolysis [93]. Similarly, abnormal τ accumulation and partial reversal by desferrioxamine was observed in aluminium-induced neurofibrillary degeneration in rabbits [94]. Also, neurofilament organisation was shown to accumulate in perikaryon following aluminium administration, suggesting an association between neurofilament phosphorylation and organisation [95].

Aluminium at physiological concentrations has been shown to cause genetic malfunction through irreversibly unwinding supercoiled DNA pockets in genome [96]. This effect was prevented by EDTA. Aluminium is not the first metal to be able to uncoil supercoiled DNA, but it is the first to relax it totally and immediately. Accumulation of aluminium in brain may also affect the regulation of RNA metabolism [97]. Interestingly, aluminium was shown to induce changes in amyloid precursor protein mRNA expression [98].

Both human and mouse neuroblastoma cells have been used as models to demonstrate aluminium uptake and toxicity. Aluminium treatment of intact neuroblastoma cells has been shown to alter neurofilament subunit phosphorylation, solubility, and proteolysis. Aluminium induced the appearance of extensively phosphorylated neurofilament isoforms in cytoskeletons of undifferentiated cells and increased levels of these isoforms in differentiated cells. Neurofilament subunits isolated from intact cells treated with aluminium were resistant to in vitro dephosphorylation and degradation [99]. These alterations were accompanied by a greater tendency of neurofilaments to form insoluble aggregates after isolation. Thus, aluminium can induce direct effects on neurofilament subunits within intact neuronal cells [99]. More recently, aluminium was shown to enhance iron uptake, inhibition of cell growth and expression of τ protein in murine neuroblastoma cells, partially mimicking the pathological hallmarks of AD [100]. Astrocytes and neurones have also been used to investigate the effects of long exposures to aluminium 1 mM (a concentration largely beyond physiological limits, unfortunately) [101]. Exposure to aluminium for 8–12 days caused strong changes in the morphology of astrocytes including shrinkage of cell bodies and retraction of processes. Exposures over 15–18 days reduced astrocytes viability by 50%, astrocyte degeneration involving the DNA fragmentation characteristic of apoptosis. Aluminium was also neurotoxic, causing first (4–6 days) abnormal clustering and aggregation, and later (8–12 days) neuronal death. Interestingly, aluminium neurotoxicity occurred in neuroglial cultures containing approximately 10% astrocytes but not in neuronal cultures containing only 1% astrocytes. Staining of co-cultured cells showed apoptotic condensation in aluminium-treated astrocytes but not in co-cultured neurones. It therefore seems that aluminium can induce the apoptotic degeneration of astrocytes, and that this toxicity is critical in determining neuronal degeneration and death [101].

Regarding investigations into aluminium occurrence in the body during AD, patients with probable AD have been shown to have statistically significant higher serum aluminium levels than patients with other types of senile dementias (alcoholic, vascular, multi-infarct) and an age-matched control group, no significant differences being found between serum aluminium of patients with non-AD senile dementias and the age-matched control group [102]. In contrast, no statistically significant differences were found between the aluminium levels in the blood, urine and CSF of another group of 15 AD patients and 20 control individuals [103]. Trabecular bone aluminium content was evaluated in seven other AD patients and 19 age-matched controls. Quantitative analysis showed a 95% significant lower aluminium content in the AD versus the non-demented group,

which does not support a hypothesis of excessive aluminium absorption and tissue accumulation in AD [104]. Patients with senile dementia (including AD) are known to have a greatly increased risk of fragility fractures. Based on the above, the suggestion of a chronic low-grade aluminium intoxication as the possible common denominator between AD and bone fragility [105] seems unlikely. As far as aluminium intoxication is concerned, however, even moderately high aluminium concentrations maintained during a short period of time were shown to produce significant haematological alterations and a depletion of body iron stores before clinical manifestations were evident [106].

3.1.3. Aluminium and lipid peroxidation

Part of aluminium neurotoxicity is due to the contribution of the Al^{3+} ion to iron-induced neuronal oxidative damage [107]. The two metals are expected to act synergistically, aluminium coordination to the neuronal membrane facilitating its attack by iron-induced free radicals [108] whereas membrane oxidation in turn increases aluminium binding, thus aggravating oxidation [109]. This vicious circle largely depends on the capacity of the two metals to compete for the negatively charged end groups of the amphiphilic molecules constituting membranes.

Since the first mention of the capacity of aluminium to enhance Fe^{2+}-induced membrane oxidative damage [108], much progress has been made in the comprehension of its involvement in this process. Using model membranes, it was first established that the Al^{3+} ion was effectively the species involved and that membrane integrity was necessary for its stimulatory effect to occur [110]. It was then shown that the aluminium prooxidant effect was concentration dependent [111]—increasing with decreasing pH and with increasing the negative charge density of the liposomes—and was significantly correlated with its capacity to promote liposome aggregation, permeability and fusion [112]. In addition, aluminium was demonstrated to cause fatty acid chain packing, thus facilitating the propagation of lipid peroxidation [112].

The effect of aluminium on lipid peroxidation was more recently confirmed to depend on metal concentration, pH, and membrane structure in liposomes, microsomes and whole homogenates of rat brains [113,114], and a more effective stimulation of peroxidation in phospholipid liposomes was observed with aluminium acetylacetonate than with aluminium chloride [115]. In addition, myelin was found to be a preferential target of aluminium-mediated oxidative damage, supporting the hypothesis that ions without redox capacity can stimulate lipid oxidation by promoting phase separation and membrane rigidification [116]. The influence of Fe^{2+} and Al^{3+} ions on phase components of phospholipid model membranes was also investigated using Laurdan fluorescence spectra and generalised polarisation measurements [117]. The results obtained were in line with the above findings in that the presence of Al^{3+} ions induced slight variations in fluorescence spectra, indicating aggravated oxidation. However, no strong influence of aluminium was observed on generalised polarisation values nor on derived phase state determinations, suggesting that the structural influence of aluminium on membranes might be less significantly marked than previously thought [117].

Apart from its impact on membranes, aluminium may also influence iron-mediated oxidation by competing with iron for the same ligands in biofluids. For example, the competition between Al^{3+} and Fe^{3+} ions for the anionic forms of carboxylic acids that abound in vivo may locally influence both the global availability of Fe^{3+} ions to membrane binding sites and the redox potential of the Fe(II)/Fe(III) couple, these two effects resulting in increased membrane oxidation. This was recently demonstrated with citrate taken as an example [118].

A final possible role for the Al^{3+} ion in peroxidation is through its interactions with the Aβ peptide. Whereas aluminium in 'physiological' concentrations does not seem to be able to compete with essential metals for a direct significant interaction with native Aβ (see Section 3.1.2), it may, however, influence the fate of Aβ in the peroxidation process in vivo. Oxidative stress and inflammation are determining components of AD genesis, and various oxidative processes are involved in Aβ neurotoxicity (see Ref. [9]). The $A\beta_{25-35}$ sequence appears to be determining in this respect in vitro [70]. However, the whole Aβ sequence specific to humans, which comprises three residues that are absent from rodent Aβ (rodents being not subject to AD), may prevail over other factors—like peptide length and $A\beta_{25-35}$ sequence—to condition human Aβ activity in oxidative stress in vivo. Aβ deposition depends on amino acid oxidation and protein cross-linking [119], His and Tyr residues (which are prone to modification by hydroxyl radicals [120]) being responsible for Aβ aggregational behaviour [121]. Thus, only human Aβ has a strong tendency to aggregate and become highly amyloidogenic in the presence of free radical generating systems [121]. Besides, whereas human $A\beta_{1-40}$ aggregates in the presence of Cu^{2+} ions at normal serum level as the pH is lowered from 7.4 to 6.6 [122], rat $A\beta_{1-40}$ or histidine-modified human $A\beta_{1-40}$s do not, indicating that Cu^{2+}–histidine coordination is required for $A\beta_{1-40}$ aggregation [122]. Finally, copper is expected to catalyse Aβ oxidation in vivo, the decrease in the content of histidine residues of human Aβ being replicated in vitro by incubating synthetic Aβ with H_2O_2 and copper, but not iron [123].

