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The solution state of aluminium(III) as relevant to experimental toxicology: recent data and new perspectives

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CONTENTS

A. INTRODUCTION

Our current knowledge of aluminium(III) toxicity* [1, 2] stems from two major experimental areas: human epidemiology and in vivo and in vitro experimental toxicology. We have learned from human epidemiology that pathological conditions such as dialysis dementia and osteomalacia [2], and non-iron deficiency microcytosis [3] are related to abnormal aluminium uptake. Remarkably, analytical and histopath-

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*The first report of the experimentally determined neurotoxicity of Al(III) was from Siem (1886), quoted by Doelken [2(a)].

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ological work [4-6] has established that Alzheimer's disease (AD) might also be linked to an aluminium dismetabolism and accumulation [7].

This framework, in which a phenomenological connection between aluminium accumulation and human pathologies is apparently established, has stimulated an impressive amount of toxicological work, mainly centered on the use as experimental animals of rabbits, which turned out to be particularly sensitive to aluminium intoxication [2].

However, the expected ability of aluminium solutions to induce in vivo and in vitro pathologies reminiscent of AD led to great expectations and to significant disappointments. In fact, systemic and intraperitoneal administration of aqueous aluminium succeeded in reproducibly inducing in the brain of rabbits a peculiar neurofibrillary degeneration (ND), consisting of the proliferation of disorganized tangles of neurofilaments in neurons of the spinal cord and hippocampus. Although the morphological and histochemical features of ND seemed originally [8] to lend credit to an implication of aluminium in the etiology of AD, the neurofilaments induced by aluminium intoxication were found to be ultrastructurally different from the helix-shaped, regular and paired neurofilaments observed in AD-affected neuronal areas; just this ultrastructural difference is a major argument against the relevance of an aluminium dismetabolism to AD [2]. Moreover, senile plaques, which represent the most important hallmark of AD [9], have never been observed in aluminium-based toxicological experiments on animals. In this connection, however, it is worth mentioning that senile plaques have recently been observed in the cerebral cortex of patients with chronic renal failure [10], i.e. in subjects known to undergo an exceptionally high aluminium burden [11].

On the basis of the above, it can be stated that, apart from analytical evidence, there is no *direct* experimental proof which supports the implication of aluminium as an etiological factor in AD. Moreover, it can be stated that no aluminium-related biological model of AD appears to have been discovered so far either in vivo or in vitro. However, the present dearth of knowledge on the biological bases of aluminium toxicity on the one hand continues to stimulate extensive experimental toxicological work and on the other motivates the necessity of properly defining the solution state of the metal centre in aqueous solutions at neutral pH values in the presence of biologically relevant ligands.

B. THE SOLUTION STATE OF ALUMINIUM(III) IN AQUEOUS MEDIA AND PROBLEMS RELATED TO EXPERIMENTAL TOXICOLOGY

Aluminium(III) in aqueous solutions is distributed among the species reported in Fig. 1; at physiological pH values, its total analytical concentration (in equilibrium with $Al(OH)_3$) is ca. 10^{-6} M.

In biological fluids, Al(III) is expected to be distributed among a variety of species (coordination compounds), some of which have been identified in the case of

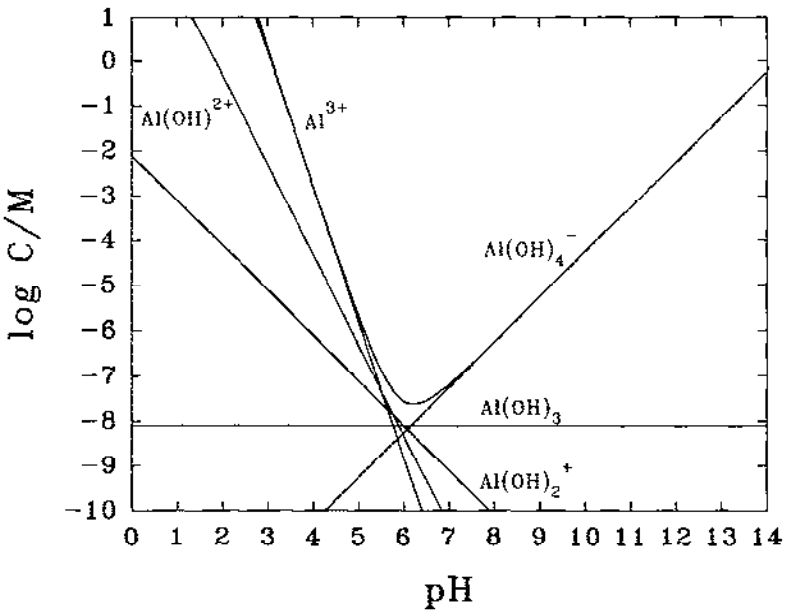

Fig. 1. Calculated solubility and speciation diagram for Al(III) in water in the absence of ligands different from H_2O and OH^-. In the formulae, metal-coordinated water molecules are omitted. (Data from ref. 12.)

