

Chemical speciation studies in relation to aluminium metabolism and toxicity

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Abstract

As a detrimental element, aluminium poses a threat to human health at two levels of contamination: (i) environmental (dietary) levels, due mainly to the release of natural stores by acid rains but also to industrial contamination (chronic intoxication), and (ii) therapeutic levels, which affect patients receiving high doses of Al-containing drugs (acute intoxication). In both cases, the bioavailability of aluminium conditions its degree of potential toxicity. Aluminium bioavailability directly depends on the chemistry of the Al^{3+} ion in vivo. In particular, the high charge-to-radius ratio of Al^{3+} , which determines its specific affinity for oxygen donors, is at the origin of its detrimental effects on macromolecular biological structures. In this context, a better understanding of the metabolism of aluminium and of its influence on physiological processes requires a knowledge of its distribution in the main biofluids. This is the *raison d'être* of speciation, either directly through experimental analysis of biological samples, or indirectly via calculations based on conditional constant analysis or computer simulation models. To realize the importance of speciation with respect to the challenge posed by aluminium toxicity, it is important to have a knowledge of all the biological implications of Al–ligand interactions in vivo. For this reason, this article begins with a review of the roles of Al–ligand interactions in aluminium metabolism and toxicity. Contributions of speciation to the understanding of the relevant problems are then treated in perspective with this background, including experimental speciation and computer-aided speciation. In spite of numerous attempts to build reliable simulation models of the distribution of aluminium in the main biofluids (e.g. blood plasma), no definitive results have yet been reached, and the few data available in this field have been established mainly on a conditional constant basis. In general, there is a sharp contrast between the recent advances made by physiologists and toxicologists and the paucity of data obtained by coordination chemists likely to interpret the corresponding phenomena.

Keywords: Aluminium speciation; Plasma aluminium; Al–ligand interactions; Computer simulation model

1. Introduction

In an exhaustive review published in 1974 on aluminium in the environment and human health, Sorenson et al. concluded that “there (was) still no need for concern by the public or producers of aluminium or its products concerning hazards to human health derived from well established and extensively used products” [1]. Twenty years later, several hundreds of articles have been published and increasing numbers appear annually on aluminium toxicity referring to its potential threat in terms of public health. In the meantime, the therapeutic administration of high amounts of aluminium has revealed the existence of a variety of symptoms specific to aluminium intoxication. In particular, the advent of long-term hemodialysis therapy in the seventies led to the discovery of the toxic effects caused by the accumulation of aluminium in patients with renal insufficiency treated by this technique. The syndrome characteristic of these patients (mainly “dialysis encephalopathy” [2], vitamin D-resistant osteomalacia [3], and microcytic anemia (see Ref. [4])) was first

attributed to direct contamination by the aluminium contained in the tap water of the dialysates [5]. Later, however, the persistence of these symptoms in spite of the purification of dialysis water, as well as their appearance in patients not undergoing dialysis, designated Al-containing phosphate binders orally administered to all uremic patients as the main cause of the intoxication [6–9]. This brought to light the notion of a significant gastrointestinal (g.i.) absorption of aluminium [5,10], earlier evidence for which in normal man [10] had been somewhat overlooked. Extensive research was then undertaken by toxicologists to identify all possible sources of aluminium contamination: pharmaceutical preparations (mainly Al-containing antacids [10–16], but also intravenous drugs, nutrients [17–20] and blood products [21], vaccines and toxoids [22,23], etc.), milk formulae [19,24], foods and drinks (e.g. tea [25]), including their additives or contaminants (e.g. coffee percolators [26–30]). A decisive incentive for this upsurge of activity regarding aluminium toxicity has been the potential implication of aluminium in Alzheimer's disease (AD), a neurodegenerative disorder affecting increased fractions of population with age, the debate about which is revived every year [31–37]. In parallel, attempts have been made by chemists [38–44] to devise effective sequestering agents capable of decorporating iatrogenic loads of aluminium with more specificity, fewer side-effects [45–49] and at a lower cost than desferrioxamine (DFO), the iron-specific ligand introduced in clinics against aluminium in 1979 [50] and which is still the treatment of choice in aluminium intoxications [40].

Thus, aluminium is now universally considered a “toxic” metal ion [51–53]. The notion of metal toxicity, however, is often misinterpreted, and needs to be put in perspective with the evolutionary context. Generally speaking, a metalloelement can be classified as: (i) “essential”, if in its absence an organism cannot live; (ii) “beneficial”, if in its absence an organism can survive but without optimized health; (iii) “neutral”, if it is present at benign levels in an organism without apparent beneficial or toxic effects; (iv) “detrimental”, if it is exclusively toxic [54,55]. The status of an element in this classification is not definitive: a species living in its usual environment into which an outer element is suddenly introduced will initially “regard” this new element as detrimental, but its surviving generations will progressively elaborate protection mechanisms which will render it neutral; then, if the element remains at a constant environmental level, the species will finally take advantage of its presence and make it beneficial, before it becomes ultimately essential (see Ref. [55]). In short, the influence of an element on life depends on its ecological age, provided that its environmental level remains constant. Above this usual level, its role will depend on the concentration it can reach *in vivo* (see Fig. 1), and all elements, be they essential, become toxic beyond a certain limit. As far as aluminium is concerned, it has obviously not become detrimental since 1974 [1] simply because of the discovery of toxicity symptoms characterised at (therapeutic or iatrogenic) *in vivo* levels well above those induced by environmental contamination. Left aside by Nature because of its extremely low bioavailability (despite its abundance and ubiquity), aluminium was probably classifiable as neutral before man started changing the conditions of his own environment. Nowadays, however, not only because of its generalised use in food processing or conditioning [29,56], but above all because of acid rains which

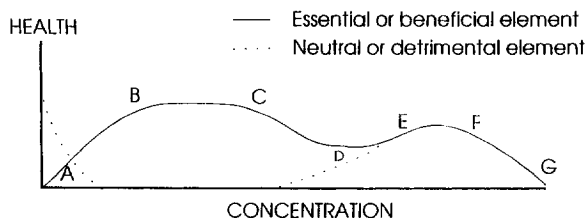


Fig. 1. The effects of increasing metal concentration upon health (from Christie and Williams [55]).

release it from soils and introduce it into the food chain [57,58], aluminium can no longer be considered neutral. What makes aluminium detrimental now is the challenge posed by the aggravation of its overall bioavailability¹ with respect to the capacity of adaptation of the human kind [55].

As Fig. 1 suggests, aluminium now constitutes a potential threat for: (i) the whole earth population, which will have to cope with constantly increasing levels of bioavailable aluminium from the environment, and (ii) more specifically, patients who are administered Al-containing drugs, especially those with renal insufficiency or peptic ulcer disease who receive high doses of metal in the long run. For all cases, the exact evaluation of the risks is directly related to a correct assessment of aluminium bioavailability. In this respect, the g.i. tract was long considered a “formidable barrier to the entry of aluminium” [10]. Being not an essential (or beneficial) metal, however, aluminium is not expected to benefit from any homeostatic process evolved to regulate its metabolism. In particular, its g.i. absorption occurs mainly by passive diffusion whenever chemical conditions in the intestinal fluid are favorable to its solubilisation and, presumably, formation of neutral complexes [59,60]. In normal individuals given a standardized diet, for example, the increase from 74% to 96% in the percentage of metal excreted in the feces when the dose of aluminium is raised from 5 mg day⁻¹ to 125 mg day⁻¹ [61] may be taken as a sign of some regulatory process, but percentages are misleading as the absolute amounts of metal actually absorbed in these circumstances are 1.3 mg day⁻¹ and 5 mg day⁻¹, respectively, and higher figures would still be expected in the presence of dietary components known to favour aluminium absorption, e.g. citrate [62]. Also, even though the kidney appears to be able to excrete all of the absorbed aluminium in healthy people under ordinary conditions [63], it is difficult to quantitate the exact limit above which its elimination capacity is exceeded. In the above study, for example, a virtual zero aluminium balance is observed in the subjects given 125 mg day⁻¹ of metal, but their serum aluminium is increased to 7 µg l⁻¹ compared with 4 µg l⁻¹ in controls [61], which may not be negligible in terms of metal retention. In this respect, it is worth recalling that even “low normal levels of serum aluminium imply potential toxicity” [64]. The above example also illustrates the limitations of the balance technique for the study of aluminium metabolism in healthy humans. Whereas applications of this technique to evaluations relative to high aluminium intakes are

¹ For a definition of bioavailability, see Ref. [133].

generally conclusive (see e.g. Refs. [65–67]), data referring to intakes within the range to which Europeans and Americans are normally exposed [1] are more open to discussion. More generally, almost all *in vivo* observations suffer from the same disadvantage: their global macroscopic data cannot provide any quantitative information to account for the complex processes that underlie them at the molecular level. Unravelling this complexity to describe the distribution of the involved reactants into all the species they form in the main biological compartments relevant to metal metabolism falls within the field of speciation.

By definition, speciation studies can operate at two levels: first, it is usually possible to discriminate between high-molar-mass (h.m.m.) and low-molar-mass (l.m.m.) fractions, and generally among the different components of the h.m.m. fraction of a given element, by means of separation techniques (see recent reviews in Ref. [68]). In contrast, the identification of the (innumerable) l.m.m. species constituting the ultrafiltrable fraction of the element is most of the time beyond direct experimental reach (see e.g. Refs. [69,70]). Speciation of this ultrafiltrable fraction can be achieved indirectly, through computer simulation, provided all total or free concentrations of the constituents of a given solution as well as the formation and solubility product constants which relate these total and free concentrations to the individual concentrations of all the complex species into which they are distributed have been determined beforehand. The main difficulty in applying this technique lies in the fact that the reactant concentration ranges that are imposed to obtain reliable constants do not always encompass those occurring *in vivo*.

Knowledge in science accrues only when there is agreement between experimental observations and theoretical hypotheses, and the advances recently made regarding aluminium in life sciences and chemistry cannot be dissociated. The present article thus begins with the main physiological observations involving Al–ligand interactions *in vivo*, especially those relative to its metabolism. It then gives a short account of the results obtained from direct experimental speciation techniques. Finally, recent progress in indirect speciation studies is described, including examples of the difficult determination of reliable formation constants for aluminium complexes as well as recent contributions of simulation studies to a better comprehension of the bioinorganic chemistry of aluminium *in vivo*.

2. Aluminium–ligand interactions and aluminium metabolism

2.1. Aluminium–ligand interactions and aluminium gastrointestinal absorption

As outlined above, the fact that aluminium has not been attributed any physiological function in the course of evolution *a priori* implies that its metabolism cannot benefit from any homeostatic regulatory process. In particular, its g.i. absorption is not expected to be based on a specific active transport system, and should thus depend in the first place on the solution chemistry of the Al^{3+} ion in the g.i. tract, i.e.: (i) solubility of the ingested salts within the g.i. pH interval; (ii) complex formation with the anionic forms of these salts, which not only induces Al^{3+} solubilisation but

may also facilitate the absorption process. In addition, aluminium may also share some absorption pathways with essential substances for which it may substitute or with which it may combine [60,71,72].

2.1.1. *Extent of aluminium absorption*

The average fraction of aluminium absorbed from a given dose is estimated to be very low [60,73,74], whole animal techniques indicating a percentage of about 1% [73]. Variations in this fraction are usually large: 0.06–27% in animals and 0.001–24% in humans [75]. They depend on the aluminium intake [73]. For example, aluminium absorption was found to be 10- to 100-fold greater when humans or animals were fed small amounts of metal (i.e. 5 mg) rather than pharmaceutical doses (i.e. 1–3 g per day for humans) [73]. Regarding this compensating effect, however, it must be borne in mind that it relates to percentages and does not equalize the absolute amounts absorbed: it may be missed that 26% of 5 mg is less than 4% of 125 mg [61] (see Introduction; see also Fig. 1 for a perspective of environmental and therapeutical concentrations). The second point of importance is that for a given dose of metal, dietary cofactors are determining [74]. These will be examined later.

2.1.2. *Site of aluminium absorption*

Definitive evidence that normal man can absorb measurable amounts of ingested aluminium was obtained by Kaehny et al. [10] with their observation of increased plasma and urine aluminium levels in healthy adults receiving Al-containing antacids. Comparatively to the other three compounds investigated, aluminium phosphate induced no significant change. As the aluminium phosphate gel was virtually insoluble at acidic pH ($4.1 < \text{pH} < 6.2$) while slightly more soluble than aluminium hydroxide under alkaline conditions ($7.5 < \text{pH} < 8.0$), it was suggested that “aluminium absorption (occurred) largely in the acidic milieu of the proximal duodenum or stomach and minimally, if at all, in the rest of the g.i. tract” [10]. Subsequently it was generally accepted that most ingested forms of aluminium are solubilised in the acidic stomach and that, on reaching the milder conditions of the duodenum, soluble hydrated Al^{3+} ions (re-)precipitate as the hydroxide or hydroxyphosphate [76] and become unavailable for absorption [74,76]. Such precipitation has recently been considered unlikely [74] because the interaction of aluminium with ligands present in intestinal secretions, and the mucus, would maintain Al^{3+} ions in solution. While the actual influence of secretory ligands may seem doubtful [76],² that of dietary ligands, effectively found of determining importance [76–78], may largely extend the fraction of the g.i. tract over which aluminium can be absorbed. In fact, various parts of the intestine (duodenum, jejunum, ileum) have a potential for aluminium absorption [60], depending on the conditions that prevail in each case. In humans on aluminium hydroxide therapy for example, the acidity of the luminal contents in the upper part of the g.i. tract is certainly the key factor for aluminium absorption

² Secretory ligands seem not sufficiently concentrated to significantly affect the solubility of high therapeutic doses of aluminium in an empty stomach, whereas lower levels of aluminium ingested in food will mainly interact with ligands of dietary origin.

[79] because of the greater solubilisation of the aluminium salt ingested. In the presence of citrate, in contrast, the role of the ligand becomes predominant, and the maximum serum concentration of aluminium is observed 4 h after administration, suggesting relatively distal small bowel absorption [80]. (Comparatively, the peak plasma concentration of aluminium in rats was observed near 40 min following gavage with aluminium citrate [76,81,82], the absorption being assigned to duodenum and proximal jejunum in that case [82].) Thus, even though proximal small bowel seems to be the site most commonly involved in aluminium absorption, physiological conditions actually determine which regions of the g.i. tract are used [72].

2.1.3. *Mechanisms of aluminium absorption*

Kinetically, transport mechanisms of chemical substances *in vivo* may involve passive transfer (simple diffusion or filtration) or specialised transport (active transport, facilitated diffusion or pinocytosis). Physiologically, intestinal absorption may be transcellular (successively through the brush-border (mucosal) membrane, the cytoplasm, and the basolateral (serosal) membrane of the enterocyte) or extracellular. Transcellular routes include lipid and aqueous routes for non-ionic and water-soluble solutes respectively, and a carrier route for hydrophilic substances too large for the aqueous route [83]. They may involve passive, facilitated and active processes [72]. The principal extracellular route is the paracellular route via the “leaky” junctions between the cells [83] (see Fig. 2). Often classified as passive diffusion [60,72], the paracellular route is rather characteristic of a solvent drag phenomenon [83].