Based on the above considerations (see Refs. [9,2]), the hypothesis of a possible protective function of Aβ in

association with copper in the CSF has recently been advanced [9]. Outside cells, free iron and copper ions are kept at extremely low levels so that they cannot trigger Fenton chemistry, extracellular production of $^{\bullet}OH$ radicals thus depending on the main ligands of these ions [124]. Compared with iron, copper triggers much faster [125] and highly site specific Fenton-type reactions, $^{\bullet}OH$ radicals reacting immediately with the ligand bound to the active metal centre [126]. Copper-mediated Fenton-type reactions can thus be deleterious or protective to surrounding biomolecules, depending on the nature of the ligand initially bound to the Cu^{2+} ion [124]. According to this hypothesis, Aβ would act as an $^{\bullet}OH$-inactivating ligand (OIL [124]), being oxidised preferably to neuronal membranes, and then being normally proteolysed [127]. The unexpected deposition of oxidised Aβ into amyloid plaques should therefore imply some interaction that would prevent its structure from being recognised by its specific protease, presumably through conformational change. The most likely event of this kind is the binding of a metal ion to the oxygen donors of the Aβ oxidised form. Among several possible candidates including Zn^{2+} [128], Al^{3+} seems well indicated to play this role [9].

3.2. Aluminium–ligand interactions and chelation therapy of aluminium intoxication

There has been no change in the clinical handling of aluminium intoxication since our last review on the topic in this journal [1]. Desferrioxamine (DFO) still is the only treatment in use, in spite of its numerous drawbacks, i.e. cost, lack of oral efficiency and side effects. In particular, its capacity to release part of the transferrin iron to make it available to iron-dependent microorganisms and enhance their pathogenicity is of major concern [129]. Yet, intensive research has been pursued by groups involved in aluminium toxicity towards the design of new aluminium sequestering agents. 3-Hydroxypyridin-4-ones (HPs) are the most promising among these. Deferiprone, in particular, the 1,2-dimethyl derivative (CP20, or L1), has been extensively investigated for its capacity to decorporate aluminium, with some success. For example, it has been shown to display a higher capacity to displace aluminium from human serum proteins (80%) than DFO (60%) at equivalent concentrations [130].[5] The kinetics of the release were also faster with L1, which took 20 min to achieve its maximum effect whereas DFO achieved only 80% of its maximum effect after 2 h [130]. Simultaneously, a comparison of the pharmacokinetics of aluminium complexes with several HPs led to contrasted results as to the redistribution of the metal following chelation therapy [131], wherefrom it was suggested that increasing ligand lipophilicity—which must be high enough to facilitate the penetration of the ligand into tissues where aluminium is stored—would increase the distribution of the metal to the brain and induce neurotoxicity. As elimination half-lives of aluminium complexes with L1(CP20) and CP94 were smaller than those of free ligands, suggesting a more rapid elimination of the former, L1 appeared as the best choice among the five HPs tested [131]. Efficacy and toxicity of DFO and other (iron and) aluminium ligands were subsequently reviewed by Kontoghiorghes [132]. Oral L1 being as effective as subcutaneous DFO in the removal of the (two) metal(s) in man and having a similar but different toxicity profile from DFO in both animals and man, the author concluded that 'the low cost and oral activity of L1 (would) make it the drug of choice for the vast majority of patients' [132].

Influences of HPs and DFO on aluminium mobilisation from tissues of aluminium-loaded rats were investigated [133]. Ligand administration decreased tissue aluminium content in the liver and in all regions of the brain under study. DFO displayed more efficacy in mobilising liver aluminium than L1(CP20) and CP94. In contrast with Allen et al.'s [131] previous suggestion (see above), the more hydrophobic of HPs (CP94) were most effective in mobilising brain aluminium, the authors concluding that orally active ligands of appropriate hydrophobicities could be extremely efficacious in mobilising brain aluminium [133]. The influence of the lipophilicity of eight HPs on their in vivo aluminium chelation activity was assessed on aluminium-loaded rabbits by reference to DFO, all ligands being administered intravenously [134]. Urine accounted for 78–98% of total aluminium excretion. No correlation was found between aluminium chelation efficacy and HP or Al(III)–HP complex lipophilicity. However, increasing HP lipophilicity increased the fraction of aluminium excreted through the bile. All the investigated HPs decorporated aluminium more efficiently than DFO [134]. The chelation of aluminium by intravenously administered HPs was then demonstrated in the rat by microdialysis [135]. The liver was suggested to be a primary site of aluminium sequestration. In contrast, little aluminium binding in the brain and little distribution of the Al(III)–HP complex into the brain were suggested to occur [135]. Oral administration of six HPs to aluminium-loaded rabbits was also shown to be more effective than intravenous DFO, but L1(CP20) and CP94 were found the least effective of the HPs examined [136]. More recently, L1 was evaluated as a possible protective treatment on aluminium-induced developmental toxicity in mice [137], but was found ineffective

[5] Compensating for the terdenticity of DFO with respect to the monodentate L1 by comparing triple concentrations for L1 with respect to DFO would even improve the result in favour of L1.

in preventing aluminium-induced maternal and embryo/fetal toxicity [137].

In a comparative study of chelating agents in rats, the effects of a series of HPs and other ligands on the distribution and excretion of aluminium in rats have been recently investigated. Although DFO plus a number of newly synthesised ligands significantly enhanced aluminium urinary excretion, only treatment with 1-(*p*-chlorobenzyl)-2-methyl-3-hydroxypyrid-4-one and 1-benzyl-2-ethyl-3-hydroxy-pyrid-4-one significantly reduced aluminium concentrations in all analysed tissues [138]. A similar study was subsequently conducted, comparing DFO to four new HPs in aluminium-loaded rats [139]. Oral administrations of 1-(*p*-methylbenzyl)-2-ethyl-3-hydroxypyrid-4-one and 1-benzyl-(4-carboxylic acid)-3-hydroxy-2-methyl-4-oxopyridine were suggested as potential alternatives of parenteral administration of DFO in aluminium removal [139].

The hypothesis that DFO and L1 might be more efficient as a combined treatment than as monotherapies in removing aluminium from the body was recently tested in a new acute rat model [140]. Oral L1 was compared with intraperitoneal DFO or L1 + DFO after a single intraperitoneal administration of aluminium chloride. DFO was found to be more effective than L1 in enhancing urinary aluminium excretion, and L1 did not increase the DFO effect in the combined treatment group, there being no statistical difference between DFO and combined therapy groups [140]. The same combined therapy was independently tested on two age groups of aluminium-loaded uraemic rats, DFO being administered subcutaneously [141]. The effect of combined administration of DFO and L1 was lower than that induced by DFO or L1 only. On the other hand, a significant reduction of aluminium was noted following joint administration of DFO and L1 in the liver of young rats, while no significant effect was seen in any of the examined tissues of adult animals [141].

Polyaminocarboxylic acids were also tested as possible aluminium sequestering agents. HEDTA and EDTA were compared to DFO in vitro and in vivo on rats, HEDTA showing an equal aluminium mobilising capacity with DFO [142]. EDDHA, HAES, HBED, citric acid and tartaric acid were subsequently compared to DFO through the same techniques: EDDHA was found to display a similar chelation potential with DFO in vitro and to significantly increase aluminium urinary excretion in rats [143].

4. Experimental speciation of aluminium in vivo

Like most metals, aluminium in biofluids mainly occurs in the form of protein-bound fractions to which experimental access is expected to be relatively straightforward [1]. By comparison, analysing its ultrafiltrable pool is considered a much more difficult task, even impossible on biological samples taken from healthy individuals because of the low 'physiological' concentration of aluminium. Despite its presumably easy evaluation, the composition of the h.m.m. fraction of aluminium in blood plasma remained uncertain and debatable for some time. It is only recently that transferrin was definitively established as the virtually exclusive protein to carry the Al^{3+} ion on its sites left vacant by iron, albumin being confined to a minor role (see Ref. [1] for references). These results, which have been lately confirmed [144] are discussed in more detail in the article ''Aluminium speciation in human serum'' Ref. [145] in this issue.

