IMPACT OF PROTEIN MALNUTRITION ON SUBCELLULAR NUCLEIC ACID AND PROTEIN STATUS OF BRAIN OF ALUMINUM - EXPOSED RATS

Prasunpriya NAYAK and Ajay K. CHATTERJEE*

Biochemistry & Nutrition Research Laboratory

Department of Physiology

University College of Science & Technology

University of Calcutta

92, A.P.C. Road Calcutta - 700 009, INDIA

(Received June 10, 1997; Accepted October 6, 1997)

ABSTRACT — Nucleic acid and protein content in various cellular fractions of different regions of the brain were investigated in male albino rats following aluminum (Al) exposure (at the dose of 15% of LD₅₀ i.p. for 28 days) on either an adequate or inadequate protein diet. It was observed that there was a decrease in homogenate DNA content in the thalamic area (Th), midbrain-hippocampal region (MH) and cerebellum (CL), but not in the cerebrum (CC) of the protein-restricted group of animals. Increased RNA content was recorded in the ribosomal and soluble fractions of CL of the adequately protein-fed animals compared to pair-fed controls. In the low-protein-fed animals, on the other hand, a decrease in RNA content was observed in the whole homogenate and nuclear fractions of CC, MH and CL, the ribosomal and soluble fractions of MH and CL, and in the mitochondrial fraction of TH. Ribonucleolytic activity was found to be increased only in the Th and CL of the adequately protein-fed group. Protein contents in the subcellular fractions of these four regions remain almost unaltered with the present dose and duration of Al-exposure; only the soluble fraction of CC and microsomal fraction of Th of the low-protein-fed group showed a significant decrease. The results of the present investigation confirm that Al has generally depressive effects on the nucleic acid metabolism of the brain and suggest that these effects are region-specific as well as dependent on dietary protein level. It is further suggested that alterations in the cellular microenvironment, caused by protein malnutrition, may play a significant role in the modification of the effects of Al in the brain.

KEY WORDS : Aluminum, Nucleic acids, Brain, Protein malnutrition

INTRODUCTION

It has been reported that brain cell nuclei of aluminum-intoxicated animals are one intracellular locus for aluminum accumulation (Karlik *et al*, 1980). In certain human neurological disorders, highly localized and increased intracellular aluminum was reported (Kruck and McLachlan, 1988). Histochemical localization and subsequent subcellular fractionation studies demonstrated that the increase in aluminum concentration occurs specifically in the chromatin (Karlik *et al*, 1980). It was also reported that aluminum does induce

considerable changes in chromatin structure at micro-molecular concentration (Walker *et al*, 1989). Although the exact mechanism of aluminum-induced neurotoxicity is still not clear, it is believed that some of the neurotoxic effects of aluminum can be partially explained by its genotoxic and subcellular effects on DNA in neurons and other cells (U.S.Pub.Health Sci. Report, 1992). Smith (1984) showed *in vitro* that micromolar concentration of aluminum has a mitogenic effect and stimulates DNA synthesis, and this was further supported by Jones *et al* (1986). From these reports it is clear that aluminum may have some effects on brain nucleic acids and protein synthesis machinery. Most of the studies pertaining to this area were done *in vitro*.

Correspondence : Ajay K. CHATTERJEE at the above address.

On the other hand, protein undernutrition was found to affect the brain weight, DNA, RNA and protein content in young rats (Thakur *et al.*, 1992).

In view of the above, the present investigation was intended to study the *in vivo* effect of aluminum on brain nucleic acids in rats on an adequate and inadequate dietary protein status.

MATERIALS AND METHODS

Animals and diets

Male albino rats of Wistar strain weighing 80-100 g were divided into four groups of equal average body weight. The animals of half of the groups were maintained on a diet containing 18% protein (casein) while those of the remaining groups were maintained on a diet containing 6% protein (casein). The 18% protein was used as considered as an adequate (normal) dietary protein level which was used on earlier occasions (Chatterjee *et al.*, 1984; Ghosh *et al.*, 1992). The 6% protein was used as an inadequate dietary protein level (protein inadequacy) to study the influence of dietary protein inadequacy. This level of dietary protein was also employed on earlier occasions (Ghosh *et al.*, 1992). The carbohydrate (amylum) contents of the above two diets were kept at 71% and 83%, respectively. The other ingredients of the diets and supply of vitamins were the same as reported elsewhere (Chatterjee *et al.*, 1976; Chatterjee *et al.*, 1984). Some of the rats from each of

the dietary regimens received 4.2 mg/kg body weight (15% of LD₅₀)/day as aluminum chloride i.p. for 28 days. The animals were maintained on these diets and the treatment was done for four weeks. The animals of the control groups were pair-fed with those of the aluminum-treated groups and received only vehicle.

Tissue collection

After the period of treatment, the animals were sacrificed by cervical dislocation and the whole brain was removed, washed with ice-cold saline, blotted dry and immediately transferred to the ice chamber. Cerebrum, thalamic area, midbrain-hippocampal region and cerebellum were separated following dissection of the frozen brain after thawing (softening) in a cold condition, and with the help of a dissecting microscope.

