CHAPTER IIB

BIOCHEMICAL EFFECTS OF IN-OVO-ADMINISTRATION OF CALCIUM
ON THE CONTENTS OF NUCLEIC ACIDS AND PROTEIN AND THE
ACTIVITIES OF DEOXYRIBONUCLEASES UNDER LEAD INTOXICATION
IN THE BRAIN OF DEVELOPING CHICK EMBRYO
INTRODUCTION

One of the most salient characteristics of lead intoxication in the developing chick embryo is the development of hydrocephalus and subsequent its reversal by the supplementation of calcium has been reported in earlier chapter. In order to investigate the possible biochemical mechanism(s) involved in the overall process, the metabolism of deoxyribonucleic acid (DNA) was studied in control and various treated groups of developing chick embryos. Researches concerning the metabolism of DNA in developing chick embryo revealed high activities of both acid and alkaline deoxyribonucleases (E.C. 3.1.4.5 and 3.1.4.6; DNAses) during the early embryonic development. With increase in the age of the animals, both the activities decreased and in old brain, acid DNases could hardly be detected, although alkaline DNase remained at a significant level (Shrivastava and Subba Rao, 1975; Subba Rao and Shrivastava, 1976). The above results tempted us to speculate that the physiological role of acid DNase may be in those types of cells proliferating during the early part of the brain development (neuronal cells), whereas
alkaline DNAse may have some role both in the early forming (neuronal as well as in late forming cells (glial cells).

In the present chapter, the contents of RNA, DNA and protein and the activities of acid and alkaline DNAse were determined in control, lead treated, calcium treated and lead and calcium co-treated groups.

MATERIALS AND METHODS

The White Leghorn fertilized eggs, procured from Uttar Pradesh Government Poultry Farm, Lucknow, India, were used throughout the study.

Chemicals

Highly polymerized calf thymus DNA and RNA and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis, Mo, U.S.A. All the other chemicals and reagents were of analytical grade.

Technique for instillation

The selection of egg, candling and injection were the same as described in Chapter I.

Treatment

The fertilized and viable eggs were divided into four groups of each 30. The instillation was performed as follows:
I Group - Received glass distilled water 0.1 ml and served as control.

II Group - Received 50 μg lead solution in 0.1 ml.

III Group - Received 500 μg calcium solution in 0.1 ml.

IV Group - Received 50 μg lead and 500 μg calcium solution in 0.1 ml.

Biochemical analysis

The brains from all the groups were carefully removed and pooled separately. Ten per cent homogenates were prepared in glass-distilled water using Potter Elvehjem Glass Teflon homogenizer. Whenever necessary, the homogenate was further diluted with water to 1%.

RNA and DNA contents were estimated by the methods of Schmidt and Thannhauser (1945).

RNA and DNA contents were estimated according to the procedure of Schmidt and Thannhauser (1945), slightly modified following the suggestions of Munro (1966). The protein content was estimated by the method of Lowry et al. (1951).

The activities of acid and alkaline DNAses were assayed according to the method of McDonald (1955).
RESULTS

The levels of RNA, DNA and protein were studied in the brain of 21 days old chick embryo under lead intoxication. The lead significantly decreases the levels of the macromolecules. The decreases noticed in the contents of RNA, DNA and protein under lead indication were 37, 30 and 44% respectively. However, the supplementation of calcium along with lead abolished the reduction in the contents of RNA, DNA and protein occurred in lead toxic group. Moreover, the calcium alone does not have any significant effect on the levels of these macromolecules (Table 2B.1).