human plasma [13], i.e. (i) aquo-, hydroxo- or aquo-hydroxo- species (Fig. 1), (ii) complexes with carboxylates (mainly citrate), (iii) complexes with metallo-proteins (mainly transferrin), and (iv) complexes with other plasmatic proteins (e.g. albumin). It should be stressed that the speciation of aluminium (i.e. the distribution of the metal among different molecular species) in human plasma is far from being entirely known to date and, in fact, a new proteic carrier has recently been identified, at least at a preliminary level [14]. In principle, the coordination sphere of aluminium is as important as its haematic level in determining the exposure risk, so that on-going attempts aimed at identifying as yet unknown potential biological carriers of aluminium might prove to be essential in the development of human aluminium toxicology (and therapy).

It is becoming clear that reliable results in experimental aluminium toxicology can be obtained only if the administered metal toxin (i) has been precisely chemically identified and (ii) is able to survive in biological fluids (first of all in plasma) or in culture media in order to reach its biological target.

Inspection of the literature before 1986 indicates that such requisites do not appear to have been considered in the extensive scientific production related to aluminium biology prior to that time (see later).

C. THE SYSTEM Al(III)/H_2O/OH^-/LIGAND AT pH 7.5: DEPENDENCE OF SPECIATION ON THE STABILITY CONSTANTS AND ON THE ANALYTICAL CONCENTRATION OF THE METAL COMPLEX

An obvious way of increasing the (analytical) concentration of aluminium in aqueous neutral solutions is the use of ligands able to give rise to relatively stable and hydrophilic complexes, e.g.

$$Al(OH)_3 + 6L^- = AlL_6^{3-} + 3OH^- \quad (1)$$

$$x\,Al(OH)_3 + 3(L\text{–}L')^- = Al(L\text{–}L')_3 + 3OH^- \quad (2)$$

The choice of monoanionic bidentate ligands, as in eqn. (2), leads to neutral complexes, which might be particularly suitable for biological and toxicological experimentation [15, 16]. The necessity of utilizing tailored complexes in experimental toxicology was first asserted in the literature in 1986 [17–19] and the consequent toxicological results indicate that this methodological breakthrough is disclosing unprecedented biological effects of aluminium.

The utilization of structurally characterized and hydrolytically stable $Al(L\text{–}L')_3$ species makes it possible to establish precise dose–response and structure–response relationships in aluminium toxicology. Moreover, the proper tailoring of the structure of the metal coordination sphere discloses the possibility of testing the role of lipophilicity–hydrophilicity of the metal toxin at constant hydrolytic stability and overall structure.

A good example of this possibility is given by $Al(acac)_3$ (acac = acetylacetonate) and $Al(malt)_3$ (malt = maltolate) (Fig. 2), which possess an identical overall structure, very similar hydrolytic stability and different lipophilicity–hydrophilicity, as evidenced from their *n*-octanol/H_2O partition coefficients (D).

In order to ensure that the chemical identity of an $Al(L\text{–}L')_3$ complex present