Assay methods

Subcellular fractions (nuclear, mitochondrial, microsomal and soluble) of the four different regions of the brain were prepared according to the method as employed elsewhere (Chatterjee *et al.*, 1970). A weighed portion of different regions of brain was homogenized in ice-cold 0.25 M sucrose solution, using an all-glass homogenizer. These homogenates were spun in an ordinary centrifuge in the cold to remove cell debris and were then subjected to differential centrifugation according to Schneider and Hogeboom (1950) in an ultracentrifuge, successively at

Fig. 1. Body weight gain of rats during the period of treatment.

3000 g for 10 min, 10,000 g for 15 min, 30,000 g for 30 min and 105,000 g for 1 hr, to separate nuclear, mitochondrial, microsomal, and ribosomal fractions, respectively. The supernatant liquid obtained after centrifugation at 105,000 g formed the soluble fraction.

Protein contents were estimated using Folin-Ciocalteu reagent after treating the tissue homogenate with sodium dodecyl sulfate following the modified Lowry method (Stevens, 1992).

Ribonucleolytic activity was measured according to the method as employed elsewhere (Ghosh *et al*, 1992). This involved homogenization of a weighed portion of brain regions in 0.1 M-acetate buffer, pH 5.0, and determination of the ribonucleolytic activity according to the method based on ultraviolet absorption of acid-soluble degradation products from RNA (Josefsson and Lagerstedt, 1962).

RNA and DNA were isolated from tissue homogenates of different regions of the brain and their subcellular fractions by the method recommended by Munro and Fleck (1969) except that DNA was extracted with 0.8 M-perchloric acid at 70°C. The RNA and DNA nucleotides were measured in the respective extracts by ultraviolet absorption (Stroev and Makarova, 1989).

Student's 't'-test was used to determine the significance of differences, if any, between the means, and probability levels of 5% or less were considered significant (Das, 1981).

RESULTS

It appears from Fig.1 that the body weight gain of aluminum-treated rats receiving an 18% protein diet

was slightly higher than that of the corresponding control group of rats. But this increase in body weight gain was not found to be statistically significant. It is further seen from Fig.1 that the body weight gain of aluminum-treated rats on a 6% protein diet was not different from that of the corresponding control group of rats.

Table 1 shows that aluminum treatment at the present dose elevated the aluminum content of all the regions of the brains of rats receiving either an 18% protein or a 6% protein diet.

The results presented in Table 2 reveal that there was no significant change in DNA content of different regions of the brain on a wet-weight basis in the adequate protein (18%)-fed group of rats following aluminum treatment. It is further revealed from Table 2 that aluminum treatment had no significant effect on the RNA content (wet-weight basis) of different regions of the brain of the adequately protein-fed group of rats. But, aluminum-treated rats fed on a 6% protein diet exhibited a significant decrease in DNA content of the thalamic area, midbrain-hippocampal region and cerebellum, and a decrease in RNA content of the cerebrum, midbrain-hippocampal region and cerebellum. Aluminum treatment had no significant effect on protein content of the different regions of the brain of either group of rats except the cerebellum of the 6% protein-fed group of rats which showed a slight increase ($P < 0.05$) in protein content.

The results presented in Table 3 demonstrate that aluminum treatment had no significant effect on the sub-cellular fractional proteins of different regions of the brain except the ribosomal fraction of the cerebrum of the adequately protein-fed group, and the soluble

Table 1. Tissue aluminum contents of different regions of the brain.

Groups of animals	ALUMINUM CONTENT (μ moles / 100 mg tissue)			
	Brain regions			
	Cerebrum	Thalamic area	Midbrain-hippocampal region	Cerebellum
18% Protein	2.42 $\pm$ 0.43	5.61 $\pm$ 0.55	4.09 $\pm$ 0.26	2.40 $\pm$ 0.37
18% Protein +Aluminum	3.96 $\pm$ 0.17 ^b	10.06 $\pm$ 0.83 ^c	8.17 $\pm$ 0.51 ^c	4.11 $\pm$ 0.49 ^a
6% Protein	2.53 $\pm$ 0.33	4.76 $\pm$ 0.73	4.45 $\pm$ 0.68	2.37 $\pm$ 0.41
6% Protein +Aluminum	3.77 $\pm$ 0.46 ^a	9.03 $\pm$ 1.02 ^b	7.68 $\pm$ 0.91 ^a	3.86 $\pm$ 0.47 ^a

Data are means of six observations $\pm$ SEM

a = $P < 0.05$ compared with respective pair-fed control,

b = $P < 0.01$ compared with respective pair-fed control,

c = $P < 0.001$ compared with respective pair-fed control.

fraction of the cerebrum and microsomal fraction of the thalamic area of the inadequately protein-fed group, which showed significant decreases in protein content.