The activities of acid and alkaline DNAses were also studied on the brain of lead-treated chick embryo. The activity of acid DNAse shows a significant decrease whereas the changes in the alkaline DNAse, under lead toxicity, was only marginal. The lead inhibits the activities of acid and alkaline DNAses by 45 and 13% respectively. However, calcium again protects the chick brain from the toxic effect of lead by restoring the activities of these enzymes at their normal levels when co-administered with lead. Calcium does not have any effect on the activities of these enzymes in vitro (Table 2B.2).
Table 2B.1
Effect of lead (50 µg) and calcium (500 µg) separately and in combination on the contents of RNA, DNA and protein in the brain of 21 days old chick embryo

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Contents*</th>
<th>RNA</th>
<th>DNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>6.5±0.22</td>
<td>1.2±0.07</td>
<td>98.45±3.3</td>
</tr>
<tr>
<td>Lead</td>
<td></td>
<td>4.095±0.13</td>
<td>0.84±0.05</td>
<td>55.6±2.7</td>
</tr>
<tr>
<td>Lead + calcium</td>
<td></td>
<td>6.3±0.26</td>
<td>1.1±0.085</td>
<td>95.39±4.01</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td>7.01±0.19</td>
<td>1.34±0.09</td>
<td>102.84±3.91</td>
</tr>
</tbody>
</table>

*The values are expressed as mg per g fresh tissue weight. All values are averages ± S.E. from eight experiments.
DNA was estimated by the diphenylamine methods, while RNA was estimated by the orcinol reaction.
Table 2B.2

Effect of lead and calcium separately and in combination on the activities of acid and alkaline DNAses in the brain of 21 days old chick embryo

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Enzyme activities</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid DNAse</td>
<td>Alkaline DNAse</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>257.05±9.2</td>
<td>1403.69±32.15</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>141.38±8.05</td>
<td>1221.17±27.8</td>
<td></td>
</tr>
<tr>
<td>Lead + Calcium</td>
<td>248.52±11.35</td>
<td>1385.41±21.43</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>261.28±7.85</td>
<td>1425.8±17.39</td>
<td></td>
</tr>
</tbody>
</table>

The reaction mixture for acid DNAse consisted of 1 mg of DNA (highly polymerized, calf thymus) in 1 ml of water, 1.5 ml of 0.2 M acetate buffer, pH 5.5 and 0.5 ml of brain homogenate. At the end of 2 hr incubation at 37°C, the reaction was terminated by adding 2 ml of 1.4 M perchloric acid and immediate chilling. The whole mixture was filtered through Whatmann 42 paper and deoxyribose was estimated in the filtrate. Activity expressed as µg of acid soluble deoxyribose phosphorus liberated per 2 hr. For alkaline DNAse 2 mg of heat-denatured DNA in 1 ml of water and 1.5 ml of Tris-HCl buffer of 0.2 M, pH 8.25 were used. Other details are just same as for acid DNAse. Values are averages ± S.D. for six experiments.
DISCUSSION

It has been reported that lead inhibits renal potassium dependent phosphatase and a large number of enzymes being single functional SH-group. On the other hand, it enhances the activities of certain enzymes like glucose-6-phosphatase dehydrogenase and lactate dehydrogenase in different species of animals. Extensive studies have also been carried out on the status of drug metabolizing enzymes under lead toxicity in rats. Moreover, lead ingestion causes alterations in the activities of various enzymes in brain tissues of male albino rats. Lead poisoning causes cerebral dysfunction and clinically defined encephalopathies and neuropathies in experimental animals. Reports are also available on the histology, morphology and biochemical alterations of renal tissues under lead toxicity (Mukherjee et al., 1982).

Trace elements in the microenvironment may alter the proliferative rates, thus modifying the number of cells available for the formation of an organ or body as a whole. Cell death and necrosis during embryogenesis, may similarly alter the size of the pool of cells available for organogenesis (Mukherjee et al., 1982).

In the present study, the lead reduces the contents of some vital macromolecules, i.e. RNA, DNA and protein.
At the enzyme level, it specifically inhibits the activity of acid DNAse in the brain of developing chick embryo, however, the simultaneous co-administration of calcium with lead abolishes the inhibition and enzyme level returns to its normal values. The activity of alkaline DNAse remains almost unaltered by lead. The finding of acid DNAse inhibition is of particular importance and deserves a special attention of the toxicologists working on lead toxicity on neural tissues. The acid DNAse is an enzyme of early embryonic development of the brain in chick and which is almost absent in aged brain. Thus, the inhibition of acid DNAse by lead clearly indicates that lead disturbs the metabolism of DNA in those types of neural cells proliferating during the early part of the brain development. Inhibition of DNA synthesis via acid DNAse, lead ions particularly arresting the proliferating activity of the brain cells and thus resulting an incomplete brain development.

