4. DISCUSSION
The results of the above experiments clearly demonstrate that biogenic amines, amino acids as well as alpha-ketoacids are unevenly distributed in cerebrum, cerebellum and brain stem of control rats. The concentration of biogenic amines has been found to be of the order of 5-HT, NE, DA, whereas, the regional distribution of free amino acids was glutamic acid, taurine, aspartic acid, GABA & glycine in all the three above mentioned regions of the brain. Exceptionally, the concentration of glycine was observed to be slightly higher than that of GABA level in cerebrum. Additionally, the present observations indicate that the distribution of alpha-ketoacids in the given areas of the brain was alpha-ketoglutaric acid, oxaloacetate & pyruvic acid. In accordance with these observations, there are various reports showing heterogeneous regional localization of amino acids, biogenic amines, and alpha-ketoacids in the brain. This regional heterogeneity in the distribution of amino acids and monoamines, seems to be of great importance with the view that this may have some implications in respect to their functional role as neurotransmitters. Since, alpha-ketoglutarate and oxaloacetate are the intermediates of tricarboxylic acid cycle, and pyruvate is an intermediate of anaerobic degradation of glucose, the uneven regional distribution of alpha-ketoacids indicate that the general metabolism of glucose and amino acids appear to be regionally compartmentalized in brain. Furthermore, the changes produced by lithium ions are again variable in respect to the different regions of the brain. These observations altogether suggest, that each region of the brain tends to function as an isolated metabolic compartment.
4.1. **Bioamine amines**: Both the catecholamines, dopamine and norepinephrine, have been found to be reduced in all the brain regions when the rats were injected for two weeks with a daily intraperitoneal dose of 3 m eq lithium/kg body weight. With other doses the decrease was, however, confined to one or two regions of the brain. The finding is partially supported by the observation of Schubert\(^{117}\), indicating that lower doses of lithium do not affect the synthesis and turnover of catecholamines in the brain. For the depletion in the steady state level of dopamine, and norepinephrine, several possibilities may exist. Either lithium inhibits the synthesis or stimulates the breakdown process of these amines in the brain. Furthermore, these metal ions might be associated with some changes in the release and uptake processes of the catecholamines in the brain.

In support to our finding regarding dopamine, the lithium administration by intraperitoneal injections for 14 days has been shown to decrease the striatal dopamine synthesis by Friedman and Gershon\(^{85}\). Some other studies\(^{159,160}\) have also shown that 2-3 weeks of lithium treatment reduces dopamine turnover and synthesis in whole brain. But, Hesketh *et al.*\(^{161}\) studied dopamine and its metabolites in rats giving lithium chloride in diet. Their results showed an increase in homovanillie acid and 3,4-dihydroxyphenylacetic acid but no significant change in striatal dopamine concentration and the activity of tyrosine hydroxylase. In contrast to Hesketh *et al.*, our results show a diminution in
the steady state level of dopamine following chronic intraperitoneal injections. This might be due to the difference in the mode of administration, since in the experiments of Heketh et al., lithium was given orally. It is pertinent to note that also Friedman et al., administering lithium by i.p. route have reported a diminution of dopamine. Our results further show that prolonged lithium treatment produces an increase in the MAO activity. However, the enzyme does not show any response to these ions in short term lithium-treated rats, as well as in vitro. It is, therefore, suggested that lithium decreases the level of dopamine by inhibiting its synthesis as well as by increasing the rate of its monoamine oxidase influenced oxidative deamination. This increased deamination of dopamine might have resulted in the elevated levels of homovanillic acid and 3,4-dihydroxyphenylacetic acid (DOPAC) as observed by Heketh et al. The picture is, however, not entirely clear since some other investigators have failed to find out any change in the dopamine turnover in whole brain following chronic lithium treatment. Overall the mechanism by which lithium affects dopamine concentration can not be explained exactly because of the inconsistency of results of various workers on dopamine metabolism.