Table 4 shows that on a wet-weight basis there was a significant increase in the RNA contents of the ribosomal and soluble fractions of the cerebrum of the adequately protein-fed group of rats on exposure to aluminum, but in the protein-restricted (6% dietary protein) group the cerebrum showed a significant decrease in RNA content in its nuclear fraction. This decrease in nuclear RNA was also observed when expressed per mg of protein (Fig.2). The increased RNA contents in the ribosomal and soluble fractions of the cerebrum of the adequately protein-fed group following aluminum treatment was also observed when RNA was expressed per mg of protein (Figs. 5 & 6). On a protein weight basis, the cerebral mitochondrial RNA of the adequate-

ly protein-fed group decreased following aluminum treatment (Fig.3). Aluminum treatment produced no significant changes in RNA contents of different fractions of the thalamic area of the adequately protein-fed group on a tissue weight basis (Table 4) as well as protein weight basis (Figs. 2-6). But the inadequately protein-fed group of rats, in response to aluminum exposure, showed significant decreases in mitochondrial RNA on a wet weight basis and soluble fractional RNA both on wet-weight (Table 4) and protein-weight basis (Fig.6). Microsomal RNA in the same group of rats was, however, elevated on a protein-weight basis (Fig.4).

On an adequate-protein (18%) diet, RNA contents of sub-cellular fractions of the midbrain-hippocampal region remained unchanged on exposure to aluminum both on a wet-weight basis (Table 4) and protein-

Table 2. DNA, RNA and Protein contents (homogenate) of different regions of the brain.

Groups of animals	Brain regions			
	Cerebrum	Thalamic area	Midbrain-hippocampal region	Cerebellum
DNA content (μ g / 100 mg tissue)				
18% Protein	78.96 $\pm$ 7.54	64.50 $\pm$ 5.74	70.27 $\pm$ 6.53	74.20 $\pm$ 6.84
18% Protein	89.54 $\pm$ 6.30	72.13 $\pm$ 6.12	76.41 $\pm$ 5.14	59.38 $\pm$ 3.16
+ Aluminum				
6% Protein	74.20 $\pm$ 3.71	58.47 $\pm$ 2.20	69.99 $\pm$ 3.75	64.70 $\pm$ 3.49
6% Protein	71.16 $\pm$ 2.30	47.55 $\pm$ 2.51 ^b	53.27 $\pm$ 3.51 ^b	47.36 $\pm$ 1.43 ^c
+ Aluminum				
RNA content (μ g / 100 mg tissue)				
18% Protein	140.46 $\pm$ 9.96	124.70 $\pm$ 9.12	164.68 $\pm$ 11.97	154.45 $\pm$ 16.03
18% Protein	154.52 $\pm$ 14.50	145.55 $\pm$ 15.33	186.67 $\pm$ 13.62	176.83 $\pm$ 17.65
+ Aluminum				
6% Protein	133.81 $\pm$ 7.16	127.86 $\pm$ 9.51	177.09 $\pm$ 9.76	179.61 $\pm$ 9.19
6% Protein	108.49 $\pm$ 6.97 ^c	113.71 $\pm$ 7.62	135.24 $\pm$ 8.75 ^b	124.68 $\pm$ 9.43 ^b
+ Aluminum				
Protein content (mg / 100 mg tissue)				
18% Protein	9.24 $\pm$ 0.16	9.28 $\pm$ 0.13	9.54 $\pm$ 0.18	9.81 $\pm$ 0.14
18% Protein	9.08 $\pm$ 0.13	9.59 $\pm$ 0.19	10.45 $\pm$ 0.30	10.18 $\pm$ 0.17
+ Aluminum				
6% Protein	10.40 $\pm$ 0.17	9.95 $\pm$ 0.15	9.31 $\pm$ 0.15	9.67 $\pm$ 0.22
6% Protein	9.81 $\pm$ 0.16	10.02 $\pm$ 0.14	9.31 $\pm$ 0.17	10.41 $\pm$ 0.17 ^a
+ Aluminum				

Data are means of six observations $\pm$ SEM

a = $P < 0.05$ compared with respective pair-fed control,

b = $P < 0.01$ compared with respective pair-fed control,

c = $P < 0.001$ compared with respective pair-fed control.

weight basis (Figs. 2-6) except that the microsomal RNA showed a significant decrease when expressed per mg protein (Fig.4). On a 6% protein diet all the subcellular fractions of the midbrain-hippocampal region except the microsomal fraction showed a significant decrease in RNA content both on a wet-weight (Table 4) and protein-weight basis (Figs. 2-6). Microsomal RNA, however, exhibited a significant

increase when expressed per mg protein (Fig.4).

In the cerebellum of adequately protein-fed rats exposed to aluminum, soluble fractional RNA increased when expressed both on a wet-weight basis (Table 4) and protein-weight basis (Fig.6). On the other hand, nuclear RNA decreased when expressed on a protein-weight basis (Fig.2). On a low-protein (6%) diet, the cerebellum of aluminum-treated rats showed

Table 3. Protein contents of subcellular fractions of different regions of the brain.

