Cyclic nucleotides are important second messengers and are involved in many cellular activities (Beebe and Corbin, 1986). Intracellular concentrations of cyclic nucleotide is regulated through modulation of their production and degradation by adenylyl cyclase and cyclic nucleotide phosphodiesterase (PDE) respectively (Sibley and Lefkowitz, 1985). Calcium dependent PDE is one of the multiple forms of cyclic 3', 5'-nucleotide PDE, discovered by Sutherland and Rall (1958). The enzyme is widely distributed. Both cytosol and membrane particles contain PDE, and their distribution varies with the types of cells and species of animals (Cheung, 1970). Phosphodiesterase is the only enzyme that catabolizes cAMP and cGMP, thereby regulating the tissue levels of cyclic nucleotides (Robison et al., 1971).

The intracellular concentrations of cyclic AMP and cyclic GMP are determined in part by the activities of one or more nucleoside 3',5'-monophosphate phosphodiesterase which catalyse the hydrolysis of the 3-phosphate band yielding the nucleotide 5'-monophosphate. Cyclic 3'-5'-adenosine monophosphate PDE was first studied in preparations from heart (Butcher and Sutherland, 1962) and further investigations of PDE activity in heart, brain, liver, fat cells and erythrocytes have been reported (Thomson and Appleman, 1971b).
SECTION - I

STUDIES ON BRAIN SPECIFIC ACIDIC PROTEINS

3. Studies on calcium dependent and calmodulin stimulated phosphodiesterase
Multiple forms of cyclic nucleotide PDE exist in various tissues, and a large family of them is activated by Ca\(^{2+}\)/Calmodulin (Beavo and Hardman, 1988). Thompson and Appleman (1971a,b) detected 3 forms of PDE in brain as well as in several other rat tissues. Monn and Christiansen (1971), Monn et al. (1972) identified as many as seven different forms of PDE in rat and rabbit tissues using starch gel electrophoresis and activity staining. Brain generally has a more complex PDE pattern. Hidaka et al. (1977) resolved human brain PDE into four activity peaks by DEAE-cellulose column.

Kakiuchi et al. (1975) estimated the level of Ca\(^{2+}\) dependent PDE in several rat tissues mainly in cortex, cerebellum, kidney and liver. Kakiuchi et al. (1975) also reported Ca\(^{2+}\) dependent PDE in a particulate fraction of rat liver, kidney and heart. Tanigawa and Shimoyoma (1976) found that in the early embryonic state of the chick embryo nearly all PDE's are Ca\(^{2+}\) dependent. Singer et al. (1978) showed that in early development rabbit foetal brain, kidney, liver and lung had the mature array of PDE.

Considerable information is available on the effects of pesticides on cyclic nucleotide phosphodiesterases. Natural and synthetic methyl xanthines have been reported to be the inhibitors of phosphodiesterase activity (Nathanson, 1984).
Rogakovich and March (1976) detected inhibition of PDE activity with nine different pesticides in the central ganglion of cockroach *Gromphadorhina portentosa*. Inhibition of adenyl cyclase and PDE with several organochlorine pesticides has been reported in rat brain synaptosomes (Kodavanti *et al*., 1988). An increase in the activity levels of PDE was reported on acute dieldrin exposure in different tissues of rats (Robert Joy *et al*., 1982).

The above review reveals that studies are entirely lacking on PDE in the developing mammal during critical stage of CNS development. Since cyclic nucleotide PDE occupies a strategic position and plays a significant role in regulating cyclic nucleotide levels in various tissues, it was felt essential to estimate changes occurring in PDE activity on experimentally induced methyl parathion toxicity in developing rats. It is hoped that this study would unravel the disruptions occurring in the molecular mechanisms in the developing CNS due to the toxic impact of methyl parathion.

**MATERIALS AND METHODS**

Developing albino rat pups (2 and 7-day-old) were injected with sublethal doses of methyl parathion. They were decapitated 48hrs after insecticide administration and
discrete areas of brain and spinal cord were separated for analysis at 0°C as described in general materials and methods.

