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Effects of Aluminum and Other Cations on the Structure of Brain and Liver Chromatin†

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ABSTRACT: The reactivity of aluminum and several other divalent and trivalent metallic cations toward chromatin from rat brain and liver has been investigated. Two criteria are used to determine the relative reactivity of these cations toward chromatin. The first involves the ability of the ions to compact the chromatin fibers to the point where chromatin precipitates. The second criterion measures the ability of cations to interfere with the accessibility of exogenous structural probes (nucleases) to chromatin. Of the divalent cations tested, nickel, cobalt, zinc, cadmium, and mercury were the most reactive toward chromatin, on the basis of their ability to induce precipitation of chromatin in the micromolar concentration range. The divalent cations magnesium, calcium, copper, strontium, and barium were much less effective, although all cations precipitate chromatin if their concentration is increased. Of the trivalent cations tested, aluminum, indium, and gallium were very effective precipitants, whereas iron and scandium were without effect at the concentrations tested. Of all the cations tested, aluminum was the most reactive. Aluminum's ability to alter the structure of chromatin was investigated further by testing its ability to interfere with nuclease accessibility. This test confirmed that aluminum does induce considerable changes in chromatin structure at micromolar concentrations. Furthermore, chromatin from cortical areas of the brain was much more sensitive to aluminum than chromatin from liver. These results are discussed in light of the known toxicity of these cations, with particular emphasis on the possible role of aluminum in Alzheimer's disease.

The transcription of a gene into mRNA by RNA polymerase is preceded by a complex series of changes in the structure of DNA as it is unfolded from a compacted chromatin fiber in order to make the coding strand accessible to RNA polymerase. Divalent cations play vital roles in these reactions. For example, magnesium ions and probably calcium ions are involved in the stabilization of DNA in chromatin fibers (Eichhorn, 1979, 1981; Walker & Sikorska, 1987a,b), and calcium and copper ions appear to be involved in the stabilization of the highest levels of chromatin structure, the chromatin loop domains (Lebkowski & Laemmli, 1982). Furthermore, zinc ions together with a second divalent cation, typically manganese or magnesium, are essential for RNA polymerase activity (Mildvan & Loeb, 1981). Given such essential roles for divalent cations, the question arises of how other metallic cations, particularly those derived from the environment and believed to be toxic, might interfere with these reactions. This area has been poorly studied, but some earlier studies have indicated that nonphysiological cations can increase the error rate of RNA polymerase (Eichhorn, 1979; Loeb & Mildvan, 1980) and can interact with DNA (Record et al., 1978; Eichhorn, 1981).

Considerable information now exists on the effects of physiological cations on chromatin structure, and, indeed, monovalent and divalent cations are routinely used to manipulate the structure of chromatin in vitro in studies designed to elucidate the structure of the chromatin fibers (Walker & Sikorska, 1986, 1987a,b; Walker et al., 1986). Using this information, it is now practical to carry out a more meaningful examination of the effects of inorganic metal cations on the structure of chromatin. In this paper, we have examined the effects of a number of cations on chromatin from rat liver [for which the bulk of structural information is available (Walker & Sikorska, 1986, 1987a,b; Walker et al., 1986)] and extended these studies into the chromatin from brain, because chromatin from neuronal nuclei is known to have several structural differences from other chromatins (Pearson et al., 1984). The data show that a number of metallic cations can cause marked changes in the degree of compaction of chromatin, resulting in its precipitation. In general, the ability of the cations to induce chromatin compaction and precipitation is related to their ionic radius and charge with aluminum, by virtue of its high charge density and low ionic radius being the most effective, particularly in brain. Since "heavy metal" cations can readily penetrate the cell nucleus (Bryan, 1980), it is conceivable that some of their toxic effects are due to disruption of the normal mechanisms of gene expression.