Groups of Animals	Protein content (mg / 100 mg tissue)				
	SUBCELLULAR FRACTIONS				
	Nuclear	Mitochondrial	Microsomal	Ribosomal	Soluble
CEREBRUM					
18% Protein	3.64 ± 0.23	2.08 ± 0.11	0.39 ± 0.03	2.00 ± 0.09	1.47 ± 0.09
18% Protein + Aluminum	3.38 ± 0.19	2.49 ± 0.17	0.35 ± 0.08	1.59 ± 0.10 ^a	1.38 ± 0.10
6% Protein	3.23 ± 0.19	1.66 ± 0.13	0.31 ± 0.02	1.10 ± 0.06	3.90 ± 0.24
6% Protein + Aluminum	3.65 ± 0.16	1.72 ± 0.14	0.35 ± 0.02	1.14 ± 0.08	2.86 ± 0.31 ^a
THALAMIC AREA					
18% Protein	4.13 ± 0.22	2.19 ± 0.17	0.46 ± 0.06	1.69 ± 0.11	1.08 ± 0.16
18% Protein + Aluminum	4.00 ± 0.26	2.37 ± 0.16	0.42 ± 0.04	1.85 ± 0.14	1.01 ± 0.08
6% Protein	4.06 ± 0.39	1.35 ± 0.13	0.53 ± 0.02	1.22 ± 0.12	2.89 ± 0.20
6% Protein + Aluminum	4.31 ± 0.28	1.04 ± 0.10	0.36 ± 0.03 ^c	1.28 ± 0.06	2.76 ± 0.22
MIDBRAIN-HIPPOCAMPAL REGION					
18% Protein	4.51 ± 0.32	1.87 ± 0.12	0.30 ± 0.05	1.52 ± 0.10	1.28 ± 0.13
18% Protein + Aluminum	4.24 ± 0.27	2.04 ± 0.17	0.42 ± 0.06	1.82 ± 0.12	1.63 ± 0.11
6% Protein	3.97 ± 0.18	1.25 ± 0.09	0.55 ± 0.04	1.31 ± 0.04	3.83 ± 0.26
6% Protein + Aluminum	3.84 ± 0.29	1.37 ± 0.11	0.45 ± 0.03	1.46 ± 0.07	3.39 ± 0.20
CEREBELLUM					
18% Protein	3.58 ± 0.24	2.32 ± 0.15	0.41 ± 0.05	1.97 ± 0.16	1.79 ± 0.08
18% Protein + Aluminum	4.09 ± 0.29	2.62 ± 0.19	0.37 ± 0.05	1.68 ± 0.13	1.61 ± 0.14
6% Protein	3.87 ± 0.31	1.39 ± 0.11	0.48 ± 0.04	1.40 ± 0.09	3.73 ± 0.17
6% Protein + Aluminum	4.31 ± 0.28	1.21 ± 0.08	0.46 ± 0.04	1.48 ± 0.06	3.32 ± 0.15

Data are means of six observations ± SEM

a = P < 0.05 compared with respective pair-fed control,

b = P < 0.01 compared with respective pair-fed control,

c = P < 0.001 compared with respective pair-fed control.

significant decreases in nuclear and soluble fractional RNAs when expressed on wet-weight tissue (Table 4). On a protein weight basis, however, there were decreases in RNA contents of nuclear, ribosomal and soluble fractions (Figs. 2,5 & 6).

On an adequate protein diet, aluminum treatment appeared to elevate the RNA/DNA and protein /DNA ratios in the cerebellum only (Figs. 7 & 8). On a low-

protein diet, on the other hand, aluminum treatment appeared to have no significant effect on the RNA/DNA ratio in any of the brain regions studied, but increased the protein/DNA ratios in the midbrain-hippocampal region, thalamic area and cerebellum (Figs. 7 & 8).

The data presented in Table 5 reveal that the ribonucleolytic activity increases in the thalamic area

Table 4. RNA contents of subcellular fractions of different regions of the brain.

Groups of Animals	RNA content (μ g / 100 mg tissue)				
	SUBCELLULAR FRACTIONS				
	Nuclear	Mitochondrial	Microsomal	Ribosomal	Soluble
CEREBRUM					
18% Protein	47.61 $\pm$ 4.16	15.34 $\pm$ 1.36	3.58 $\pm$ 0.34	28.47 $\pm$ 2.82	33.36 $\pm$ 3.48
18% Protein + Aluminum	45.27 $\pm$ 2.23	11.76 $\pm$ 1.77	4.24 $\pm$ 0.52	40.54 $\pm$ 4.10 ^a	46.70 $\pm$ 3.88 ^a
6% Protein	49.15 $\pm$ 3.11	8.32 $\pm$ 0.64	5.99 $\pm$ 0.77	3.63 $\pm$ 0.47	45.48 $\pm$ 3.57
6% Protein + Aluminum	28.47 $\pm$ 2.13 ^c	9.66 $\pm$ 1.06	5.94 $\pm$ 0.82	3.69 $\pm$ 0.22	42.71 $\pm$ 3.70
THALAMIC AREA					
18% Protein	14.37 $\pm$ 1.83	15.27 $\pm$ 1.35	7.86 $\pm$ 1.54	19.46 $\pm$ 1.68	53.40 $\pm$ 4.37
18% Protein + Aluminum	18.85 $\pm$ 2.20	13.89 $\pm$ 1.37	8.52 $\pm$ 1.47	22.61 $\pm$ 1.83	57.74 $\pm$ 3.28
6% Protein	20.33 $\pm$ 2.56	13.29 $\pm$ 1.69	6.23 $\pm$ 0.47	13.25 $\pm$ 1.24	47.88 $\pm$ 2.94
6% Protein + Aluminum	23.44 $\pm$ 1.15	8.77 $\pm$ 0.70 ^a	7.37 $\pm$ 1.01	16.46 $\pm$ 1.49	26.02 $\pm$ 3.05 ^c
MIDBRAIN-HIPPOCAMPAL REGION					
18% Protein	30.51 $\pm$ 1.46	15.98 $\pm$ 0.97	13.89 $\pm$ 0.96	24.47 $\pm$ 1.94	68.19 $\pm$ 4.32
18% Protein + Aluminum	32.91 $\pm$ 2.55	17.24 $\pm$ 0.96	15.83 $\pm$ 1.10	27.02 $\pm$ 1.67	77.60 $\pm$ 4.63
6% Protein	37.43 $\pm$ 2.33	21.22 $\pm$ 1.46	14.38 $\pm$ 1.17	22.09 $\pm$ 1.92	63.88 $\pm$ 2.94
6% Protein + Aluminum	24.40 $\pm$ 1.29 ^c	11.50 $\pm$ 0.87 ^c	16.40 $\pm$ 1.53	16.38 $\pm$ 0.08 ^a	43.64 $\pm$ 2.57 ^c
CEREBELLUM					
18% Protein	38.69 $\pm$ 2.82	9.10 $\pm$ 1.11	3.50 $\pm$ 0.33	37.27 $\pm$ 3.13	55.18 $\pm$ 4.76
18% Protein + Aluminum	31.50 $\pm$ 1.86	10.20 $\pm$ 1.17	4.77 $\pm$ 0.71	40.63 $\pm$ 3.72	75.83 $\pm$ 7.13 ^b
6% Protein	34.35 $\pm$ 2.62	14.12 $\pm$ 1.03	7.24 $\pm$ 0.53	26.42 $\pm$ 1.65	72.44 $\pm$ 3.42
6% Protein + Aluminum	21.49 $\pm$ 1.81 ^b	10.78 $\pm$ 0.93	6.52 $\pm$ 0.41	21.45 $\pm$ 1.83	35.13 $\pm$ 1.86 ^c