Assay of Phosphodiesterase activity

Cyclic nucleotide hydrolysing enzyme PDE was assayed by following the method of Cheung (1971). The method is essentially that of Butcher and Sutherland employing snake venom as a source of 5'-nucleotidase. Cyclic AMP was used as the substrate. The inorganic phosphate liberated was determined by the method of Fiske-Subba Rao (1925). The reaction mixture of 0.5ml contained 40mM Tris-chloride, pH 8.0, 0.1mM MnCl₂, 2mM cAMP and an appropriate concentration of enzyme. The reaction was initiated by the addition of cAMP. At the end of 10min, at 30°C the tubes containing the reaction mixtures were transferred to boiling water bath for 2min to terminate the reaction. After thermal equilibrium to 30°C 0.05ml of snake venom (1mg/ml) was added for a second 10min incubation. The reaction was stopped by the addition of 0.05ml of 55% TCA, which was followed by 0.75ml of water and 1.5ml of 2.5% ammonium molybdate in 5N H₂SO₄. Denatured proteins were removed by centrifugation and optical density was read at 660nm. The protein content was determined by the method of Lowry et al. (1951).
The subunit pattern of PDE was detected by polyacrylamide gel electrophoresis developed by Davis (1964) and Ornstein (1964). After the run gels were cut into 2mm bits and each bit was homogenised in 1ml of Tris-HCl buffer pH 8.0. The activity levels of PDE was determined calorimetrically as described above.

RESULTS

The data after statistical analysis is presented in the tables (6-7) and figs. (9-10). From the data it is clear that PDE activity showed a considerable decrease in all the compartments of the brain of 2nd and 7th day-old rat pups.

Characteristically 2-day-old pups showed lower PDE activity compared to 7-day-old animals. In 7-day-old pups the activity levels of PDE was comparatively more indicating that PDE becomes more efficient physiologically as development progresses. Methyl parathion exposure has brought about substantial decrease in the activity of PDE in all the regions of the CNS in 2nd and 7th day-old pups (Tables 6-7).

The isozymic spectrum of PDE studied in the discrete areas of CNS showed considerable disruptions during methyl parathion induced toxicity. In general three isozymal forms of PDE could be resolved in all the compartments of brain
(Figs. 9-10). It is clear from the figs. (9-10) that a considerable inhibition in the activity of isozymal forms has occurred due to methyl parathion treatment. The relative electromobility of the isozymes was not altered during pesticide poisoning.

In the 2nd day-old rat pups in the cerebral cortex one out of the three isozymes showed slight stimulation in the activity on methyl parathion exposure, whereas the slow and fast moving forms were considerably inhibited (Fig. 9A).

In the brain stem of 2nd day-old rat pups all the three isozymes exhibited inhibition during methyl parathion toxicity. The slowest moving form showed the highest reduction in the activity on methyl parathion administration (Fig. 9B).

In the spinal cord only the fastest moving isozyme of PDE showed considerable inhibition on methyl parathion exposure (Fig. 9C).

In general only three isozymes could be detected even in 7-day-old rat pups both in control and experimental animals (Figs. 10A-C).

In the cerebral cortex only the fastest moving form was inhibited considerably on methyl parathion exposure (Fig. 10A) whereas the slowest moving form in the brain stem
exhibited substantial suppression of the activity (Fig.10B).

In the spinal cord of 7th day-old developing rats change in the activity levels of the three isozymes was comparatively of lesser magnitude. Only the fastest moving form showed suppression of the activity on methyl parathion exposure (Fig.10C).

Thus considerable disruptions of the isozymic pattern of PDE was observed on inducing methyl parathion toxicity.

DISCUSSION

Considerable disruptions of the isozymic pattern of PDE was observed on inducing methyl parathion toxicity. The suppression in the activity levels of PDE found in the present study could be due to the decline in the level of the modulator protein CaM, in the experimental animals (pl. see tables 2-3 and Figs. 5-8). This suggests existence of a novel mechanism mediated by CaM in the inhibition of PDE. Earlier studies in mammals also support the above suggested CaM mediated inhibitory mechanism in suppressing the activity of PDE in the nervous tissue during insecticide induced toxicity (Peter Evans and Antony, 1986).

