DISCUSSION

Regional distribution of the enzymes:

From various tables and figures given in the previous section, it is clearly evident that all the three glycolytic enzymes, namely, hexokinase, phosphofructokinase and pyruvate kinase have been distributed differently in different regions of the adult rat brain. When the enzyme activities in whole homogenates are taken into consideration, the pattern of regional distribution seems to vary for each enzyme. The distribution of hexokinase with the decreasing order of its activity is as follows: cerebrum, cerebellum, midbrain and medulla oblongata. This type of distribution pattern was not observed in the case
of phosphofructokinase. As already mentioned elsewhere, the highest PFK activity was found in the midbrain region followed by the cerebellum. However, the difference between the enzyme activities of these two regions is hardly one unit. The distribution pattern of PFK activity can, thus, be summarised as: midbrain cerebellum cerebrum medulla oblongata. Pyruvate kinase was also found differentially distributed in different regions of the brain. Its distribution pattern is: cerebellum cerebrum midbrain medulla oblongata. These variations in the distribution pattern of the enzymes of the same metabolic pathway, i.e. glycolytic pathway indicate that the ratios of the enzymes of the same pathway also vary from region to region. Buell et al. (1958) observed different enzyme activity ratios between phosphofructokinase and hexokinase in many regions of rabbit brain. A striking similarity in the various distribution patterns of the three enzymes in the brain is that in the medulla the enzyme activities are lower and in all other regions anterior to it the enzyme activities are higher.

It is generally accepted that a substance concentrated in a specific functional unit in the
brain must be closely related to the function of the region (Nishimura et al., 1963). It was suggested that the relative levels of glycolytic enzymes in a particular structure reflect its relative capacity for glycolysis (Sugden and Newsholme, 1973). Therefore, relatively lower levels of the three glycolytic enzymes in the medulla, as observed in the present studies, indicate that the region possesses relatively lower capacity for glycolysis when compared with the anterior regions of the brain like cerebrum, cerebellum and midbrain. Chesler and Himwich (1944a) found that the rate of glycolysis was higher in the cerebral cortex than in the brain stem. It was also found in the adult cats and dogs that the glycolytic rates were the highest in the caudate nucleus and cerebral cortex, with lower rates in each succeeding caudal portion of the neuraxis (Chesler and Himwich, 1944b). It was also observed in the same study that glycolysis of the medulla and cord decreased progressively with age, and so concluded that the part of the brain exhibiting the highest glycolytic rate advances in a rostral direction as growth proceeds. This might be one of the reasons for the higher activities of
hexokinase, phosphofructokinase and pyruvate kinase in the cerebrum and cerebellum of the adult rat. Maker et al. (1976) pointed out that those regions of the brain with higher metabolic requirements have higher activity of enzymes in glycolytic series and the citric acid cycle as well as higher levels of respiration. Therefore, higher activities of the glycolytic enzymes in the cerebrum and cerebellum, as observed here, indicate that the rate of glucose metabolism is higher in these regions, whereas, in the medulla the metabolic rate is very much lower.

The existence of differential distribution of the metabolic activity in the brain can be explained by correlating the differences in the metabolic activities observed in the gray and the white matter of the brain. Wilson and Wilkin (1977) found that white fibre tracts are notably low in hexokinase which reflects relatively low capacity for energy generation via glucose metabolism and suggested that the relatively higher levels of hexokinase in gray regions were due to relatively higher concentration of the enzyme in nerve terminal which are expected to be abundant in gray regions.
(Wilkin and Wilson, 1977). It was also found that the rate of glucose utilisation is higher in the gray matter than in the white and the local cerebral blood flow to the gray matter is greater than to the white (Sokoloff, 1975; Des Rosiers, 1974). Several studies in man and experimental animals showed that the white cerebral (or cerebellar) cortex consumes 3 to 5 times as much oxygen as the white matter (Seisjö, 1978; Himwich et al., 1941). Tyler and van Harreveld (1942) showed that oxygen uptake is the highest in the pallium and lowest in the medulla. All these studies indicate that the white matter possesses the lowest metabolic rate than does the gray. As it is known that the medulla possesses relatively the highest proportion of the white matter (Himwich et al., 1941) and the cerebral hemispheres and cerebellum are rich in the gray matter, it can probably be concluded that in the medulla the metabolic rate may be lower, where as in the cerebrum and cerebellum it may be higher.