According to Powell and Thompson [74], many of the experimental studies carried out on aluminium absorption have been flawed, in particular because of overlooked aluminium precipitation in solutions at physiological pH, or because of the inappropriate use of buffers (e.g. phosphate). Also, few authors [60,71,72] have considered the implications of the status of aluminium as a detrimental element for its absorption mode (see above). It is generally recognised that there is a large uptake of aluminium by the gut but then little absorption, much of the metal being retained in the mucosa [74]. Adsorption of aluminium by the overlying insoluble mucus and precipitation of its salts in the lumen thus combine to limit its absorption [74]. In kinetic terms, the absorption phenomenon may be perceived as a biphasic process, consisting of a rather fast mucosal uptake of the metal followed by its gradual release into the blood [60]. The aluminium compound absorbed is usually not the compound ingested, and concentration dependence may be confused with species dependence. As pointed out by van der Voet [72], the main problem in most studies is the lack of knowledge of the complex species formed, hence a lack of basis for any useful comparison and extrapolation to the human situation. The diversity of the suggested mechanisms as reported in the recent reviews by this author [60,72] largely reflects this: in the duodenum, a non-saturable mechanism and a vitamin D-dependent saturable mechanism for which aluminium may compete with calcium [84]; in the duodenum (at pH 8.5!), an energy-dependent transport, calcium channels possibly providing an additional entry site [85]; in the jejunum, an energy-dependent, carrier-mediated mechanism [86]; in the jejunum, interaction with the energy-

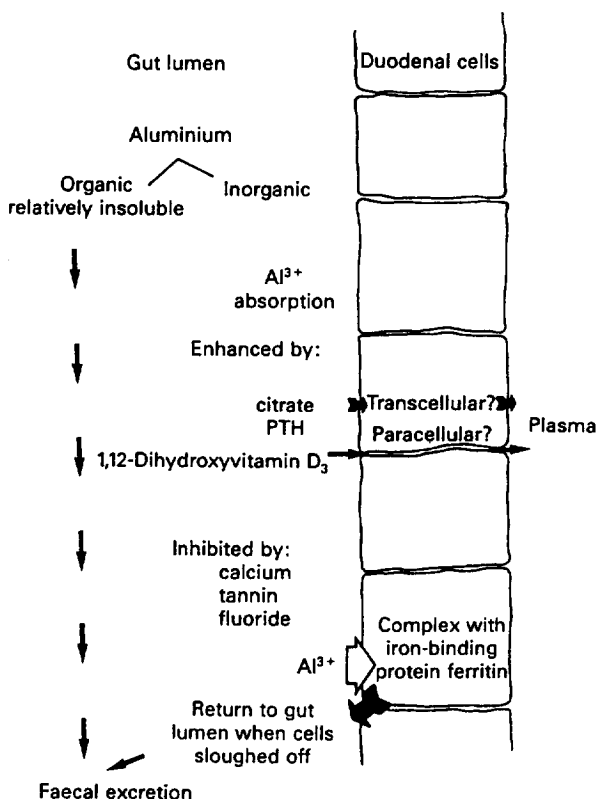


Fig. 2. Gastrointestinal absorption of aluminium (from Lote and Saunders [91]).

dependent calcium-transporting system [87]; in the jejunum, an energy-independent, sodium-dependent, paracellular pathway mediated process [88]; in the small intestine, an energy-independent diffusion for aluminium administered as chloride and an energy-dependent active transcellular transfer for aluminium citrate [71,89]; in the proximal bowel, paracellular pathway for aluminium citrate [82]. All this led van der Voet to conclude that each aluminium species most probably has its own absorption mechanism [72], an assertion which illustrates the need for speciation data relevant to the g.i. tract.

As detrimental metals may at least partially share absorption pathways with chemically similar essential metals [71,60], investigating potential interactions of aluminium with such essential metals may shed some light on the possible mechanisms of its own absorption. This has been done for calcium and iron, aluminium intestinal absorption apparently depending on the status of the absorbing organism in these two metals [60]. In particular, tests relative to calcium performed in different sections of the small intestine and involving vitamin D [84] and parathyroid hormone (PTH) [90] (see Refs. [60,72,91] and references therein) led to the suggestion that aluminium interacts with the calcium-transporting system. In addition, the

calcium level in the intestine is thought to be absolutely critical for protection against aluminium [92], and it may not be fortuitous that the increase in aluminium absorption in AD patients is paralleled by reduced calcium absorption [93]. Similarly, evidence has been provided for a common route of absorption of aluminium and iron in rats [94], iron depletion markedly increasing absorption and cellular uptake of aluminium. This result confirmed the negative relationship previously established between aluminium and iron absorption [95]. Recently, however, these conclusions have been questioned on the argument that the small size of the Al^{3+} ion [96] will not favour substitution for the larger Ca^{2+} ion nor for the Fe^{2+} ion, as it is Fe^{2+} (and not Fe^{3+}) which was shown to reduce aluminium absorption [74,95]. As constantly stressed by Martin [58,59,96,97], the Al^{3+} ion does indeed bear a greater resemblance to Mg^{2+} than to Ca^{2+} (or Fe^{2+}). Interestingly, the serosa-to-mucosa Mg^{2+} flux in all segments of the small intestine is purely passive and probably restricted to the paracellular pathway [98] via a solvent drag mechanism [83]. The fact that aluminium was found to reduce the flux of water across the intestinal membrane in a concentration-dependent manner *in vitro* [99] may be indicative of a similar mechanism. The investigation of a possible interaction of aluminium with magnesium absorption is warranted.

2.1.4. *Effects of dietary components*

Aluminium absorption may depend on the intraluminal presence of essential metals, but also on that of ligands like citrate [72]. Since its discovery by Slanina *et al.* [62], the role of citrate as an enhancer of aluminium absorption has been repeatedly confirmed in many circumstances [76,77,80,81,100–112]. The origin of this effect was long thought to lie in the formation of the neutral species $[\text{Al-Cit}]^0$ in the $1 < \text{pH} < 4$ interval [59,62,113,114]. The real situation seems to be more complex. Recently, Froment *et al.* [82] have shown that enhanced aluminium absorption following administration of aluminium citrate occurs in the proximal bowel via a paracellular pathway due to the opening of cellular junctions subsequent to complexation of free calcium by citrate. *A priori* citrate may thus favour aluminium absorption through three mechanisms: (i) by maintaining the Al^{3+} ion in solution, but other dietary acids can do so [76,78,82] which do not produce the same effect; (ii) by neutralising the charge of the Al^{3+} ion in the $1 < \text{pH} < 4$ interval, but absorption in the stomach is unlikely; (iii) by chelating endogenous mucosal calcium, which renders the paracellular pathway of the bowel more accessible by opening the “leaky” junctions between enterocytes. This last mechanism currently seems the most likely [74,106].

In addition to citrate, other compounds such as ascorbate, gluconate, lactate, malate, oxalate and tartrate have been shown to elevate the pH of precipitation of the aqueous Al^{3+} ion from the usual value of 4.5 to above 8 *in vitro* [76]. All of these dietary constituents are thus *a priori* capable of enhancing the absorption of aluminium by solubilising more of its salts. This has been experimentally confirmed in rats: in healthy animals [109], aluminium retention was significantly increased in most tissues, with ascorbate and citrate showing the highest rate of metal accumulation; in uremic animals, ascorbate, citrate and lactate significantly raised the elimina-

tion of aluminium into urine, presumably because of enhanced g.i. absorption of the element [110]. The administration of lactate also increased the g.i. absorption of aluminium in mice [115]. A systematic investigation of the influence of common dietary constituents (lactate, tartrate, gluconate, malate, succinate, ascorbate, citrate and oxalate) on the absorption (and retention) of aluminium from drinking water and diet was recently carried out with mice [108]: all these compounds significantly increased the aluminium levels in bone, aluminium concentrations also being raised in brain by lactate, gluconate, malate, citrate and oxalate, in spleen by gluconate and ascorbate, and in the kidney by gluconate and oxalate.

Among substances that can associate with the aqueous Al^{3+} ion, silicic acid would play a particular role with regard to aluminium absorption. Based on the geographic relation observed between AD and aluminium in drinking water [116], it has been proposed that the interaction of aluminium with silicic acid restricts the absorption of the metal in the g.i. tract [117–119] and that the classification of silicon as an essential trace element might only be due to its capacity to limit the bioavailability of aluminium [120]. These points are developed in Birchall's chapter in this special issue.

2.2. *Aluminium–ligand interactions and aluminium excretion*

Because of the complex relationship between concentration and protein binding of aluminium in plasma³, it is difficult to assess quantitatively the role of the kidney in the excretion of the metal [91]. Aluminium renal clearance decreases with increasing aluminium plasma level. However, since this level in healthy subjects is low ($10 \mu\text{g l}^{-1}$ or less), it seems likely that the kidneys play an important role in aluminium excretion [91]. Calcium metabolism would be involved in this process through calcium-regulating factors such as PTH and vitamin D [60].

Ligands capable of significantly binding aluminium in plasma are expected to interact with its renal excretion. For example, citrate and ascorbate have been shown to enhance aluminium urinary excretion in rabbits [103], as does ascorbate in man [121] and also ascorbate, citrate and lactate in uremic rats [110] (see above). In line with this, dietary citrate increased retention of metal in rats fed aluminium, but increasing citrate did not enhance aluminium accumulation linearly (i.e. citrate favoured aluminium excretion) [122]. To avoid the complications of altered g.i. absorption, Lote et al. [123] investigated the effect of citrate on aluminium excretion in rats using intravenous administration of both metal and citrate: (i) plasma aluminium concentrations were higher in the groups given aluminium chloride than in those receiving aluminium citrate, this indicating a higher volume of distribution for the citrate form (i.e. a greater capacity to leave the plasma); (ii) the ultrafiltrability of plasma aluminium following administration of aluminium chloride was about 1% whereas it reached nearly 80% with aluminium citrate, values which compare well with the ultrafilterability of the same salts in aqueous solutions (1% and 97%,

³ In an absolute sense, the Al–protein binding in plasma increases with increased plasma aluminium concentration, but, at least up to about 200 ng ml^{-1} , the corresponding fraction decreases [123].

respectively)⁴; (iii) urinary aluminium excretion *in vivo* was found to be much greater for aluminium citrate than for aluminium chloride. Similarly, citrate (unlike maltol) coadministered with aluminium to rats intraperitoneally led to lower aluminium serum levels than aluminium alone [124]. This result was also interpreted as being due to the capacity of citrate to enlarge the ultrafiltrable fraction of aluminium in serum. A more recent study in which aluminium was injected intraperitoneally into mice with in turn citrate, malate, tartrate and isocitrate reached a similar conclusion [125]. Hepatic and renal aluminium concentrations were found to be low for the Al–citrate mixture while hepatic aluminium concentrations were considerably higher for the other three organic acids. Compared with the sole administration of metal, urinary excretion of aluminium was at a high level with all organic acids. As aluminium citrate added to control serum *in vitro* was present only as l.m.m. fraction whereas the metal occurred as both l.m.m. and h.m.m. fractions when added with other acids, it was suggested that the aluminium l.m.m. fraction was excreted into the urine, a significant part of the h.m.m. fraction being transferred to the liver [125].

The above consensus on the role of citrate as a mobiliser of aluminium into its l.m.m. (ultrafiltrable) fraction has lately been questioned. In rats previously loaded with aluminium, perfusion of citrate increased the aluminium excretion rate above the basal state, this increase being directly related to that of the citrate excretion rate [126]. However, as aluminium excretion rose more slowly than the plasma citrate level and as there was no significant change in plasma aluminium fractions with citrate infusion, it has been suggested that the increased excretion of aluminium is independent of the aluminium filtered load and may be the result of changes in the handling of the metal within the kidney. 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. In other words, the additional l.m.m. aluminium mobilised by citrate would be excreted as it is formed.

2.3. *Aluminium–ligand interactions and aluminium distribution in vivo*

The aluminium which reaches the systemic blood circulation and is not excreted through urine accumulates into tissues where it is strongly bound. Accordingly, the concentration of aluminium in blood reflects recent exposure rather than the total body burden of the metal [62,127]. Animal experiments have shown that the increase in blood concentrations after a 10-week administration of various aluminium compounds was invariably accompanied by a retention of the metal in brain and bone tissues [128]. In addition, a positive aluminium balance has been reported in non-uremic patients who showed a moderate increase in plasma aluminium after treatment with Al-containing antacids [129]. Aluminium–ligand interactions play a determining role in the processes involved. A microdialysis study of the aluminium distribution into brain and liver of rats and rabbits following intravenous aluminium lactate or citrate at $500 \mu\text{mol Al kg}^{-1}$ ($13.5 \text{ mg Al kg}^{-1}$) was done by Yokel et al.

⁴ Even though the phosphate buffer used in these latter determinations may have interfered with the results, citrate clearly maintains aluminium in an ultrafiltrable form.

[130]. Tissue/blood dialysable aluminium ratios (TBR) for liver were found near unity, suggesting unhindered diffusion of aluminium between blood and liver. In contrast, brain TBRs were less than unity, demonstrating a partial blood–brain barrier (BBB) to aluminium. The brain TBR relative to aluminium lactate was superior to that for aluminium citrate, thus predicting a shorter transit time in brain extracellular fluid for aluminium lactate than for aluminium citrate: aluminium lactate would more rapidly cross membranes than aluminium citrate, enabling it to more rapidly leave the blood and pass through the extracellular space. These results show that the chemical form of aluminium may have an extremely important role beyond the issue of its oral bioavailability. For example, maltol is a strong enhancer of aluminium accumulation in serum, brain and bone, in a dose-dependent manner [124]. Glutamate has also been suggested as a possible carrier for aluminium deposition in brain [131].

In connection with aluminium tissue distribution, it has been suggested that transferrin (Tf) is an important mediator of aluminium uptake by cells through the receptor-mediated endocytic process responsible for iron uptake, while citrate, although important in enhancing *g.i.* absorption of aluminium, is largely irrelevant to cellular uptake or transcellular transport at physiological pH [132].

3. Aluminium–ligand interactions and aluminium toxicity

3.1. Aluminium–ligand interactions and neurodegenerative disorders

A general theme in aluminium toxicity is accelerated cell death. Mechanisms have been described to account for this phenomenon from both acute and chronic aluminium challenges in which aluminium associations with both extracellular surfaces and intracellular ligands are implicated [133]. Most concern about aluminium toxicity refers to its derived neurodegenerative disorders, with a special mention to the potential implication of aluminium in the pathogenesis of AD [37].

3.1.1. Aluminium and its access to brain

Throughout the central nervous system (CNS), the function of the BBB is dependent on the integrity of a continuous layer of cell membranes that substances can cross primarily in one of two ways: (i) by transmembrane diffusion, depending mainly on the lipophilicity of the substance; (ii) by specific saturable transport systems (e.g. glucose) [134]. Although it does not disrupt the integrity of the membranes, aluminium may directly alter the BBB function. It is known to bind to BBB endothelial cells and to be deposited around the blood vessels in patients with dementia. Aluminium also increases the passage of many compounds thought to cross the BBB by transmembrane diffusion. In particular, it enhances the transport of many peptides in the blood-to-brain direction. This enhancement is not explained by a direct Al–peptide interaction, however, and would be due to an effect of aluminium on the very diffusion process. A similar effect could account for the transport of aluminium glutamate to brain evidenced in rats [131]. Aluminium also selectively affects satura-

ble transport systems (e.g. it inhibits the transport of enkephalins from brain to blood).

A most significant work in respect of the access of aluminium to brain refers to Tf and its receptor [135]. Cells in the brain possess a specific high-affinity receptor for Tf that is independent of the metal being transported. This Tf-TfR system is postulated to be the route whereby the brain can access iron from the general circulation. It has been demonstrated [135] that a metal ion other than iron is capable not only of binding to Tf but also of utilising this interaction to gain access to cells in the brain via the Tf-TfR system. Thus, aluminium may be capable of interfering with normal cellular iron homeostasis and could disrupt iron-dependent cellular processes (e.g. oxidative phosphorylation) in the CNS. Interestingly, ferritin isolated from the brains of AD subjects has a 6-fold higher aluminium content than normal age-matched controls [136], which further suggests that aluminium is accessing the same cellular regulatory routes as iron. Even the levels of aluminium found associated with brain ferritin *in vivo* may adversely affect ferritin function [137]. As iron is a well established inducer of ferritin synthesis, aluminium may overload the tissue with ferritin by indirect stimulation. Brain is a highly aerobic tissue and in the presence of oxygen iron from ferritin produces hazardous free radicals [138]. Colocalisation of aluminium and iron in the brain would thus favour oxidative damage (see later).

3.1.2. *Aluminium and Alzheimer's disease*

Alzheimer's disease is characterised by the presence of large numbers of senile plaques and neurofibrillary tangles (NFTs) in the brain [139]. Neither neuritic plaques nor NFTs, however, are unique to AD, being present to varying degrees in normal ageing and other neurological diseases [140]. Both structures may contain a fibrous protein-carbohydrate complex known as amyloid, whose principal component is a peptide known as β -amyloid (or A4 amyloid). It is likely that β -amyloid results from the proteolytic cleavage of the amyloid precursor protein (APP) produced by neurones and encoded by a gene on chromosome 21. It seems that abnormal APP metabolism is fundamental to the disease [141], and the ability of the peptide to aggregate and form fibrils is critical [142]. Senile plaques, which have a filamentous structure, comprise both neuronal and non-neuronal elements. The neuronal components consist of degenerative and regenerative neurites. The non-neuronal components consist of amyloid protein and extracellular deposits such as aluminosilicates [143]. Diffuse deposits of β -amyloid in the extracellular neuronal space are considered as precursors of the senile plaques [27,140]. Neurofibrillary tangles (NFTs) 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 in severely affected areas of the AD brain, marking the site of the original neurone. Abnormally phosphorylated τ , a protein normally associated with microtubules and rapid axonal transport, is the main component of PHFs [144]. Intracellular NFTs do not contain demonstrable amyloid, but amyloid is deposited onto the extracellular NFTs, which do not contain τ protein (possibly stripped away during neurone degeneration). Brain deposits of

aluminium have been identified in patients with AD, located within the senile plaques [143] and NFT-containing neurones [145] characteristic of the disease.