These observations, which demonstrate that aluminum has the highest affinity for chromatin of all the ions we tested, are important in relation to the known involvement of this cation

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in a number of diseases and are particularly relevant in light of the observations of McLachlan's group on the role of aluminum in the changes in gene expression associated with Alzheimer's disease (Crapper McLachlan et al., 1984; Crapper McLachlan & Van Berkum, 1986; McLachlan et al., 1988a,b).

Materials and Methods

Isolation of Nuclei. Nuclei were isolated from livers and brain cortex of 200-g male Specific-pathogen-free Sprague-Dawley rats bred at this facility. Four grams of tissue was homogenized in 20 mL of 0.25 M sucrose, 50 mM Tris-HCl (pH 7.5), 70 mM KCl (80 mM total ionic strength), 5 mM $MgCl_2$, and 0.2 mM phenylmethanesulfonyl fluoride, and nuclei were isolated as described previously (Sikorska et al., 1980; Walker & Sikorska, 1986). In some experiments, 0.5 mM aluminum chloride was included in the isolation buffer.

Isolation of Chromatin Fragments. In all experiments, nuclei were resuspended at a concentration of 0.8–1.0 mg/mL DNA in 10 mM Tris-HCl (pH 8.0), 75 mM KCl, 0.5 mM $MgCl_2$, and 0.2 mM phenylmethanesulfonyl fluoride. Digestions were carried out for 10 min at 30 °C in the presence of 50 μg/mL deoxyribonuclease I (DNase I; Sigma Chemical Co., St. Louis, MO) as previously described (Walker & Sikorska, 1986). In some experiments, digestions were continued for up to 60 min. The reactions were stopped by rapid cooling in ice/water. The suspension was then centrifuged at 25000*g* for 15 min in the 50 Ti rotor of a Beckman L8-70 ultracentrifuge at 5 °C to generate a supernatant, S1, containing 40–50% of total nuclear chromatin as soluble oligonucleosome fragments ranging in size from mononucleosomes to 100-mers.

Sedimentation Analysis of Chromatin Fragments. Sucrose density gradient ultracentrifugation was used to analyze the effects of cations on the sedimentation coefficient ($s_{20,w}$) of chromatin oligomers as described previously (Walker & Sikorska, 1987b). Chromatin fragments were generated by carrying out digestions, as described above, in the presence of different concentrations of monovalent cation (K^+). Gradient centrifugation and data analysis were carried out as described by Walker and Sikorska (1987b). Briefly, 0.5 mL of S1 supernatant was loaded onto 14 mL of 8–35% (w/w) sucrose gradient and centrifuged in the SW40 rotor at 40 000 rpm for 3.25 h. Following centrifugation, fractions were collected and taken for DNA size analysis and refractive index determination (Walker & Sikorska, 1987b). The data are plotted as a log–log plot of $s_{20,w}$ vs ionic strength for chromatin fragments with a mean size of 60 nucleosomes.

Effects of Cations on Chromatin Solubility. Aliquots (5–25 μL) of solutions of various cations were added to 1 mL of S1 supernatant (containing approximately 1.2–1.5 mg of DNA as chromatin), and the mixture was incubated on ice for 30 min. After this time, the samples were centrifuged at 25000*g* for 15 min at 5 °C as described above, to generate a supernatant, S2, and a pellet, P2. The amounts of DNA in S2 and P2 were determined by the diphenylamine reagent (Walker et al., 1977), and the data are expressed as the percentage of the chromatin remaining in the supernatant.

Effects of Cations on the Accessibility of Chromatin to Nucleases. Isolated nuclei were digested with DNase I as described above or with micrococcal nuclease (MNase, 50 units/mL, in a buffer containing 10 mM Tris-HCl, pH 8.0, 70 mM KCl, and 0.2 mM phenylmethanesulfonyl fluoride) for various lengths of time in the presence or absence of the concentrations of aluminum chloride indicated in the figure legends. The reactions were stopped by the addition of EDTA to 1 mM followed by rapid cooling on ice. S1 supernatants were generated by centrifugation as described above, and the amounts of DNA released into S1 and remaining in the pellet P1 were determined by the diphenylamine reagent.