The importance of the regulation of RNA and DNA metabolism during fetal growth and differentiation is obvious (Wasserman and Rosso, 1977). A number of agents, including metals, also that inhibit RNA and DNA synthesis also inhibit the fetal growth and are known to be teratogenic (Scott et al., 1971; Harvey and Srebnik, 1978; and Ivytup et al., 1978). Thirtyone metal salts have been screened in an in vitro system for potential teratogens. Nine metal salts decrease the fidelity of DNA synthesis and were considered
to be teratogenic (Scrover and Loeb, 1976).

The simultaneous co-incorporation of Ca\(^{++}\) with Pb\(^{++}\) abolishes the incidence of deformities produced by the lead alone. At the cellular level, the decrease in acid DNAse activity in brain of lead-treated group was almost abolished by the co-administration of calcium with lead. Moreover, other teratogenic effects, e.g. hydrocephalus, beak and leg deformities, short, neck, microphthalmia and anophthalmia were not produced when calcium was administered along with lead and the chick embryos were almost similar to control.

There are several mechanisms which may be postulated for the interaction of calcium with lead. High dietary intake of calcium may decrease lead absorption through intestine or increases the excretion rate of lead or interfere with transport of the toxic metal through blood, thereby decreasing the availability of this toxic metal in target tissue. It may also be possible that divalent cations may competitively compete for the general active binding site of the enzymes where lead binds.

Interactions of calcium with lead have been reviewed by Mahaffey et al. (1972). Low intake of calcium increases the susceptibility of rats to lead toxicity, while, on the other hand, high dietary intake of calcium inhibits lead absorption from the intestine of rats (Kostial et al., 1971). At the cellular level, calcium also appears to have protective
effects (Chatterjee et al., 1971). Acute intraperitoneal injection of lead acetate to the day old white male Leghorn for two days caused growth retardation and severe alterations in the activities of several enzymes, viz. acid phosphatase, alkaline phosphatase glucose-6-phosphatase, succinic dehydrogenase, glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase and acetylcholinesterase in different tissues. Calcium supplementation to the lead treated group of chicks showed a very good protective effects with respect to the above mentioned enzymes (Mukherjee et al., 1982).

Inorganic lead added to the biological systems in vitro is known to inhibit Ca\(^{++}\) transport activities in whole cells of brain, liver and in mitochondria isolated from heart and brain (Pound et al., 1982). Recently, it has been shown that lead has two effects on mitochondrial Ca\(^{++}\) metabolism. By virtue of its affinity for Ca carrier sites (calmodulin), it inhibits the unidirectional uptake of Ca\(^{++}\) and, when accumulated in the mitochondria, it induces a loss of Ca\(^{++}\) already present (Kapoor and Van Rossum, 1984). Moreover, Habermann et al. (1983) demonstrated that among the many heavy metal ions, Pb is a fully potent substitute for Ca\(^{++}\) in every calmodulin-dependent reactions.

Calmodulin is a tissue protein which binds Ca\(^{++}\) and mediates some of its effects. The protein is widely
distributed in plants and animals and its structure is well preserved phylogenetically. Calmodulin possesses four domains each of them able to bind one Ca\(^{++}\) ions. Depending on Ca\(^{++}\) load, it regulates many intracellular events (Cheung, 1982). Calmodulin stimulates the activity of the soluble brain cyclic nucleotide brain phosphodiesterase protein kinase, Ca-ATPase and several other enzyme systems (Cheung \textit{et al.}, 1978).

Thus, the present study tempted the author to speculate that lead hampers the transport and metabolism of Ca\(^{++}\) in the target tissue. Once the metabolism of calcium is impaired by lead, all the important biological events mediated by Ca\(^{++}\) might be adversely affected. However, this impairment in the Ca\(^{++}\) metabolism by lead may be reversed adequately by supplying the excess of calcium (10 fold) to the target sites or tissue which replaces the Pb\(^{++}\) competitively and specifically from the site of action.