The decrease in norepinephrine level as observed in our experiments reflects a possible correlation with the decrease in dopamine concentration which acts as a precursor for norepinephrine synthesis in vivo. Simultaneously, there are several reports showing the increased reuptake and breakdown of
norepinephrine. Corrodi et al.\textsuperscript{165} observed an increased rate of
disappearance of brain norepinephrine after inhibition of tyrosine
hydroxylase in the lithium-treated animals. A shift in brain
metabolism of noradrenaline from extracellular methylation (by COMT)
towards intraneuronal deminination (by monoamine oxidase) has been
reported by Schildkrout et al.\textsuperscript{166}. Lithium-induced decrease in the
rate of release of H\textsuperscript{3}-norepinephrine was observed during electrical
stimulation of brain\textsuperscript{167}. Colburn et al.\textsuperscript{168} have reported enhanced
uptake \textit{in vitro} of H\textsuperscript{3}-norepinephrine into synaptosomes (pinched-off-nerve endings). This finding has not been confirmed, and there
is evidence that uptake of H\textsuperscript{3}-norepinephrine in intact brain\textsuperscript{166} or
brain slices\textsuperscript{167} may not be altered by prior treatment of rats with
lithium chloride. However, these observations together suggest
that lithium may tend to keep NE in a presynaptic location, while
increasing catabolism, particularly by oxidative deminination\textsuperscript{169}.
The view of Shaw\textsuperscript{170} that lithium generally causes an increase in
intraneuronal metabolism together with increased reuptake, seems
to be closer to our findings. Since we have observed an increase
in the MAO activity following chronic lithium treatment, this might
reflect a support for the increase in intraneuronal metabolism
after lithium treatment as suggested by Shaw\textsuperscript{170}. Though the results
of various workers are very much conflicting, even it can be
reasonably assumed that decrease in the level of norepinephrine
seems to be primarily related to the decrease in the concentration
of its precursor, the dopamine. Whereas, the increased reuptake
of norepinephrine\textsuperscript{168}, and its intraneuronal breakdown\textsuperscript{169}, following
stimulation of MAO, in lithium-treated rats might be equally
responsible for this decrease. The net effect of lithium ions
would be decreased availability of dopamine and norepinephrine at the central synapses, an interpretation consistent with the antismanic effect of lithium as propounded by Schildkraut\textsuperscript{171}.

The decrease in dopamine and norepinephrine might be secondary to the lithium-induced increase in the level of aspartic acid (as observed in our experiments), which has been reported to diminish the level of these amines when administered to the experimental animals\textsuperscript{183}.

On the other hand, during this investigation the behaviour of indolealkylamines, the 5-HT, has been observed to be quite different from the catecholamines. The level of this amine shows elevation in cerebellum following the prolonged treatment of rats with 3 m eq lithium/kg body weight. However, the increase was observed in cerebellum as well as brain stem when the rats were acutely treated with these metal ions. The finding is in accordance with the earlier report of Perez-Card et al.\textsuperscript{172}, that repeated administration of lithium salts, increases the brain hydroxyindoleacetic acid by about 80 per cent and brain serotonin by 15-20 per cent. Also the report of Sheard and Aghajanian\textsuperscript{32} is in agreement with our observation. This shows an elevation in 5-HT metabolism following lithium administration. The elevation in serotonin concentration is suggested to be the secondary effect of lithium-induced increase in brain and plasma level of tryptophan, which on the other hand, promotes the rate of synthesis of 5-hydroxytryptamine\textsuperscript{172}. The plasma/brain level of tryptophan has been reported to be 5:1, by McGeer et al.\textsuperscript{173}. This was further
suggested by Perez-Cruet et al. that lithium has a general effect on the amino acid metabolism and the increased brain level of this tryptophan possibly results from an increased brain permeability.

