INTRODUCTION

The past decade has seen an enormous proliferation in the number of publications dealing with 'biology and pharmacology' of lithium ion. The recognition of lithium as a substance of biological importance can be traced to 1949, the first year in which 14 publications appeared in the literature (Johnson and Cade, 1975), although lithium itself was discovered in 1817. However, the specific use of lithium can be traced back to as early as 2nd century A.D. (Kline, 1973). He has noted that the first indication of the antimanic properties of lithium salts may be found in the writings of Soranus of Ephesus, who lived in early 2nd century A.D., as recorded by Caelius Aurelianus in the fifth century A.D. Though lithium made a brief
appearance in psychiatric usage in the late nineteen twenties, it was not until 1949 the specific anti-manic properties of lithium were established when J.F.J. Cade in Australia was conducting studies to test a hypothesis regarding the etiology of manic-depressive illness. The solubility of lithium urate led Cade to give animals a lithium salt in order to decrease the nephrotoxicity of uric acid in his search for a connection between purine metabolism and behaviour. Serendipitously, he noted a quieting effect in the animals and so was encouraged to try lithium clinically. In 1949, he reported striking responses among severely disturbed manic patients.

After Cade's report (1949) which led to the pharmacological rehabilitation of lithium, the turning point in the fortunes of lithium therapy came in 1954 when Mogens Schou and his associates took up a study to examine the claim that lithium possessed a specific anti-manic action and reported the significant anti-manic potential of lithium in their first report (Schou et al., 1954). This report stimulated afresh the interest of clinicians in lithium therapy and opened up many different lines of investigation.
Initially lithium was used for its therapeutic action, and the possibilities of a prophylactic effect were not formally considered although the action was implicit in the reports. Later on Schou et al. (1954) noted briefly some degree of protection against a recurrence of the manic phase following lithium treatment.

It was not until 1963 that a report on the prophylactic effect on recurrent depression was published (Hartigan, 1963). At the same time as, and quite independently of Hartigan, Ba astrup also observed the similar effect of lithium (Baastrup, 1964). Since then many reports have been appearing which established fully two of the clinical uses:

i) the therapeutic use in mania; and

ii) the prophylactic use against relapses of mania and depression in recurrent manic-depressive disorder of both bipolar and unipolar types (Schou, 1968; Gershon, 1970; Birch et al., 1977; Quitkin et al., 1978; Coppen et al., 1978; Schou, 1979).

There is also fairly good evidence that lithium may be of use also in other affective disorders such as recurrent schizo-affective disorder (Smulevitch et al., 1974; Perris, 1978), some cases
of depression like nonmanic depression (Tupin, 1972) periodic pathological aggressiveness (Sheard, 1975; Goetzl et al., 1977; Miller, 1978), and even in tyrotoxicosis (Temple, 1972; Kristensen, 1976; Merry 1977). The research in lithium therapy has stimulated investigations in many other clinical and biochemical aspects also. Of those, an important study is to investigate the influence of lithium on carbohydrate metabolism in brain.

**Lithium effects on carbohydrate metabolism:**

An important field of investigation which came up due to the increased application of lithium in the treatment of manic-depressive illness, is lithium's involvement in a variety of side effects such as nausea (Schou et al., 1954; Furlong, 1973), vomiting (Cade, 1949; Strömgren and Schou, 1964; Dias and Hocken, 1972), diarrhoea (Cade, 1949; Prien et al., 1972), renal effects like polydispia and polyuria (Gutman et al., 1971; Brightwell et al., 1973), hand tremor (Schou et al., 1970), and many other neuromuscular manifestations such as ataxia (Rogers and Whybrow, 1971; Prien et al., 1972), choreoathetotic movements (Peters, 1949), muscular hyper-irritability and hyper active deep
tendon reflexes (Schou et al., 1968). Another important side effect is weight gain, which in some cases leads the patient to refuse to continue the lithium treatment (Plenge et al., 1969; Schou et al., 1970; Dempsey et al., 1976). The weight gain after prolonged lithium administration may be secondary to the lithium effects on several enzymes involved in the carbohydrate metabolism, on glycogen synthesis, on glucose tolerance and on hormonal balance (Mellorup and Raefaelsen, 1975). The mechanism of these side effects of lithium is not known, but could be traced to an action of the drug on the central nervous system (Glesinger, 1954) and could in part be related to the particular distribution and accumulation of lithium in the brain (Ebadi et al., 1974).

Shopsin and Gershon (1973) noted that the effect of the lithium ion on carbohydrate metabolism has been studied since 1924. A clear-cut relationship among a specific psychiatric illness, lithium and changes in carbohydrate metabolism could not emerge from investigations in man because of the diagnostic and methodological problems. However, data are accumulating to suggest that lithium treatment of psychiatric patients is accompanied by decreased tolerance to glucose (Heninger and Mueller,
1970; Shopsin, et al., 1972). Implications are that this disturbance is related, whether directly or indirectly, to physiological effect of the lithium ion rather than a specific psychiatric illness (Shopsin et al., 1972).