Aluminium directly causes aggregation of β -amyloid, the major component of senile plaques [146]. Although the mechanism of this process is still unclear, it has been shown that aluminium binds directly to the peptide and changes its conformation [147], and that silicate can reverse both binding and conformation [148]. The capacity of aluminium to form polynuclear species acting as crosslinkers may play a role [149]. Interestingly, this aggregation was also induced by a radical generation system involving metal-induced oxidation of histidine, tyrosine and methionine residues [150,151].

Evidence for the selective accumulation of aluminium within NFT-bearing neurones was obtained in subjects with AD [152], the site of aluminium deposition being the NFT itself. Iron was also detected within NFTs, without any other metallic element being consistently present⁵. Aluminium induces NFTs in the perikaryon of neurones in vivo and in culture. In vitro, the binding of aluminium to neurofilament subunits causes a conformational change of the molecule (intrafilamentous reaction), and aluminium compounds strongly stimulate the interaction between neurofilaments (interfilamentous reaction) [153]. Maltol, whose aluminium complexes may penetrate neuronal cells at neutral pH and low concentrations [33], promotes the effects of the metal [154]: the Al–maltol mixture induced tangle formation in a dose-dependent manner, and the longer the treatment with a fixed dose, the greater the effects. The addition of citrate to the culture medium reduced tangle formation without affecting already formed tangles (see also Ref. [124]).

AD-like changes in τ protein processing have recently been found in the brain of renal dialysis patients following prolonged exposure to aluminium [155], although none of the clinical features of dialysis encephalopathy was seen in that group. The most prominent biochemical change was the white matter accumulation of τ protein endogenously truncated at Glu-391. Since truncation at Glu-391 is characteristic of τ protein in the core of the PHF, white matter changes could be early events in PHF formation. More recently still, it has been shown that PHF τ and AlCl_3 co-injected into rat brain form aggregates which persist longer than PHF τ alone, and that neurones near the injection site acquire PHF τ -like properties [156]. Aluminium would thus bind to PHF τ , induce these proteins to aggregate and retard their proteolysis [156].

Aluminium at physiological concentrations may also alter the biological functions of DNA [157]. One important neurotoxic action of aluminium in AD-affected neocortex may be to increase the binding of histones, particularly H1⁰, to DNA, which results in increased compaction of chromatin and reduced transcription [34,158,159]. Moreover, aluminium at physiological concentration has recently been shown to cause genetic malfunction through irreversibly unwinding supercoiled DNA pockets in the genome [157], this effect being prevented by EDTA. Aluminium is

⁵ It was thus suggested that the beneficial effect of DFO on AD patients, which was attributed to chelation and removal of aluminium from the brain, might as well be due to iron sequestration as an inducer of oxygen free radicals (see above and later) [152].

not the first metal to be able to uncoil the supercoiled DNA [160], but it is the first to relax supercoiled DNA totally and immediately. Accumulation of aluminium in brain may also affect the regulation of RNA metabolism [161].

3.1.3. *Aluminium and lipid peroxidation*

Aluminium has been shown to exert both oxidant and antioxidant effects in mouse brain membranes [162]. Its stimulatory effect on the peroxidation of brain homogenates, microsomes and myelin confirmed an earlier report that Al^{3+} could increase Fe^{2+} -induced peroxidation in liposomes and erythrocytes [138]. This prooxidant action was suggested to occur through interactions of the Al^{3+} ion with membranes, subtle changes in the rearrangement of lipids increasing the availability of fatty acids to free radical attack or facilitating the propagation of lipid peroxidation. In particular, membrane integrity was shown to be necessary for the manifestation of the effect. In contrast, Al^{3+} ions exerted an antioxidant action at much lower concentration, presumably through a replacement of iron at cellular binding sites.

In a more recent study with liposomes [163], the binding of Al^{3+} to the membrane was confirmed as an important step in Al-mediated stimulation of Fe^{2+} -induced lipid peroxidation. The aluminium prooxidant effect increased with decreasing pH and with increasing negative charge density of the liposomes, and was significantly correlated with the capacity of the Al^{3+} ion to promote liposome aggregation, permeability and fusion. In addition, aluminium was demonstrated to cause fatty acid chain packing. Confirmation of these results was obtained by another group, who observed a marked stimulation of Fe^{2+} -induced liposomal peroxidation at pH 7.4, depending on the concentration of Al^{3+} [164]. The lag phase of the reaction was shortened by the addition of aluminium in a dose-dependent fashion, this addition also resulting in a marked increase of the turbidity of the liposomal suspension at pH 7.4 (but not at pH 5 nor at pH 7.4 in the absence of the liposomes). It was suggested that Al^{3+} promotes the aggregation of the liposomes, and a good correlation was found between the stimulatory effect of Al^{3+} and the turbidity change of the suspension [164].

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

For the last 15 years the only clinical treatment in use against aluminium intoxication has been DFO [50]. DFO is beneficial for the therapy of aluminium accumulation in the bone of patients with renal failure: bone histological studies before and after DFO infusion revealed differences similar to those observed between uremic patients with and without aluminium accumulation in bone [165]. DFO is capable of mobilising aluminium from tissue stores [166]: in patients on chronic hemodialysis, an intravenous infusion of 4 g DFO led not only to an elevation of total plasma aluminium levels but also to a marked increase in the ultrafiltrable fraction of aluminium (from 20% to 32%) [166]. A similar effect was observed following an intravenous administration of 100 mg per kg body weight DFO to patients on maintenance hemodialysis, whose total serum aluminium increased by more than 2-fold while the proportion of ultrafiltrable aluminium increased by more than 4-fold

[167] (Table 1). Reversal of osteomalacia and microcytic anemia and improvement of encephalopathy were reported in a child on continuous ambulatory peritoneal dialysis given intraperitoneal DFO (150 mg to each 750 ml dialysate) [168]. Improvement of anemia was also observed following DFO infusion in hemodialysis patients with Al-induced bone disease [169]. DFO therapy continues to remove aluminium for an extended period of time. Because of possible side effects, the end point to treatment in severely intoxicated patients (at maximum clinical improvement rather than after total aluminium removal) may be difficult to define.

Potential substitutes for DFO have been experimentally investigated during the last ten years, sometimes with confusing results. For example, several potential antidotes (DFO, salicylate, citrate, EDTA, nitrilotriacetic acid (NTA), L-cysteine, *N*-acetyl-L-cysteine and dimercaptosuccinic acid (DMSA)) against acute aluminium intoxication have been tested in mice [170]. Citrate was found to be the most effective in reducing aluminium lethality, in increasing the urinary and fecal excretion of aluminium, and in reducing the concentration of metal found in most of the tissues studied [170]. A similar series of chelating agents (DFO, L-cysteine, salicylate, citrate, oxalate, malonate, succinate, malate and aurin tricarboxylate) was tested by the same group against acute aluminium toxicity in rats and mice [171]. Based on therapeutic index (LD_{50}/ED_{50}) and effectiveness (LD_{50} of treated mice/ LD_{50} of controls) calculations, malate, succinate, oxalate and malonate showed the best results, especially malate and succinate. Salicylate, L-cysteine, but also, more surprisingly, DFO and citrate were found to be relatively less effective. In another study of the same group comparing the relative efficacy of citrate, malate, malonate, oxalate, succinate and DFO against the toxicity of aluminium in mice [172], malate and succinate were the most effective in terms of survival at 14 days, whereas the largest excretion of aluminium was induced by malate and DFO in urine and by citrate in the feces. Malonate, oxalate, and succinate had no overall beneficial effects, and citrate was said to be the most effective agent of those tested in the prevention of acute aluminium intoxication [114,172]. Intraperitoneal citrate, malate, succinate and DFO were also administered for 2 weeks to mice which had previously received intraperitoneal aluminium nitrate at a daily dose of $0.27 \text{ mmol kg}^{-1}$ for 5 weeks [173]. All four chelating agents significantly increased the fecal and urinary excretion

Table 1

Aluminium ultrafiltration in control and dialysis patients (from Leung et al. [167])

Subject	<i>n</i>	Total Al ($\mu\text{g/l}$)	Al _{UF} (%)
Healthy controls	10	7.8 ± 5.8	14.5 ± 3
Patients with normal renal function	24	14.0 ± 5.6	16.2 ± 4
Renal-dialysis patients	20		
Pre-DFO		140 ± 62^a	19.7 ± 7^a
Post-DFO		$311 \pm 117^{a,b}$	$85.8 \pm 9^{a,b}$

Results are means $\pm$ SD. Al_{UF} = proportion of serum aluminium ultrafiltered; *n* = number of subjects;

^a $P < 0.001$ as compared with controls; ^b $P < 0.01$ as compared with pre-DFO values.

of aluminium and reduced its concentration in various organs and tissues, with citrate being the most effective. Citrate, malate and succinate were proposed as possible alternatives to DFO in aluminium toxicity [173]. More recently, the relative effectiveness of DFO, L1, citrate and succinate in mobilising aluminium and promoting its excretion were compared in female uremic rats which had previously received aluminium nitrate [174]. Although the daily amounts of aluminium excreted into urine by L1-treated rats were significantly higher than those of the controls, most animals died during the period of treatment. None of the compounds tested significantly altered the concentration of aluminium in bone, kidney and brain under the conditions of the study, whereas only DFO and succinate significantly reduced the levels of aluminium in spleen. L1 and citrate significantly reduced the liver aluminium burden.

A series of potential Al-sequestering ligands have also been tested in terms of aluminium solubilisation, distribution in an octanol–aqueous system and mobilisation of Tf-bound aluminium [39]. When possible, the octanol–aqueous distribution coefficient of each Al–ligand complex was also determined to predict the likelihood of redistribution within or excretion from the intact animal. Aluminium complexes were usually more hydrophilic than their parent ligands. As a conclusion, 3-hydroxy-4-pyridinones and di- and trihydroxamic acids were proposed as promising compounds. In Yokel's recent review on the topic [40], some carboxylic acids have been suggested as possible DFO substitutes; however, as these can increase aluminium absorption and the aluminium body burden, it was concluded that no satisfactory alternative to DFO has yet been shown.

4. Experimental speciation of aluminium in vivo

As is the case with all metals (see Ref. [68] for a review), the characterisation of the protein-bound fraction of aluminium in biofluids is relatively easy. In contrast, the identification of the ultrafiltrable species presents more difficulties [175] and remains largely beyond direct experimental reach [69,70]. First, the number of small ligands (especially with oxygen donors) capable of competing for aluminium in vivo is very large, the situation being further complicated by the possible formation of mixed-ligand complexes [176]. Second, aluminium complexes with these ligands are generally colourless, thus identification by spectrophotometric methods is not possible. Third, separation of the individual complexes by conventional methods is tedious and time consuming, and, above all, there may be alteration of the species during the fractionation [175]. Therefore, most of the experimental data available on aluminium speciation in biofluids refer to the h.m.m. fraction.

Despite the easy evaluation of the aluminium binding to protein, there are large variations in the values reported for its extent (8–98%) [91]. In general, the findings are based on ultrafiltration experiments, and the ultrafiltrability of aluminium in plasma depends on its concentration. Increasing the concentration of the metal is said sometimes to decrease its ultrafilterability (Ref. [91] and references therein),

sometimes to increase it [177,123]. This discrepancy may arise from the basis of comparison used (see footnote 3).

Initially, Elliott et al. [4] proposed that 60 to 70% of aluminium in plasma was bound to h.m.m. proteins, 10 to 20% to albumin, the remaining 10 to 30% being ultrafiltrable, whereas Lundin et al. [178] estimated that about half the plasma aluminium was bound to a protein of a molar mass greater than 8 kg mol^{-1} and the rest was ultrafiltrable. King et al. [179] using gel filtration chromatography described aluminium binding in serum as being associated with large proteins (e.g. α_2 -macroglobulin), albumin, and possibly smaller proteins and inorganic anions. Combining gel filtration chromatography with UV difference spectroscopy, Trapp was the first to characterise the association between aluminium and Tf in addition to albumin [180], and to indicate that at least one of the two specific iron sites was involved in the Al–Tf binding (CO_2 requirement). By contrast with previous findings [179], Gardiner et al. using gel permeation chromatography found no evidence of a fraction with a molar mass higher than 100 kg mol^{-1} in human blood serum [175], and concluded that (i) albumin and Tf were the major proteins to bind aluminium, (ii) a slow exchange of aluminium occurred between species, (iii) the l.m.m. aluminium fraction was made up of mainly inorganic complexes, (iv) the pH of the serum determined the level of this fraction (10% of the total aluminium at pH 7.4).

The notion of Tf as the predominant plasma Al-binding protein was introduced by Cochran et al. [181] from gel filtration analysis of human post-dialysis plasma. Both albumin and Tf were distinguished by immuno-diffusion in the fractions containing aluminium, but whereas adjacent fractions showed a highly reproducible Al:Tf ratio, no consistent relationship was found between aluminium and albumin. Two Al-binding sites were characterised on Tf by spectrophotometric titration, and an affinity constant was estimated near $2.5 \times 10^{15} \text{ M}^{-1}$ [182]. It was concluded that Tf was the major, and probably sole, protein carrier of aluminium in plasma, a small amount of metal probably being associated with phosphate and citrate. Transferrin was confirmed to be the major Al-binding protein in both normal subjects and uremic patients in a comparative study [183] in which 46% of total serum aluminium was found to be ultrafiltrable ($< 10 \text{ kg mol}^{-1}$) for 30 healthy individuals and 33% for 30 patients with chronic renal failure. The presence of an h.m.m. aluminium complex attributed to Tf was also confirmed in rat serum by gel filtration chromatography [177]; in addition, aluminium was shown to be associated with citrate, and Tf and citrate were suggested to interact in the binding of aluminium [177]. Contrasting with the above studies, competitive assays of aluminium binding to Tf in the presence of citrate and albumin at molar ratios corresponding to those found in normal plasma revealed that a considerable amount of aluminium was not bound to Tf [184]. Taking a concentration of $5 \mu\text{M}$ as a typical value observed for the plasma of patients on hemodialysis [185], the competitive binding assays indicated that $\sim 60\%$ of it was bound to Tf, $\sim 34\%$ to albumin and the remainder to citrate. It was therefore suggested that, although Tf at pH 7.4 is the major Al-binding component of plasma, an appreciable amount of aluminium present in patients on hemodialysis may be bound to albumin.

More recently, Favarato et al. [186] using size exclusion chromatography resolved up to five h.m.m. aluminium complexes in the sera of hyperaluminemic individuals, against one to three in normal serum. In normal subjects, Tf (and possibly albumin) would thus be the major aluminium carrier(s). With aluminium concentrations above 200 nM, other proteins such as α_2 -macroglobulin, immunoglobulins, hepatoglobin and also a fraction provisionally designated as albindin are involved in aluminium binding and transport, with different affinities [186]⁶. Incidentally, DFO treatment significantly decreased the proportion of aluminium bound to albumin–Tf but increased that bound by albindin [187]. From the kinetic point of view, a major problem of iron chelation therapy is the slow rate of the release of the Tf-bound iron [188]. The fact that the rate constants for the release of aluminium from Al_2Tf are 2 to 4 orders of magnitude larger confirms that Tf-bound aluminium is effectively a possible target for sequestering ligands [189].

Comparatively to the aluminium protein fraction, experimental data relative to the ultrafiltrable pool of the metal are extremely limited. Evidence for an Al–citrate association in rat serum has been produced [177], but attempts to investigate the speciation of the Al–citrate system in biological samples by HPLC proved unsuccessful [70]. The aluminium binding to citrate in plasma has recently been confirmed by proton NMR [190]. More interesting experimental data have been obtained relative to Al–citrate speciation in the g.i. tract. Partitioning studies *in vitro* between water and ethyl acetate revealed that uncomplexed aluminium exhibited maximum partitioning into the ethyl acetate phase at pH 2.5 [106]. When complexed with citrate, aluminium exhibited partitioning over a much broader pH range (2.5–8.0). *In vivo* rat gut perfusion studies carried out at pH 4, 6 and 8 have shown that citrate increases aluminium plasma levels as well as soluble aluminium in the perfusate at all three pH values [106]. These results, interpreted in terms of absorption of soluble aluminium species by passive diffusion (or rather solvent drag), confirm the mechanism by which citrate facilitates aluminium g.i. absorption (see above).