Our results show an increase in serotonin-level in cerebellum and brain stem during the acute study but following the administration of lithium for a longer period this amine was found to be elevated only in the cerebellum. Moreover, the magnitude of increase in cerebellum was higher following acute treatment than that observed in chronic lithium-treated rats. As regards the regional variations in the effects produced by lithium, nothing can be clearly suggested at present, and such observations need further elaboration. It is likely that these lithium-influenced changes in cerebellum and brain stem might be associated with the selective regional brain permeability to the tryptophan.

Our data on serotonin after lithium administration are in contrast to those of Corrodi et al. and Ho et al. The former authors found a decreased serotonin-depletion after inhibition of the amine synthesis with alpha-propyldopacemide ($H_{22-54}$) and suggested that lithium decreases the rate of serotonin synthesis. The possible explanation of discordance might be: 1) difference in experimental conditions; in the experiment of Corrodi the lithium was added to the food, 2) the dose of $H_{22-54}$, which blocks serotonin synthesis in normal rats.
might not completely inhibited the synthesis in lithium treated rats, 3) \( \text{H}_2\text{O} \) which inhibits both tryptophan hydroxylase and tyrosine hydroxylase as reported by Carlsson et al., also depletes brain catecholamines, which in turn influence the rate of serotonin synthesis. On the other hand the latter authors found that prolonged administration of lithium to the rats produces a marked reduction in 5-HT levels in hypothalamus and brain stem but no significant changes in the other brain regions. These authors also found that lithium treated animals show a decreased accumulation of brain 5-HT after monoamine oxidase inhibition and suggested that lithium decreases the rate of serotonin synthesis. Since, these authors measured the rate of synthesis of 5-hydroxytryptamine by accumulation of this amine after pargyline, a decreased accumulation of 5-HT in lithium-treated animals may simply reflect a decreased storage capacity in serotonergic granules. Since monoamine oxidase accomplishes the transformation of serotonin to 5-hydroxyindoleacetic acid, the increase in the activity of this enzyme should have resulted into a decreased concentration of 5-HT. Therefore, lithium-induced elevation of 5-HT level in the cerebellum, is not in consonance with the aforementioned findings. The anomaly can be explained in the light of the earlier report that the synthesis rather than the breakdown of 5-hydroxytryptamine was more potently stimulated by lithium ions. This increase in the activity of MAO following the chronic treatment with lithium chloride might result in the increased intraneuronal demination of the amine which in turn result in the increased rate of synthesis of 5-HT as reported by Sheard and Aghajanian.
4.2. **Amino acids:** A considerable literature exists on the pools of free amino acids in brain tissues. Free amino acids are of considerable interest as the source from which proteins and neurohormones are synthesized and as that to which the end products of their degradation return. Free amino acids also participate in the regulation of metabolic homeostasis, are part of the ionic environment, and act as substrates for oxidative phosphorylation. Most amino acids have some physiological and pharmacological actions at various levels of the whole nervous system, and some of them are accepted as neurochemical transmitters.

Much attention has been focussed in recent years on the metabolic transformations of glutamate, for which brain tissue is better equipped enzymatically than other tissues of the body. Glutamine serves as a depot for glutamate in brain. Glutamate can be formed from alpha-ketoglutarate and ammonia by the action of glutamate dehydrogenase. Aspartic acid is formed from glutamate by the action of glutamate-oxaloacetate transaminase, whereas, GABA is formed from glutamate via the reaction of glutamate decarboxylase. Glutamate can enter the citric acid cycle as a substrate liberating ammonia; conversely, glutamate and other amino acids can be synthesized from ketoacids and ammonia.