Many investigators have studied the influence of lithium on the metabolism of glycogen, glucose and other metabolites of carbohydrate metabolism in several animals. But very few studies have been conducted on these aspects in rat brain (vide table). Plenge et al. (1969) found that 500-1200 μmoles of lithium chloride (LiCl) injected intraperitoneally (i.p.) increases glycogen content of rat brain and diaphragm. Afterwards it was shown that 9-20 μ moles of LiCl injected intracisternally by the suboccipital route increases glycogen content after 2½ hr. only in the brain but not in the diaphragm (Plenge et al. 1970). In the same study it was also found that an increase in the brain glycogen content could be first observed 18 hr. after injecting 1200 μmoles of LiCl per rat. De Feudis (1973) also reported that lithium increases the glycogen deposition in brain.

Very few reports have been published so far on lithium effects on rat brain glucose metabolism.
Comparison of results of earlier reports on the effects of lithium on carbohydrate metabolism

<table>
<thead>
<tr>
<th>Dose (μmoles/rat)</th>
<th>Results</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Plenge et al. 1969 500 - 1200</td>
<td>Glycogen content increased after i.p. injection.</td>
<td>It was not specified if the dose was per kg body weight or per rat. Time after injection, when the change was observed in glucose content, was not given.</td>
</tr>
<tr>
<td>Plenge et al. 1970 9 - 20</td>
<td>Glycogen content increased 2 1/2 hr. after i.c. injection.</td>
<td>I.c. injection is of little importance in lithium experiments.</td>
</tr>
<tr>
<td>1200</td>
<td>Glycogen content increased. The effect was first found 18 hr. after i.p. injection</td>
<td>Serum [Li⁺] was not given at this particular dose.</td>
</tr>
<tr>
<td>Plenge, 1976</td>
<td>Acute doses Glucose activity increased 2 hr. after i.p. injection</td>
<td>Abstract only was available in which the dose was not specified. Acute doses result in serum Li⁺ concns which are higher than the pharmacological levels.</td>
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(contd...)
| Plenge, 1978 | 400 | Glucose conc. increased 1, 2 and 4 hr. after i.p. injection. G-6-P conc. increased 1 and 8 hr. after injection, but not after 2 hr. F-1, 6-P2 conc. increased after 2 1/4 hr. Glycogen conc. increased after 1, 2, 4 and 8 hr. of treatment. | Long-term treatment. No metabolite conc. was measured 12 hr. after injection. |
Plenge (1976) reported some acute lithium effects on rat brain glucose metabolism in vivo; the concentrations of the brain glucose, lactate, and glycogen were found increased 2 hr. after the injection of comparatively higher dose of LiCl. More or less similar observations were made in a long term study (Plenge, 1978). Brain glucose concentration was increased 1, 2 and 4 hr. after i.p. injection of LiCl, in rats maintained for 15 days at a dose level of 400 μmoles per rat. Increase in glycogen concentration was maintained even 8 hr. after the injection.

Although these studies report apparently similar observations, the parameters were greatly varied from one experiment to the other; for example dose range was varied, in some cases the doses of LiCl being very high and uncomparable with those levels usually employed during lithium therapy; dose schedule was also found varied from one study to the other. It is noted that in all these studies whole brain was used.

Lithium effects on enzymes related to carbohydrate metabolism:

Any effect of lithium on metabolism in general, might be primarily due to the fact that
certain enzymes are affected by this ion (Mellerup et al., 1974). A number of enzymes are being reported to be affected by the lithium. Among those are several belonging to, or related to carbohydrate metabolism.

In the absence of any specific study of lithium effects on glycolytic enzymes in rat brain, the observations of a few published reports which primarily dealt with either commercially available enzymes, purified enzyme preparations or enzymes in some systems other than brain are reviewed below.

Birch (1970) has pointed out a possible effect of lithium on magnesium-dependent enzymes. Subsequently he and his associates reported (1974) significant inhibition of pyruvate kinase, hexokinase and alkaline phosphatase at normal pharmacological lithium concentrations. Whereas the enzymes glucose-6-phosphate dehydrogenase and 3-phosphoglycerate kinase were unaffected at the same lithium concentrations. But all these enzyme studies were performed with the commercially available ones. Much earlier than this study, lithium has been reported to inhibit the activity of rabbit muscle pyruvate kinase (Kachmar and Boyer, 1953). But the concentration
of lithium used was 100 mmol/l, far in excess of the plasma level. Effects of physiological and pharmacological doses of lithium ion on three enzymatic reactions namely hexokinase, pyruvate dehydrogenase and L-alanine aminotransferase were investigated (Kadis, 1974) and it was reported that the results were in agreement with those of Birch et al. (1974).