5. Speciation calculations on aluminium distribution *in vivo*

In the absence of direct experimental data, the analysis of the ultrafiltrable fraction of aluminium must be based on calculations. Regardless of the sophistication of the technique employed, these calculations must satisfy two criteria: relevance and reliability. The notion of relevance implies (i) the use of a correct mode of representation of one or several variables as a function of characteristic parameters, i.e. (mainly) the choice of an appropriate simulation program, and (ii) a realistic selection of the most important reactants present in the involved biofluid in relation to the problem being investigated and a correct estimation of their concentrations, i.e. the constitution of a correct model. Once a relevant simulation model has been constituted, the reliability of the calculations run with it is a direct function of the thermodynamic data used. A rigorous selection, or, better, determination under appropriate condi-

⁶ And also possibly an 8 kg mol^{-1} polypeptide [186].

tions, of stability and solubility product constants is thus required for all the complexes possibly formed between the reactants included in the model. This latter aspect carries a particular weight in the case of aluminium, because of the difficulty in studying its complexes.

5.1. *Modes of calculations and simulation models*

There are basically two modes of quantifying the influence of ligands on the distribution of a metal ion in a biofluid: (i) implicitly, by using effective [191] (or conditional [192]) stability constants⁷ for a direct comparison between ligands (but these constants are difficult to evaluate properly), or by using simpler conditional pH-dependent constants for calculating equilibrium concentrations of the “free” (aquated) metal ion in the relevant medium; (ii) explicitly, by using a simulation model. It is to be noted that if the implicit mode allows an absolute classification of ligands by order of potential binding capacity (which, for example, may be sufficient to select an exogenous chelating agent), the use of a simulation model is necessary to estimate the relative importance of complexes from various endogenous ligands coexisting *in vivo* (for more details, see Ref. [193]).

Gastrointestinal fluid and blood plasma are the two main biofluids for analyses relative to aluminium metabolism. The basic difficulty in studying the influence of a given ligand on the distribution of a metal ion in the g.i. fluid lies in the diversity of the nutrients ingested among individuals, which prevents any standardisation of the diet composition. Also, the variation of pH along the g.i. tract from about 2 to 8 must be taken into account. Usually the distribution of the metal is therefore calculated in an empty stomach, its percentage in each complex being plotted as a function of pH. This simplified graphical representation is extendable to three dimensions, which makes it possible to analyse the influence of a third reactant on the interactions between metal and ligand [194].

From a computational point of view, blood plasma seems easier at first sight because its pH is fixed at 7.4. It is in fact a much more complicated problem, because most of its innumerable components occur at virtually constant concentrations within known limits and all must therefore be taken into account *a priori*. A first difficulty is thus to select the most important reactants for the question to be solved. Another important difficulty is to choose how to consider metal–protein equilibria in the calculations. Practical association constants calculated for metal–protein interactions (see below) are far from true thermodynamic data. Results of calculations directly including these must thus be regarded simply as indications of potential mobilising capacities [195], as are “pM” values. Fortunately, a simulation program, ECCLES [196], exists in which free reactant concentrations may be used as input data and thus avoid this difficulty [197]. This is not an immediate advantage for aluminium, which, as a detrimental metal, suffers no regulation of its free concen-

⁷ Which take into account not only the binding between the metal and ligand in question but also the ligand affinity for the proton and other metal ions as well as the metal affinity for other ligands present in the biological medium.

tration in plasma, but this problem has been circumvented [198]. Another advantage of ECCLES is the great number of species that can be accommodated simultaneously.

5.2. Formation constant determinations

Investigating aluminium complex formation in aqueous media is not an easy task. The first difficulty lies in the very strong tendency of the Al^{3+} ion to hydrolyse. The fact that hydrolysis equilibria must be taken into account throughout virtually the whole pH range accessible to glass electrode potentiometry greatly complicates the calculations relative to complex formation with other ligands. The slow kinetics of the hydrolysis process is an additional problem from the experimental point of view, especially with ligands with a relatively low affinity for aluminium. It is thus particularly important (although often overlooked) that measurements relative to Al–ligand interactions be referred not only to ligand–proton but also to Al–hydroxide equilibrium constants determined under identical experimental conditions. Secondly, aluminium complexes tend to polymerise. Not only does this make the discrimination of the “best” set of constants within the reactant concentration range investigated more difficult, but also close attention must be paid to the applicability of such constants under physiological conditions (see later); another factor to be taken into account is the temperature-dependence of metastable polymerisation equilibria (i.e. conclusions drawn at 25 °C may not apply at 37 °C). Finally, electrically neutral aluminium complexes are particularly prone to precipitation (e.g. $\text{Al}(\text{OH})_3$, AlPO_4). This makes the experimental study of their stabilities a real challenge and has often led to soluble forms being ignored in discussions concerning the distribution of aluminium *in vivo*, despite their potential significance. Keeping all these difficulties in mind may help in understanding how speciation data pertinent to living systems may sometimes be so controversial.

5.2.1. Aluminium hydrolysis

In very acidic aqueous media ($\text{pH} < 3$), aluminium exists in the form of the $\text{Al}(\text{H}_2\text{O})_6^{3+}$ ion. As the pH increases, rapid and reversible [199] deprotonation of the solvating water molecules gives rise successively to $[\text{Al}(\text{OH})(\text{H}_2\text{O})_5]^{2+}$, $[\text{Al}(\text{OH})_2(\text{H}_2\text{O})_4]^+$, $\text{Al}(\text{OH})_3(\text{H}_2\text{O})_3$ and $[\text{Al}(\text{OH})_4(\text{H}_2\text{O})_2]^-$. Small polynuclear species such as $[\text{Al}_2(\text{OH})_2(\text{H}_2\text{O})_8]^{4+}$, $[\text{Al}_3(\text{OH})_4(\text{H}_2\text{O})_{10}]^{5+}$ may also form less rapidly, and more slowly still the large polymeric ion $[\text{Al}_{13}\text{O}_4(\text{OH})_{24}(\text{H}_2\text{O})_{12}]^{7+}$. Clearly the main technical difficulty in investigating aluminium hydrolysis arises from the very slow reaction process, which never seems to reach true equilibrium. The precise conditions of hydrolysis (e.g. composition of solutions and rate at which they are combined, agitation, temperature) determine the nature and quantity of transient polymeric species, colloidal particles, or amorphous solid phases formed [199]. The diverse approaches to tackle this problem are probably at the origin of the discrepancies observed among different groups. Also, the degree of cooperativity in the successive mononuclear equilibria [200] adds indeterminacy as to the intermediate species $\text{Al}(\text{OH})_2^+$ and $\text{Al}(\text{OH})_3$. At 25 °C a consensus exists on Baes and Mesmer's selection (written in the simplified manner) [199]: $\text{Al}(\text{OH})^{2+}$, $\text{Al}(\text{OH})_2^+$,

$\text{Al}(\text{OH})_3$, $\text{Al}(\text{OH})_4^-$, $\text{Al}_2(\text{OH})_2^{4+}$, $\text{Al}_3(\text{OH})_4^{5+}$, and $\text{Al}_{13}(\text{OH})_{32}^{7+}$, but other species such as $[\text{Al}_{2n}(\text{OH})_{5n}]^{n+}$ [201,202] have also been suggested. Only one investigation has been performed at 37 °C, in which the influence of the kinetic factor on the stoichiometries of the potential hydroxides and the accuracy of their formation constants was assessed within extreme conditions [203]. The practical choice of a specific operational protocol was made so as to obtain the closest similarity with the kinetic conditions relative to Al–ligand interactions; the corresponding constants therefore apply in those specific conditions only.

5.2.2. Aluminium complexation

Competition for the Al^{3+} ion between hydroxide and other ligands is governed by thermodynamic and kinetic factors. From the thermodynamic point of view, the high charge-to-radius ratio of the Al^{3+} ion predicts its preference for electrostatic rather than covalent binding [59,97] (i.e. for “hard”–“hard” associations in the HSAB terminology). Thus, aluminium forms stable complexes with ligands containing negatively charged organic functional groups [204], especially strongly basic O atoms (phenolate O^- , carboxylates, phosphonate O^-), even though size may become determining when N atoms are appropriately spaced to form five-membered or six-membered metallocycles (e.g. EDTA, DTPA). Kinetically, Al^{3+} is regarded as a “sluggishly labile” metal ion and the exchange of bound ligands is thought to roughly follow the aquo exchange rate [205].

It is beyond the scope of this review to make a critical evaluation of the formation constants of all aluminium complexes thus far identified. Selected values of these can be found in recent constant tables (see e.g. Ref. [204], Vol. 6, 1989) or databases [206–208], or in other recent reviews [38,44,209]. Nevertheless, a few ligands are of particular relevance to aluminium speciation in vivo, and a quick examination of the available data relative to these may help to trace the origin of conflicting speciation models. A good example of these is citrate. Given its importance in aluminium metabolism (see above), citrate is one of the ligands whose interactions with aluminium have been the most studied [198,210–216]. If we disregard an initial partial study at 25 °C [214], the first investigation of the system at 37 °C in NaCl 0.15 mol dm⁻³ [212] led to the characterisation of MLH, ML, ML(OH) and of $\text{ML}_2(\text{OH})$ as a minor species, no mention being made of the pH interval investigated nor of the metal hydrolysis constants used (if any)⁸. At 25 °C in NaCl 0.6 mol dm⁻³ (2 < pH < 8), Öhman and Sjöberg [210] using hydrolysis constants determined under appropriate conditions [217] reported the existence of MLH, ML, ML_2 and $\text{M}_3\text{L}_3(\text{OH})_4$ (the latter being shown to be predominant over $\text{M}_2\text{L}_2(\text{OH})_2$ in 1:1 metal:ligand ratio experiments at 3 < pH < 4). At 25 °C in KNO_3 0.1 mol dm⁻³, Motekaitis and Martell [211] using hydrolysis constants selected from the literature found MLH, ML, ML(OH) in the 2.5–8 pH range. A larger number of species, MLH, ML, ML_2H , ML_2 , $\text{ML}_2(\text{OH})$ and $\text{ML}_2(\text{OH})_2$, was found by Gregor and Powell [213] with an excess of citrate over aluminium at 25 °C in 0.10 mol dm⁻³ (pH < 7.0); owing to this excess, aluminium hydroxides with equilibrium constants

⁸ Considering citrate as a trianion.

taken from Ref. [217] did not account for more than 0.03% of the metal in solution. The second study performed at 37 °C in NaCl 0.15 mol dm⁻³ (1.7 < pH < 9) using hydrolysis constants specifically determined for those conditions (see above) [198] reported the existence of MLH, ML, ML₂, ML₂(OH), ML₂(OH)₂, M₂L₂(OH)₂ and M₃L₃(OH)₄. More recently, Öhman [215] published a complement to his first investigation [210], characterising in addition (i) ML(OH) and ML(OH)₂ from “time zero” data in slightly acidic solutions (3 < pH < 7) and (ii) M₃L₃(OH)₇ at equilibrium in slightly alkaline solutions (7 < pH < 9.5). In the third study at 37 °C in 0.15 mol dm⁻³ (2.0 < pH < 6.6), Findlow et al. [216] using hydrolysis constants adapted from the literature found MLH, ML, ML(OH), ML(OH)₂, ML₂ and ML₂(OH), but mentioned no polynuclear species.

From the kinetic point of view, Jackson [212] mentioned that long delay times (20 min) were necessary to attain equilibrium. Öhman and Sjöberg [210] reported up to 6 h for equilibration in “less acidic solutions” at “low” ligand-to-metal ratios. Motekaitis and Martell [211] observed “variable” equilibration times, excluding from the calculations cases where the potential drift continued beyond 4 h. In contrast, owing to the high ligand-to-metal ratios in their experiments, Gregor and Powell [213] used no data for which the pH equilibrium time exceeded 10 mn. On the other hand, Venturini and Berthon [198] noted that the 2 min frequency of additions in the above-mentioned protocol was almost always sufficient to reach true equilibrium. Similarly, Findlow et al. [216] using data taken at 5, 10 and 15 min after titrant additions reported little observable difference in their final results. Although equilibration times appear to depend mainly on the respective metal and ligand concentrations, which determine the nature and proportion of aluminium hydroxides, it thus seems that equilibrium is reached much faster at 37 °C than at 25 °C, which confirms that conclusions drawn at 25 °C may not apply at 37 °C. Even at the same temperature, however, results from different sources are still far from concurring: the distribution profiles plotted in Fig. 1 of Gregor and Powell [213] are very expressive in this respect, and even apparently small differences in the experimental cell may diversely affect aluminium simulated distributions *in vivo*. Regarding the indetermination between ML(OH) and M₂L₂(OH)₂ or/and M₃L₃(OH)₄ for example, ML(OH) will always be favoured in blood plasma while in the g.i. tract M₃L₃(OH)₄ may well predominate (see later).

Another ligand of considerable physiological importance whose aluminium complexes are still more badly characterised than those of citrate, in spite of their high stability, is phosphate. The reason for this is the insolubility of the neutral AlPO₄ over a large pH interval⁹. The first systematic investigation of the Al–phosphate system by ion exchange and pH titration [218] in very acidic media (pH < 2) confirmed Bjerrum and Dahm’s pioneering work [219] indicating that the three anionic forms H₂PO₄⁻, HPO₄²⁻ and PO₄³⁻ were all potential ligands of aluminium. The existence of M₂L, M₂L(OH), M₂L(OH)₂, MLH, MLH₂ and ML₂H₄ was postulated but no formation constants were calculated for these. ²⁷Al and ³¹P NMR studies at very acidic pH (1) led to the qualitative characterisation of MLH₃, MLH₂

⁹ Covering in particular the almost complete physiological range.

and ML_2H_4 complexes, with two binuclear species and a series of polymeric $\text{M}(\text{LH}_x)_n$ species with $n \geq 2$ [220]. More recently, a reinvestigation of the system with the same technique [221] suggested MLH_3 , ML_2H_6 , MLH_2 , ML_2H_4 , MLH and ML_2H_2 to be the major species present under similar conditions. The first quantitative study of Al–phosphate equilibria was that of Jackson and colleagues [222,223], who calculated formation constants for MLH_2 , MLH and ML_2H complexes from potentiometric measurements at 37 °C in NaCl 0.15 mol dm⁻³ ($2 < \text{pH} < 4$). A subsequent study in our laboratory by the same technique and under the same conditions extended the pH range investigated up to 11 for a series of experiments with metal-to-ligand ratios less than unity, while experiments with metal-to-ligand ratios equal or greater than unity were reported to precipitate near pH 3 [224]. For the first time, a constant was estimated for the ML complex whereas MLH_2 , MLH , M_2L , $\text{M}_2\text{L}(\text{OH})_2$ and $\text{M}_2\text{L}(\text{OH})_3$ were the main species characterised. These results were later questioned by Duffield et al. [225], who observed the presence of a precipitate at all metal-to-ligand ratios and calculated lower stability constants for five of the above complexes [224] still thought to be physically significant¹⁰. It seems, however, that either there was no precipitate in Daydé's experiments [226] and the stoichiometries of the proposed complexes were (and would still be) correct, or a precipitate has really been overlooked and not only are the constants questionable, but the species stoichiometries too. From speciation model considerations, Harris [227] recently called both Daydé et al.'s [224] and Duffield et al.'s [225] results into question, and in new experiments in which precipitation has effectively been observed above pH ~ 3 [228], species stoichiometries have been found different from those characterized previously [224]. Interestingly, the constant established by Harris [227] for $\text{ML}(\text{OH})$ from linear free energy relationships (LFERs) has since then been shown to be overestimated by two orders of magnitude [229].

These two examples illustrate well the difficulties encountered in investigating aluminium l.m.m. complex equilibria in solution, and such cases of discrepancy are many (see e.g. Refs. [78,209,216]) even though space is too limited to discuss more of these here (for a recent review see Ref. [205]). Another problem that deserves some comment is the determination of practical values for the association constants of aluminium with proteins. The large variations observed in protein-bound aluminium fractions measured experimentally (see above section) are a potential source of conflicting results.