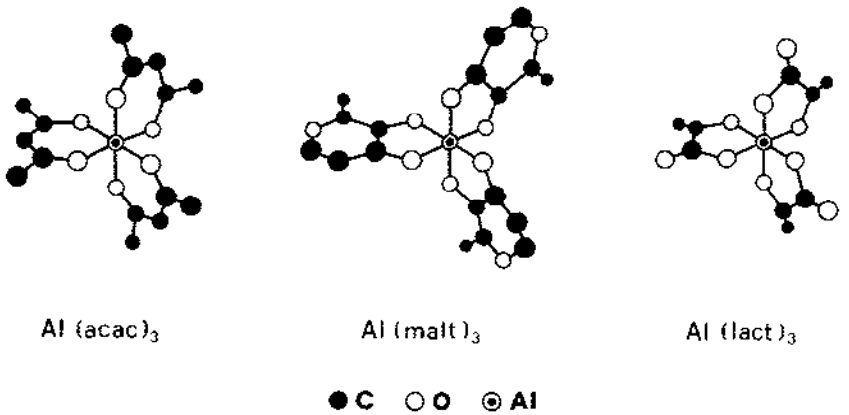

Fig. 2. Molecular structures of Al(III) synthetic toxins. Hydrogen atoms are omitted. For $Al(acac)_3$, $K_s = 10^{22.3}$ [20] and $D = 2.4$ [16]. For $Al(malt)_3$, $K_s = 10^{22.5}$ [12(b)] and $D = 7.8 \times 10^{-2}$ [16].

in solutions to be injected into experimental animals or to be administered to cell cultures be preserved, both the hydrolytic stability of the metal complex and the total analytical concentration of aluminium should be considered. The effect of these two parameters on the existence of the $Al(L-L')_3$ species as a function of pH is depicted in Figs. 3–6 (data worked out from ref. 12).

The information on the solubility of aluminium as a function of pH and on the metal speciation given by Figs. 3 and 4 is based on thermodynamic data, and hence on the assumption of a rapid attainment of the equilibria involved in defining the overall speciation pattern. Note, however, that this assumption is unrealistic in the pH range from ca. 5 to ca. 8 (see later): as a consequence, a given species, e.g. $Al(L-L')_3$, can possibly exist in this pH range, even in contrast with the predictions based on thermodynamic grounds.

Inspection of Fig. 3 shows that the species $Al(lact)_3$ is not expected to exist in water in the neutrality range under equilibrium conditions. On the contrary, the corresponding diagram referring to $Al(malt)_3$ (Fig. 4) indicates that aluminium exists just in this molecular form from pH 6 to 9. However, the stability range for $Al(malt)_3$ undergoes a marked reduction with the decrease of the analytical concentration of aluminium (Fig. 6). Thus, in, for example, a 10^{-4} M $Al(malt)_3$ solution, not only is the window reduced to ca. 1.5 pH units, but the actual concentration of $Al(malt)_3$ is, in fact, only 0.5×10^{-4} M. Finally, in a 10^{-5} M solution, $Al(malt)_3$ is expected to

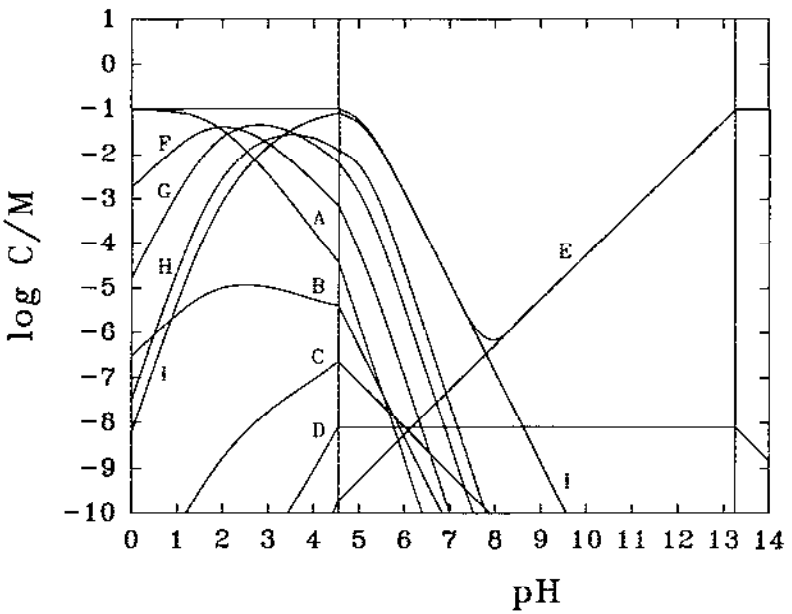

Fig. 3. Speciation of Al(III) as a function of pH in a solution containing $Al(lact)_3$, 0.1 M (analytical concentration); log $K_s = 10^{5.8}$ M, $T = 25°C$. A, Al^{3+}; B, $Al(OH)^{2+}$; C, $Al(OH)_2^+$; D, $Al(OH)_3$; E, $Al(OH)_4^-$; F, $Al(lact)^{2+}$; G, $Al(lact)_2^+$; H, $Al(lact)_3$; I, $Al(lact)_2(OH)$.