The general inhibition of PDE as detected in the present study presumably results in the accumulation of
cAMP. The cAMP thus accumulated can be envisaged to play a vital role in altering neuronal function in the conditions of insecticide induced toxicity. A similar accumulation of cAMP and consequent reduction in PDE was reported earlier due to the impact of different classes of antipsychotics and insecticides (Peter Evans and Antony, 1986; Robert Joy et al., 1982; Bodnaryark, 1982; Kodavanti et al., 1988).
Table 6: Changes in the activity levels of phosphodiesterase in discrete areas of CNS of control and methyl parathion treated 2-day-old rat pups

<table>
<thead>
<tr>
<th>Region of the tissue</th>
<th>Control</th>
<th>MPT</th>
<th>Percentage in change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>10.0±2.12</td>
<td>7.67±1.13</td>
<td>-23.3 *</td>
</tr>
<tr>
<td>Brain stem</td>
<td>11.2±0.51</td>
<td>8.25±1.07</td>
<td>-26.34 *</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>11.5±0.50</td>
<td>9.40±1.03</td>
<td>-18.26 *</td>
</tr>
</tbody>
</table>

Values are (Mean ± S.D. of 6 observations) expressed as μmoles of inorganic phosphate liberated/g wet wt. of tissue/min.

Sign '-' indicates a decrease over the controls.

* P < 0.01.
Table 7: Changes in the activity levels of phosphodiesterase in discrete areas of CNS of control and methyl parathion treated 7-day-old rat pups

<table>
<thead>
<tr>
<th>Region of the tissue</th>
<th>Control</th>
<th>MPT</th>
<th>Percentage in change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>14.50±1.77</td>
<td>11.28±0.89</td>
<td>-22.21 *</td>
</tr>
<tr>
<td>Brain stem</td>
<td>13.22±1.51</td>
<td>9.75±1.92</td>
<td>-26.25 *</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>11.7±1.57</td>
<td>9.35±0.95</td>
<td>-20.08 *</td>
</tr>
</tbody>
</table>

Values are (Mean ± S.D. of 6 observations) expressed as μmoles of inorganic phosphate liberated/g wet wt. of tissue/min.

Sign '-' indicates a decrease over the controls.

* P < 0.01.
**Fig. 9A:** Polyacrylamide gel electrophoresis for the molecular heterogeneity of phosphodiesterase in 0.05M Tris-HCl buffer, pH 8.9 in cerebral cortex of 2-day-old rat pups.

Solid lines with closed circles represent the electrophoretic pattern of phosphodiesterase in the control cerebral cortex.

Broken lines with open circles represent the electrophoretic pattern of phosphodiesterase in the methyl parathion treated cerebral cortex.

**Fig. 9B:** Polyacrylamide gel electrophoresis for the molecular heterogeneity of phosphodiesterase in 0.05M Tris-HCl buffer, pH 8.9 in brain stem of 2-day-old rat pups.

Solid lines with closed circles represent the electrophoretic pattern of phosphodiesterase in the control brain stem.

Broken lines with open circles represent the electrophoretic pattern of phosphodiesterase in the methyl parathion treated brain stem.

**Fig. 9C:** Polyacrylamide gel electrophoresis for the molecular heterogeneity of phosphodiesterase in 0.05M Tris-HCl buffer, pH 8.9 in spinal cord of 2-day-old rat pups.

Solid lines with closed circles represent the electrophoretic pattern of phosphodiesterase in the control spinal cord.

Broken lines with open circles represent the electrophoretic pattern of phosphodiesterase in the methyl parathion treated spinal cord.
Fig.10A: Polyacrylamide gel electrophoresis for the molecular heterogeneity of phosphodiesterase in 0.05M Tris-HCl buffer, pH 8.9 in cerebral cortex of 7-day-old rat pups.

Solid lines with closed circles represent the electrophoretic pattern of phosphodiesterase in the control cerebral cortex.

Broken lines with open circles represent the electrophoretic pattern of phosphodiesterase in the methyl parathion treated cerebral cortex.

Fig.10B: Polyacrylamide gel electrophoresis for the molecular heterogeneity of phosphodiesterase in 0.05M Tris-HCl buffer, pH 8.9 in brain stem of 7-day-old rat pups.

Solid lines with closed circles represent the electrophoretic pattern of phosphodiesterase in the control brain stem.

Broken lines with open circles represent the electrophoretic pattern of phosphodiesterase in the methyl parathion treated brain stem.

Fig.10C: Polyacrylamide gel electrophoresis for the molecular heterogeneity of phosphodiesterase in 0.05M Tris-HCl buffer, pH 8.9 in spinal cord of 7-day-old rat pups.