Except for the evidence of an aluminium–citrate association in rat serum through gel filtration chromatography [146] and its confirmation in blood plasma by proton NMR [147], there were no direct experimental data available at the time of our previous review on the distribution of aluminium into its ultrafiltrable fraction in vivo [1]. Since then, the work of Bantan et al. on the topic [148] has increased our knowledge significantly. Because of the low plasma aluminium level in the normal state—which prevents any analysis of the l.m.m. pool of the metal (see above)—the serum of healthy volunteers was spiked with aluminium nitrate in order to obtain Al^{3+} concentrations within the range observed in haemodialysed patients [148]. Using fast protein liquid chromatography coupled with electrothermal atomic absorption spectrometry or electrospray mass spectrometry, the main ligands individually bound to aluminium in its l.m.m. fraction were identified to be citrate and phosphate. In addition, as in some samples the same mass spectra characteristic of citrate and phosphate were found for fractions eluted around the retention time specific to citrate, the formation of an aluminium–citrate–phosphate ternary complex was presumed [148]. However, both percentage and distribution of the l.m.m. aluminium fraction were found to vary among individuals. (For example, some of these had no presumed ternary complex, one had no phosphate binary species, another had citrate binary species only.) Therefore, whereas it can definitely be concluded from the above study [148] that, in addition and to a comparable extent to citrate, phosphate also contributes to the ultrafiltrable fraction of aluminium in blood plasma, the variability observed in the ligands found from an individual to another, especially relative to the citrate plus phosphate mixture, suggests that serum extraneous factors may have interfered with the aluminium complexation analysis. Even in individuals classified as healthy, silent inflammatory foci may cause the systemic release of components of the inflammatory response that are likely to induce the aggregation of a number of circulating chemical species [149,150]. Given the physico-chemical properties of the Al^{3+} ion (espe-

cially prone to colloid formation in vivo [51,151]), it cannot thus be excluded that some samples used in the above study may have contained variable factors capable of triggering the formation of colloidal aluminium aggregates involving citrate and phosphate. Such aggregates could well correspond to the presumed aluminium–citrate–phosphate ternary complex, now scavenging all phosphate binary species (samples VII and VIII), now being too large to escape microultrafiltration (< 30 kDa) and thus leaving only citrate species in excess in solution (sample VIII).

5. Computer-aided speciation of aluminium in vivo

Speciation has been officially defined as 'the identification and determination of the concentration of all the species into which an element is distributed in a given solution in a state of dynamic equilibrium' [152]. De facto, this definition precludes the use of classical analytical techniques to analyse labile l.m.m. metal pools in vivo since the separation/concentration procedures required for the characterisation of individual complex species would perturb relevant equilibria. The only possible speciation technique for labile l.m.m. metal pools in vivo must therefore be indirect, through calculations based on simulation models (i.e. computer-aided speciation). Simulation models are fed with: (i) total and/or free concentrations of the reactants (metals, ligands, acid) as determined from biological samples by direct analytical techniques, and (ii) the parameters that relate these total and free concentrations to those of the individual species to be identified, i.e. formation and solubility product constants. Obtaining reliable results from such calculations therefore requires: (i) the choice of an appropriate simulation program, and (ii) the use of reliable constants.

Technical considerations leading to the choice of a simulation program have been developed in our previous review [1]. It is only worth reiterating here that while the use of conditional constants (or their equivalent 'pM' values) may be sufficient to compare the binding capacities of exogenous chelating agents, the use of a simulation model is absolutely necessary to estimate the relative importance of complexes from various endogenous ligands that coexist in vivo [153]. Another technical point worth mentioning here is the preference to be given to free over total metal concentrations whenever proteins must be taken into account in the calculations, their presence being implicitely accounted for to obtain exact species percentage distributions [154,155]. Regarding formation and solubility product constants, these should always be determined beforehand under experimental conditions as close as possible to those of the biofluid under investigation, not only in terms of temperature and ionic strength, but also of reactant concentrations so that the species identified in vitro be relevant to the problem posed in vivo. For aluminium, meeting this condition often amounts to a daunting challenge.

5.1. Formation constant determinations

Attention was called in our previous review [1] to the difficulties inherent in the determination of aluminium complex formation constants. Given the still generalised lack of awareness of the main aspects of the problem, these need to be summarised again. Glass electrode potentiometry undoubtedly constitutes the most efficient technique to determine metal complex stability constants provided one abides by a few basic principles. Determining metal–ligand complex equilibria in aqueous medium first requires to know with great accuracy the pK_w specific to the temperature and ionic medium used. This value must be worked out during the calibration of the electrode pair, special attention being paid to the pH interval within which the response of the glass electrode is rigorously Nernstian. The second stage in the required strategy is the determination of the constants that rule the interactions of the ligand with the proton *and* of the metal with the hydroxide anion. With 'classical' metals, ligand–proton interactions are systematically investigated, but (almost) never metal–hydroxide interactions since metal hydroxides are—with the few exceptions of very weak ligands—negligible, and the use of the hydrolysis constants available in the literature (Baes and Mesmer [156] are providential in this respect!) can, as a consequence, only reflect negligible errors in the calculations. With aluminium, things are considerably different. Because of its very high polarising power, the Al^{3+} ion has such a high tendency to hydrolyse that, above pH 4, its hydrolysis equilibria must absolutely and accurately be taken into account in the calculations—with, conversely to the classical case, the few exceptions of very strong ligands that render hydroxides negligible. Also, instead of reasoning in the same way as with other metals for which metal–ligand interactions are considered first in the coordination sphere, then water at low pH or OH^- at higher pH,[6] aluminium hydrolysis even requires for a correct analysis of metal–ligand interactions that these be considered in terms of progressive substitution of the ligand for water at very low pH and OH^- as from very acidic pH ($pH < 2$ in some cases, e.g. oxalate). Therefore, adapting aluminium hydrolysis constants from the literature as many authors do induces a double risk for the assessment of aluminium–ligand equilibria: (i) from a strict equilibrium point of view, the risk is to introduce

[6] Even though all metal ions are primarily hydrated in aqueous solution.

an error equivalent to that which would result from adapting ligand protonation constants; (ii) given the slow process of hydrolysis whose kinetics is specific to every ionic medium and temperature, the additional and even more important risk is to introduce a systematic bias in the calculations that will be reflected in the form of one or several 'computer complexes'. The only way to limit errors in the investigation of aluminium–ligand complexation (especially with weak ligands) is to adopt a reference protocol selected during the experimental study of aluminium hydrolysis, and then replicate it in the presence of any ligands [157]. The problem is even more complicated by the poor solubility of $Al(OH)_3$ that coexists with virtually all ligands. The only way to prevent the interference of $Al(OH)_3$ precipitation with the calculations relative to aluminium–ligand interactions consists in simulating the distribution of all the complexes in all the titrations after a first provisional determination of the formation constants, so as to check if the pH limits of solubility predicted by the simulation do effectively correspond to those experimentally observed.

Only when all these precautions have been taken is it possible to tackle the problems of the influence of the medium and temperature on metastable polymerisation equilibria (e.g. conclusions drawn at 25 °C may not apply at 37 °C) and of the possible precipitation of poorly soluble aluminium–ligand complexes (see for example the well-known phosphate example [158–162]!). Once again, "keeping all these difficulties in mind may help to understand how speciation data pertinent to living systems may sometimes be so controversial" [1].

It is beyond the scope of this article to critically review all determinations of aluminium complex formation constants published since 1995. The following analysis will thus be restricted to the main ligands involved in simulated distributions of aluminium in the principal body fluids.