In our experiments the glutamic acid has been found to be lowered only in brain stem showing that lithium does not seem to significantly influence the steady state level.
of this amino acid by altering any major pathway related to synthesis and breakdown of this amino acid. The decrease in glutamic acid might be secondary to its conversion to GABA or biosynthesis of glutamine by transformation of glutamic acid or its utilization in the synthesis of aspartate. The present results show an increase in the level of GABA and aspartic acids in cerebrum, cerebellum and brain stem of lithium-treated rats. But it is not possible to correlate this finding with the decrease in glutamate concentration in brain stem, because in such a condition the depletion should have been observed in all the regions of the brain. Additionally, increased aspartic acid level in the brain of lithium-treated rats might have in turn decreased the level of glutamate, since the administration of aspartic acid to the animals has been reported to cause a significant decrease in the levels of several amino acids including glutamate. Various conflicting results are reported in literature regarding the brain concentration of glutamate. Some of the reports showing lithium-induced decrease in the brain glutamate, are supporting our results. Contrariwise, lithium has also been shown to increase the level of glutamic acid in the brain. At this stage, nothing can be precisely stated regarding the mechanism involved, with respect to the depletion of glutamate observed in our experiments. Simultaneously, glycine has been found to be elevated only in the cerebrum during this study. This amino acid is known to be synthesized through the demethylation of
choline and is transformed to serine and threonine. The enhanced glycine level might be related to the demethylation of choline in order to provide a methyl group for several biological reactions such as in the metabolism of catecholamines during CGT influenced increase in the formation of DOPAC as reported by Hasketh et al. Presently, it is very difficult to assess exactly the factors responsible for the decrease in glutamic acid and increase in glycine level, in only one out of three regions of the brain studied in this investigation. The selective regional permeability of these amino acids or their metabolites in the brain may be one of the possible mechanisms.

The increase in the level of oxaloacetate and alpha-ketoglutarate as observed in our experiments shows that lithium increases the rate of tricarboxylic acid cycle by increasing the concentrations of the intermediates involved in the cycle. Because all the reactions of the citric acid cycle are freely reversible, an increase in the level of two of its intermediates, might reflect a proportional increase in the concentration of all the metabolites taking part in the cycle. Furthermore, most of the amino acids are known to be cataolised to intermediates of tricarboxylic acid cycle. Therefore, the increase in alpha-ketoglutaric acid and oxaloacetic acid might be related to the lithium-induced changes in the general metabolism of amino acids as suggested by Perez-Crust et al. On the other hand, lithium has been found to increase glucose uptake in isolated
rat muscle. In experiments with acute administration of large dose to the rats, lithium was found to increase both the uptake and the concentration of glucose in the brain. Furthermore, lithium has been shown to increase brain glucose concentration and the metabolites metabolically close to glucose, such as glucose-6-phosphate in the glycolytic chain, and 6-phosphogluconate in the pentose phosphate pathway, were also reported to be increased. It is possible that the increase in the brain levels of alpha-ketoglutaric acid and oxaloacetic acid might be secondary to the lithium-induced increase in the steady state level of glucose in the brain.