Solid lines with closed circles represent the electrophoretic pattern of phosphodiesterase in the control spinal cord.

Broken lines with open circles represent the electrophoretic pattern of phosphodiesterase in the methyl parathion treated spinal cord.
SECTION – I

STUDIES ON BRAIN SPECIFIC ACIDIC PROTEINS

4. Changes in total RNA and proteins during methyl parathion exposure
INTRODUCTION

The brain is highly complex and intricately organised (Kandel and Schwartz, 1985). The role and functions of RNA in the brain tissue of various species has been well recognised (Kandel and Schwartz, 1985; Edward Glassman and John 1972; Grant and Meherle, 1973). There is evidence in literature that in the resting mammalian cell, protein and RNA exist in dynamic equilibrium, the unstable molecules are continuously removed and simultaneously replaced (John Gaito, 1972). Cote and Kremzner (1983) showed that functional changes in nerve cells are accompanied by changes in nucleic acid and protein metabolism.

It was known that the RNA content of brain underwent an alteration in diverse type of situations. Moderate stimuli tended to cause a small increase, whereas intense and prolonged stimuli diminished the total amount of RNA (Talwar et al., 1961). Lajtha (1971) reported an increase in RNA concentration of the vestibular ganglion cells on rotatory stimulation of the animals. Vraajensen (1957) has found by histological technique evidence for an increased content of RNA in the earlier stages of a functional stress. Perzmer (1966) summarized the results obtained by different groups of Russian investigators concerning the changes in brain RNA metabolism during excitation and inhibition of nervous activity. These results indicated an increase in RNA
synthesis during excitation of nerve cells and a preponderance of RNA breakdown during the inhibition of nerve cells. It has been found that increased production of RNA molecules would result in increased protein synthesis and a hyperfunction of the cells. It has also been postulated that the release of transmitter substance like ACh during sensory stimulation results in an increase in RNA synthesis (Rappoport and Daginawala, 1970).

Brain is known to possess a high rate of protein synthesis and turnover (Davison, 1967), but this dynamic nature of proteins is markedly heterogeneous (Marks and Lajtha, 1971). The fact that the rate of RNA synthesis is higher in brain during early neonatal period is supported by the findings of Orrego (1967). According to Orrego (1967) there was a fivefold decline in the apparent rate of RNA synthesis from third day of age to maturity. It has been demonstrated that the increase in protein content during the late prenatal period coincided with the appearance of continuous activity and mature patterns in the electroencephalogram and was preceded by the critical period of morphological and biochemical changes (Naiyara Yasmeen, 1989).

According to Lajtha (1971) many factors influence protein turnover and these include effects of hormones,
drugs, pathological and nutritional state of the animal. Roberts et al. (1970) reported that mitochondrial protein synthesis may be more responsible to environmental control in immature brain than in the adult brain.

Pesticides are known to alter protein, lipid and nucleic acid metabolism (Moorthy et al., 1985). Organophosphorous insecticides have an effect on protein metabolism in addition to their specific inhibition of cholinesterase enzymes (Clouet and Welsh, 1963). Tayyaba et al. (1981) reported regional alterations in the brain levels of DNA, RNA, DNAse, RNAse qualitatively in male adult albino rats by organophosphorous compounds. Sivaprasad Rao and Ramana Rao (1979) reported an increase in the total proteins and free amino acid content in muscle, gill and liver tissues of fish *Tilapia mossambica* on methyl parathion exposure. *In vitro* studies using labelled amino acids also confirmed increased protein synthesis, specially more in liver than any other tissue in *Tilapia mossambica* exposed to malathion (Kabeer Ahamed, 1979). Earlier studies also reported an increased incorporation of amino acids into microsomal proteins (Dehlinger and Schmiks, 1972; Kato et al., 1965).

However, considerable decrease in protein synthesis was found in various regions of methyl parathion treated
pregnant rats viz., maternal brain and in maternal viscera, placenta and whole embryos (15 days old) and in fetal brain and viscera (Gupta et al., 1984). Inhibition of protein synthesis has been reported by organochlorine insecticides in biological systems like Hella cells, mouse peritoneal macrophages, sea urchin embryo, Crithidia fasiculata and Tetrahymena pyriformis (Saxena et al., 1981). Decreased levels of DNA RNA and proteins were found in Diazinon exposed liver homogenates of Zebra fish Brachydaniorerio cyprinidae and Channa punctatus (Kaushal Kumar and Ansari, 1984; Jyothi et al. 1989). Shakuntala and Srihari (1980) found a decrease in total protein levels in the livers of vacor treated rats. Radha Pant et al. (1982) found decrease in protein content due to high proteolytic activity induced by fungicide hexachlorobenzene has been reported. Inhibition of protein synthesis has been reported in the brain of dichlorovos treated suckling pups.