5.1.1. Aluminium–citrate interactions

Citrate is undoubtedly the most important l.m.m. ligand of aluminium in vivo. Logically, its interactions with the Al^{3+} ion have been extensively investigated. A detailed review of these is available in our previous review [1] and their structural aspects are discussed in the article 'Speciation and structural aspects of interactions of Al(III) with small biomolecules' [163] in this special issue. Aluminium–citrate interactions are therefore only evoked here for their direct relevance to aluminium speciation in vivo. The main problem on the topic, which was initially evoked by Öhman [164], concerns the slow formation of the $M_3L_3(OH)_4$ at 25 °C in 0.6 M NaCl that takes more than 20 h to reach true equilibrium [164]. From 'time zero' data extrapolated from potentiometric measurements—carried out for a unique metal-to-ligand ratio equal to one with 0.01 M concentrations—constants were calculated for the two MLH_{-1} and MLH_{-2} species, which then slowly converted into the more stable $M_3L_3(OH)_4$ [164]. Based on these observations, Öhman called attention to the bias introduced in constants derived from continuous potentiometric titrations that would reflect an intermediate behaviour, questioning their possible use in complexation models [164]. These results have lately been confirmed at 25 °C in 0.2 M KCl using a more dilute concentration for both reactants (0.004 M), the time necessary to reach equilibrium being observed over 30 h [165]. (NMR measurements were also made at higher concentrations (0.04 and 0.1 M) [165]. Except for the MLH_{-2} species characterised in both studies at high pH values, the species identified for the Al(III)–citrate system (MLH, ML, MLH_{-1}, ML_2, ML_2H_{-1}, ML_2H_{-2}, $M_3L_3H_{-4}$) confirm those found in a former study carried out at 37 °C in 0.15 M NaCl (in which $M_2L_2H_{-2}$ had been definitely preferred to MLH_{-1} on numerical and graphical grounds) [166].

The actual problem posed by the more recent determinations [164,165] is that of the relevance of thermodynamic data to speciation calculations relative to biofluids. Although life is incompatible with the notion of thermodynamic equilibrium, living processes often operate for energetic reasons at stationary states near to equilibrium [167]. It is on this observation that the use of equilibrium constants for speciation calculations relative to in vivo conditions is based. In the present case, however, the non-equilibrium state is of chemical origin, and the data to be used for in vivo applications must be adapted to the different situations encountered. This is no easy task. For extracellular fluids like blood plasma for example, the $M_3L_3H_{-4}$ species that would be fully formed at equilibrium will be negligible given the extremely low metal-to-ligand ratio whereas in the gastrointestinal fluid where the metal-to-ligand ratios are favourable to its formation, equilibrium will never be reached. For this reason, it is impossible with this system to conform to thermodynamic rules and, as was recently recommended [157,165], stability constants determined under conditions near to equilibrium have to be used. It is worth noting, however, that the slow electrode responses described at 25 °C were not observed at 37 °C [166]. A deep reinvestigation of the system under physiological conditions in the light of the above developments would be of interest. It is underway in our group.

5.1.2. Aluminium–phosphate interactions

Phosphate is *the* other ligand of importance for aluminium in vivo. It is therefore unfortunate that its interactions with the Al^{3+} ion can be experimentally investigated within the short pH 2–4 window only. The case was treated in our previous review [1]. The only

attempt to extend the pH limit of investigation up to 11 [158] yielded questionable results [159] that were then provisionally corrected with the finding of MLH_2, $(MLH)_{n=1,3}$, ML_2H_3, $(ML)_{n=1,3}$ and M_2L complexes [162]. Such a large number of species identified within the narrow 2–4 pH interval suggests that many others (involving various degrees of hydrolysis) are likely to form at higher pH values. Before future experimental determinations can be carried out, the problem has been temporarily circumvented for a limited number of species (MLH, ML, MLH_{-1}) using linear free energy relationships relative to data determined at 25 °C [168].

The formation of aluminium ternary complexes with citrate and phosphate at 25 °C has recently been mentioned [169]. It is difficult to comment on these results not yet published. The only remark that can be made a priori is that investigating ternary complex equilibria in mixtures composed of a binary system in which a slow polymerisation reaction takes place (and reflects on the corresponding formation constants [164,165]) and another affected by a precipitation process that limits the useful pH range to values below 4 is an extremely difficult task. The first obvious risk is to take previously unidentified binary phosphate species for ternary 'computer complexes'. Adding to this the fact that the authors use aluminium hydrolysis constants adapted from the literature cannot help (see above). Whatever they may be, these results will have to be independently confirmed from experimentally determined Al(III)–phosphate equilibria before they can be used for in vivo applications.

5.1.3. Aluminium interactions with other ligands

A number of dietary acids of relatively minor importance for aluminium distribution in blood plasma may, however, significantly influence aluminium gastrointestinal absorption (see Section 2.2.4). Among these, succinate [170], tartrate [171] and malate [172] have recently been investigated for their interactions with the Al^{3+} ion.

For succinate, formation constant determinations were based on two independent series of potentiometric titrations using different equipments and reactant solutions. Comparisons of the results (Table 1) with those of previous studies are to be found in Ref. [170]. The most puzzling result was the characterisation of a highly condensed polynuclear species, $M_8L_4H_{-12}$, whose fundamental structure $(M_2LH_{-3})_4$ corresponds to that of the $(M_2H_{-5})_n$ hydroxide series in which two hydroxide ions are replaced by a succinate dianion.

For tartrate, three independent series of titrations were used to determine the formation constants reported in Table 1. Comparisons of these constants with results obtained in previous studies are developed in Ref. [171]. Because of experimental conditions (37 °C; 0.15 M NaCl) more favourable to the stability of both tartaric acid and aluminium tartrates than those (25 °C; 0.6 M NaCl) used in Marklund and Öhman's study [173], not only was the $M_2L_2H_{-n}$ series already found by these authors confirmed, but also the MLH_{-1} species was characterised for the first time [171].

Table 1
Formation constants of Al(III) complexes with succinate, tartrate and malate under physiological conditions (37 °C; 0.15 M NaCl)

System	Species	log β	Reference
Proton–succinate	LH	5.145±0.001	[170]
	LH_2	9.067±0.001	
Al(III)–succinate	ML	3.753±0.014	[170]
	MLH	7.234±0.015	
	M_2LH_{-2}	−1.103±0.013	
	$M_3L_2H_{-1}$	9.114±0.028	
	$M_3L_2H_{-3}$	1.385±0.037	
	$M_4L_3H_{-6}$	−4.442±0.033	
	$M_8L_4H_{-12}$	−12.155±0.021	
Proton–tartrate	LH	3.838±0.001	[171]
	LH_2	6.600±0.001	
Al(III)–tartrate	ML	3.788±0.016	[171]
	MLH_{-1}	1.165±0.030	
	$M_2L_2H_{-1}$	7.976±0.015	
	$M_2L_2H_{-2}$	5.347±0.023	
	$M_2L_2H_{-3}$	1.009±0.022	
	$M_2L_2H_{-4}$	−5.101±0.116	
Proton–malate	LH	4.604±0.001	[172]
	LH_2	7.792±0.001	
Al(III)–malate	MLH	7.032±0.023	[172]
	ML	4.519±0.019	
	ML_2H	10.981±0.131	
	MLH_{-1}	1.268±0.049	
	M_2LH_{-2}	0.564±0.033	
	M_2LH_{-3}	−3.054±0.017	
	$M_2L_2H_{-3}$	1.779±0.041	
	$M_2L_2H_{-4}$	−4.462±0.105	
	$M_2L_3H_{-1}$	12.789±0.049	
	$M_3L_4H_{-4}$	10.132±0.061	
	$M_4L_4H_{-5}$	10.537±0.045	

For malate, again two series of independent titrations were used for constant determinations. Whereas protonation constants were found to be remarkably reproducible from one series to the other (within 0.01 log units), the situation was more difficult for Al(III)–malate complexes [172], many complexes coexisting within restricted pH and concentration ranges so that the least variation of experimental data led to large deviations in the refined constants. These difficulties have been commented in Ref. [172]. From a chemical point of view, it is interesting to note that aluminium complexation schemes of both succinate and tartrate (the two extreme structures with malate being the intermediate) are represented in the characterised complexes (see Table 1): it is the case of M_2LH_{-3}, of which succinate $M_8L_4H_{-12}$ is derived, and also of the $M_2L_2H_{-n}$ series specific to tartrate.