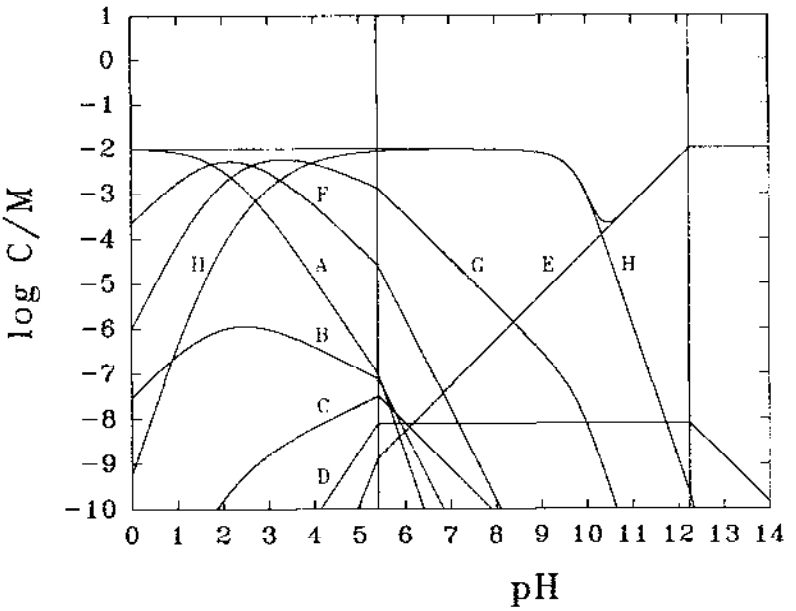

Fig. 4. Speciation of Al(III) as a function of pH in a solution containing Al(malt)$_3$, 0.1 M (analytical concentration); log $K_s = 10^{22.5}$, $T = 25$°C. A, Al^{3+}; B, $Al(OH)^{2+}$; C, $Al(OH)_2^+$; D, $Al(OH)_3$; E, $Al(OH)_4^-$; F, $Al(malt)^{2+}$; G, $Al(malt)_2^+$; H, $Al(malt)_3$.

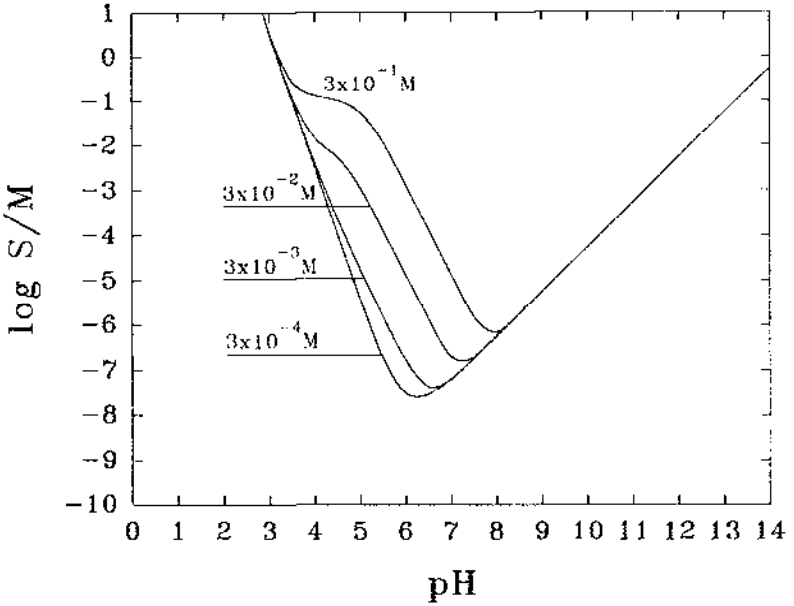

Fig. 5. Solubility of Al(III) in the presence of lactate as a function of ligand analytical concentration and of pH; $T = 25$ °C.