The only constant available for Al–albumin complexation was calculated by Bertholf et al. [230] using a chelex competitive binding assay. After appropriate corrections allowing for aluminium hydrolysis [59], the dissociation constant reported (1.96 μM) yields a logarithmic stability constant of 12.1 [198], a value difficult to reconcile with chemical considerations [59] as well as with other findings [181,183] (see above section), and which must, therefore, be considered as a maximum for simulations relative to plasma in patients on hemodialysis [184].

Being predominant in normal plasma, Al–Tf associations are much better known quantitatively. The first value calculated for an Al–Tf binding constant was that of

¹⁰ $\text{M}_2\text{LH}(\text{OH})_3$ was the only species to be discarded out of the six reported by Daydé et al. [224].

Berthoin et al. [230] in parallel to that of albumin. Once corrected for aluminium hydrolysis [59], the dissociation constant ($0.515 \mu\text{M}$) yields a logarithmic stability constant of 12.68, which compares well with other values determined simultaneously [181,182] or more recently [184,185,231]. In the same year, Cochran et al. reported for the Al–Tf constant 13 (13.3–13.9; with 23.7 for Fe^{III} –Tf) [181] and 15.40 (with 22.48 for Fe^{III} –Tf) [182]. More recently, conditional step-stability constants (at pH 7.4 and 27 mM HCO_3^-) have been assessed by Martin et al. as $\log K_1 = 12.9$ and $\log K_2 = 12.3$ (for Al–Tf and Al_2 –Tf, respectively) from UV spectra using NTA and citrate as competing ligands to control the very low concentrations of free metal ion [231]. Another study carried out under identical conditions in all except that Hepes buffer was used instead of Tris resulted in $\log K_1 = 13.5$ and $\log K_2 = 12.5$ [185], this difference remaining unexplained. The latest results have been obtained from a spectrophotometric study of aluminium binding to Tf in the presence and absence of citrate and albumin [184]. Apparent association constants have been calculated at pH 7.4: $\log K_1 = 12.23$ and $\log K_2 = 11.76$, which reduce with both increasing and decreasing pH. As these constants have been determined without NTA in the presence of concentrations of NaCl and HCO_3^- , close to those present in normal serum, their authors consider these, apparently with good reason, as the appropriate ones for speciation studies.

5.3. Speciation calculations and aluminium metabolism

In physiological applications of indirect chemical speciation, the knowledge of the biological milieu is not always sufficient to allow the constitution of a simulation model. In such cases, the rational use of conditional stability constants remains an irreplaceable tool to reveal the most salient features of the system. Many calculations in the recent reviews of Bruce Martin on aluminium bioinorganic chemistry [58,59,96,232–234] are reference examples of this kind of approach. By definition, however, the use of simulation models which explicitly take account of more variables is preferable whenever the criteria of relevance and reliability can be reasonably satisfied. Such applications have thus far been virtually limited to the g.i. fluid and blood plasma.

5.3.1. Gastrointestinal fluid

As expressed in a previous section, the fraction of ingested aluminium that is solubilised and dissociated in the acidic stomach is normally expected to (re-)precipitate as the hydroxide or hydroxyphosphate on reaching the duodenum [74]. However, the occurrence of dietary acids in the g.i. tract may impair this protective phenomenon [76,77]: the aluminium complexes formed by the anions of these acids may be stable enough to maintain a significant amount of the metal in solution, and some of these complexes, or other species arising from other nutrients [205], can cross the g.i. membrane. Some of these effects have been analysed by computer-aided speciation, neutral complexes thought to readily diffuse through the membrane receiving particular attention.

First, simulations have been run to assess the influence of a series of dietary acids on the pH range over which $\text{Al}(\text{OH})_3$ and AlPO_4 can totally dissolve [78], which substantiated previous observations on the effect of some of these acids on $\text{Al}(\text{OH})_3$ precipitation [76] (Table 2). Also, distribution profiles of aluminium citrate complexes have frequently been presented in support of the notion of aluminium absorption through the neutral complex $[\text{AlCta}]^0$ [59,78,198,205]. Recent considerations [74,82] tend to reduce the impact of this neutral species on the aluminium absorption process (see previous section on aluminium absorption), but do not minimise the influence of aluminium complexation by citrate in general. In contrast, Partridge et al. [106] have demonstrated that other Al–citrate complexes also can significantly elevate the partitioning of the metal from water to ethyl acetate in the pH range of the intestine (and increase aluminium plasma levels in gut-perfused rats). Comparing their results with distribution curves taken from Motekaitis and Martell's study of the Al–citrate system [211], Partridge et al. [106] suggested that the anionic complex

Table 2

pH intervals between 1 and 8 within which $\text{Al}(\text{OH})_3$ or/and AlPO_4 can totally dissolve in the presence of dietary acids (from Berthon and Daydé [78])

Acid	Al concentration (mol dm ⁻³)	pH range	
		$\text{Al}(\text{OH})_3$	AlPO_4
None	0.005	< 3.9	1–8
	0.05	< 3.6	< 2.4/7.7 <
	0.5	< 3.2	< 1.8
Citric acid	0.005	1–8	1–8
	0.05	< 3.7	< 2.4/7.7 <
	0.5	< 3.2	< 1.8
Malic acid	0.005	< 7.0	1–8
	0.05	< 3.9	< 2.4/7.7 <
	0.5	< 3.2	< 1.8
Oxalic acid	0.005	< 6.4	1–8
	0.05	< 3.8	< 2.4/7.7 <
	0.5	< 3.2	< 1.8
Succinic acid	0.005	< 4.3	1–8
	0.05	< 3.7	< 2.4/7.7 <
	0.5	< 3.2	< 1.8
Tartaric acid	0.005	< 5.8	1–8
	0.05	< 3.7	< 2.4/7.7 <
	0.5	< 3.2	< 1.8
Aspartic acid	0.005	< 4.7	1–8
	0.05	< 3.8	< 2.4
	0.5	< 3.4	< 1.8
Glutamic acid	0.005	< 4.7	1–8
	0.05	< 4.2	< 2.4
	0.5	< 3.4	< 1.8

Acid concentrations: citrate 0.0312 M, malate 0.0466 M, oxalate 0.0278 M, succinate 0.01 M, tartrate 0.02 M, aspartate 0.06 M, glutamate 0.068 M.

$[\text{MLH}_{-1}]^-$ was responsible for this phenomenon. However, the concentrations used for these calculations ($0.002 \text{ mol dm}^{-3}$ for aluminium and citrate; see Ref. [211] and their Fig. 8) are far from those employed in their own experiments (0.1 mol dm^{-3} for aluminium and 0.1 mol dm^{-3} or 0.2 mol dm^{-3} for citrate), and simulations run with their experimental concentrations and thermodynamic data determined under physiological concentrations [198] (results not shown here) indicate that $[\text{MLH}_{-1}]^-$ (or $[\text{M}_2\text{L}_2\text{H}_{-2}]^{2-}$), but also $[\text{M}_3\text{L}_3\text{H}_{-4}]^{4-}$ and $[\text{ML}_2]^{3-}$, depending on the metal-to-ligand ratio, are present within the extended partitioning range ($1.5 < \text{pH} < 8$). Surprisingly, this confirms that anionic (hence water-soluble) Al–citrate complexes would enhance partitioning from water into an organic phase.

Thus, solubility seems to be the major requirement for increased aluminium absorption in the presence of citrate [106]¹¹. However, this does not exclude the possibility that other ligands may induce the same net effect through formation of neutral aluminium complexes. For example, such species have been shown to be significant over relatively large pH intervals with tartrate and succinate, ligands which have been shown to increase bone aluminium levels in mice [108]. Fig. 3 shows the distribution of $0.0005 \text{ mol dm}^{-3}$ aluminium in the presence of 0.02 mol

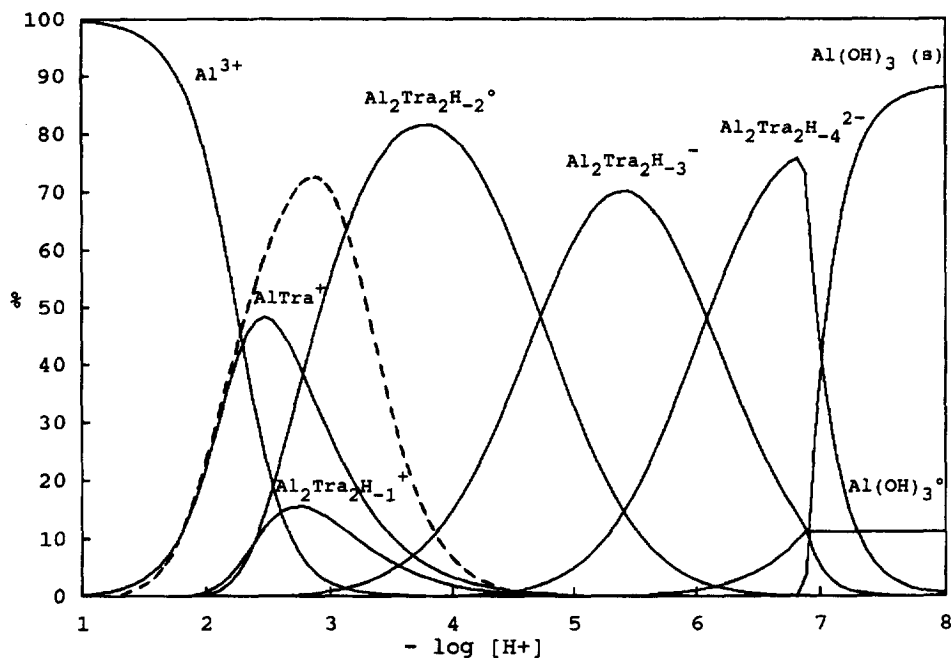


Fig. 3. Distribution profiles of Al complexes with tartrate in the g.i. fluid. Reactant concentrations are $C_{\text{Al}} = 0.0005 \text{ mol dm}^{-3}$ and $C_{\text{Tra}} = 0.02 \text{ mol dm}^{-3}$. The broken line represents the percentage of the AlCta^0 complex under identical conditions ($C_{\text{Al}} = 0.0005 \text{ mol dm}^{-3}$ and $C_{\text{Cta}} = 0.02 \text{ mol dm}^{-3}$). From Daydé and Berthon [237].

¹¹ Presumably because of the demonstrated role of citrate in the opening of cellular junctions [82].

dm^{-3} tartrate, which corresponds to 2.7 mg of aluminium (about half the environmental aluminium ingested daily [235]) and 0.6 g of tartrate (the average amount in a glass of white wine [236]) in 200 cm^3 of water. Under these conditions, the percentage of the neutral complex $[\text{Al}_2\text{Tra}_2\text{H}_{-2}]^0$ reaches 81.6% (2.2 mg of aluminium) near pH 4 and remains over 10% from pH 2.5 to 5.5. Interestingly, with 0.05 mol dm^{-3} aluminium (a single dose of an antacid such as Maalox® in the above volume of water) the same concentration of tartrate neutralises 34.7% of metal (about 93 mg) in the same form, and 3.45% with 0.5 mol dm^{-3} aluminium, which shows that above a certain aluminium concentration, the absolute amount of metal neutralised remains constant whatever the intake, whereas the corresponding pH range slowly shifts towards more acidic values. Fortunately tartrate is much more sensitive than citrate to the competition of phosphate, which limits the formation of soluble neutral species provided the phosphate concentration is at least as great as that of aluminium [237]¹².

The potential effect of succinate is similar, although less important. As Fig. 4 shows, a concentration of 0.01 mol dm^{-3} of succinate (approximately the amount present in a glass of wine [236] diluted to 200 cm^3 in water) can neutralise up to 64.2% of $0.0005 \text{ mol dm}^{-3}$ aluminium (1.7 out of 2.7 mg in the same volume of

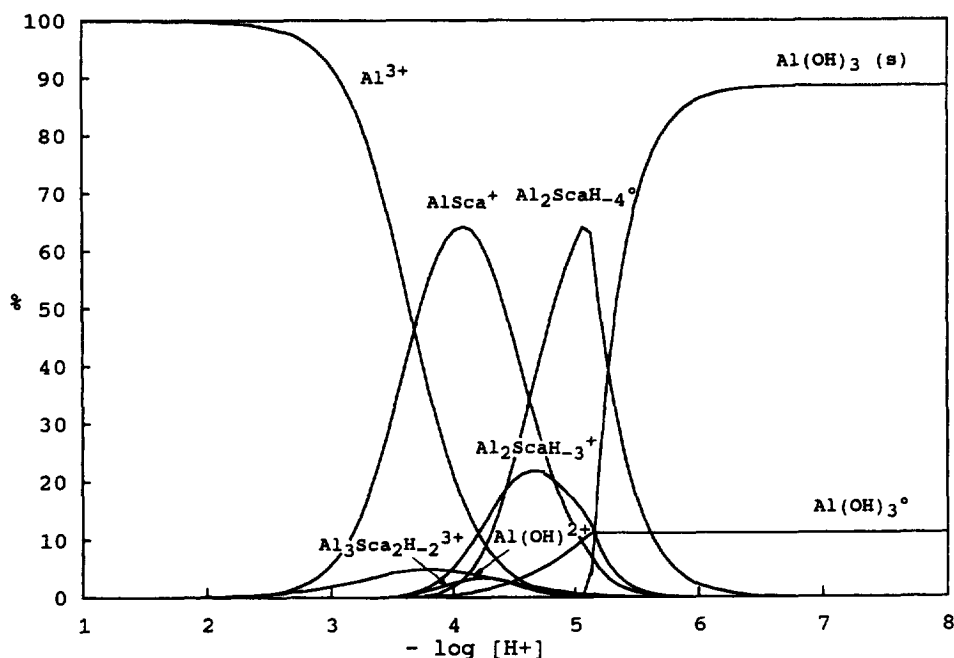


Fig. 4. Distribution profiles of Al complexes with succinate in the g.i. fluid. Reactant concentrations are $C_{\text{Al}} = 0.0005 \text{ M}$ and $C_{\text{Sca}} = 0.01 \text{ M}$. From Venturini-Soriano et al. [238].

¹² With therapeutic aluminium, this condition can in fact be fulfilled only if the metal is administered as AlPO_4 .

water) around pH 5, the maximum amount of aluminium which can be neutralised being about 8 mg from a concentration of $0.005 \text{ mol dm}^{-3}$ aluminium onwards (i.e. approx. one-fifth of that neutralised by an equivalent concentration of tartrate) [238]. Like tartrate, succinate is very sensitive to the competition of phosphate.

Concerning the relative toxicities of Al-containing antacids, the better safety of aluminium phosphate over $\text{Al}(\text{OH})_3$ previously suggested to be due to the poorer solubility of the former [10] has recently been attributed to the combination of the coating protection of solid AlPO_4 with that of the non-absorbable $\text{Al}_2\text{PO}_4(\text{OH})_2$ complex [78]. However, as the validity of this interpretation depends on stability constants which have recently been questioned [225,227], it should be considered with caution until the situation is clarified.

Other percent distributions of aluminium in the presence of phosphate at different pH values and with various concentrations and Al:PO₄ concentration ratios have also been calculated by Duffield et al. [225]. They should also be considered with caution as the constants used by these authors are equally open to question [227,228]. The Al–phosphate system is highly controversial. It has even been said that “the most likely pathogenic route of aluminium (was) via the phosphate complex” [222]. Incidentally, Jackson has also used computer simulation under g.i. conditions to assess the influence of maltol on aluminium toxicity [239]. The mobilisation of 90% of aluminium in the form of the neutral $[\text{Al}(\text{maltol})_3]^0$ species near pH 7 at the concentrations used (0.1 mol dm^{-3} aluminium and 0.3 mol dm^{-3} maltol) is expected to significantly enhance aluminium absorption. However, phosphate is once again expected to reduce this enhancing effect.

Finally, regarding the claimed reducing effect of silicate towards aluminium absorption [117–119], Vobe and Williams’ calculations [240] predict that it is impossible for significant Al–silicate l.m.m. complexes to coexist in the presence of citrate and phosphate in the intestinal fluid.

5.3.2. Blood plasma

Because of its central physiological role, blood plasma is the biofluid in which aluminium speciation has been the most extensively investigated. Corresponding results have been analysed mainly in terms of electrical charge for the predominant complexes into which aluminium is distributed: cationic or anionic species tend to be excreted via the renal system, while those which are electrically neutral induce tissue retention before they are ultimately excreted through the bile. Simulation models developed so far differ in (i) the number of metals and ligands which can be accommodated, (ii) the consideration of either total or free aluminium concentrations, and (iii) the stoichiometries and stability constants of the complexes taken into account.