There were also a few studies on the effects of lithium on some glycolytic enzymes in serum and red blood cells, the results of which contradict the above reports. Agar et al. (1975) reported that either 0.2 mM or 2 mM lithium did not affect the activities of phosphofructokinase, phosphoglycerate kinase and pyruvate kinase in red blood cells. But 2 mM lithium reduced enolase activity. Pscheidt and Meltzer (1975) also found no effect of lithium carbonate on serum pyruvate kinase activity in manic-depressive patients at the serum lithium concentration of 0.9-1.2 mEq/l. And it was also found that lithium concentration of 0.5, 1.0, 2.0 and 10 mEq/l in vitro did not inhibit the pyruvate kinase activity of six serum samples.
Mechanism of action of lithium on enzymes:

Birch (1970) proposed 'diagonal relationship' of lithium with magnesium and calcium. From this point of view he postulated that the 'normothymotic' effect of lithium (Schou, 1968b) might be due to an interference with the metabolism or binding of magnesium. Since many enzymes are magnesium dependent, several of the effects of lithium have been explained later on by its competition with magnesium and the other divalent cation, calcium (Birch, 1973). The inhibition of various nucleotide requiring, magnesium-dependent enzymes' activities was explained by the formation of 'magnesium-lithium-nucleotide complexes' (Birch and Goulding, 1975; Birch, 1975, 1974).

Apart from the interaction of lithium with magnesium, it has also been suggested that lithium competes with other monovalent cations such as Na⁺, K⁺, and NH₄⁺ for sites on an enzyme to alter the rate at which that enzyme processes its substrate (Suelter, 1976).

Lithium effects on brain electrolytes:

Since the lithium induced changes in carbohydrate metabolism result in changes in electrolyte
metabolism (Mellerup and Plenge, 1976; Mellerup et al., 1974) and also since lithium alters several calcium and magnesium dependent 'synthesis and release' functions of the neuron by competing with these ions (Lipton, 1978), the studies on lithium interaction with other monovalent cations such as Na+ and K+ in brain are also reviewed.

There is considerable evidence, from both in vivo and in vitro studies that sodium transfer takes place from intracellular and extracellular sites due to lithium (Ljungberg and Paalzow, 1969; Baer et al., 1970). King et al. (1969) in their in vivo studies showed that brain sodium concentration in LiCl-treated mice was lower than in untreated controls, but not significantly. However, Baer et al. (1970) found that chronic lithium administration to rats resulted in a significant decrease in the brain sodium concentrations. Similar findings were reported by Ho et al. (1970): chronic administration of lithium led to an initial rise of lithium and a concomitant decrease in intracellular sodium in the cerebral cortex. This is further supported by the findings of Kjeldsen et al. (1973) who reported that sodium ion levels in isolated slices of rat brain cortex are lowered when the tissue
is maintained in a medium rich in lithium ions. Birch and Jenner (1973) reported an in vivo decrease in whole brain sodium following lithium treatment of rats for about 28 days at a dose of about 1 mEq/kg body weight. Bond et al. (1975) and Edelfors (1975) also reported the decrease in brain sodium concentration after lithium treatment.

Effects on potassium concentration after lithium treatment are quite ambiguous. King et al. (1969) reported that brain potassium concentration and the uptake of radioactive potassium remained the same in lithium treated mice. But Ho et al. (1970) reported decrease in intracellular potassium in the cerebral cortex of rat and this was supported later on by the findings of Kjeldsen et al. (1973) that the presence of lithium ions in an in vitro nutrient medium caused a fall in tissue concentrations of potassium ions in isolated slices of rat brain cortex. The discrepancy in these results might be related to the species variations and/or different doses administered.

Similarly, reports on lithium effects on brain magnesium are controversial. King et al. (1969) reported significantly elevated magnesium concentration
after lithium in mice; whereas in rats, lithium apparently leads to a reduction in brain magnesium (Birch and Jenner, 1973).

These effects of lithium on the electrolytes could easily be hypothesized to result in several changes in the neural activity. In fact, many reports are available which show that lithium affects the neural activity of the nerve cells. It has been found that entry of lithium into cells is much higher than its exit. It exits from cells at up to only one eighth the rate of sodium which it thus tends to displace (Williams and Györy, 1976). It has also been reported that lithium interferes with the sodium pump (Giacobini and Stepita-Klauco, 1970; Ploeger, 1974). By interfering with the sodium pump, lithium might induce depolarisation (Giacobini and Stepita-Klauco, 1970). Membrane permeability to potassium can also be increased by lithium, even at intracellular levels to increase the membrane potential slightly and to stabilise the cell (Patridge and Thomas, 1976).