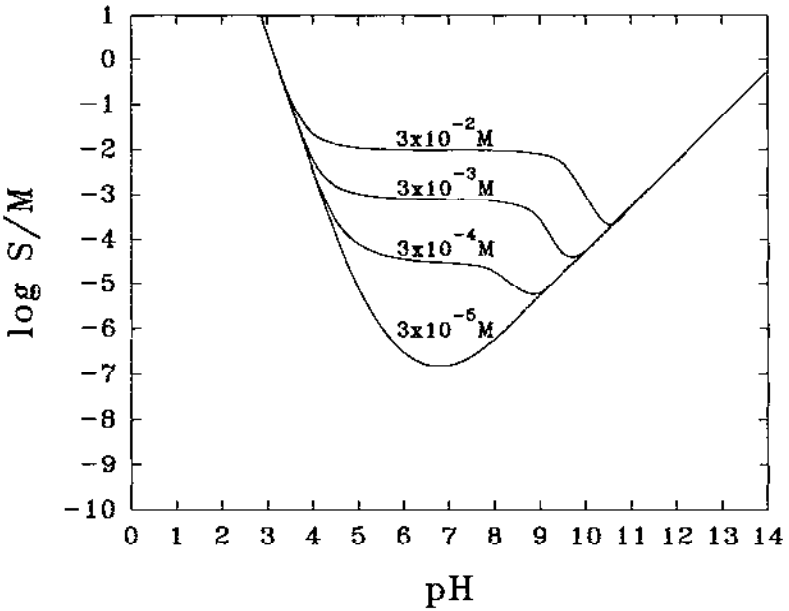

Fig. 6. Solubility of Al(III) in the presence of maltolate as a function of ligand analytical concentration and of pH; $T = 25°C$.

be completely dissociated. On the other hand, as observed above, the intrinsic inertness of aluminium complexes around pH 7 (see next section) makes not unexpected the occurrence of metastable solutions of, for example, $Al(acac)_3$ or $Al(malt)_3$ at analytical concentrations at which they are predicted to be either partially or totally hydrolysed. Specific work in this connection will be quite useful to experimental toxicologists.

D. THE CHEMICAL INERTNESS OF ALUMINIUM(III) COMPLEXES AT PHYSIOLOGICAL pH VALUES: SPECIFIC IMPLICATIONS IN EXPERIMENTAL TOXICOLOGY

The kinetic behaviour of octahedral aluminium complexes has been studied under a variety of conditions [21]. Most of the investigated complexes were β-carbonylenolate neutral species isotopically exchanging their ligands and we are not aware of kinetic studies carried out in water in the neutrality range. Moreover, complex formation reactions at pH ca. 7 are intrinsically difficult to study owing to the low solubility of Al(III).

As a matter of fact, it is a common experience [22], that any equilibration reaction involving aluminium species at pH 7.5 requires long, sometimes undefined times. On the other hand, in view of the expected dissociative character of any ligand substitution reaction, the rate of reactions occurring inside the coordination sphere of aluminium must be expected to be limited by the rate of ligand dissociation.

This kinetic feature of aluminium complexes in water in the neutrality range implies fundamental consequences:

(i) its inertness under physiological pH conditions explains the known irrelevance (see, for example, ref. 23) of the metal centre in biologically "useful" processes;

(ii) AlL_6 and $Al(L-L')_3$ complexes utilized in toxicology may possibly be present in the administered solutions even at analytical concentrations at which thermodynamics predict their decomposition to give eventually solid $Al(OH)_3$;

(iii) the rate of uptake of environmental aluminium by biological systems is expected to be intrinsically "slow" (unless occasional favourable acidic conditions are operative);

(iv) the rates of aluminium transfer from biological carrier to receptor and from receptor to carrier are expected to be generally "slow"; and

(v) if a given aluminium complex is to be prepared in situ upon mixing a solution of an aluminium species with that of the proper ligand, this operation should be made under acidic conditions, and the pH should be adjusted to the physiological value only at the presumed end of the reaction (this implies that the desired complex must be stable in acid medium).

E. THE SYSTEM Al^{3+}–H_2O–OH^-–LACTATE. A CASE HISTORY OF A COMMONLY EMPLOYED METAL TOXIN

In aluminium experimental toxicology, two basic approaches can be noticed in the "chemical" administration strategy, i.e. (i) use of an "inorganic" compound of aluminium (e.g. chloride) to be dissolved in water, followed (or not) by pH adjustment to pH 7.5 (see, for example, ref. 24) and (ii) use of carboxylates such as aluminium lactate (see, for example, ref. 25), tartrate [26], citrate [27] and gluconate [28].

In case (i), it is obvious that the administered toxin is $Al(OH)_3$, but in case (ii), the nature of the administered species is a very open question. On the basis of thermodynamic predictions, in all these cases aluminium solutions are expected to be unstable towards $Al(OH)_3$ at pH 7.5 (see Fig. 3), while experience tells us that solutions of $Al(lact)_3$ down to 5 mM appear, in fact, metastable in neutral solutions.