Amino acids are also of interest for aluminium distribution in vivo, although to a lesser extent account

being taken of the poor affinity of the Al^{3+} ion for the nitrogen atom. The first report of formation constant determinations for complexes of aluminium with amino acids was by Djurdjevic and Jelic with their investigation of the Al(III)–glycine and –alanine systems using aluminium and ligand concentrations in the respective 0.5–5 and 0.5–50 mM ranges [174]. Aluminium complexation with alanine was then found to be negligible by Marklund and Öhman [175] in the 2–5 and 5–12 mM metal and ligand concentration ranges, respectively. Based on higher metal and ligand concentrations (1–10 and 5–30 mM, respectively) a series of constant determinations was subsequently made under physiological conditions (37 °C; 0.15 M NaCl) for aluminium complexes with glycine, serine, threonine, histidine, and aspartic and glutamic acids [176] over a large pH range using the experimental protocol defined in a previous aluminium hydrolysis study [157]. The resulting constants, to be found in Table 2, were simultaneously refined together with the aluminium hydroxides in each system as recommended in the previous hydrolysis study [157]. (Fig. 1, relative to histidine chosen as an example, shows how close simulated formation curves based on aluminium hydroxides only (b) are to those which take the ML constant into account (c) and how close both of these (b) and (c) simulations are to the experimental curves (a), with the exception of the lowest metal-to-ligand ratio (+) titration.) These values, however, seem to be reliable for the following reasons. Relative to the constant of the ML complex with glycine taken as a reference, the extra stability of the ML complexes with aspartic and glutamic acids is, respectively, 1.54 and 1.46, which roughly corresponds to the value 1.51 found for acetate at 25 °C with a ionic strength equal to one [177]. In addition, the formation constants corresponding to the bonds between aluminium and the diprotonated forms of aspartate and glutamate (1.58 and 1.46, respectively) compare well with that of the aluminium complex with the monoprotonated form of salicylate (1.64 [176]). Commenting on these results, Bruce Martin concluded that aluminium complexation begins to become distinguishable from hydrolysis at ligand concentrations greater than 20 mM and recommended the use of up to 0.5 M (!) amino acids [178]. More recently, he co-signed a new investigation carried out at 25 °C in 0.2 M KCl [179] in which potentiometric titrations were limited to pH ranges where potentials were considered at equilibrium when varying by less than 0.1 mV in 40 s. [180]. Unfortunately, to increase free concentrations of ligands and favour complexation, the aluminium concentration was uniformally taken as 1 mM while ligand concentrations were varied from 10 to 40 mM, this implying metal-to-ligand ratios well beyond normally acceptable limits for systematic errors. (Mathematically speaking, errors are minimum for a ligand total concentration divided in three approximately equal

Table 2
Formation constants of Al(III) complexes with some amino acids

System	Species	log β	Experimental conditions	Reference
Al(III)–glycine	ML	6.23±0.10	37 °C; 0.15 M NaCl	[176]
	ML	5.91±0.10	25 °C; 0.20 M KCl	[179]
	MLH_{-1}	1.08±0.09		
	M_2LH_{-1}	4.35±0.09		
Al(III)–serine	ML	5.97±0.05	37 °C; 0.15 M NaCl	[176]
	ML	5.66±0.11	25 °C; 0.20 M KCl	[179]
	MLH_{-1}	0.62±0.23		
	M_2LH_{-1}	3.75±0.11		
Al(III)–threonine	ML	5.71±0.08	37 °C; 0.15 M NaCl	[176]
	ML	5.51±0.12	25 °C; 0.20 M KCl	[179]
	MLH_{-1}	0.94±0.15		
Al(III)–histidine	ML	7.08±0.20	37 °C; 0.15 M NaCl	[176]
Al(III)–aspartic acid	MLH_2	14.48±0.04	37 °C; 0.15 M NaCl	[176]
	MLH	11.24±0.03		
	ML	7.77±0.02		
	MLH	11.76±0.06	25 °C; 0.20 M KCl	[179]
	ML	7.87±0.04		
	MLH_{-1}	3.30±0.03		
	MLH_{-2}	−2.32±0.07		
Al(III)–glutamic acid	MLH_2	14.74±0.04	37 °C; 0.15 M NaCl	[176]
	MLH	11.07±0.06		
	ML	7.69±0.03		
	MLH	10.88±0.22	25 °C; 0.20 M KCl	[179]
	ML	7.29±0.04		
	MLH_{-1}	2.55±0.03		
	M_2L	9.46±0.15		

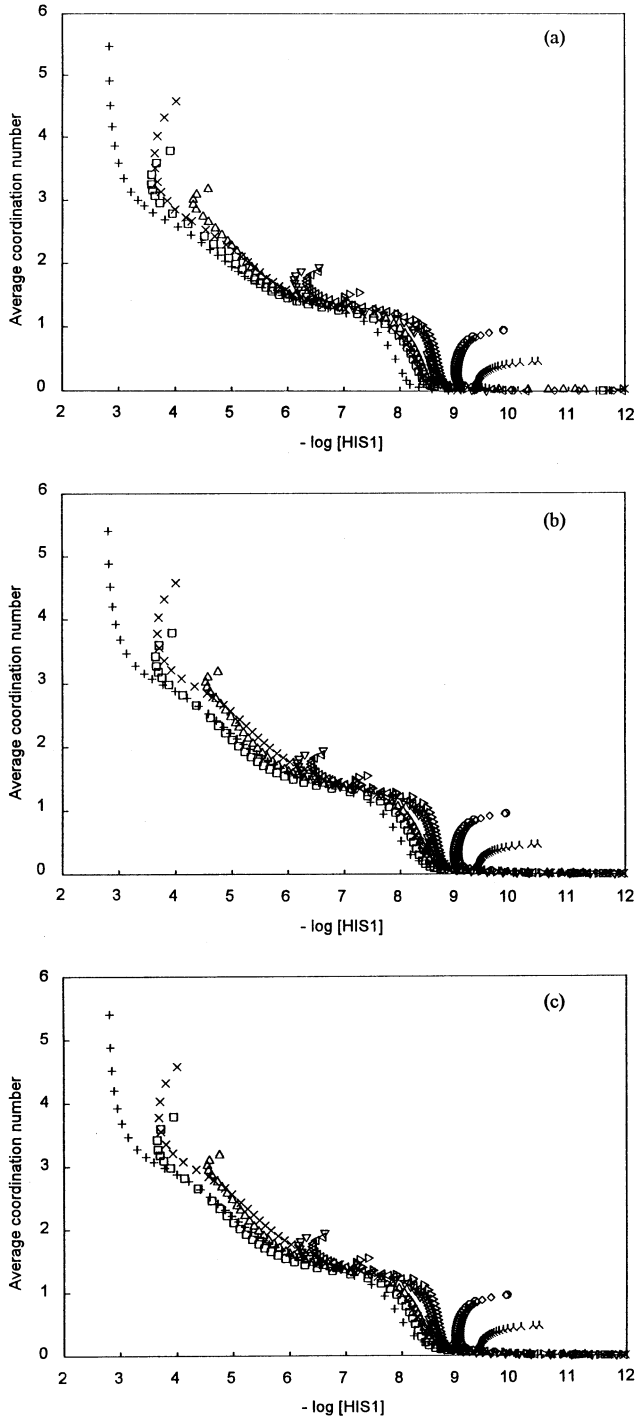

Fig. 1. Formation curves relative to the Al(III)–histidine system: (a) experimental; (b) simulated on the basis of Al(III) hydroxides only; and (c) taking the ML constant in Table 2 [176] into account.

free, protonated, and complexed fractions—a metal-to-ligand ratio of ≤ 10 being usually considered maximum (see comments on figure 1 above). In spite of this disadvantage combined with the use of hydrolysis constants adapted from the literature, constants could be calculated with some coherence, which compare reasonably well with the values obtained under physiological conditions [176] (aluminium complexation being confirmed by NMR at much higher metal and ligand concentrations [179]—see Ref. [163]).