Controversies in Lithium research:

Lithium application in the psychiatry is a subject of controversy even after the appearance of several hundreds of reports in the medical journals
which clearly brought out the beneficial effects in manic-depressive patients. Till the beginning of this decade, even the therapeutic effect of lithium in mania has been questioned by Blackwell and others (1969). The controversy over the therapeutic effect in mania seems to be resolved when the Food and Drug Administration (FDA) of the U.S. Department of Health, Education and Welfare had approved clinical uses of lithium in 1970. Johnson and Cade (1975) pointed out that the approval was only partial since the FDA announcement stated that "... the sole indication for the use of lithium carbonate at this time is for control of manic episodes of manic-depressive psychosis..." and thus the prophylactic properties were not acknowledged or even mentioned. Later on, the prophylactic efficacy of lithium in recurrent bipolar affective illness has also been acknowledged by two U.S. Committees namely, the Neuropsychopharmacology Advisory Committee to the FDA in 1974 and the American Psychiatric Association (APA) Task Force on lithium therapy in 1975.

The current controversy in the lithium research is concerned with the prophylactic action
of lithium against unipolar depression. In concurrent with the FDA Advisory Committee's position, APA Task Force concluded, "there is persuasive evidence from controlled studies that it is also effective in unipolar depressive illness. However, the inexactness of the definition of unipolar illness and the relatively small number of patients studied to date indicate the need for additional evaluation of lithium in disease." Prien (1979) has mentioned that even after reexamination of the issue of lithium's effectiveness in unipolar illness, the FDA Advisory Committee concluded in 1976 that there was still insufficient evidence to warrant change in the approved indications for lithium therapy. Pioneers in the lithium research like Prof. Schou (1979) has compared the results of presently available reports on the prophylactic efficacy of lithium and cyclic antidepressants in unipolar affective illness, and opined that the evidence already available is sufficient to justify the prophylactic use of lithium in unipolar patients too.

Scope and aim of the present work:

It is known that the brain is almost entirely dependent on the utilisation of glucose for its
biochemical energy (McIlwain, 1959; Balázs, 1970; Schwartz et al., 1979) and thus glycolysis is the most important metabolic pathway in this tissue. Therefore, the present studies are aimed at to find out the effects of lithium on the activities of the key enzymes of glycolysis, namely hexokinase (ATP: D-hexose-6-phosphotransferase, E.C.2.7.1.1.) phosphofructokinase (ATP: D-fructose-6-phosphate 1-phosphotransferase, E.C. 2.7.1.11) and pyruvate kinase (ATP: Pyruvate phosphotransferase, E.C. 2.7.1.40). Manifestation of any seemingly undetectable effects can best be reflected in the refined gene expression like the synthesis and activity of key enzymes which are proteins.

Some of the reports on lithium distribution studies in brain indicate that lithium has been found distributed differentially among different parts of the brain (Ebadi et al., 1974; Bond et al., 1975; Edelfors, 1975). So, degree of changes (if any) in enzyme activities due to lithium administration may vary from one region of the brain to the other.

Moreover, it is well established that the brain tissue exhibits structural and functional heterogeneity. Hence, different regions of the
brain differ in their metabolic properties, thus establishing 'metabolic compartmentation' within the brain (Balázs and Cremer, 1973; Berl et al., 1975; Shaffi and Habibulla, 1977a, 1977b; Raju and Habibulla, 1979). So certain pattern of regional distribution in enzymatic activities could be observed in brain. If any such distribution is observed then the question arises how the differentially distributed lithium affects the regional distribution of the enzyme in the brain.

With these considerations the effects of lithium on carbohydrate metabolism in different regions of the brain ought to have been studied. But so far, very few reports on the effects of lithium on glucose metabolism (Plenge, 1976, 1978) and glycogen synthesis (Plenge et al., 1970) in the rat brain are available. All these studies have been conducted on the whole brains and no attempt has been made to observe the effects in different regions of the brain. And also, very few reports on the effects of lithium on hexokinase, phosphofructokinase, pyruvate kinase and some other glycolytic enzymes appeared in the literature (Birch, 1974; Kadis, 1974; Kachmar and
Boyer, 1953). Almost all of these studies are based on the studies either on commercial enzyme preparations or on partially purified enzymes (mostly of muscle preparations).

Therefore the present studies aim at firstly to find out the distribution of the activities of hexokinase, phosphofructokinase and pyruvate kinase in four gross anatomical regions of adult rat brain, namely, cerebral hemispheres, cerebellum, brain stem (medulla oblongata + pons) and the rest, the mid brain region (diencephalon), and secondly the effects of lithium on the activities of these enzymes and their regional distribution.