Additionally, in the experiments carried out by us, the in vitro as well as in vivo activities of glutamate-oxaloacetate transaminase, were observed to be increased by lithium ions in cerebrum, cerebellum and brain stem. Since this enzyme is responsible for carrying out the transformation of oxaloacetate to aspartic acid, the increase in brain aspartic acid concentration as observed by us, is likely to be secondary to the lithium-influenced elevation of oxaloacetate and its rapid transformation to aspartic acid by preactivated glutamate-oxaloacetate transaminase. The view is further supported by the coincidental observation, that the increase in the level of aspartic acid as well as activation of glutamate-oxaloacetate transaminase have been observed only after prolonged administration of lithium chloride. Whereas, both these changes were absent in the acutely treated rats.
In contradistinction to other ketoacids, the level of pyruvic acid was found to be reduced following chronic as well as acute treatment of rats with lithium chloride. There might be several possibilities for the decrease in the level of this ketoacid, such as: i) its increased entry into tricarboxylic acid cycle, ii) activated conversion to oxaloacetate through the action of pyruvic carboxylase, iii) increased transformation to lactate or alanine, iv) its reduced formation from glucose through glycolytic pathway. The first and second assumptions seem to be more convenient in this regard, because they are partially supported by our results showing that lithium induces increase in the brain levels of alpha-ketoglutaric acid as well as oxaloacetate. The pyruvic acid formed from glucose is likely to be utilized at higher rates as a substrate of TCA cycle or as a substrate for pyruvic carboxylase to form oxaloacetate. The increased formation of oxaloacetate will itself result in the increased rate of TCA cycle as mentioned previously. The third alternative seems to be less convincing because of the finding of Fleige\textsuperscript{190} regarding the lithium-induced decrease in the brain lactate concentration after one hour of the injection to the experimental animals. Moreover, the enzyme, glutamate-pyruvate transaminase which brings about the formation of alanine from pyruvate, did not show any response to the lithium ions in vitro or in vivo, during the present investigation. Therefore, the increased transformation of pyruvate to alanine also seems to be insignificant. Alternately, lithium may block the synthesis of pyruvate from glucose by inhibiting some step in the glycolytic pathway. But the reports of various workers have shown
that lithium have stimulatory effect on the glucose metabolism\textsuperscript{106, 107}, glycogen synthesis\textsuperscript{108} and the phosphate uptake intracellularly\textsuperscript{109}. Therefore, it would not be appropriate to assume that lithium diminishes the level of pyruvate by decreasing its formation from glucose by triggering some step in the glycolytic pathway. Finally, it seems appropriate to suggest that decreased brain pyruvate level in lithium-treated rats might result through its activated utilization in TCA cycle. Furthermore, the rate of consumption of pyruvate seems to be much higher than the rate of its formation in the brain.

The elevated level of GABA in our experiments might be related to lithium-influenced activation of glutamate decarboxylase or inhibition of GABA-transaminase. The possibility of interaction of lithium with the transamination process is more likely, because the long term increase in GABA has been reported\textsuperscript{51} to decrease the brain synthesis of the former enzyme. Since GABA is a well known inhibitory neurotransmitter\textsuperscript{23b}, the increase in its steady state level is likely to be connected with the therapeutic effect of lithium in manic symptoms.

4.3. Enzymes: The polar side chains of protein molecules lead to interaction with other electrolytes in solution, and it is, therefore, natural that metal ions often have a pronounced effect on the catalytic activity of proteins. The various roles that metal ions may play in enzymic reactions range from weak ionic strength effects to highly specific associations in, for example, these enzymes\textsuperscript{192}. In the latter group, the metal ions remain firmly bound to the enzyme itself and participate in the enzyme reaction. In the absence of metal ions the activity of these metal containing enzymes approaches zero. Another group comprises
the so-called metal ion-activated enzymes. A good deal of speculation and many hypothetical reaction mechanisms have been advanced for enzymes belonging to this group. Several metal ions have been described as positive and negative modifiers of various enzyme systems\textsuperscript{191-196}. It is further known that metals form a part of active site of the enzyme\textsuperscript{193,194}. Additionally, they are known to interact also with the allosteric sites leading to modulation of the enzyme activity\textsuperscript{194,195}. In some cases metals are found to chelate some regulatory substances like amino acids thus alters the activity of enzymes\textsuperscript{191,192}. In this investigation the effect of lithium ions on various enzymes has been studied. Since metals may act as modifiers of enzyme activity in several ways, it is difficult to assess the exact nature of action of lithium ions.