Aluminium interactions with exogenous ligands meant to sequester the metal in vivo are by definition

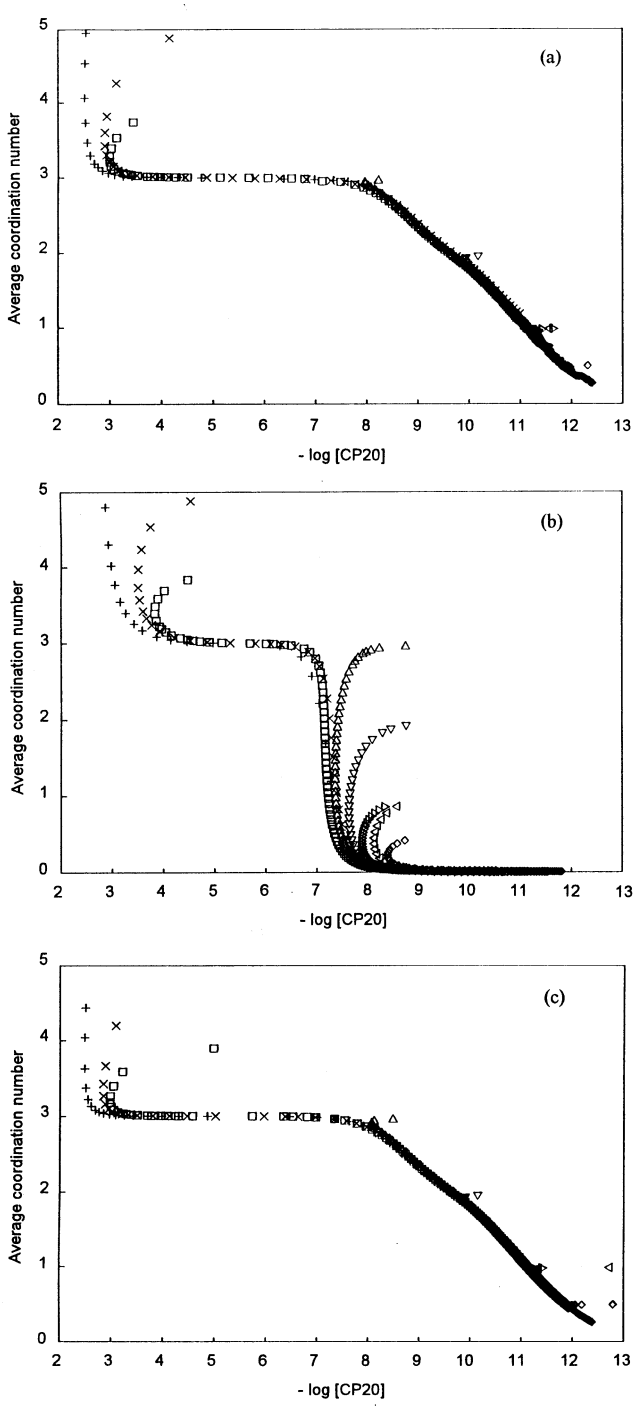

Fig. 2. Formation curves relative to the Al(III)–CP20 system: (a) experimental (from Ref. [181]); (b) simulated on the basis of Al(III) hydroxides only; and (c) taking all formation constants into account (from Ref. [181]).

easier to investigate as the common property of all of these substances is their high aluminium binding capacity, which makes hydrolysis minor or even negligible under the experimental conditions used. This was recently confirmed with a study of five of these ligands (2,3-dihydroxybenzoic acid, 4,5-dihydroxy-1,3-benzenedisulphonic acid (Tiron), 3-hydroxy-1,2-dimethyl-4(1*H*)-pyridone (CP20, or L1), 3-hydroxy-1,2-diethyl-4(1*H*)-pyridone (CP94) and DFO), which was carried out under physiological conditions [181]. Fig. 2 is shown to illustrate the sharp contrast between simulated formation curves relative to CP20, a strong ligand, and histidine (see Fig. 1).

5.2. Speciation calculations and aluminium metabolism

Relatively few speciation calculations relative to aluminium metabolism have been carried out since our previous review on the topic [1]. As some other articles in this issue specifically focus on speciation data relative to structural aspects of aluminium complexation [163], only the calculations relevant to in vivo applications will be treated here.

5.2.1. Gastrointestinal conditions

As emphasised in Section 2.1, there are two possible modes of aluminium intoxication, depending on the amounts of metal ingested and duration of contamination. Whereas chronic environmental contamination may ensue from low aluminium dietary intakes but exceeding our natural capacities of accommodation, acute iatrogenic intoxication may afflict patients receiving high oral doses of aluminium-containing drugs (see Fig. 1 of Ref. [1]). Simulations have recently been run to assess the influence of three dietary acids on these two types of aluminium contamination [170–172].

The first case that was (re-)investigated in detail is succinic acid [170]. As already demonstrated for many dietary acids [42], succinate should solubilise a significant fraction of aluminium in the gastrointestinal tract [170]. For an aluminium level of 0.0005 M considered to reflect normal dietary exposure, 0.01 M succinate (approximate concentration in a glass of wine) shifts the pH of precipitation of $Al(OH)_3$ from $\sim$4.3 to 4.9, a concentration of 0.05 M pushing it further to pH 5.4. The percentage of aluminium present as the neutral complex $M_4L_3H_{-6}$ reaches 60 and 82% (1.6 and 2.2 mg) for these two succinate concentrations, respectively. Fortunately, the restricted pH range over which this effect is acting (pH $\leq$ 6 even for higher succinate levels) should limit its consequences in the small bowel. In the presence of food also, the average dietary intake of phosphate (0.05 M) is expected to make it entirely negligible [170].

The simultaneous occurrence of succinate with higher levels of aluminium such as those resulting from the therapeutic administration of aluminium hydroxide is predicted to aggravate the situation. Firstly, even though the percentage of aluminium bound to succinate decreases, its absolute amount actually increases. Secondly, because of the polynuclearity of the neutral $M_4L_3H_{-6}$, its own percentage also tends to increase with the concentration of aluminium at higher concentrations of succinate. Even though the pH of aluminium hydroxide precipitation progressively returns back to its value in the absence of ligand, this cannot compensate for the succinate effect in favour of aluminium absorption since soluble succinate complexes coexist with solid $Al(OH)_3$ within a significant pH interval that progressively extends to cover up to the whole range of occurrence of $M_4L_3H_{-6}$ (Fig. 3). The only protection is expected to be afforded by physiology: the higher the aluminium concentration, the smaller the pH range within which $M_4L_3H_{-6}$ spans, hence the shorter the section of the gastrointestinal tract in contact with it. Protection is also expected from dietary phosphate. However, in the presence of high therapeutic aluminium concentrations, this protection may become insufficient. At the highest aluminium concentration considered (0.5 M), for example, phosphate cannot reduce the fraction of metal bound to succinate. Aluminium phosphate should effectively precipitate, but entirely at the expense of solid aluminium hydroxide, and the percentages of metal present in the form of $M_4L_3H_{-6}$ are calculated perfectly identical to those in the absence of phosphate. The phosphate protective effect, however, should be somewhat restored with time, as the concentration of aluminium decreases with stomach emptying. Most logically also, complete safety is expected from the administration of aluminium phosphate, the $M_4L_3H_{-6}$ species being negligible at all concentrations investigated [170].