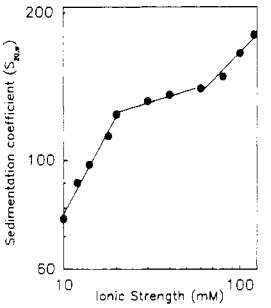

FIGURE 1: Effect of ionic strength on the sedimentation coefficient of chromatin. Nuclei were isolated from liver in buffer containing different concentrations of KCl to give the final ionic strengths indicated. DNase I digestions were carried out at the same ionic strength and the samples loaded onto sucrose gradients (also at the same ionic strength). Centrifugation was carried out as described under Materials and Methods. Gradients were fractionated, and the sedimentation coefficients ($s_{20,w}$) for chromatin oligomers with an average size of 60 nucleosomes were calculated for each ionic strength.

Results

Effects of Cations on Chromatin Structure. Mono- and divalent cations exert profound effects on chromatin structure in vitro as indicated by considerable changes in the sedimentation coefficient of chromatin fibers in the ultracentrifuge (Walker & Sikorska, 1987a,b). The data in Figure 1 show the results of an experiment designed to illustrate the effects of monovalent cations on chromatin structure. As the ionic strength was increased, the sedimentation coefficient also increased, indicating that the chromatin fibers were becoming more compact. The curve has a characteristic triphasic shape which can be interpreted in terms of the folding of the 10-nm polynucleosome chain into the higher order 30-nm chromatin fiber and the subsequent compaction of the latter fiber (Walker & Sikorska, 1987b). At higher ionic strengths, the sedimentation coefficient continued to increase as the fibers became more compact until the chromatin eventually precipitated. This occurred, in these experiments, at about 150–170 mM monovalent cation (K^+ or Na^+). The divalent cation Mg^{2+} also induced similar changes in chromatin structure but at much lower concentrations, with precipitation occurring above about 2 mM (Walker & Sikorska, 1986). Calcium ions as well as cobalt and copper ions, on the other hand, induced precipitation at even lower concentrations (Walker & Sikorska, 1986; Borochov et al., 1984). Precipitation is, therefore, the end product of a series of conformational changes, induced by the cation and resulting in the chromatin fiber becoming increasingly more compact. Furthermore, this phenomenon can be used as a convenient assay for determining the relative effects of various cations on chromatin structure.

Effects of Mono-, Di-, and Trivalent Cations on Chromatin Solubility. Figure 2 shows the effect of mono-, di-, and trivalent cations, in the concentration range of 0–500 μM, on the solubility of chromatin. There was a marked variation in the ability of the cations to cause precipitation, with the monovalent cations being essentially ineffective at these low concentrations. Among the divalent cations, nickel was a much more effective precipitant than the larger barium ion. Moreover, whereas divalent cations such as cobalt and nickel were very effective precipitants, trivalent cations of similar size such as scandium and iron were ineffective at this concentration. It should be noted, however, that at higher concentrations all cations eventually precipitate chromatin (data not shown). Of the cations tested, aluminum was a particularly

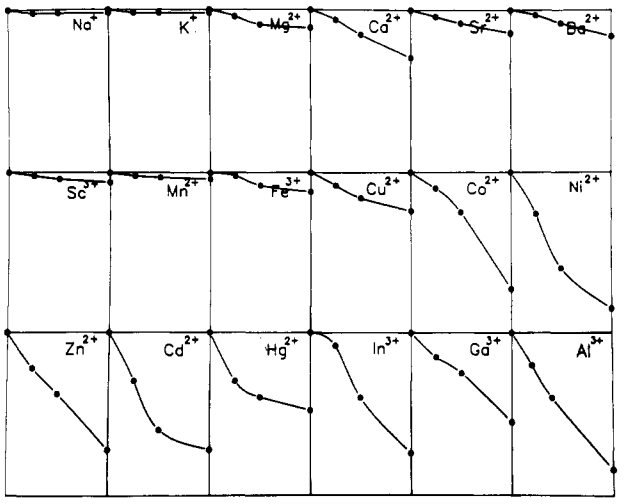

FIGURE 2: Effect of various cations on chromatin solubility. Chromatin fragments were prepared from liver as described under Materials and Methods, and the ability of each of the cations, in the concentration range of 0–500 μM, to precipitate chromatin was tested. Each unit within the chart has the same axes as Figure 3 (y axis = percentage of chromatin remaining in solution, 0–100%; x axis = Al^{3+} concentration, 0–500 μM).