The results show an increase in the activity of monoamine oxidase in cerebrum, cerebellum as well as brain stem with 3 m eq lithium/kg body weight. However, this stimulation has not been detected in acute as well as in vitro studies. Previous report\textsuperscript{152}, revealing no changes in the MAO activity in vitro when the enzyme prepared from beef renal cortex mitochondria, in accordance with our finding. Moreover, lithium carbonate at concentration of $1.1 \times 10^{-4}$ - $1.1 \times 10^{-3}$M, has recently been reported to stimulate monoamine oxidase in rat brain homogenates by 33-44 per cent\textsuperscript{174}. Although this report is in contradiction to our finding as well as some of previous reports\textsuperscript{152} but this shows a responsiveness of monoamine oxidase to lithium. The conflicting results seem to be due to the different experimental
conditions, the substrates and methodology. The increase, in the activity of monoamine oxidase in our case only after prolonged lithium injections, does not seem to be the direct interactions of these metal ions with the enzyme reaction itself because lithium ions were found to be ineffective in producing any change in the enzyme activity during in vitro experiments. Similarly the enzyme activity remained unaltered in the brain of acutely treated animals. Therefore, it seems possible that lithium-influenced changes in the normal level of some of the regulatory substances in the brain (as seen for amino acids and monoamines), might have affected the activity of monoamine oxidase in chronically treated rats. Or else, it is likely that lithium stimulates the synthesis of monoamine oxidase at the ribosomal level.

The activity of glutamate-oxaloacetate transaminase has been found to be increased in homogenates of different brain regions by in vitro addition of lithium chloride. However, this effect could not be seen in vivo, in cases where the animals were administered this metal ion according to the acute schedule. This might be due to the fact that the concentration of lithium in vitro remains much higher than that available in different brain regions following acute intraperitoneal injections. Furthermore, the GOT was found to be stimulated in all the specified brain areas after prolonged treatment with lithium. It is likely that the concentration of these ions in the brain of chronically administered rats, becomes sufficient enough to activate this enzyme. As regards the mechanism of lithium-induced activation, any of the aforementioned possi-
lities may exist. The in vivo activation of GCT in chronically treated rats is valuable in the sense, that this accomplishes the elevated levels of aspartic acid, which in turn may have some implications in respect to the therapeutic effects of lithium in manic depressive psychosis.

The results further show a decrease in the activity of acetylcholinesterase in the homogenates of cerebrum, cerebellum and brain stem of acutely lithium treated rats, while prolonged treatment by lithium does not change the enzyme activity. There is possibility that the metal ion modify the active conformation of the enzyme by interacting with some allosteric site. But this interaction seems to be transient and after a longer period of treatment the brain might have developed resistance to this effect of lithium ions. During in vitro experiments the enzyme did not show any response with lower concentrations of lithium ions but at higher concentrations there has been observed a significant decrease in the enzyme activity. Also, the aforementioned allosteric interactions of lithium ions with the enzyme might be, similarly responsible for the inhibition of acetylcholinesterase in vitro. Since acetylcholinesterase is a hydrolyzing enzyme the decrease in its activity should result in an accumulation of acetylcholine in acutely treated rats. Therefore, the present results contradict with the earlier observation of Kroll and Goldberg, which showed a depletion of brain acetylcholine in the rats acutely treated with lithium chloride. But lithium has also been found to inhibit the acetylcholine synthesizing enzyme, the cholineacetyltransferase in vitro. Our finding
regarding the inhibition of the latter enzyme are supported by the previous reports that lithium inhibits the release and synthesis of acetylcholine\textsuperscript{\textsuperscript{119},120}. It is tempting to explain the contradiction with the observation of Krell and Goldberg by presuming that synthesis rather than the breakdown of acetylcholine has been more significantly affected by lithium ions \textit{in vivo}. The decrease in the activity of acetylcholinesterase was about 40 per cent at 100 mM concentration of lithium. By further increasing the concentration, there was seen a tendency towards increase in the enzyme activity. That is at higher concentrations of lithium instead of inhibition an activation was observed. But this activation was statistically insignificant. This effect might be due to the development of higher ionic or osmotic concentrations at relatively higher concentrations of lithium (see $Na^{+}$, $K^{+}$-ATPase).