Tartaric acid has also been (re-)investigated [171]. Tartaric acid is ubiquitous in fruits and more generally

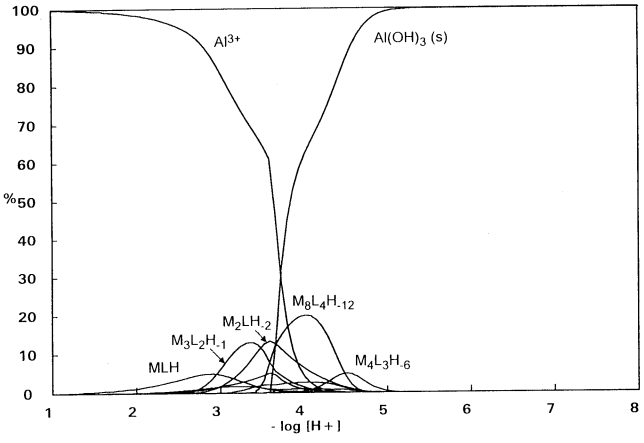

Fig. 3. Distribution profiles of aluminium–succinate complexes in the gastrointestinal fluid. Aluminium and succinate concentrations are 0.05 and 0.01 M, respectively (from Ref. [170]).

food and beverages where it is used as an acidulent. It may therefore have important repercussions on aluminium bioavailability. Under conditions of normal aluminium dietary exposure (0.0005 M), tartrate is expected to exert a critical influence on aluminium hydroxide solubility as a concentration as low as 0.01 M is predicted to shift the pH of precipitation of $Al(OH)_3$ from 4.29 to 6.66 and a concentration of 0.1 M to 7.59. This value, which corresponds to the pH of distal ileum, implies that tartrate can solubilise aluminium over the whole small intestine. Even more important in terms of potential aluminium toxicity is the fact that the two neutral species MLH_{-1} and $M_2L_2H_{-2}$ are calculated to represent up to 85% of aluminium (i.e. 2.3 g of metal) at pH 3.66 in the presence of 0.01 M tartrate and, although stomach cells are not fit for absorption, these two species span over the 1.5–7.5 pH range with $M_2L_2H_{-2}$ still standing for 10% of aluminium at pH 6, pH of the duodenum. Also, in contrast with succinate, the amount of neutral aluminium is maximum from the lowest tartrate concentrations. Tartrate thus appears as potentially capable of significantly increasing aluminium bioavailability under normal dietary conditions. Its influence is especially worth taking into account as, even though dietary phosphate (0.05 M) can notably reduce the fraction of tartrate-neutralised aluminium, it cannot nullify it as it does with succinate (see earlier discussion) [171].

For aluminium concentrations in the therapeutic range as in the case of aluminium hydroxide treatment, for example, the influence of tartrate varies with the aluminium level. The more the tartrate for a given aluminium level, the larger the fraction of neutral aluminium. Also, the more the aluminium for a given tartrate concentration, the lower the neutral aluminium percentage but the higher the neutral aluminium absolute amounts. As an apparent compensation for this predicted aggravation of aluminium bioavailability with the increase in aluminium levels, the pH of precipitation of aluminium trihydroxide regresses towards its value in the absence of ligand, but—as with succinate (see earlier discussion)—solid $Al(OH)_3$ cannot prevent the formation of the soluble neutral species MLH_{-1} and $M_2L_2H_{-2}$ with which it coexists within progressively larger pH intervals. Once again, physiology is the only source of protection for the reasons already developed for succinate. This is more especially true as the protective effect usually expected from phosphate rapidly vanishes as aluminium levels are raised, being utterly insignificant for aluminium 0.5 M, and it is only with an identical level of phosphate (0.5 M) that neutral aluminium significantly decreases for any tartrate concentration. This implies that phosphate should remain highly concentrated during stomach emptying for a significant protection to be afforded. Even aluminium phosphate administration would not be totally safe in the presence of tartrate: with a moderate level of aluminium phosphate of 0.05 M, a tartrate concentration as low as 0.01 M is predicted to mobilise more than 11 mg of metal into soluble neutral forms, which is by far larger than the average total ingested daily under normal dietary conditions (5 mg). Care should therefore be taken to the ingestion of tartaric acid simultaneously to therapeutic aluminium in any of its marketed chemical forms [171].

Malic acid has also been lately investigated [172]. Its ubiquitous occurrence in fruits and in industrial foods and drinks makes it a potential enhancer of aluminium bioavailability under usual conditions of alimentation. Simulations relevant to these conditions indicate that the influence of malate upon aluminium bioavailability can effectively be significant. For the average dietary exposure of aluminium (0.0005 M), a malate concentration as low as 0.01 M can shift the pH limit of $Al(OH)_3$ solubility from 4.29 to 6.95. An increase in malate concentration up to 0.05 M can extend this limit to 7.65, and 0.1 M malate—its concentration in 200 ml of cherry juice, for example—is expected to shift it up to 7.93, i.e. near the higher pH limit of the ileum. These results, which fully substantiate Partridge et al.'s former observations [42], definitely confirm the potential capacity of malic acid to maintain aluminium hydroxide solubility throughout the whole small intestine. In addition, even though stomach cells are not fit for absorption and electrically neutral Al(III)–malate complexes may remain sufficiently polar not to be able to freely diffuse into cell membranes, it is worth noting that: (i) in the presence of 0.01 M malate, the two neutral species ML_2H and MLH_{-1} contain up to 39% of aluminium (i.e. 1.1 mg of metal) near pH 3–3.5, this percentage reaching 49 (1.3 mg) and 59% (1.6 mg) with 0.05 and 0.1 M malate, respectively; and (ii) one of these species, MLH_{-1}, represents a significant fraction of total aluminium over the whole duodenal 4–7 pH range [182] (see Fig. 4).

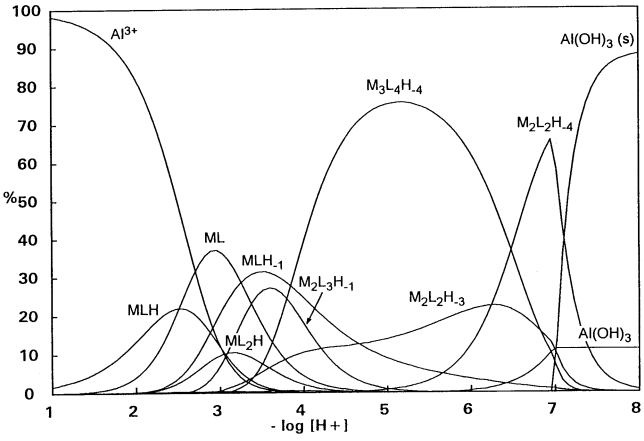

Fig. 4. Distribution profiles of Al(III)–malate complexes in the gastrointestinal fluid. Aluminium and malate concentrations are 0.0005 and 0.01 M, respectively (from Ref. [172]).

Aluminium charge neutralisation by malate, however, is expected to be markedly inferior to that due to tartrate and, to a lesser extent, to succinate (see above). Also, average dietary phosphate (0.05 M) significantly reduces malate-neutralised aluminium but, as with tartrate, does not nullify it as it did with succinate.