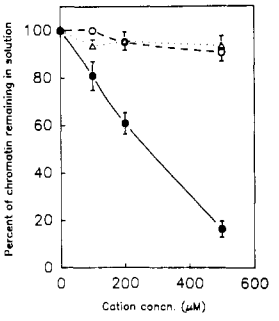

FIGURE 3: Concentration curve for the precipitation of chromatin by trivalent cations. The standard precipitation test was used as described under Materials and Methods at the concentrations of cation indicated. Each point is the mean ± SEM for three to five observations. (△) Sc^{3+}; (○) Fe^{3+}; (●) Al^{3+}.

effective precipitant as shown in Figure 3, with significant amounts of chromatin being precipitated at concentrations in the 50–100 μM range. In general, the ability of the cations to precipitate chromatin was increased in the higher groups (2b and 3) of the periodic table (increased valency). Within each group, the smaller cations were generally the most effective. Moreover, it should be remembered, on the basis of the data in Figure 1, that the cation could be inducing more subtle, physiologically relevant, changes in chromatin structure at concentrations an order of magnitude lower than those required to produce precipitation. Thus, of all the cations tested, aluminum was the most effective inducer of precipitation, although a number of the "heavy metals" were also very reactive toward chromatin.

Effects of Aluminum on the Sensitivity of Chromatin to Nucleases. Exogenous nucleases, such as DNase I and MNase, have been successfully used as probes to gain information about the structure of the chromatin fibers (Walker & Sikorska, 1986, 1987a,b; Walker et al., 1986). In general, the more compact the fiber, the less accessible it is to exogenous nucleases. Thus, chromatin isolated under low ionic strength conditions where the fibers are fully relaxed is rapidly degraded by these nucleases, whereas chromatin isolated under physiological conditions is much more resistant. Figure 4 shows the results of a series of experiments carried out on chromatin from brain and liver in which DNase I digestions

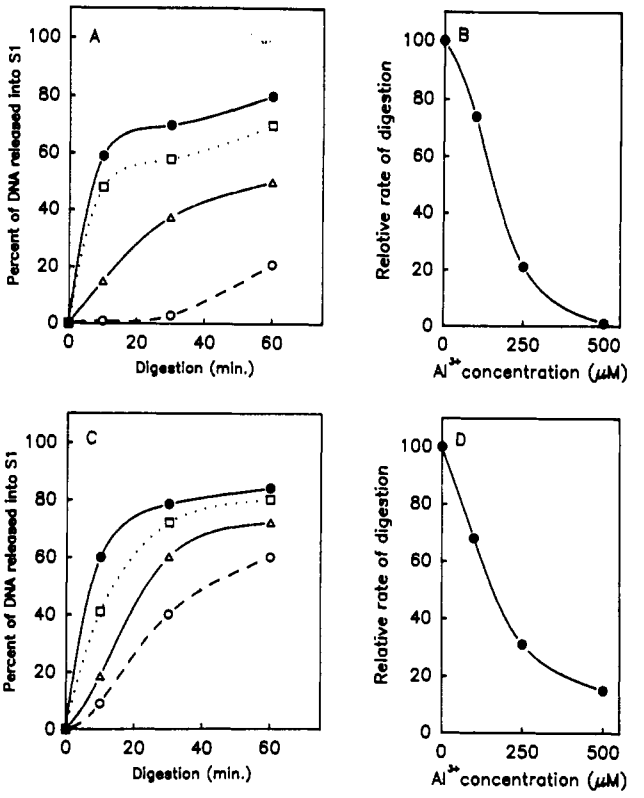

FIGURE 4: Effect of aluminum on the sensitivity of chromatin to DNase I. Nuclei were isolated from brain (A and B) and liver (C and D) as described under Materials and Methods. Digestions were carried out with DNase I for the times indicated in the presence of 0 (●), 100 (□), 250 (△), or 500 (○) μM aluminum (A and C). In panels B and D, the extent of digestion after 10 min is plotted against Al^{3+} concentration to illustrate the degree of inhibition for brain and liver chromatin, respectively.