Data are means of six observations $\pm$ SEM

a = P < 0.05 compared with respective pair-fed control,

b = P < 0.01 compared with respective pair-fed control,

c = P < 0.001 compared with respective pair-fed control.

and cerebellum of the adequately protein-fed group of animals in response to aluminum exposure. But no significant changes were observed when the activity was expressed as unit per mg protein. The ribonucleolytic activities in other regions of the brain were not altered in response to aluminum treatment.

DISCUSSION

The present studies demonstrate that the body weight gain of rats receiving either an 18% protein diet or a 6% protein diet is independent of aluminum treatment. This is evident from the fact that the body weight gain of aluminum-treated rats receiving either an 18% protein diet or a 6% protein diet was not different from that of the corresponding control group of rats (Fig.1).

It would appear from the Table 2 that, following aluminum treatment of rats fed on an 18% protein, the increases in the aluminum contents of the cerebrum, thalamic area, midbrain-hippocampal region and cerebellum were 63%, 79.3%, 99.8% and 71.3% respectively, while on a 6% protein diet such increases in the respective regions of the brain were 49%, 89.7%, 72.6% and 62.9%. This shows that inadequate dietary protein status reduces the ability of different regions of the brain, barring the thalamic area, to concentrate aluminum.

Preferential protection of the brain from the nutritional insult is not absolute (Ahmed and Rahman, 1975). This has been further elucidated by the present investigation. Restricted-protein-fed animals showed a significant decrease in the DNA content of homogenate of all the brain regions, except the cerebrum, in response to aluminum exposure. Depressing effects of aluminum on DNA synthesis (Blair *et al*, 1989, Zatta *et al*, 1994) of slowly dividing cells of the brain, increased sensitivity of DNA (particularly mitochondrial DNA) to oxidative damage as in Alzheimer's disease (Mecocci *et al*, 1994) or aluminum-induced increased activity of acid DNase (Suzuki *et al*, 1988) may be implicated as the probable cause of this decreased DNA content. On the other hand, no significant alteration in homogenate DNA contents of the brain regions of the adequately protein-fed group suggested that the impairing effect of aluminum depends on the dietary protein status. The 20% decrease, though statistically insignificant, in the DNA content of cerebellum on an adequate protein diet following aluminum exposure cannot be ignored.

Thus, as far as the change in DNA content is concerned, the cerebrum can withstand the aluminum exposure in either dietary protein adequacy or inadequacy, but the cerebellum cannot, in any dietary protein status.

Fig. 2. Nuclear RNA content of different regions of the brain in response to aluminum (Al) exposure. b = $P < 0.01$ compared with respective pair-fed control, c = $P < 0.001$ compared with respective pair-fed control.

Fig. 3. Mitochondrial RNA content of different regions of the brain in response to aluminum (Al) exposure. a = $P < 0.05$ compared with respective pair-fed control, c = $P < 0.001$ compared with respective pair-fed control.

Fig. 4. Microsomal RNA content of different regions of the brain in response to aluminum (Al) exposure. a = $P < 0.05$ compared with respective pair-fed control.

Brain Nucleic Acids and Aluminum Exposure.

Fig. 5. Ribosomal RNA content of different regions of the brain in response to aluminum (Al) exposure. a = $P < 0.05$ compared with respective pair-fed control, b = $P < 0.01$ compared with respective pair-fed control.

Fig. 6. Soluble RNA content of different regions of the brain in response to aluminum (Al) exposure. a = $P < 0.05$ compared with respective pair-fed control, b = $P < 0.01$ compared with respective pair-fed control, c = $P < 0.001$ compared with respective pair-fed control.