The number of reactants which can be included in a model depends on the capacities of the program used. Being designed to accommodate as many of these as necessary, ECCLES [196] is certainly the best choice in this respect. Another determining advantage of ECCLES is the choice it allows between total and free metal concentrations as input data (see above). It may thus seem surprising that among the groups of authors of the different models published [209,224,225,227,241],

notably the four running ECCLES [209,224,225,227], only one [224] used the free concentration of aluminium. This stems from the status aluminium as a detrimental metal (see Introduction). For essential (and beneficial) trace metals, the “buffering” of their free concentrations by proteins as well as the solubility products of their poorly soluble salts are sufficiently well known to allow the calculation of an interval within which their free concentrations may vary without affecting their percentage distributions [197]¹³. For aluminium, this interval was yet to be defined, and the group whose calculations were based on the free metal concentration [224] actually used the total concentration approach first [198]. On this occasion [198], albumin was quantitatively confirmed to be a negligible ligand of aluminium in plasma compared with Tf, and the free Al^{3+} concentration was shown to vary linearly with the total concentration up to $10^{-16} \text{ mol dm}^{-3}$; above this limit, the free concentration decreased more rapidly than the total concentration, because of the growing influence of the $[\text{Al}_2\text{PO}_4(\text{OH})_2]^+$ complex. Although the stability constant [225,227] and even the stoichiometry [228] of that complex have later been questioned, the prediction that the fraction of protein-bound aluminium should decrease with increasing plasma aluminium concentration has been experimentally validated in rats [177,242].

The use of the total concentration of aluminium in the calculations run by all groups (exclusively or not) allows a comparison of the values obtained for the protein-bound aluminium fraction (Table 3). These results can be divided into two groups: 57% [224] and 63% [209] on the one hand, and 83% [241], 80% [225] and 81% [227] on the other hand, to be compared with $54 \pm 7\%$ determined experimentally on healthy individuals [183]. As practical constants for Al–Tf and Al_2 –Tf ($K_1 = 10^{12.9}$ and $K_2 = 10^{12.3}$ [231], or $K_1 = 10^{13.5}$ and $K_2 = 10^{12.5}$ [185]) as well as Al–albumin ($K = 10^{12.1}$ [59,198,230]) complexes are common to all these authors (supposedly for Ref. [225]), the discrepancies observed mainly arise from the different sets of constants selected for l.m.m. complexes.

The ECCLES model published by Daydé et al. [224] highlights the importance of the soluble forms of the two poorly soluble salts, aluminium trihydroxide and monophosphate (Table 3)¹⁴. As already expressed, the constant for $\text{Al}(\text{OH})_3$ has been determined under specified kinetic conditions [203]. The principle of that strategy was to obtain approximate data rather than no data at all. Of course, the clear disadvantage of such an approach is that it can always be questioned from a purely thermodynamic point of view. The situation with AlPO_4 (even more difficult) has been discussed in a previous section.

Concerning its applications, Daydé et al.’s model [224] was the first to interpret the positive effect of citrate on aluminium urinary excretion [113,198] observed in many animal studies [103,110,114,122–125,170,172]. Also, its prediction that tartrate

¹³ As there is a large excess of ligands over these metals in vivo: (i) the complexed fraction of each ligand is negligible and its free concentration depends on its interactions with protons only; and (ii) polynuclear complexes are insignificant.

¹⁴ It is noteworthy that considering $\text{AlCta}(\text{OH})$ (with a constant of $10^{5.25}$ for AlCtaH_{-1} as rejected as a second choice by Venturini and Berthon [198]) instead of $\text{Al}_2\text{Cta}_2(\text{OH})_2$ in the calculations would lead to 30% of AlCtaOH at the expense of other species shown in Table 1.

Table 3

Simulated distributions of l.m.m. aluminium in blood plasma (from Orvig and Berthon [205])

Protein-bound Al	Al l.m.m. fraction	References
57%	Al(OH) ₃ [51%] AlPO ₄ [41.5%] Al ₂ PO ₄ (OH) ₂ [7.2%]	[224] ^a
63%	AlPO ₄ [62%] AlCta(OH) [23%] AlPO ₄ CtaH [10%]	[209]
83%	Citrate [98%] Hydroxide [2%] (phosphate not considered)	[241]
80%	AlCta(OH) ₂ [94%] AlCta(OH) [3%] AlPO ₄ [1.5%] AlOxa(OH) ₂ [1.4%]	[225]
81%	AlPO ₄ (OH) [80%] AlCta(OH) [10%] Al(OH) ₃ [4%] Al(OH) ₄ [3%] AlPO ₄ [2%]	[227]

^a It is noteworthy that considering AlCta(OH) with a constant of $10^{5.25}$ for AlCtaH₋₁ (as rejected as a second choice by Venturini and Berthon [198]) instead of Al₂Cta₂(OH)₂ in the calculations would lead to 30% of AlCtaOH at the expense of other species.

was unable to significantly mobilise plasma aluminium [237] was recently validated in mice (urinary aluminium levels with tartrate being 7% of citrate and 8% of DFO) [125]. A more recent application of this model [238] predicts a poor capacity for succinate to mobilise plasma aluminium, which agrees with the latest (negative) conclusions of Domingo's group [174] as to the possible use of succinate as a substitute to DFO and with Yokel's suggestion [39] that the possible protective effect of intraperitoneal succinate against aluminium toxicity in mice may not be due to aluminium complexation. These results show that no compensation similar to that provided by citrate can be expected from tartrate or succinate for their aggravating influence on aluminium absorption (see above section).

Jackson [209] also used ECCLES to simulate the distribution of plasma aluminium considered as its total concentration, before and after equilibrium with Tf. The calculations were based on constants selected from the literature for complexes of naturally occurring organic and inorganic anions, among which phosphate plays a determining role. In particular, a series of values have been estimated for hydroxo complexes of mono- and diprotonated phosphate considered as independent ternary species, and the fraction of aluminium reported as AlPO₄ in Table 3 represents in fact the sum of Al(HPO₄)OH and Al(H₂PO₄)(OH)₂ percentages. Under these conditions, the aluminium distribution was found to be independent of total aluminium concentration within the 10^{-13} – 10^{-3} mol dm⁻³ range, with the free concentration

being 10^{-9} less. The plasma mobilizing indices (PMIs)¹⁵ [243] for various potential sequestering agents *in vivo* were also evaluated, among which DFO was found to be the most efficient aluminium mobiliser. Later, the influence of maltol on aluminium speciation in blood plasma was also investigated with this model [239]. Even with protein binding being disregarded, the percentage of aluminium present as $[\text{Al}(\text{maltol})_3]^0$ was predicted to be low, whereby it was concluded that the neurotoxicity of that species was probably an order of magnitude greater than previously thought.

A simpler model of blood plasma (50 mM Tf vacant sites, 100 mM citrate, pH 7.4 at 25 °C) was developed [241,244] to compare the efficacy of a series of 28 ligands as possible aluminium binders. Citrate constants were taken from Öhman [215]. Phosphate was excluded from the model because of a lack of reliable formation constants. Applications of speciation computer modelling to animal studies were discussed. It was found that 3-hydroxy-4-pyridinone was more effective at complexing aluminium than EDTA, maltol or catechol [241].

Duffield et al. [225], using ECCLES, found the ultrafiltrable fraction of aluminium to exist almost exclusively as the $\text{AlCta}(\text{OH})_2$ species (Table 3). The formation constant used for this complex, (presumably) taken from a previous study [216], has been questioned [44,227]. No $\text{Al}_3\text{Cta}_3(\text{OH})_4$ was reported in that work [216] although aluminium and citrate concentrations were equal to, or greater than, those used by other authors who characterised it [198,210,215], and the maximum pH investigated was 6.6, a value at which $\text{Al}_3\text{Cta}_3(\text{OH})_4$ should be predominant and $\text{AlCta}(\text{OH})_2$ still negligible [210,215]. The constant for $\text{AlCta}(\text{OH})_2$ (−0.59) may therefore have been overestimated, as would indicate a comparison with the value calculated by Öhman (−2.6) at 25 °C and with that of −2 found at 37 °C by Venturini during preliminary refinements [245]. This constant thus needs confirmation under physiological conditions. The stoichiometries and constants of the complexes used in this model for the Al–phosphate system have been questioned recently [228] (see above) and also need reinvestigation.

The latest plasma aluminium model, calculated by Harris also using ECCLES, represents the critical synthesis of all the above studies after consideration of their respective weaknesses [227]. For each Al–ligand system, complex formation constants have been directly selected among available experimental data or have been extrapolated from LFERs involving chemically similar ligands. In this way, composite sets were elaborated for hydroxide, citrate and phosphate. $\text{AlPO}_4(\text{OH})$ was predicted to be the predominant species in the ultrafiltrable fraction of aluminium, citrate and hydroxide complexes contributing to a much lesser extent (Table 3). Since then, however, it has been demonstrated that the constant for the $\text{AlPO}_4(\text{OH})$ complex derived by Harris from the LFER approach was overestimated by two orders of magnitude [229] (see above).

The influence of silicic acid on aluminium distribution in blood plasma has also been investigated. Through his careful method of considering conditional equilibria,

¹⁵ Ratio by which the l.m.m. fraction of the metal is increased in the presence of a given ligand with respect to normal blood plasma [243].

Martin reached the conclusion that the free Al^{3+} concentrations permitted by six aluminosilicates are competitive with those from citrate and Tf at $10\text{ }\mu\text{M}$ or greater total aluminium concentrations. He therefore postulated that supersaturation of solutions containing Al^{3+} bound to abnormally phosphorylated protein in the brain and bathed in excess silicic acid might induce the formation of the aluminosilicates found in the senile plaques of AD [233]. On the other hand, Vobe and Williams [240] using simulation models have concluded that l.m.m. Al–silicate interactions are negligible in blood plasma.

As Table 3 shows, much work remains to be done before general agreement can be obtained on plasma aluminium speciation.

5.3.3. Other biofluids

Aluminium speciation studies relative to other natural biofluids are scarce. Findlow et al. [216] investigated the distribution of aluminium in human and bovine milk, in which $[\text{AlCta}(\text{OH})_2]^{2-}$ was found to be predominant. Taking account of the considerations developed about this complex in the above section, these results should be taken with caution. This comment also applies to more recent versions of the same models as well as to other simulated distributions of aluminium in saliva and a pre-term infant parenteral nutritive mixture, published by the same group after its investigation of the Al–phosphate system [225] (see above).

5.4. Speciation calculations and aluminium toxicity

The detrimental influence of aluminium on normal metabolism in other biofluids has been assessed from conditional stability constant analyses. In the cerebrospinal fluid (CSF) for example, where the concentrations of citrate and Tf are low to non-existent, other small molecules such as catecholamines may become significant aluminium ligands. In a first study by Kiss et al. [246], it was estimated from pH 7.0 conditional constant comparisons that catecholamines should occur in 3000-fold excess over citrate and in 400-fold excess over ATP to compete for aluminium in this medium. More recently, however, after an experimental investigation of the complex equilibria in the Al–ATP system, it has been stated by the same authors that at comparable ligand concentrations in neutral solutions catecholamines would bind aluminium more strongly than ATP, and that the resulting inability of ATP to withdraw Al^{3+} from catecholamines in the CSF might be deleterious [246]. In the same manner, it was anticipated that if Al^{3+} can penetrate red cells, 2,3-diphosphoglycerate (its likely predominant small ligand) would bind it strongly enough to equalize aluminium concentrations on both sides of the cell membrane [247].

6. Conclusion and prospects

The objective of this work was to review in parallel (i) the biological aspects of Al–ligand interactions relative to aluminium metabolism and toxicity, and (ii) the

chemical speciation data likely to substantiate on a molecular basis the physiological processes thus characterised.

On both fronts, significant progress has been made during these last years, with decisive advances in the biological field regarding in particular: (i) the interaction of aluminium with protein of which it retards proteolysis [156]; (ii) the alteration by aluminium of the biological functions of DNA [157]; (iii) the involvement of aluminium in the lipid peroxidation process [163,164]; (iv) the exact role of citrate in aluminium absorption [82]; (v) the influence of diverse dietary compounds on aluminium absorption [108,110], tissue distribution [130], and excretion [123–125]; (vi) the selection of potential Al-sequestering agents likely to favour aluminium urinary excretion [39,174].

Some progress has also been made on the chemical side, particularly in the understanding of the relative capacities of several ligands to mobilise aluminium in vivo, based on conditional constants considerations [58,234,246]. However, except maybe certain limited applications relative to simple Al–ligand systems in the g.i. fluid [59,78,198,237], no agreement has yet been reached by solution coordination chemists on definite simulation models relative to the distribution of aluminium in the main biofluids (e.g. blood plasma) because of a lack of certainty on the complex equilibria of a few critical Al–ligand systems (e.g. Al–citrate, Al–phosphate; see Table 3). New determinations of the complex formation constants of these systems under physiological conditions will thus be necessary in the near future. In the longer run, much fundamental work will have to be done, in particular regarding CSF and intracellular neuronal fluids, not only relative to Al–ligand equilibria but firstly about the standard composition of these media with which to build new simulation models.

References

- [1] J.R.J. Sorenson, I.R. Campbell, L.B. Tepper and R.D. Lingg, *Environ. Health Perspect.*, 8 (1974) 3.
- [2] A.C. Alfrey, J.M. Mishell, J. Burks et al., *Trans. Am. Soc. Artif. Intern. Organs*, 18 (1972) 257.
- [3] M.K. Ward, T.G. Feest, H.A. Ellis, I.S. Parkinson, D.N.S. Kerr, J. Herrington and G.L. Goode, *Lancet*, 1 (1978) 841.
- [4] H.L. Elliott, A.J. Mac Dougall and G.S. Fell, *Lancet*, 1 (1978) 1203.
- [5] A.C. Alfrey, *N. Engl. J. Med.*, 310 (1984) 1113.
- [6] S.P. Andreoli, J.M. Bergstein and D.J. Sherrard, *N. Engl. J. Med.*, 310 (1984) 1079.
- [7] A.C. Alfrey, A. Hegg and P. Craswell, *Am. J. Clin. Nutr.*, 33 (1980) 1509.
- [8] A.B. Sedman, N.L. Miller, B.A. Warady, G.M. Lum and A.C. Alfrey, *Kidney Int.*, 26 (1984) 201.
- [9] J.G. Heaf and L.P. Nielsen, *Miner. Electrolyte Metab.*, 10 (1984) 345.
- [10] W.D. Kaehny, A.P. Hegg and A.C. Alfrey, *N. Engl. J. Med.*, 296 (1977) 1389.
- [11] K.A. Carmichael, M.D. Fallon, M. Dalinka, F.S. Kaplan, L. Axel and J.G. Haddad, *Am. J. Med.*, 67 (1984) 1137.
- [12] J.G. Heaf and F. Melsen, *Nephron*, 40 (1985) 246.
- [13] G.B. Van der Voet and F.A. de Wolff, *J. Toxicol. Clin. Toxicol.*, 24 (1986–87) 545.
- [14] E.-M. Haram, R. Weberg and A. Berstad, *Scand. J. Gastroenterol.*, 22 (1987) 615.
- [15] P. Allain, Y. Mauras, N. Krari, J. Duchier, A. Cournot and J. Larchevêque, *Br. J. Clin. Pharmacol.*, 29 (1990) 391.
- [16] L.W. Fleming, A. Prescott, W.K. Stewart and R.W. Cargill, *Lancet*, I (1989) 433.