were carried out in the presence of different concentrations of aluminum chloride at a monovalent cation ionic strength at which the chromatin was folded up into the 30-nm chromatin fiber but was still accessible to the nuclease (Walker & Sikorska, 1986).

In the absence of aluminum, digestion of chromatin proceeded rapidly, reaching a plateau after about 20 min with 75–80% of the chromatin being released into supernatant, S1. Both brain and liver chromatins were digested with similar kinetics. A significant inhibition of the initial stages of digestion was observed in the presence of the lowest concentration of Al^{3+} tested (100 μM). This was more pronounced at higher concentrations (Figure 4B,D), with both the rate of digestion and the total extent of digestion being inhibited. Furthermore, chromatin prepared from the cortical areas of the brain appeared more sensitive to Al^{3+}-induced alterations in chromatin structure than chromatin from liver.

Since these digestions were terminated with a chelating agent, which solubilizes all released chromatin, the results cannot be explained by the precipitation of released chromatin fragments during digestion. Therefore, the observation that digestion did not go to completion in the presence of higher concentrations suggested that the Al^{3+} ions were inducing irreversible changes in chromatin structure, possibly leading to precipitation of chromatin in nucleo. To study this further, nuclei were prepared in the presence of 0.5 mM aluminum chloride, but the exogenous nuclease digestions were carried out in its absence, after the nuclei were washed to remove any carry-over aluminum. In these experiments, MNase was used as the exogenous nuclease probe, and the results are shown in Figure 5. Exposure of chromatin to Al^{3+} during isolation

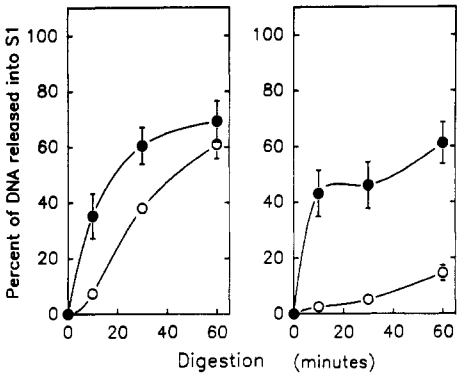

FIGURE 5: Effect of aluminum in the isolation buffer on the subsequent accessibility of chromatin to micrococcal nuclease. Nuclei were isolated from liver (left panel) and brain (right panel) in buffer containing no aluminum chloride (●) or 500 μM aluminum chloride (○). Digestions were carried out in the absence of aluminum for the times indicated. Each point is the mean ■ SEM for four to eight observations.

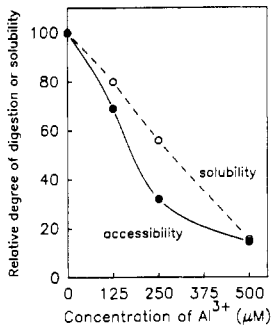

FIGURE 6: Comparison of the effects of aluminum on nuclease accessibility and chromatin solubility. The solubility data were derived from Figure 2 and the accessibility data from Figure 4B.

of the nuclei significantly reduced the accessibility of liver chromatin to MNase and rendered brain chromatin almost completely resistant to digestion by MNase under these conditions.

Aluminum ions had no direct inhibitory effect on the activity of the enzymes toward naked DNA (data not shown), confirming that the cation acts by changing the extent of nuclease digestion by changing the conformation of the substrate, chromatin, and not by inhibiting the enzymes.