Fig. 7. RNA / DNA ratio of different regions of the brain in response to aluminum (Al) exposure. a = $P < 0.05$ compared with respective pair-fed control.

Fig. 8. Protein / DNA ratio of different regions of the brain in response to aluminum (Al) exposure. a = $P < 0.05$ compared with respective pair-fed control, b = $P < 0.01$ compared with respective pair-fed control.

Aluminum induces cross-linking of histone H1 to DNA (Savory *et al*, 1991) and thus causes a shift toward a more condensed state as observed in Alzheimer's disease affecting the cerebral cortex (Martin, 1988). In rats and rabbits, however, no aluminum-induced histone DNA affinity was observed (Martin, 1988). Aluminum may also induce phosphorylation of histone proteins as a consequence of increased levels of cyclic nucleotides and these phosphorylated histones serve as a crucial binding site of aluminum in chromatin (Martin, 1991). In spite of all this, brain cells are able to maintain their chromatin unaffected or least affected when adequate protein is supplied in the diet. The present investigation shows that protein restriction not only inhibits the mitogenic and DNA synthesis-stimulating effect of aluminum (Smith, 1984; Jones, *et al*, 1986) but also causes a reduction in DNA content. Thus, protein restriction probably causes a change in the cellular microenvironment which might induce aluminum to interact with DNA differently, and activate DNase in certain regions of the brain, particularly the midbrain-hippocampus, thalamic area and cerebellum.

RNA contents of the different regions of the brain were found to be decreased in aluminum-treated rats of the low-protein-fed group only, though with certain

specificity. In most cases a decrease in homogenate RNA content was associated with the decrease in nuclear RNA content. This observation suggests that aluminum decreases RNA synthesis, which may be due to aluminum-induced inhibition of RNA polymerase (Crappier McLachlan *et al*, 1983) or due to an aluminum-induced shift towards a more compact structure limiting its accessibility to RNA polymerase activity (Kruck and McLachlan, 1988). Contrary to this, the adequately protein-fed group showed no alteration in RNA of almost all the subcellular fractions of all the regions, except that the ribosomal and soluble fractions of the cerebrum and thalamic area, and the soluble fraction of the cerebellum showed a significant increase in RNA content. This increase may partially be attributed to the decreased activity of RNase, as observed in the thalamic area and cerebellum (Table 5). These increases in RNA contents of ribosomal and soluble fractions also suggest that aluminum may also induce some modification on RNA at a post-transcriptional level. The RNA present in the soluble fraction is mainly composed of transfer RNA. After synthesis, these RNA molecules migrate from the nucleus to the cytoplasm. This is a phenomenon that occurs in response to a need for protein synthesis in the cytoplasm. It is possible that

Table 5. Ribonucleolytic activities of different regions of the brain.

Groups of animals	RIBONUCLEOLYTIC ACTIVITY			
	Brain regions			
	Cerebrum	Thalamic area	Midbrain-hippocampal region	Cerebellum
	Units / 100 mg tissue			
18% Protein	127.76 ± 1.14	121.55 ± 1.23	123.13 ± 1.14	118.44 ± 1.68
18% Protein	131.03 ± 1.78	131.32 ± 2.32 ^b	124.60 ± 2.09	134.85 ± 1.65 ^c
+ Aluminum				
6% Protein	132.33 ± 1.16	127.59 ± 1.19	126.97 ± 1.06	125.05 ± 2.28
6% Protein	128.61 ± 1.24	123.47 ± 1.26	124.82 ± 1.28	122.79 ± 2.18
+ Aluminum				
	Units/mg protein			
18% Protein	13.84 ± 0.30	13.12 ± 0.25	12.91 ± 0.20	12.08 ± 0.26
18% Protein	14.45 ± 0.37	13.69 ± 0.30	12.00 ± 0.52	13.22 ± 0.34
+ Aluminum				
6% Protein	12.74 ± 0.25	12.84 ± 0.28	13.65 ± 0.18	12.95 ± 0.34
6% Protein	13.13 ± 0.28	12.34 ± 0.37	13.42 ± 0.29	11.79 ± 0.18
+ Aluminum				

Data are means of six observations ± SEM

a = P < 0.05 compared with respective pair-fed control,

b = P < 0.01 compared with respective pair-fed control,

c = P < 0.001 compared with respective pair-fed control.

aluminum may have a role in such migration of RNA molecules, and this effect of aluminum may be modulated by the protein dietary level. Accordingly, such an interplay of factors may be suggested as contributory to the variable or diverse effects that aluminum exhibits on the level of the soluble RNA of different regions of the brain in relation to adequacy and inadequacy of the dietary protein level.