Under therapeutic conditions as with the ingestion of aluminium hydroxide, several changes are predicted. Firstly, as was already observed with succinate and tartrate, the more the malate for a given aluminium level, the larger the fraction of neutral aluminium. Second, as was the case for tartrate but not for succinate, the fraction of neutralised aluminium should uniformally decrease as aluminium increases for a given malate concentration. The absence of neutral polynuclear species in the Al(III)–malate system, which strengthens the importance of this phenomenon over the whole percentage range observed, explains the significant decrease in the absolute amounts of neutralised metal at highest aluminium levels for a given malate concentration [172]. Finally, as already observed for succinate and tartrate, the pH of precipitation of aluminium trihydroxide is expected to regress towards its original value in the absence of ligand, which should a priori limit the impact of malate on aluminium solubility. Even though some complexes coexist with solid $Al(OH)_3$, these are not electrically neutral, and the polynuclear hydroxo species—especially $M_3L_4H_{-4}$ and $M_4L_4H_{-5}$—combine with solid $Al(OH)_3$ to reduce the pH range of existence of the mononuclear neutral species towards more acidic values as the aluminium concentration is raised (Fig. 5). This effect has interesting consequences from the physiological point of view too. The more acidic and the narrower the pH interval spanned by ML_2H and MLH_{-1}, the shorter the section of the gastrointestinal tract in contact with these species. In other words, the more aluminium, the less hazardous its interactions with malate with respect to its gastrointestinal absorption. Therefore, contrary to tartrate and to a lesser extent succinate, malate is not expected to induce much more important risk in the presence of high therapeutic aluminium than low dietary aluminium levels. In addition, aluminium monophosphate is expected to precipitate at therapeutic aluminium concentrations in the presence of dietary phosphate, but a significant percentage of total metal (10%) is still neutralised near pH 3.4 for a metal concentration of 0.005 M in the presence of 0.1 M malate (i.e. 2.7 mg), and even though this percentage then regularly decreases with increasing aluminium levels, the absolute amounts of neutral metal remain physiologically significant. However, the potential risk resulting from the interaction of malate with therapeutic aluminium hydroxide in the presence of phosphate is expected to be at least one order of magnitude less than with tartrate and, to a lesser extent, succinate. When aluminium is administered as its phosphate, the protection is maximum for low malate concentrations, but quickly deteriorates as malate concentrations increase (e.g. 12.2 mg of neutral aluminium for the moderate level of 0.05 M aluminium phosphate in the presence of 0.1 M malic acid). In this respect, the situation is less safe than with succinate even though it is much more favourable than with tartrate (54.3 mg of neutralised aluminium for the same concentrations). Attention is therefore globally called to the possible risk of increased aluminium absorption in the case of ingestion of malic acid simultaneously to that of therapeutic aluminium salts [172].

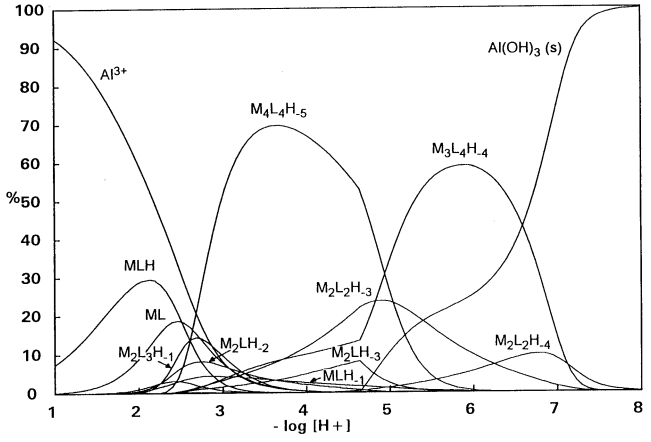

Fig. 5. Distribution profiles of Al(III)–malate complexes in the gastrointestinal fluid. Both aluminium and malate concentrations are 0.05 M (from Ref. [172]).

5.2.2. *Blood plasma conditions*

Because of the central physiological role of blood plasma, virtually all aluminium speciation studies—and even aluminium complex constant determinations—refer to its conditions, even though many of these constant determinations are carried out under conditions far from the physiological medium. The controversial situation resulting from the large discrepancies affecting all of these results was discussed in detail in our previous review [1]. Little has been done since then regarding the most important ligands of aluminium in blood plasma except the works on citrate and phosphate discussed in Sections 5.1.1 and 5.1.2. As noted in these sections, corresponding results need to be independently confirmed before they can be used for reliable applications of aluminium computer-aided speciation in vivo. It can therefore be again concluded that "much work remains to be done before general agreement can be obtained on plasma aluminium speciation" [1].

Regardless of the composition of the most realistic simulation model of aluminium in blood plasma, comparative capacities of exogenous ligands to mobilise the metal into its ultrafiltrable fraction can always be obtained from simulations based on the free aluminium concentration estimated from protein binding [166].

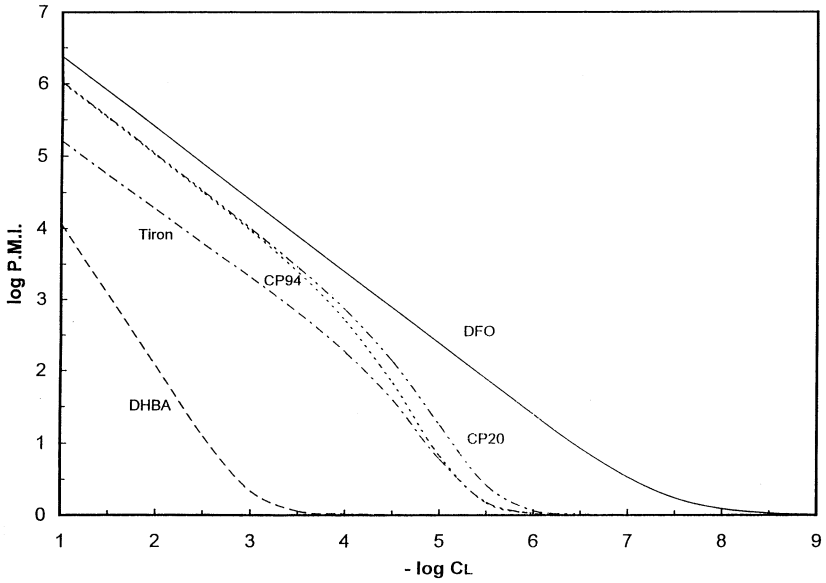

Fig. 6. Aluminium mobilising power of the five ligands under normal blood plasma conditions (pH 7.4) as predicted by computer simulation (from Ref. [181]).

This was recently done for the five aluminium–sequestering ligands evoked in Section 5.1.3 [181]. The results can be seen in Fig. 6 where they are expressed in terms of plasma mobilising index (ratio by which the ultrafiltrable fraction of aluminium is increased in the presence of the drug with respect to normal plasma) [183] as a function of logarithmic ligand total concentrations in plasma. DFO appears to be globally superior to other ligands within the whole ligand concentration range investigated. However, its superiority is not determining with respect to the two HPs. For these two ligands, a PMI value equivalent to that of DFO is obtained for a ligand concentration only ~ 2.3 times greater than that of DFO for DFO concentrations above $\sim 10^{-4}$ M. With respect to the above reported (Section 3.2) study by Yokel et al. [134] in which the authors used triple concentrations of HPs to compensate for the terdenticity of DFO, it can be seen that the HPs are superior to DFO by a factor of 1.3 (which can also be obtained by translating the PMI curves for the two HPs by 0.5, logarithmic value of 3, in Fig. 6), which perfectly substantiates the observations made by these authors [134] (see also Ref. [184]).

6. Conclusion and prospects

The objective of the present review was to update the data contained in our previous work on the same topic [1]. The contents of this previous work have therefore been followed as close as possible to examine in parallel: (i) the diverse biological aspects of the influence of aluminium–ligand interactions on aluminium bioavailability, metabolism and toxicity, and (ii) the chemical speciation data likely to substantiate on a molecular basis the observations made in this context.

Significant progress has been made on both fronts during the last five years, again with a substantial advantage to the biological aspects. In particular, the role of silica was recently confirmed to be decisive for preventing aluminium absorption from drinking water [7,44]. Knowledge about the role of dietary ligands on aluminium gastrointestinal absorption [36,38,41,45,46], excretion [53,54] and tissue distribution [39], and of competing metal ions on aluminium absorption [47,57,58], has also accrued. Very intensive research has also been pursued on the design of new aluminium sequestering agents [130,132–143].

Some progress has also been made on the chemical side as to computer-aided speciation applications to gastrointestinal [170–172] and blood plasma [181] conditions. More determinations of complex formation constants involving aluminium and ligands likely to influence the fate of aluminium in vivo will be necessary before its main metabolic pathways can be identified. In the longer run, much fundamental work will have to be done, in particular, regarding CSF and intracellular neuronal fluids, not only relative to aluminium–ligand equilibria but first and foremost to the physiological composition of these media so as to provide experimental data on which new simulation models can be based.

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