In order to shed light on this fundamental aspect of aluminium toxicology, a thorough investigation of the solution state of $Al(lact)_3$ has been carried out in these laboratories [29, 30], based on the X-ray single crystal analysis of the compound and on IR and ^1H and ^{13}C NMR spectroscopic studies of its solutions in D_2O.

The molecular structure of $Al(lact)_3$ is depicted in Fig. 7.

The carboxylate is, in fact, a molecular complex, the solid state lattice of which is defined by monomeric $Al(lact)_3$ units. The ligand chelates the metal centre by employing the carboxylate and the α-hydroxo oxygen atoms, with remarkably short Al–O bonds [29]. The IR spectrum of the complex displays the ν_{CO} bands at 1613 and 1403 cm^{-1} ($\Delta\bar{\nu} = 210\ cm^{-1}$), in good agreement with the IR criterion on possible metal–carboxylate bonding modes proposed by Deacon and Phillips [31]. On the

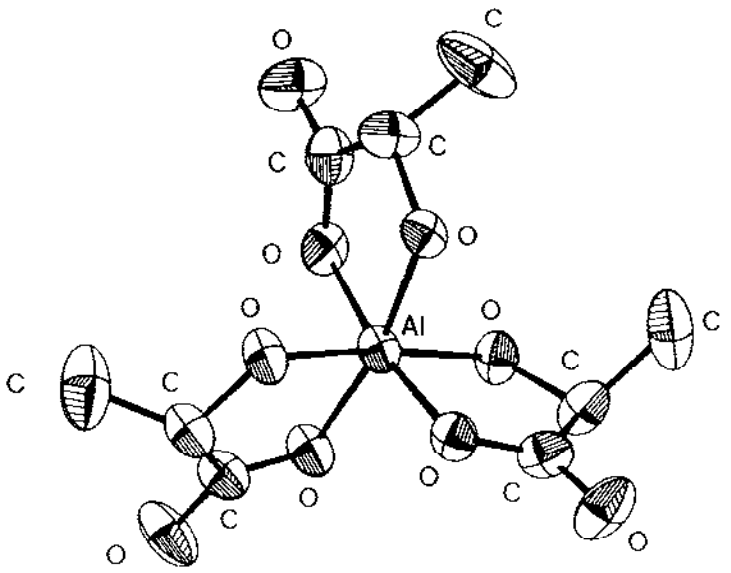

Fig. 7. Molecular structure of Al(lact)$_3$ in the solid state. (Reproduced from ref. 28 with permission.)

basis of this analytical tool, and of ^{1}H and ^{13}C NMR measurements, it turned out to be possible to establish precise features of the solution state of *dissolved* Al(lact)$_3$. Thus, at autogenous pH value (ca. 3.5), the spectral data suggest the presence of a mixture of Al(lact)$_2$(OH)(OH$_2$), Al(lact)$_2$(OH$_2$)$_2^+$ with still some Al(lact)$_3$.

Under these conditions, free and aluminium-bound lactate are seen to exchange at room temperature as shown by ^{1}H NMR at 90 MHz. At 400 MHz at 4°C, the spectra reveal the presence of free lactate and of (at least) two carboxylate complexes with methyl resonances at 4.53 and 4.46 ppm vs. $(CH_3)_3$–Si–$(CH_2)_2$–COO^-. In neutralized solutions, most of the lactate ligand appears to be released from the metal coordination sphere and the solution state is dominated by *metastable* hydroxo-aquo complexes, one of which might be Al(OH)$_3$(OH$_2$)$_3$ [30]. In any case, the toxicologically relevant conclusion is that *most of the lactate is not present inside the aluminium coordination sphere in neutral solutions* and that the metal is present as metastable hydrophilic species, which are the real toxins administered to experimental animals or to cell cultures. It has to be observed that our conclusions are not in agreement with those reached previously [32] on the basis of ^{27}Al NMR. In fact, this analytical tool, although interesting in principle, has to be considered with some suspicion if used alone, i.e. not supported by other parallel tools. The (often) very broad shape of the aluminium resonance bands and their intricate dependence on coordination changes inside the metal sphere make this spectrometry of somewhat limited interest in water solution state investigations.