The present observations further indicate an increase in the activity of acetylcholinesterase \textit{in vitro}, when the assay mixtures were containing calcium and magnesium ions. The reports of Fries\textsuperscript{196}, Nachmansohn\textsuperscript{197} and others\textsuperscript{199-203} have also reported that calcium chloride and magnesium chloride increase the maximal rate of hydrolysis of acetylcholine. The extent of increase in the maximal rate, however, is not the consistent finding among the investigators\textsuperscript{197-203}. The reported variations are most probably due to the different sources of acetylcholinesterase, as well as different assay conditions and methods. Furthermore, in our experiments lithium has been found to
interfere with the magnesium and calcium-induced activation of acetylcholinesterase. Since lithium has been reported to increase the serum level of calcium and magnesium$^{102}$, some change produced by elevated levels of these ions, on the activity of acetylcholinesterase or other enzyme systems in vivo might be valuable pharmacotheapeutically.

The widespread occurrence of non-adrenergic, non-cholinergic nerves in autonomic nervous system of vertebrates, are well recognised$^{204,205}$. Evidence has been presented that purine nucleotide, probably adenosine-5'-triphosphate (ATP), is the neurotransmitter in some of these neurons$^{206-209}$ and they have, therefore, been termed purinergic$^{210}$. Lithium induced activation of $Na^+$, $K^+$-ATPase in cerebrum, cerebellum and brain stem as observed in our case in in vivo might have produced a reducing effect on the steady state brain concentration of ATP. Simultaneously a decrease in the activity of $Mg^{++}$-ATPase only in brain stem (about -35 per cent), might produce an elevation in the normal level of ATP. Since the former enzyme is more strongly activated by lithium ions, it can be said that probably a long term treatment of animals with the clinical doses of lithium is likely to diminish the level of ATP in brain; or at least the availability of this inhibitory transmitter at the synapses, where $Na^+$, $K^+$-ATPase possesses considerable activity. This in turn might be correlated with the therapeutic effect of lithium in manic depressive psychosis.
In support of our observation regarding Na⁺, K⁺-ATPase as mentioned above, the stimulation of this enzyme \textit{in vivo} has also been observed by Gutman \textit{et al.}\textsuperscript{211} after chronic treatment with lithium in the medulla and papilla of the kidney. Furthermore, \textit{in vitro} addition of lower concentrations of lithium to the brain homogenates has been found to stimulate the Na⁺, K⁺-ATPase, while the higher concentrations induce a decreasing tendency in the activity of this enzyme. Such a dual effect of lithium ions on the Na⁺, K⁺-ATPase is in accordance with the previous reports\textsuperscript{211,212}. The inhibition of this enzymatic activity in the kidney, medulla and papilla was observed in the guinea pig kidney with high concentrations of urea and sodium\textsuperscript{213}. This \textit{in vitro} inhibition of Na⁺, K⁺-ATPase at higher concentrations of lithium may, therefore, be due to the development of a higher ionic or osmotic concentration. The concentration of lithium which reaches to the different brain regions during intraperitoneal injections is not sufficient enough to produce inhibition of this enzyme (as observed \textit{in vitro} at higher concentrations). Therefore, the present observation regarding the stimulation of Na⁺, K⁺-ATPase by lithium ions \textit{in vivo} seems to be appropriate.

Previous studies of Tobin \textit{et al.}\textsuperscript{214} and Han \textit{et al.}\textsuperscript{215} indicate that lithium is capable of substituting for either sodium or potassium in certain of the reactions of isolated Na⁺, K⁺-ATPase. In particular, lithium stimulates Na⁺, K⁺-ATPase activity in the presence of sodium and relatively low concentration of potassium\textsuperscript{213}. This indicate that lithium stimulates the enzyme by replacing the sodium. In support of this view several reports\textsuperscript{215-217} exist in
literature showing that lithium and sodium share common characteristics with respect to their effects on isolated $Na^+$, $K^+$-ATPase. These workers also reported that lithium is incapable of fully activating the ATPase reaction in the presence of potassium, magnesium and ATP.