- [17] A.B. Sedman, G.L. Klein, R.J. Merritt, N.L. Miller, K.O. Weber, W.L. Gill, H. Anand and A.C. Alfrey, *N. Engl. J. Med.*, 312 (1985) 1337.
- [18] B. Messing, A. Pfeiffer, J.L. Gineston, P. Chappuis, P. Leflon, A. Buisine and J.L. Terrier, *Presse Méd.*, 15 (1986) 1425.
- [19] M. McGraw, N. Bishop, R. Jameson, M.J. Robinson, M. O'Hara, C.D. Hewitt and J.P. Day, *Lancet*, 1/8473 (1986) 157.
- [20] W.W.K. Koo and L.A. Kaplan, *J. Am. Coll. Nutr.*, 7 (1988) 199.
- [21] G.S. Fell, A. Shenkin and D.J. Halls, *Lancet*, 1/8477 (1986) 380.
- [22] S. Shirodkar, R.L. Hutchinson, D.L. Perry, J.L. White and S.L. Hem, *Pharmaceut. Res.*, 7 (1990) 1282.
- [23] A. Lione, *N. Engl. J. Med.*, 314 (1986) 923.
- [24] N. Bishop, M. McGraw and N. Ward, *Lancet*, I (1989) 490.
- [25] K.R. Koch, M.A. Bruno Pougnet, S. de Villiers and F. Monteagudo, *Nature*, 33 (1988) 311.
- [26] A. Lione, P.V. Allen and J.C. Smith, *Food Chem. Toxicol.*, 22 (1984) 265.
- [27] M.H. Gault and L. Purchase, *Can. Med. Assn. J.*, 147 (1992) 845.
- [28] J.M. Duggan, J.E. Dickeson, P.F. Tynan, A. Houghton and J.E. Flynn, *Med. J. Aust.*, 156 (1992) 604.
- [29] A. Lione, *Nutr. Rev.*, 42 (1984) 31.
- [30] L. Fishbein, in G. Berthon (Ed.), *Handbook of Metal-Ligand Interactions in Biological Fluids, Bioinorganic Medicine, Vol. 1*, Marcel Dekker, New York, 1995, pp. 626–628.
- [31] D.P. Perl, *Neurobiol. Aging*, 7 (1986) 550.
- [32] L. Liss and D.J. Thornton, *Neurobiol. Aging*, 7 (1986) 552.
- [33] D.R. Crapper McLachlan, *Neurobiol. Aging*, 7 (1986) 525.
- [34] D.R. Crapper McLachlan, W.J. Lukiw and T.P.A. Kruck, *Can. J. Neurol. Sci.*, 16 (1989) 490.
- [35] R. Preston Mason and R.D. Besdine, *J. Am. Med. Assoc.*, 270 (1993) 1868.
- [36] D.G. Munoz, *Can. Med. Assoc. J.*, 151 (1994) 268; D.R.C. Crapper McLachlan, *ibid.*, 268; T.P.A. Kruck, *ibid.*, 269; W.F. Forbes and J.F. Gentleman, *ibid.*, 270.
- [37] D.R. McLachlan, W.J. Lukiw and T.P.A. Kruck, in G. Berthon (Ed.), *Handbook of Metal-Ligand Interactions in Biological Fluids, Bioinorganic Medicine, Vol. 2*, Marcel Dekker, New York, 1995, pp. 935–944.
- [38] C. Orvig, in G. Robinson (Ed.), *Coordination Chemistry of Aluminum*, VCH, New York, 1993, pp. 85–121.
- [39] R.A. Yokel, A.K. Datta and E.G. Jackson, *J. Pharmacol. Exp. Ther.*, 257 (1991) 100.
- [40] R.A. Yokel, *J. Toxicol. Environ. Hlth.*, 41 (1994) 131.
- [41] D.A. Allen, C. Orvig and R.A. Yokel, *Toxicology*, 92 (1994) 193.
- [42] R.A. Yokel, in G. Berthon (Ed.), *Handbook of Metal-Ligand Interactions in Biological Fluids, Bioinorganic Medicine, Vol. 2*, Marcel Dekker, New York, 1995, pp. 1288–1296.
- [43] A. Evers, R.D. Hancock, A.E. Martell and R.J. Motekaitis, *Inorg. Chem.*, 28 (1989) 2189.
- [44] A.E. Martell, R.J. Motekaitis and R.M. Smith, *Polyhedron*, 9 (1990) 171.
- [45] T.P.A. Kruck, W. Kalow and D.R. Crapper McLachlan, *J. Chromatogr.*, 341 (1985) 123.
- [46] J.L. Domingo, *J. Toxicol. Clin. Toxicol.*, 27 (1989) 355.
- [47] T.P.A. Kruck, E.A. Fisher and D.R.C. McLachlan, *Clin. Pharmacol. Ther.*, 48 (1990) 439.
- [48] C. Borgna-Pignatti, P.D. Stefano and A.M. Broglia, *Lancet*, 1 (1984) 681.
- [49] J.A. Walker, R.A. Sherman and R.P. Eisinger, *Am. J. Kidney Dis.*, 6 (1985) 254.
- [50] P. Ackrill, A.J. Ralston, J.P. Day and K.C. Hodge, *Lancet*, ii (1980) 692.
- [51] H. Sigel and A. Sigel (Eds.), *Metal Ions in Biological Systems*, Vol. 24, Marcel Dekker, New York, 1988.
- [52] M. Nicolini, P.F. Zatta and B. Corain (Eds.), *Aluminium in Chemistry, Biology and Medicine*, Cortina International, Verona (Raven Press, New York), 1991.
- [53] Ciba Foundation Symposium 169, *Aluminium in Biology and Medicine*, Wiley, Chichester, 1992.
- [54] R.B. Martin, in G. Berthon (Ed.), *Handbook of Metal-Ligand Interactions in Biological Fluids, Bioinorganic Chemistry, Vol. 2*, Marcel Dekker, New York, 1995, pp. 827–833.
- [55] G.L. Christie and D.R. Williams, in G. Berthon (Ed.), *Handbook of Metal-Ligand Interactions in Biological Fluids, Bioinorganic Medicine, Vol. 1*, Marcel Dekker, New York, 1995, pp. 29–37.
- [56] J. Bjorksten, L.L. Yaeger and T. Wallace, *Int. J. Vitam. Nutr. Res.*, 58 (1988) 462.

- [57] R.J.P. Williams, *Rec. Trav. Chim. Pays-Bas*, 106 (1987) 401.
- [58] R.B. Martin, *Accounts Chem. Res.*, 27 (1994) 204.
- [59] R.B. Martin, *Clin. Chem.*, 32 (1986) 1797.
- [60] G.B. Van der Voet, in R.L. Isaacson and K.F. Jensen (Eds.), *Vulnerable Brain and Environmental Risks*, Vol. 2, Plenum, 1992, pp. 35–48.
- [61] J.L. Greger and M.J. Baier, *Food Chem. Toxicol.*, 21 (1983) 473.
- [62] P. Slanina, W. Frech, L.-G. Ekström, L. Löf, S. Slorach and Å. Cedergrén, *Clin. Chem.*, 32 (1986) 539.
- [63] A.C. Alfrey, *Kidney Int.*, 29 Suppl. 18 (1986), S8.
- [64] G.A. Trapp, *Kidney Int.*, 29 Suppl. 18 (1986) S12.
- [65] J.M. Cam, V.A. Luck, J.B. Eastwood and H.E. de Wardener, *Clin. Sci. Molec. Med.*, 51 (1976) 407.
- [66] E.M. Clarkson, V.A. Luck, W.V. Hynson, R.R. Bailey, J.B. Eastwood, J.S. Woodhead, V.R. Clements, J.L.H. O'Riordan and H.E. de Wardener, *Clin. Sci.*, 43 (1972) 519.
- [67] J.E. Gorsky, A.A. Dietz, H. Spencer and D. Osis, *Clin. Chem.*, 25 (1979) 1739.
- [68] G. Berthon (Ed.), *Handbook of Metal-Ligand Interactions in Biological Fluids*, Bioinorganic Chemistry, Vol. 2, Marcel Dekker, New York, 1995, Part 5.
- [69] P.M. Bertsch and M.A. Anderson, *Anal. Chem.*, 61 (1989) 535.
- [70] A.K. Datta, P.J. Wedlund and R.A. Yokel, *J. Trace Elem. Electr. Hlth Dis.*, 4 (1990) 107.
- [71] G.B. Van der Voet, M.F. Van Ginkel and F.A. de Wolff, *Toxicol. Appl. Pharmacol.*, 99 (1989) 90.
- [72] G.B. Van der Voet, in *Ciba Foundation Symposium 169, Aluminum in Biology and Medicine*, Wiley, Chichester, 1992, pp. 109–22.
- [73] J.L. Greger, *Annu. Rev. Nutr.*, 13 (1993) 43.
- [74] J.J. Powell and R.P.H. Thompson, *Proc. Nutr. Soc.*, 52 (1993) 241.
- [75] M. Wilhelm, D.E. Jäger and F.K. Ohnesorge, *Pharmacol. Toxicol.*, 66 (1990) 4.
- [76] N.A. Partridge, F.E. Regnier, J.L. White and S.L. Hem, *Kidney Int.*, 35 (1989) 1413.
- [77] B.A. Molitoris, D.H. Froment, T.A. Mackenzie, W.H. Huffer, and A.C. Alfrey, *Kidney Int.*, 36 (1989) 949.
- [78] G. Berthon and S. Daydé, *J. Am. Coll. Nutr.*, 11 (1992) 340.
- [79] G.B. Van der Voet and F.A. de Wolff, *J. Appl. Toxicol.*, 6 (1986) 37.
- [80] R. Weberg and A. Berstad, *Eur. J. Clin. Invest.*, 16 (1986) 428.
- [81] D.H. Froment, B. Buddington, N.L. Miller and A.C. Alfrey, *J. Lab. Clin. Med.*, 114 (1989) 237.
- [82] D.P. Froment, B.A. Molitoris, B. Buddington, N. Miller and A.C. Alfrey, *Kidney Int.*, 36 (1989) 978.
- [83] N.J. Birch, in G. Berthon (Ed.), *Handbook of Metal-Ligand Interactions in Biological Fluids*, Bioinorganic Chemistry, Vol. 2, Marcel Dekker, New York, 1995, pp. 773–779.
- [84] A.J. Adler and G.M. Berlyne, *Am. J. Physiol.*, 249 (1985) G209.
- [85] M. Cochran, G. Goddard and N. Ludwigson, *Toxicol. Lett.*, 51 (1990) 287.
- [86] M. Feinroth, M.V. Feinroth and G.F. Berlyne, *Miner. Electrolyte Metab.*, 8 (1982) 29.
- [87] S.D. Provan and R.A. Yokel, *Res. Commun. Chem. Pathol. Pharmacol.*, 59 (1988) 79.
- [88] S.D. Provan and R.A. Yokel, *J. Pharmacol. Exp. Ther.*, 245 (1988) 928.
- [89] G.B. van der Voet and F.A. de Wolff, *Arch. Toxicol.*, 55 (1984) 168.
- [90] S. Costantini, R. Giordano, A. Ioppolo, A. Mantovani, P. Ballanti, P. Mocetti and E. Bonucci, *Pharmacol. Toxicol.*, 64 (1989) 47.
- [91] C.J. Lote and H. Saunders, *Clin. Sci.*, 81 (1991) 289.
- [92] R.J.P. Williams, in Ref. [72].
- [93] J.A. Edwardson, in Ref. [72].
- [94] J.B. Cannata, I. Fernández-Soto, M.J. Fernández-Menéndez, J.L. Fernández-Martín, S.J. McGregor, J.H. Brock and D. Halls, *Kidney Int.*, 39 (1991) 799.
- [95] G.B. van der Voet and F.A. de Wolff, *Toxicol. Appl. Pharmacol.*, 90 (1987) 190.
- [96] T.L. McDonald and R.B. Martin, *Trends Biochem. Sci.*, 13 (1988) 15.
- [97] R.B. Martin, in H. Sigel and A. Sigel (Eds.), *Metal Ions in Biological Systems*, Vol. 24, Marcel Dekker, New York, 1988, pp. 1–57.
- [98] U. Karbach, in G. Berthon (Ed.), *Handbook of Metal-Ligand Interactions in Biological Fluids*, Bioinorganic Medicine, Vol. 1, Marcel Dekker, New York, 1995, pp. 373–385.
- [99] K.A. Renton, K.L. Manchester and T.A. Kilroe-Smith, *J. Inorg. Biochem.*, 50 (1993) 21.

- [100] K.P. Nordal, E. Dahl, K. Sørhus, K.J. Berg, Y. Thomassen, J. Kofstad and J. Halse, *Pharmacol. Toxicol.*, 63 (1988) 351.
- [101] B.B. Kirschbaum and A.C. Schoolwerth, *Human Toxicol.*, 8 (1989) 45.
- [102] B.B. Kirschbaum and A.C. Schoolwerth, *Am. J. Med. Sci.*, 297 (1989) 9.
- [103] B. Fulton and E.H. Jeffery, *Fund. Appl. Toxicol.*, 14 (1990) 788.
- [104] C.R. Nolan, J.R. Califano and C.A. Butzin, *Kidney Int.*, 38 (1990) 937.
- [105] J.A. Walker, R.A. Sherman and R.P. Cody, *Arch. Intern. Med.*, 150 (1990) 2037.
- [106] N.A. Partridge, F.E. Regnier, W.M. Reed, J.L. White and S.L. Hem, *Clin. Sci.*, 83 (1992) 425.
- [107] J.L. Greger and C.F. Powers, *Toxicology*, 76 (1992) 119.
- [108] J.L. Domingo, M. Gómez, D.J. Sánchez, J.M. Llobet and J. Corbella, *Res. Commun. Chem. Pathol. Pharmacol.*, 79 (1993) 377.
- [109] J.L. Domingo, M. Gómez, J.L. Llobet and J.M. Corbella, *Kidney Int.*, 39 (1991) 598.
- [110] J.L. Domingo, M. Gómez, J.L. Llobet, D. del Castillo and J. Corbella, *Nephron*, 66 (1994) 108.
- [111] A.W. Nestel, A.M. Meyers, J. Paiker and H.B. Rollin, *Nephron*, 68 (1994) 197.
- [112] C.A. Ecelbarger, G.G. Mac Neil and J.L. Greger, *J. Agr. Food Chem.*, 42 (1994) 2220.
- [113] S. Daydé, M. Venturini and G. Berthon, *J. Inorg. Biochem.*, 36 (1989) 348.
- [114] J.L. Domingo, M. Gómez, J.M. Llobet and J. Corbella, *Lancet*, II (1988) 1362.
- [115] M.T. Colomina, M. Gómez, J.L. Domingo, J.M. Llobet and J. Corbella, *Res. Commun. Chem. Pathol. Pharmacol.*, 77 (1992) 95.
- [116] C.N. Martyn, C. Osmond, J.A. Edwardson, D.J.P. Barker, E.C. Harris and R.F. Lacey, *Lancet*, I (1989) 59.
- [117] J.D. Birchall and J.S. Chappell, *Lancet*, I (1989) 953.
- [118] J.D. Birchall, *Lancet*, 342 (1993) 299.
- [119] J.A. Edwardson, P.B. Moore, I.N. Ferrier, J.S. Lilley, G.W.A. Newton, J. Barker, J. Templar and J.P. Day, *Lancet*, 342 (1993) 211.
- [120] J.D. Birchall and C. Exley, in E. Merian and W. Haerdi (Eds.), *Metal Compounds in Environment and Life, Science and Technology Letters*, Northwood, 1992, p. 411.
- [121] J.L. Domingo, M. Gómez and C. Richart, *Lancet*, 338 (1991) 1467.
- [122] C.A. Ecelbarger and J.L. Greger, *J. Nutr.*, 121 (1991) 1755.
- [123] C.J. Lote, H.C. Saunders, J.A. Wood and A. Spencer, *Clin. Sci.*, 83 (1992) 431.
- [124] M.F. Van Ginkel, G.B. Van der Voet, P.C. d'Haese, M.A. de Broe and F.A. de Wolff, *J. Lab. Clin. Med.*, 121 (1993) 453.
- [125] T. Maitani, H. Kubota, N. Hori, K. Yoshihira and M. Takeda, *J. Appl. Toxicol.*, 14 (1994) 257.
- [126] M. Cochran, V. Chawtur, J.W. Phillips and B. Dilena, *Clin. Sci.*, 86 (1994) 223.
- [127] N.W. Boyce, S.R. Holdsworth, N.M. Thomson and R.C. Atkins, *Nephron*, 45 (1987) 164.
- [128] P. Slanina, W. Frech, A. Bernhardson, A. Cedergren and P. Mattsson, *Acta Pharmacol. Toxicol.*, 56 (1985) 331.
- [129] M. Kaye, *Clin. Nephrol.*, 20 (1983) 208.
- [130] R.A. Yokel, V. Lidums, P.J. McNamara and U. Ungerstedt, *Toxicol. Appl. Pharmacol.*, 107 (1991) 153.
- [131] R. Deloncle, O. Guillard, F. Clanet, P. Courtois and A. Piriou, *Biol. Tr. Elem. Res.*, 25 (1990) 39.
- [132] S.J. McGregor, J.H. Brock and D. Halls, *Biol. Met.*, 4 (1991) 173.
- [133] C. Exley and J.D. Birchall, *J. Theor. Biol.*, 159 (1992) 83.
- [134] W.A. Banks and A.J. Kastin, *Neurosci. Biobehav. Rev.*, 13 (1989) 47.
- [135] A.J. Roskams and J.R. Connor, *Proc. Natl. Acad. Sci. USA*, 87 (1990) 9024.
- [136] J. Fleming and J.G. Joshi, *Proc. Natl. Acad. Sci. USA*, 84 (1987) 7866.
- [137] J.T. Fleming and J.G. Joshi, *Neurobiol. Aging*, 12 (1991) 413.
- [138] J.M.C. Gutteridge, G.J. Quinlan, I. Clark and B. Halliwell, *Biochim. Biophys. Acta*, 835 (1985) 441.
- [139] S.L. Mera, *Med. Lab. Sci.*, 48 (1991) 283.
- [140] M.R. Rowan, *Proc. Nutr. Soc.*, 52 (1993) 255.
- [141] W.E. van Nostrand, S.L. Wagner, W.R. Shankle, J.S. Farrow, M. Dick, J.M. Rozemuller, M.A. Kuiper, E.C. Walters, J. Zimmerman and C.W. Cotman, *Proc. Natl. Acad. Sci. USA*, 89 (1992) 2551.
- [142] G.J. Pike, A.J. Walencewicz, C.G. Glabe and C.W. Cotman, *Brain Res.*, 563 (1991) 311.