Summary of the Effects of Cations on Chromatin Structure. The accessibility of chromatin to exogenous nucleases is generally a more sensitive indicator of cation-induced changes in chromatin structure as indicated in Figure 6. This graph summarizes the cation concentration dependent changes in accessibility and solubility of chromatin and indicates that Al^{3+} interferes with nuclease accessibility to chromatin at lower concentrations than it causes precipitation. However, both parameters follow similar trends and indicate, quite clearly, that aluminum ions can induce considerable changes in chromatin structure both in nucleo and in vitro.

Discussion

The data presented indicate quite clearly that nonphysiological "heavy metal" cations can interact with chromatin and cause considerable changes in its structure. At sufficiently high concentrations, this can lead to actual precipitation of chromatin in nucleo. The trivalent cation aluminum and the divalent cations nickel and cobalt, together with cadmium, zinc, and mercury, are particularly effective. However, some metal cations were quite ineffective at the concentrations tested. In order to rationalize the relative effectiveness of the different

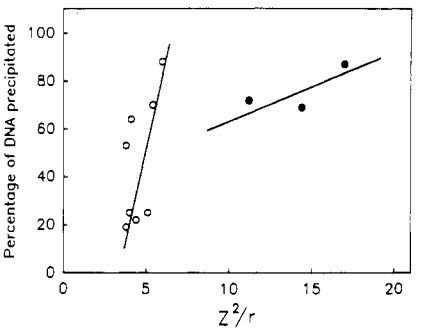

FIGURE 7: Ability of divalent (○) and trivalent (●) cations to precipitate chromatin as a function of ionic index. The values of the ionic index Z^2/r (where Z = charge and r = crystal ionic radius) were derived from Nieboer and Richardson (1980).

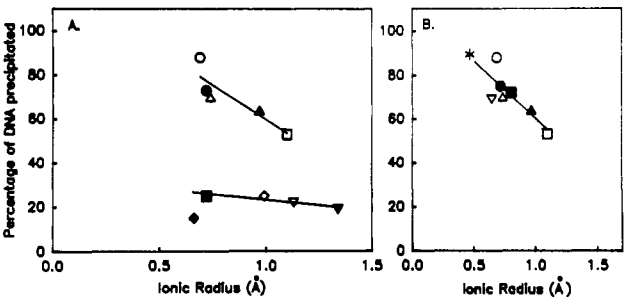

FIGURE 8: Ability of cations to precipitate chromatin as a function of ionic radius. In (A), the ability of divalent ions to precipitate chromatin (using the 500 μM data from Figure 2) is plotted against ionic radius for all the divalent cations tested. In (B), only those cations (di- and trivalent) given greater than 50% precipitation are plotted against ionic radius. Symbols in (A): (○) Ni; (●) Co; (△) Zn; (▲) Cd; (□) Hg; (■) Cu; (▽) Sr; (▼) Ba; (◇) Ca; (◆) Mg. Symbols in (B): (*) Al; (○) Ni; (●) Co; (■) In; (▽) Ga; (△) Zn; (▲) Cd; (□) Hg.

cations, the data from Figures 2 and 3, for those cations that caused greater than 20% of chromatin to be precipitated, are plotted in Figure 7 as a function of ionic index (Z^2/r). The ionic index is a measure of a cation's ability to form ionic bonds with biological macromolecules (Nieboer & Richardson, 1980). The divalent and trivalent cations fall into two distinct groups, with each group showing a good correlation between reactivity toward chromatin and increased ionic index, particularly for the divalent cations. This analysis indicates that the effective cations do, indeed, induce changes in chromatin structure by binding ionically to specific targets.