Preliminary data obtained on the solution state of aluminium tartrate, citrate

and gluconate reveal that analogous situations occur with tartrate and gluconate at pH 7.5, while metal-coordinated citrate appears to be *still significantly* present in solution upon starting from aluminium citrate, after neutralization.

F. $Al(lact)_3$, $Al(acac)_3$ AND $Al(malt)_3$: SPECIATION AND DIFFERENTIATED BIOLOGICAL EFFECTS

On the basis of the rationale illustrated in the previous paragraphs, an articulated in vivo and in vitro toxicological programme has been developed in these laboratories.

Different aluminium species have been (and are being) tested towards:

(i) animals (rabbit and rat);

(ii) cell cultures (bacteria, human cells, murine neuroblastomas, erythrocytes); and

(iii) enzymes (trypsin).

In view of the character of this review, mainly centered on coordination chemistry arguments, only the main results will be outlined briefly, with particular emphasis on the *biological role of the metal speciation.*

(i) Animals [33–37]

$Al(acac)_3$ is found to be at least 100 times more cardiotoxic than $Al(lact)_3$ to rabbit upon intravenous (i.v.) administration (total doses ca. 0.3 mg per kg body weight [35]). $Al(malt)_3$ is not cardiotoxic under comparable conditions. $Al(acac)_3$ and $Al(malt)_3$ considerably increase the permeability of the blood–brain barrier in the rat to ^{14}C-labelled sucrose when administered (i.v.) at pH 7.5 [36]. On the other hand, $Al(lact)_3$ (i.v.) is ineffective in this connection.

(ii) Cell cultures [37–41]

$Al(acac)_3$ is strongly mutagenic to *S. typhimurium* but the analysis of the effect is difficult owing to a similar behaviour exhibited by Hacac. $Al(malt)_3$ and $Al(lact)_3$ are ineffective, although $Al(lact)_3$ was found to be quite reactive with purified DNA. $Al(acac)_3$ and $Al(malt)_3$ are genotoxic to mammalian cell cultures (CHO line) in which they produce a remarkable sister chromatid exchange (SCE assay) [37].

$Al(acac)_3$ and $Al(malt)_3$ are considerably cytotoxic to murine neuroblastomas ($LD_{50} \approx 180\ \mu M$ and 280 μM, respectively); on the other hand, and most remarkably, $Al(lact)_3$ is not cytotoxic but cytostatic (from 10 to 100 mM) and its action produces a spectacular differentiating effect (Fig. 8), which transforms the globular probe cells into differentiated cells bearing fairly complex neuritic expansions [41].

$Al(acac)_3$ at mM levels induces rapid, profound and irreversible morphological damages (echino-acanthocytosis) to washed suspended rabbit, rat and human erythrocytes [38–40], while $Al(malt)_3$ and $Al(lact)_3$ are ineffective, as are Hacac and $Fe(acac)_3$, which were both used for molecular control tests. This biophysical effect

Fig. 8. Differentiating effect caused by aqueous $Al(lact)_3$ at pH 7 on murine neuroblastomas (magnification $\times 25000$).

is accompanied by a large increase in aluminium concentration inside the erythrocytes ghosts (from 11 to 5700 ppm, dry weight, in rabbit erythrocytes) [40] and by an increase of osmotic fragility [38]. A marked decrease of membrane fluidity, as revealed by ESR measurements after labelling with suitable spin labels, is observed in the ghosts of the echinoacanthocytes produced [40].

(iii) Enzymes [42]

Aluminium administered under controlled speciation conditions inhibits some enzymatic activities, mainly those which are Mg^{2+}-dependent [23].

We find that $Al(lact)_3$ at μM levels at pH 7 strongly inhibits trypsin (a serinprotease), an effect which is not reversed by the action of mM EDTA. This observation appears particularly remarkable in that it might be related to the uncontrolled protein proliferation observed in the manifestation of the two major hallmarks of AD, i.e. neurofilament degeneration and development of the "amiloidic" material in senile plaques.

G. CONCLUSIONS

The molecular solution state of aluminium at neutral pH values strongly affects the quality and the intensity of the biological response to specific aggression by the metal centre.

Targeted toxicological and enzymological experiments must rely on very tight speciation control.

Investigations on the reactivity of aluminium with biologically relevant molecules at neutral pH values are highly recommended. Special care in obtaining structural and not only kinetic and thermodynamic results will be particularly useful for shedding light on the molecular basis of aluminium toxicity.

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