Thus from the above review it is evident that the impact of methyl parathion on RNA and protein levels has not been studied in the discrete brain areas in a developing mammal. Hence the present study was proposed.

MATERIALS AND METHODS

Developing albino rat pups (2 and 7 days old) were injected with sub lethal doses of methyl parathion as
described in general materials and methods of this thesis. They were decapitated 48hrs after insecticide administration and regions like cortex, brain stem and spinal cord were separated for analysis at 0°C as described earlier.

Ribonucleic acid

Ribonucleic acid (RNA) was extracted by the method of Schmidt-Thannhauser-Schneider (1957) and estimated by orcinol colour reaction following the colorimetric procedure as described by Glick (1964). The RNA content was expressed per unit weight of the tissue.

Total Proteins

The total protein content was determined in the brain tissue of control and methyl parathion administered albino rat pups, by the method of Lowry et al. (1957) utilizing folin-ciocalten phenol reagent. The optical density of colour development was read in spectrophotometer.

RESULTS

The data after statistical analysis presented in tables (8-11). A considerable increase in the content of RNA (Tables 8-9) and proteins (Tables 10-11) was found in all the regions studied as a function of methyl parathion administration. In 2-day-old rat pups the levels of RNA
increased significantly in cortex (+61.14%), brain stem (+55.46%) and spinal cord (+36.00) (Table 8). Even in 7-day-old rats the RNA content increased +37.32%, +40.68%, and +27.78% in cortex, brain stem and spinal cord respectively (Table 9).

Remarkable increase in the total protein content of brain and spinal cord was observed as a result of methyl parathion administration (Tables 10-11). In 2-day-old rat pups the protein content increased significantly in cortex (+78.69%), brain stem (+30.00%) and spinal cord (+19.38%) (Table 10). Even in 7-day-old rats the protein content increased +89.55, +24.81% and +12.12% in cortex, brain stem and spinal cord respectively (Table 11).

Parallel to protein content, the body weight of the animal also increased on methyl parathion exposure (Table 12). The percentage change in body weight was +8.3%, and +10.8% in 2 and 7-day-old pups respectively on methyl parathion exposure.

**DISCUSSION**

The remarkable increase observed in the total RNA content in different regions of CNS could be due to the direct stress caused by the pesticide exposure. Earlier reports substantiate the present findings. For instance,
Tayyaba et al. (1981) reported regional alterations in the brain levels of DNA, RNA, DNase, RNase qualitatively in male adult albino rats by organophosphorous compound metasystox. It was also suggested that DNA concentration significantly decreased in all brain regions, whereas the level of RNA decreased in cerebrum and increased in rest of the brain parts (Tayyaba et al., 1981). Decreased content of DNA and RNA also reported in the liver homogenates of diazinon exposed zebra-fish Brachdanio rerio.

The relative richness of the tissue RNA represents an index of functional activity and it grossly reflects the importance of protein synthesis (Mandel and Jacob, 1971). The relation between RNA and protein synthesis is well established (Van Sande, 1972). Increased production of RNA molecule is known to result in increased protein synthesis and a hyperfunction of the cells (Lajtha, 1971). From the present study it is also clear that the protein synthesis gets stimulated on methyl parathion administration (Tables 8-9). This significant increase (on methyl parathion administration) could be due to the stress induced by the pesticide exposure.

Earlier investigations also reported effects of organophosphorous insecticide on protein metabolism, in addition to their specific inhibition of cholinesterase
enzyme (Clouet and Welsh, 1963). Siva Prasad Rao and Ramana Rao (1979) reported an increase in the total proteins and free amino acid content in muscle, gill and liver tissues of methyl parathion exposed fish *Tilapia mossambica*. In vitro studies using labelled amino acids also confirmed increased synthesis of proteins in the various tissues of *Tilapia mossambica* exposed to malathion (Kabeer Ahamed, 1979).