However, some cations with similar ionic radius and charge are much less effective than others, and this is examined more closely in Figure 8A for all of the divalent cations tested. The data are plotted as a function of ionic radius, and the results falls into two distinct groups—one group (Ni, Co, Zn, Cd, and Hg) is very reactive toward chromatin, whereas the second group (Mg, Cu, Ca, Sr, and Ba) is much less reactive. Within each group, reactivity decreases with increased ionic radius, but clearly the radius of the ion is not the only factor contributing to reactivity. In general, the cations in the most reactive group belong to the borderline and class B group of ions according to the heavy-metal classification of Nieboer and Richardson (1980), with the exception of Cu^{2+}, and the less reactive cations belong to the class A group. This same order is reflected in the general toxicity of metal cations (Nieboer & Richardson, 1980). Therefore, although size and charge are important, ions with an increased covalent index (Nieboer & Richardson, 1980) are the most effective. Thus, in general, the cations that are the most reactive toward chromatin have a relatively high covalent index, but within this group, the

cations with the smallest ionic radius are the most effective (Figure 8B). This suggests that nonionic bonds (for example, interaction with nucleotide bases of DNA, aromatic amino acid side chains of proteins, and sulfhydryl groups) are involved in the interaction between the cation of chromatin but that small size is important for the cations to reach their binding sites in chromatin.

Little is known about the exact mechanisms by which cations interact with chromatin. Monovalent cations are believed to effect changes in chromatin structure by neutralization of the charge repulsion between the negative charged phosphates on the DNA backbone. However, divalent cations such as magnesium and calcium can effect changes in chromatin structure at much lower ionic strengths which suggests that they have more specific binding sites. Other cations such as zinc play essential roles in very specific reactions, such as the binding of regulatory proteins and transcription factors to their regulatory DNA sequences (deHaseth et al., 1977; Miller et al., 1985; Saavedra, 1988) or the activation of RNA polymerase (Mildvan & Loeb, 1981). The actual binding sites of nonphysiological cations on chromatin are still unknown. They may be sites to which cations do not normally bind, or they may act by displacing physiological cations, such as calcium, magnesium, or zinc, and disrupt the normal processes which these cations are involved in. In addition, they may bind to sites on DNA which would disrupt the binding of incoming cationic proteins (Record et al., 1978). It is also conceivable that different cations may interact at different sites. A number of cations, particularly aluminum, have been shown to bind directly to DNA (Record et al., 1978; Karlik et al., 1980a,b). Alternatively, they may bind to specific chromatin proteins that either are involved in the maintenance of chromatin structure or are involved in the enzymic reactions of gene transcription, such as RNA polymerase (Mildvan & Loeb, 1981).

Of all the cations tested, aluminum was the most reactive toward chromatin. Moreover, nuclei from the neuron-rich areas of the brain were much more sensitive to this cation, suggesting that chromatin which has a very short linker region between nucleosomes may be more susceptible to aluminum-induced alterations in chromatin structure. Aluminum has been shown to accumulate in the brains of people suffering from Alzheimer's disease (Crapper et al., 1980; Perl & Brody, 1980). The cation appears to be concentrated both in the neurofibrillary tangles (Candy et al., 1986) and in the nucleus. Moreover, the concentration of the cation in the nucleus could achieve levels which, based upon the present study, would seriously disrupt the normal structure of chromatin. Indeed, McLachlan's laboratory has shown that there are both marked alterations in chromatin structure and a concomitant decrease in specific gene expression in brain tissue from Alzheimer patients (Crapper McLachlan et al., 1986). Taken together, all of these data suggest that localized increases in aluminum in the nuclei of neurons could lead either to severe alterations in the structure of chromatin or to its actual precipitation in nucleo, both of which would prevent the genes embodied in this altered chromatin from being expressed. Further work is required to gain an understanding of the exact mechanisms by which these highly reactive cation induces changes in the structure of chromatin.

Acknowledgments

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Registry No. Nickel, 7440-02-0; cobalt, 7440-48-4; zinc, 7440-66-6; cadmium, 7440-43-9; mercury, 7439-97-6; magnesium, 7439-95-4; calcium, 7440-70-2; copper, 7440-50-8; strontium, 7440-24-6; barium, 7440-39-3; aluminum, 7429-90-5; indium, 7440-74-6; gallium, 7440-55-3; iron, 7439-89-6; scandium, 7440-20-2.

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