In the cerebellum specifically, no significant change in RNA content (except an increase in soluble fraction) was observed in spite of the decrease in DNA content. This causes a significant increase in the RNA/DNA ratio, which suggests that the decrease in DNA content may be ascribed to the effect of aluminum on the non-neuronal cells. Aluminum treatment increased the protein/DNA ratios in the midbrain-hippocampal region and thalamic area of the inadequately protein-fed group only, while it elevated the protein/DNA ratio in the cerebellum of both the adequate and inadequate protein-fed groups of animals (Figs. 7 & 8). It appears therefore that, of all the regions of the brain, the cerebellum becomes more sensitive to aluminum treatment in terms of the protein/DNA ratio, which is altered on an adequate as well as inadequate protein status, and the midbrain-hippocampal and thalamic protein/DNA ratios become sensitive to aluminum treatment only on an inadequate protein status. The increased protein/DNA ratios suggest accumulation of protein in the respective brain regions which might result from decreased degradation of proteins due to aluminum treatment. Whether the decreased degradation of proteins is a result of aluminum-induced inhibition of the activity of protein-degrading enzymes needs to be ascertained by further studies.

It was demonstrated on several occasions that the activity of ribonucleases influences the cellular concentrations of RNA (Ghosh *et al.*, 1992, Sajdel-Sulkowska and Marotta, 1984, Thakur *et al.*, 1992). Cho and Kim (1991a) demonstrated that aluminum interrupts the formation of the alkaline-ribonuclease-inhibitor complex and suggested that accumulation of aluminum in the brain might affect the regulation of RNA metabolism. Cho and Kim (1991b) also demonstrated that aluminum activates the acid RNase in the brain, which in turn may change the regulation of RNA metabolism. In the present investigation, ribonucleolytic activity (acid RNase) of the different regions of the brain appears to show a relation with the relative changes of the regional RNA contents as well as subcellular RNA distribution in the regions. It is therefore possible that the relative effect of aluminum on alkaline and acid RNase

may be one of the contributory factors for diverse changes that were observed in the RNA content of different regions of the brain and their subcellular fractions on an adequate and inadequate dietary protein status.

The translatability of mRNA has been reported to decrease (Nicholls *et al.*, 1990), though the poly(A) RNA yields increased significantly as an effect of aluminum induction (van Berkum *et al.*, 1986). San-Marina and Nicholls (1992) showed that higher doses of aluminum cause a significant increase in labelled amino acid incorporation into a protein synthesizing system. This increase in amino acid incorporation was observed only in the polysomal fraction of the rat brain. Decreased capability of protein synthesis has been reported in murine neuroblastoma cells as a consequence of aluminum exposure (Martin, 1991). In the present investigation no alteration in protein content was observed either in the total homogenate or in the subcellular fractions of either of the dietary groups in response to aluminum exposure. The μg RNA/mg of protein is a measure of protein-synthesizing capacity, and ribosome, which is a site of protein synthesis, showed decreased μg RNA/mg protein in the midbrain-hippocampal region and cerebellum of aluminum-treated rats on a 6% protein diet (Fig. 5). This might suggest that aluminum reduces the protein-synthesizing capacity of the midbrain-hippocampal region and cerebellum on a low-protein diet. On the other hand, ribosome in the cerebrum of aluminum-treated rats receiving an adequate protein diet increased μg RNA/mg protein (Fig. 5), suggesting enhanced protein-synthesizing capacity.

It is concluded from the present investigation that aluminum *in vivo* decreases the synthesis of nucleic acids and also increases its degradation, particularly in RNA in the brain depending on the region. Aluminum *in vivo* appears to also have diverse effects on the protein-synthesizing capacity of the brain depending on the region. These effects of aluminum on the brain are particularly apparent in protein malnutrition.

ACKNOWLEDGEMENT

The authors are thankful to the University Grants Commission, New Delhi, for providing a Research Fellowship to P. Nayak.