Thus the increase in the levels of proteins and RNA observed in the present study could be due to the stimulation of protein synthetic machinery of the developing brain. An increase in the concentration of total proteins on methyl parathion exposure, in the present study appears to be specific to the stress caused by methyl parathion and reflects a pathological process leading to hyperfunctioning of neurons (Lajtha, 1971).
Table 8: Changes in the levels of total RNA in discrete areas of CNS of control and methyl parathion treated 2-day-old rat pups

<table>
<thead>
<tr>
<th>Region of the tissue</th>
<th>Control</th>
<th>MPT</th>
<th>Percentage in change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>2.476±0.215</td>
<td>3.99±0.302</td>
<td>+ 61.14 #</td>
</tr>
<tr>
<td>Brain stem</td>
<td>2.322±0.239</td>
<td>8.61±0.183</td>
<td>+ 55.46 #</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>1.794±0.240</td>
<td>2.44±0.261</td>
<td>+ 36.00 *</td>
</tr>
</tbody>
</table>

Values are (Mean ± S.D. of 6 observations) mg of RNA/gm wet weight of tissue.

Sign '+' indicates an increase over controls.

#  P > 0.005;  * P < 0.02.
Table 9: Changes in the levels of total RNA in discrete areas of CNS of control and methyl parathion treated 7-day-old rat pups

<table>
<thead>
<tr>
<th>Region of the tissue</th>
<th>Control</th>
<th>MPT</th>
<th>Percentage in change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>3.575±0.289</td>
<td>4.915±0.261</td>
<td>+37.32 #</td>
</tr>
<tr>
<td>Brain stem</td>
<td>3.388±0.321</td>
<td>4.710±0.215</td>
<td>+40.68 #</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>2.692±0.215</td>
<td>3.440±0.289</td>
<td>+27.78 *</td>
</tr>
</tbody>
</table>

Values are (Mean ± S.D. of 6 observations) expressed as mg of RNA/g wet weight of tissue.

Sign '+' indicates an increase over controls.

# P > 0.005; * P > 0.02.
Table 10: Changes in the levels of total proteins in discrete areas of CNS of control and methyl parathion treated 2-day-old rat pups

<table>
<thead>
<tr>
<th>Region of the tissue</th>
<th>Control</th>
<th>MPT</th>
<th>Percentage in change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>30.5±2.33</td>
<td>54.5±4.10</td>
<td>+ 78.69 @</td>
</tr>
<tr>
<td>Brain stem</td>
<td>30.0±1.32</td>
<td>39.0±3.61</td>
<td>+ 30.00 @</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>32.25±2.2</td>
<td>38.5±1.9</td>
<td>+ 19.38 *</td>
</tr>
</tbody>
</table>

Values are (Mean ± S.D. of 6 observations) are expressed as mg protein/g wet tissue.

Sign '+' indicates an increase over controls.

@ P < 0.005; * P > 0.01.
Table 11: Changes in the levels of total proteins in discrete areas of CNS of control and methyl parathion treated 7-day-old rat pups

<table>
<thead>
<tr>
<th>Region of the tissue</th>
<th>Control</th>
<th>MPT</th>
<th>Percentage in change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>33.5 ±2.96</td>
<td>63.5 ±4.10</td>
<td>+ 89.55 @</td>
</tr>
<tr>
<td>Brain stem</td>
<td>32.25 ±2.76</td>
<td>40.25 ±3.34</td>
<td>+ 24.81 *</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>34.0 ±2.40</td>
<td>38.12 ±2.90</td>
<td>+ 12.12 *</td>
</tr>
</tbody>
</table>

Values are (Mean ± S.D. of 6 observations) expressed as mg protein/gm wt. of tissue.

Sign '+' indicates an increase over the controls.

@ $P < 0.005$; * $P > 0.01$. 
Table 12: Changes in the body weights of control and methyl parathion treated developing rat pups

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>MPT</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>9.4±0.4</td>
<td>10.2±0.6</td>
<td>+ 8.3 *</td>
</tr>
<tr>
<td>7</td>
<td>14.3±0.5</td>
<td>15.8±0.6</td>
<td>+ 10.8 *</td>
</tr>
</tbody>
</table>

Values are expressed in 'g' are Mean ± S.D. of 25 observations.

Sign '+' indicates an increase over controls.

* P > 0.001.