The \textit{in vitro} activity of $Mg^{++}$-ATPase is found to be lowered at higher concentrations of lithium, while at lower concentrations the inhibition of the enzyme has not been observed in the present investigation. The antagonistic effect of a particular metal ion against the other is determined by certain steric-chemical factors — the size and charge\textsuperscript{192}. Since $Mg^{++}$ ions are heavier, as well as having more electrical energy associated with them as compared with lithium ions, the replacement of former ions by the latter seems to be affected by some steric factors, at the lower concentrations of lithium chloride. However, at higher concentrations lithium overcomes this effect thereby the inhibition in the activity at $Mg^{++}$-ATPase was observed. On the other hand, the inhibition of this enzyme \textit{in vivo}, as observed in brain stem, is likely through the formation of some chelated compounds by lithium in this particular brain region.

Several investigators suggested that the antimanic action of lithium involves membrane $Na^+$, $K^+$-ATPase or its functional correlate, the cell membrane sodium pump\textsuperscript{214},\textsuperscript{218},\textsuperscript{219}. This hypothesis is primarily based upon the observation that sodium transport is altered in affective disorders\textsuperscript{210},\textsuperscript{225}, and that the enzyme system is sensitive to these cations\textsuperscript{214},\textsuperscript{215},\textsuperscript{217},\textsuperscript{227},\textsuperscript{232}.\textsuperscript{2}
The \textit{in vivo} activation of $\text{Na}^+$, $\text{K}^+$-$\text{ATPase}$ in cerebrum, cerebellum and brain stem and inhibition of $\text{Mg}^{++}$-$\text{ATPase}$ in brain stem might, therefore, bear some relation to the \textit{psychopharmacological action} of lithium ions.

Current hypothesis about mania and depression is that they correspond to greater or lesser availability of biogenic amines at central synapses\textsuperscript{121} or to changes in electrolyte distribution\textsuperscript{233}. The mode of action of lithium salts, which seem to be effective at least in mania, has been related to both the hypotheses\textsuperscript{101}.

Several amino acids are known to act as \textit{excitatory and inhibitory neurotransmitters}. GABA, glycine and taurine function as the \textit{inhibitory transmitters}, whereas, glutamic acid and aspartic acid are the \textit{excitatory transmitters} in the mammalian brain and spinal cord\textsuperscript{234}. On the other hand, biogenic amines such as dopamine, norepinephrine and 5-hydroxytryptamine are well known as \textit{excitatory neurotransmitters}. Though nothing can be said exactly about the \textit{psychopharmacological action} of lithium ions, it can be surmised that lithium acts in a \textit{biphasic manner}\textsuperscript{113}; on the one hand it relieves hyperexcitation - the manic phase of manic depressive psychosis, while on the other hand this cation is partially helpful in relieving depression. It is likely that lithium exerts its \textit{therapeutic action} by alterations in the level of excitatory and inhibitory neurotransmitters. It has been found that prolonged administration of lithium, in the range of clinical doses, increases the level of GABA, glycine, aspartic acid and 5-hydroxytryptamine, thereby increasing the availability of these
transmitters at the neurons. The results of this investigation also indicate, a decrease in the level of norepinephrine, dopamine and glutamic acid. This effect, in turn, will decrease the availability of these neurotransmitters at the synapses. Furthermore, the *in vivo* activation of Na⁺, K⁺-ATPase might reflect the decreased availability of ATP at the site of purinergic neurons. It is suggested that lithium-induced increase in the availability of GABA, glycine (inhibitory transmitters) together with the decreased availability of excitatory transmitters such as dopamine, norepinephrine and glutamic acid might reduce hyperexcitability of neuronal structures during the excitatory phase of manic depressive psychosis. Whereas, the increased availability of 5-hydroxytryptamine and aspartic acid (excitatory neurotransmitters) might alleviate depressive phase of this disease by inducing excitatory effect on certain sluggish neuronal structures.