REFERENCES

Ahmed, G. and Rahman, M. A. (1975) : Effects of

- undernutrition and protein malnutrition on brain chemistry of rats. *J. Nutr.*, **105**, 1090-1103.
- Blair, H. C., Finch, J. L., Avoiele, R., Slatopolsky, E. and Teitelbaum, S. L. (1989) : Micromolar aluminum levels reduce ^3H thymidine incorporation by cell line UMR 106-01. *Kidney Int.*, **35**, 1119-1125.
- Chatterjee, A. K., Basu, J., Dutta, S. C., Sengupta, K. and Ghosh, B. B. (1976) : Effects of L-lysine administration on certain aspects of ascorbic acid metabolism. *Int. J. Vit. Nutr. Res.*, **46**, 286-290.
- Chatterjee, A. K., Jamdar, S. C. and Ghosh, B. B. (1970) : Effect of riboflavin deficiency on *in vivo* incorporation of C_{14} amino acid into liver protein of rats. *Br. J. Nutr.*, **24**, 635.
- Chatterjee, A. K., Sadhu, U., Dalal, B. B. and Chatterjee, T. (1984) : Studies on certain drug-metabolizing enzymes in deoxypyridoxine treated rats. *Jap. J. Pharmacol.*, **34**, 367-373.
- Cho, S. W. and Kim, G. Y. (1991a) : Aluminum interrupts the formation of alkaline-ribonuclease inhibitor complex from bovine brain. *Eur. J. Biochem.*, **202**, 107-111.
- Cho, S. W. and Kim, G. Y. (1991b) : Activation of acid ribonuclease from bovine brain by aluminum. *J. Biochem.*, **110**, 802-805.
- Crapper McLachlan, D. R., Farnell, B., Galin, H., Karlik, S., Eichorn, G. and DeBoni, U. (1983) : *In Biological Aspects of Metal Related Disease* (Sarkar B, ed.), pp.209-218, Raven Press, N.Y.
- Das, D. (1981) : *Statistics in Biology and Psychology*, pp. 54-158, Academic Publishers, Calcutta.
- Ghosh, S., Chatterjee, A. K. and Gupta, M. (1992) : Impact of lead toxicity in brain metabolisms of nucleic acid and catecholamine in protein malnourished rats. *J. Nutr. Sci. Vitaminol.*, **38**, 451-462.
- Jones, T. R., Autonetti, D. L. and Reid, T. W. (1986) : Aluminum ions stimulate mitosis in murine cell in tissue culture. *J. Cell Biochem.*, **30**, 31-39.
- Josefsson, L. and Lagerstedt, S. (1962) : Characterization of ribonuclease and determination of its activity. *Meth. Biochem. Anal.*, **9**, 39-74.
- Karlik, S. J., Eichorn, G. L. and Crapper McLachlan, D. R. (1980) : Molecular interaction of aluminum with DNA, *Neurotoxicology*, **1**, 83-88.
- Kruck, T. P. A. and McLachlan, D. R. (1988) : Mechanism of aluminum neurotoxicity - relevance to human disease *In Metal Ions in Biological System* (Siegel, H. ed.), pp. 285-314, Marcel Dekker Inc., New York.
- Martin, R. B. (1988) : Bioinorganic chemistry of aluminum *In Metal Ions in Biological System* (Siegel, H. ed.), Vol. 24, pp.1-50, Marcel Dekker Inc., New York.
- Martin, R. B. (1991) : Aluminum in biological system *In Aluminum in Chemistry, Biology and Medicine* (Nicolini, M, Zatta, P.F., Corain, B. ed.), pp. 3-20, Cortina International, Verona.
- Mecocci, P., Macharvey, U. and Beal, M. F. (1994) : Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann. Neurol.*, **36**, 747-751.
- Munro, H. N. and Fleck, A. (1969) : Analysis of tissues and body fluids for nitrogen constituents *In Mammalian Protein Metabolism*. (Munro, H. N. ed.), Vol. 3, pp. 433-525, Academic Press, New York.
- Nicholls, D. M., Spears, G. M., Asina, S. and Miller, A. C. (1990) : Translability of mRNA from brain of infant rabbits exposed chronically to aluminum, *Int. J. Biochem.*, **22**, 119-125.
- San Marina, S. and Nicholls, D. M. (1992) : Some effects of aluminum on rat brain protein synthesis. *Comp. Biochem. Physiol.*, **103**, 585-591.
- Sajdel-Sulkowska, E. M. and Marotta, C. A. (1984) : Alzheimer's disease brain : alterations in RNA levels and ribonuclease-inhibitor complex. *Science*, **225**, 947-949.
- Savory, J., Herman, H. M., Katsetos, C. D. and Willis, M. R. (1991) : Aluminum and neurodegenerative disorders. *In Aluminum in Chemistry Biology and Medicine*, (Nicolini, M. Zatta, P. F., Corain, B. ed.), pp. 45-52, Cortina International, Verona.
- Schneider, W. C. and Hogeboom, G. H. (1950) : Intracellular distribution of enzymes V. Further studies on the distribution of cytochrome c in rat liver homogenates. *J. Biol. Chem.*, **183**, 123-128.
- Smith, J. B. (1984) : Aluminum ions stimulate DNA synthesis in quiescent cultures of Swiss 3T3 and 3T6 cells. *J. Cell Physiol.*, **118**, 298-304.
- Stevens, L. (1992) : Buffers and the determination of protein concentration *In Enzymatic Assay - A Practical Approach* (Eisenthal, R., Danson, M. J.ed), pp. 316-335, IRL Press, Oxford.
- Stroev, E. A. and Makarova, V. G. (1989) : Nucleic acids *In Laboratory Manual in Biochemistry*, pp. 66-73, Mir Publishers, Moscow.
- Suzuki, H., Takeda, M., Nakamura, Y., Tada, K., Hariguchi, S. and Nishimura, T. (1988) : Activities of lysosomal enzymes in rabbit brain with experi-

- mental neurofibrillary changes, *Neurosci. Lett.*, **89**, 234-239.
- Thakur, M. L., Van, T. T., Srivastava, U. S., Radhakrishnamurthy, R. (1992) : Protein and RNA biosynthesis in various cellular fractions of the brain of undernourished rats. *J. Nutr. Biochem.*, **3**, 217-223.
- U. S. Public-Health Science Report (1992) : Toxicological Profile of Aluminum and Its Compounds pp.1-99.
- Van Berkum, M. F., Wong, Y. L., Lewis, P. N. and McLachlan, D. R. (1986) : Total and poly (A) RNA yields during an aluminum encephalopathy in rabbit brains. *Neurochem. Res.*, **11**, 1347-1359.
- Walker, P. R., LeBlanc, J. and Sikroska, M. (1989) : Effects of aluminum and other cations on the structure of brain and liver chromatin. *Biochemistry*, **28**, 3911-3915.
- Zatta, P. F., Zambenedetti, P. and Masiero, S. (1994) : Effects of aluminum lactate on murine neuroblastoma cells. *Neurotoxicology*, **15**, 789-798.