- [143] J.M. Candy, A.E. Oakley, J. Klinowsky, T.A. Carpenter, R.H. Perry, J.R. Atack, E.K. Perry, G. Blessed, A. Fairbairn and J.A. Edwardson, *Lancet*, i (1986) 354.
- [144] M. Kawahara, K. Muramoto, K. Kobayashi and Y. Kuroda, *Biochem. Biophys. Res. Commun.*, 189 (1992) 1317.
- [145] D.P. Perl and A.R. Brody, *Science*, 208 (1980) 297.
- [146] M. Kawahara, K. Muramoto, K. Kobayashi, H. Mori and Y. Kuroda, *Biochem. Biophys. Res. Commun.*, 198 (1994) 531.
- [147] C. Exley, N.C. Price, S.M. Kelly and J.D. Birchall, *FEBS Lett.*, 324 (1993) 293.
- [148] G.D. Fasman and C.D. Moore, *Proc. Natl. Acad. Sci. USA*, 91 (1994) 11232.
- [149] T.P. Flaten and R.M. Garruto, *J. Theor. Biol.*, 156 (1992) 129.
- [150] T. Dyrks, E. Dyrks, T. Hartmann, C. Masters and K. Bayreuther, *J. Biol. Chem.*, 267 (1992) 18210.
- [151] T. Dyrks, E. Dyrks, C.L. Masters and K. Bayreuther, *FEBS Lett.*, 324 (1993) 231.
- [152] P.F. Good, D.P. Perl, L.M. Bierer and J. Schmeidler, *Ann. Neurol.*, 31 (1992) 286.
- [153] J.F. Leterrier, D. Langui, A. Probst and J. Ulrich, *J. Neurochem.*, 58 (1992) 2060.
- [154] D. Langui, A. Probst, B. Anderton, J.-P. Brion and J. Ulrich, *Acta Neuropathol.*, 80 (1990) 649.
- [155] C.R. Harrington, C.M. Wischik, F.K. McArthur, G.A. Taylor, J.A. Edwardson and J.M. Candy, *Lancet*, 343 (1994) 993.
- [156] R.-W. Shin, V.M.-Y. Lee and J.Q. Trojanowski, *J. Neurosci.*, 14 (1994) 7221.
- [157] K.S. Jagannatha Rao, B.S. Rao, D. Vishnuvardhan and K.V.S. Prasad, *Biochim. Biophys. Acta*, 1172 (1993) 17.
- [158] W.J. Lukiw, T.P.A. Kruck and D.R. Crapper, *Neurotoxicology*, 8 (1987) 291.
- [159] W.J. Lukiw, B. Krishnan, L. Wong, T.P.A. Kruck, C. Bergeron and D.R. Crapper McLachlan, *Neurobiol. Aging*, 13 (1991) 115.
- [160] S. Takenaka, T. Ihara and M. Takagi, *J. Mol. Recogn.*, 3 (1990) 156.
- [161] S.-W. Cho and G.-Y. Kim, *Eur. J. Biochem.*, 202 (1991) 107.
- [162] P.I. Oteiza, C.G. Fraga and C.L. Keen, *Arch. Biochem. Biophys.*, 300 (1993) 517.
- [163] P.I. Oteiza, *Arch. Biochem. Biophys.*, 308 (1994) 374.
- [164] T. Ohyashiki, T. Karino and K. Matsui, *Biochim. Biophys. Acta*, 1170 (1993) 182.
- [165] H.H. Malluche, A.J. Smith, K. Abreo and M.C. Faugere, *N. Engl. J. Med.*, 311 (1984) 140.
- [166] H.-K. Stummvoll, H. Graf and V. Meisinger, *Miner. Electrolyte Metab.*, 10 (1984) 263.
- [167] F.Y. Leung, A.B. Hodsman, N. Muirhead and A.R. Henderson, *Clin. Chem.*, 31 (1985), 20.
- [168] S.P. Andreoli, D. Dunn, W. DeMyer, D.J. Sherrard and J.M. Bergstein, *J. Pediatr.*, 107 (1985) 760.
- [169] C. Tielemans, F. Collart, R. Wens, J. Smeyers-Verbeeke, I. Van Hooff, M. Dratwa and D. Verbeelen, *Clin. Nephrol.*, 24 (1985) 237.
- [170] J.L. Domingo, J.M. Llobet, M. Gómez and J. Corbella, *Res. Commun. Chem. Pathol. Pharmacol.*, 53 (1986) 93.
- [171] J.M. Llobet, J.L. Domingo, M. Gómez, J.M. Tomás and J. Corbella, *Pharmacol. Toxicol.*, 60 (1987) 280.
- [172] J.L. Domingo, M. Gómez, J.M. Llobet and J. Corbella, *Human Toxicol.*, 7 (1988) 259.
- [173] J.L. Domingo, M. Gómez and J.M. Llobet, *J. Toxicol. Clin. Toxicol.*, 26 (1988) 67.
- [174] M. Gómez, J.L. Domingo, D. del Castillo, J.M. Llobet and J. Corbella, *Hum. Exp. Toxicol.*, 13 (1994) 135.
- [175] P.E. Gardiner, M. Stoeppler and H.W. Nürnberg, in P. Brätter and P. Schramel (Eds.), *Trace Element — Analytical Chemistry in Medicine and Biology*, Vol. 3, W. de Gruyter, Berlin, New York, 1984, pp. 299–310.
- [176] P.A. Arp and W.L. Meyer, *Can. J. Chem.*, 63 (1985) 3357.
- [177] M.F. van Ginkel, G.B. van der Voet, H.G. van Eijk and F.A. de Wolff, *J. Clin. Chem. Clin. Biochem.*, 28 (1990) 459.
- [178] A.P. Lundin, C. Caruso, M. Sass and G.M. Berlyne, *Clin. Res.*, 26 (1978) 636A.
- [179] S.W. King, M.R. Wills and J. Savory, *Res. Commun. Chem. Pathol. Pharmacol.*, 26 (1979) 161.
- [180] G.A. Trapp, *Life Sci.*, 33 (1983) 311.
- [181] M. Cochran, D. Patterson, J.H. Coates and P.T.H. Coates, in P. Brätter and P. Schramel (Eds.), *Trace Element — Analytical Chemistry in Medicine and Biology*, Vol. 6, W. de Gruyter, Berlin, New York, 1984, pp. 311–322.

- [182] M. Cochran, J. Coates and S. Neoh, *FEBS Lett.*, 176 (1984) 129.
- [183] H. Rahman, A.W. Skillen, S.M. Channon, M.K. Ward and D.N.S. Kerr, *Clin. Chem.*, 31 (1985) 1969.
- [184] S.J.A. Fatemi, F.H.A. Kadir and G.R. Moore, *Biochem. J.*, 280 (1991) 527.
- [185] W.R. Harris and J. Sheldon, *Inorg. Chem.*, 29 (1990) 119.
- [186] M. Favarato, C.A. Mizzen and D.R. Crapper McLachlan, *J. Chromatogr.*, 576 (1992) 271.
- [187] M. Favarato, C.A. Mizzen, M.K. Sutherland, B. Krishnan, T.P.A. Kruck and D.R. Crapper McLachlan, *Clin. Chim. Acta*, 207 (1992) 41.
- [188] P.M. May and R.A. Bulman, in G.P. Ellis and G.B. West (Eds.), *Progress in Medicinal Chemistry*, Vol. 20, Elsevier Science, New York, 1983, pp. 224–336.
- [189] H.M. Marques, *J. Inorg. Biochem.*, 41 (1991) 187.
- [190] J.D. Bell, G. Kubal, S. Radulovic, P.J. Sadler and A. Tucker, *Analyst*, 118 (1993) 241.
- [191] J. Schubert, *Annu. Rev. Nucl. Sci.*, 5 (1955) 369.
- [192] A. Ringbom, *Complexation in Analytical Chemistry*, Wiley, New York, 1963.
- [193] P.M. May, in G. Berthon (Ed.), *Handbook of Metal–Ligand Interactions in Biological Fluids*, *Bioinorganic Chemistry*, Vol. 2, Marcel Dekker, New York, 1995, pp. 1184–1194.
- [194] C. Blaquière and G. Berthon, *Inorg. Chim. Acta*, 135 (1987) 179.
- [195] G. Berthon, B. Hacht, M.-J. Blais and P.M. May, *Inorg. Chim. Acta*, 125 (1986) 219.
- [196] P.M. May, P.W. Linder and D.R. Williams, *J. Chem. Soc. Dalton Trans.* (1977) 588.
- [197] P.M. May, P.W. Linder and D.R. Williams, *Experientia*, 32 (1976) 1492.
- [198] M. Venturini and G. Berthon, *J. Inorg. Biochem.*, 37 (1989) 69.
- [199] C.F. Baes, Jr. and R.E. Mesmer, *The Hydrolysis of Cations*, R.E. Krieger, Malabar, FL, 1986.
- [200] R.B. Martin, *J. Inorg. Biochem.*, 44 (1991) 141.
- [201] P. Charlet, J.P. Deloume, G. Duc and G. Thomas-David, *Bull. Soc. Chim. Fr.* (1984) I-222.
- [202] P.L. Hayden and A.J. Rubin, *Sep. Sci. Tech.*, 21 (1986) 1009.
- [203] M. Venturini and G. Berthon, *J. Chem. Soc. Dalton Trans.* (1987) 1145.
- [204] A.E. Martell and R.M. Smith, *Critical Stability Constants*, Plenum, New York, Vols. 1–6, 1974–1989.
- [205] C. Orvig and G. Berthon, in G. Berthon (Ed.), *Handbook of Metal–Ligand Interactions in Biological Fluids*, *Bioinorganic Chemistry*, Vol. 2, Marcel Dekker, New York, 1995, 1266–1280.
- [206] L.D. Pettit and H.K.J. Powell, *IUPAC Stability Constants Database*, Academic Software, Otley, UK.
- [207] NIST Standard Reference Database 46, Gaithersburg, MD.
- [208] P.M. May and K. Murray, *Talanta*, 38 (1991) 1419.
- [209] G.E. Jackson, *Polyhedron*, 9 (1990) 163.
- [210] L.O. Öhman and S. Sjöberg, *J. Chem. Soc. Dalton Trans.* (1983) 2513.
- [211] R.J. Motekaitis and A.E. Martell, *Inorg. Chem.*, 23 (1984) 18.
- [212] G.E. Jackson, *S. Afr. J. Chem.*, 35 (1982) 89.
- [213] J.E. Gregor and H.K.J. Powell, *Aust. J. Chem.*, 39 (1986) 1851.
- [214] K.S. Rajan, S. Mainer, N.L. Rajan and J.M. Davis, *J. Inorg. Biochem.*, 14 (1981) 339.
- [215] L.O. Öhman, *Inorg. Chem.*, 27 (1988) 2565.
- [216] J.A. Findlow, J.R. Duffield and D.R. Williams, *Chem. Spec. Bioavail.*, 2 (1990) 3.
- [217] L.O. Öhman and W. Forsling, *Acta Chem. Scand., Ser. A*, 35 (1981) 795.
- [218] J.E. Salmon and J.G.L. Wall, *J. Chem. Soc.* (1958) 1128.
- [219] N. Bjerrum and C.R. Dahm, *Z. Phys. Chem. Bodenstein Festband* (1931) 627.
- [220] J.W. Akitt, N.N. Greenwood and G.D. Lester, *J. Chem. Soc.* (1971) 2450.
- [221] M.A. Wilson, P.J. Collin and J.W. Akitt, *Anal. Chem.*, 61 (1989) 1253.
- [222] G.E. Jackson and V.V.K. Qubeka, *Proc. XXV ICCS*, Nanjing, China, July 1987, p. 685.
- [223] G.E. Jackson and K.V.V. Vayi, *S. Afr. J. Chem.*, 41 (1988) 17.
- [224] S. Daydé, M. Filella and G. Berthon, *J. Inorg. Biochem.*, 38 (1990) 241.
- [225] J.R. Duffield, K. Edwards, D.A. Evans, D.M. Morrish, R.A. Vobe and D.R. Williams, *J. Coord. Chem.*, 23 (1991) 277.
- [226] S. Daydé, PhD Thesis, Université de Toulouse, 1990.
- [227] W.R. Harris, *Clin. Chem.*, 38 (1992) 1809.
- [228] N. Alliey and G. Berthon, unpublished results.

- [229] L.O. Öhman and R.B. Martin, *Clin. Chem.*, 40 (1994) 598.
- [230] R.L. Bertholf, M.R. Wills and J. Savory, *Biochem. Biophys. Res. Commun.*, 125 (1984) 1020.
- [231] R.B. Martin, J. Savory, S. Brown, R.L. Bertholf and M.R. Wills, *Clin. Chem.*, 33 (1987) 405.
- [232] R.B. Martin, *J. Inorg. Biochem.*, 28 (1986) 181.
- [233] R.B. Martin, *Polyhedron*, 9 (1990) 193.
- [234] R.B. Martin, in M. Nicolini, P.F. Zatta and B. Corain (Eds.), *Aluminium in Chemistry, Biology and Medicine*, Raven Press, New York, 1991, pp. 3–20.
- [235] R.L. Bertholf, M.R. Wills and J. Savory, in H.G. Seiler, H. Sigel and A. Sigel (Eds.), *Handbook of Toxicity of Inorganic Compounds*, Marcel Dekker, New York, 1988, pp. 55–64.
- [236] J. Ribereau-Gayon, E. Peynaud, P. Ribereau-Gayon and R. Sudraud, *Sciences et Techniques du Vin*, Dunod, Paris, 1977.
- [237] S. Daydé and G. Berthon, in E. Merian and W. Haerdi (Eds.), *Metal Compounds in Environment and Life, Science and Technology Letters*, Northwood, UK, 1992, pp. 401–409.
- [238] M. Venturini-Soriano, N. Alliey and G. Berthon, unpublished results.
- [239] G.E. Jackson and C.B. Steyn, *S. Afr. Tydskr. Chem.*, 44 (1991) 110.
- [240] R.A. Vobe and D.R. Williams, *Chem. Spec. Bioavail.*, 4 (1992) 85.
- [241] D.J. Clevette and C. Orvig, *Polyhedron*, 9 (1990) 151.
- [242] C.J. Lote, J.A. Wood and H.C. Saunders, *Clin. Sci.*, 82 (1992) 13.
- [243] P.M. May and D.R. Williams, *FEBS Lett.*, 78 (1977) 134.
- [244] D.J. Clevette, W.O. Nelson, A. Nordin, C. Orvig and S. Sjöberg, *Inorg. Chem.*, 28 (1989) 2079.
- [245] M. Venturini, Thèse de 3^{ème} Cycle, Université de Toulouse, 1987.
- [246] T. Kiss, I. Sóvágó and R.B. Martin, *Inorg. Chem.*, 30 (1991) 2130.
- [247] I. Sóvágó, T. Kiss and R.B. Martin, *Polyhedron*, 9 (1990) 189.