It was observed in the present studies that the distribution of the particulate and the soluble hexokinase activities varied from one region to the other. It means that the ratio of the particulate
to the soluble hexokinase is not constant in all the regions of the brain. Several investigators pointed out that the particulate hexokinase is located in the neurones and the soluble fraction in the glial cells (Bigl et al., 1971; Kellogg, et al., 1974). If this assumption is taken into consideration, it can be proposed that the observed variations in the ratios of the enzyme activities between the particulate and the soluble fractions might be due to the regional variations in the neuronal to glial cell ratio.

Phosphofructokinase is known to be exclusively cytoplasmic enzyme (Kurata et al., 1972). Therefore, much of the enzyme activity has to be recovered in post-microsomal supernatent fraction. But the enzyme is quite unstable and after a longer period of centrifugation much of the activity will be lost. In the present investigations, the recovery of the enzyme in the cytosolic fraction is not satisfactory. Because of the heavy losses in the enzyme activity after the centrifugation, the cytosolic enzyme activity values obtained for each of the brain regions may not represent the exact in vivo values. Hence interpretation of the regional variations of the cytosolic PFK has been partially dealt with in the present studies.
Pyruvate kinase was mistaken as being present entirely in the cytoplasm of the nerve tissue (Johnson, 1960; Brunngraber et al., 1963). Later on, it was shown by Tamir et al. (1972) that in the cerebrum PK is present in microsomes, synaptosomes and synaptic vesicles as well as in the cytoplasm. Subcellular distribution of this enzyme in the other regions of the brain has not been done so far. The data of the present studies clearly suggest that pyruvate kinase is present in all the regions of the brain studied, in a membrane-associated form as well as in the cytoplasm.

Brain hexokinase is known to exist in two isoenzymic forms, namely, type I and type II. Type I is relatively heat-stable when compared to type II. However, in the absence of glucose, about 20% to 30% of the initial activity of type I was lost after incubation for a period of about 5-45 minutes (Katzen and Schimke, 1965). It was found in the present work also that HK activity decreased in almost all the tissue extracts of all the brain regions up to 30% after 1 hr. incubation at 45°C. The activity decrease might be due to the loss of initial activity of type I. Thus,
HK activity found in all the brain regions consists almost totally of type I. In the particulate and the soluble fractions of the regions also type I isoenzyme is predominant. The barely detectable amount of type II in the whole brain which was shown on gel electrophoretograms (Katzen et al., 1970) might be too low to determine in the tissue extracts by the conventional spectrophotometric enzymatic assays. Therefore, it may be concluded that the pattern of distribution of hexokinase in various regions of the brain is nothing but the distribution of type I isozyme.

**Lithium effects on the enzymes:**

It was observed in the present studies that lithium treatment at the dose of 1 mmol/kg body weight did not result in any significant change in hexokinase activity in any part of the brain studied. This observation differs with that reported by Birch et al. (1974) in their in vitro studies. This discrepancy might be due to the different type of the enzyme used in the later study. As already mentioned elsewhere, Birch et al. (1974) used commercially available enzyme which is most probably rabbit muscle hexokinase.
The lack of any effect of lithium on hexokinase might be because the dose used (1 mmol/kg) which is sufficient to maintain rats for longer periods without any toxicity, might be too low to affect particularly hexokinase activity in the brain 12 hr after the injection.

It was shown that the uptake of glucose into brain was enhanced shortly after the injection of lithium. As a result, the concentration of glucose in brain was increased 4 hr after lithium treatment. But at 8 hr the brain glucose concentration returned to normal level (Plenge, 1978). So it can be speculated that any effect of lithium on hexokinase might be primarily related to its effect on the uptake of glucose into the brain. Thus, even if any change in the enzyme activity would have occurred immediately after the lithium treatment due to increased glucose concentration, the effect must have been nullified 12 hr after the treatment because of the return to normal levels of glucose concentration.

It can be seen in the tables given in the 'results' section that hexokinase activities in the soluble fractions of all the brain regions increased in the lithium treated rats, though insignificantly.
Comparatively, cerebellum showed the highest increase in the enzyme activity (30%) and lowest being observed in the medulla oblongata (nearly 1%). The particulate fractions of all the brain regions, except that of the medulla, also showed very small increases in hexokinase activities. These minor increases in the enzyme activities, even though statistically insignificant, are not to be ignored since any minor change might have its own physiological importance in the nervous tissue.

It was reported earlier that lithium caused a significant accumulation in the particulate fraction of rat brain both in vivo and in vitro (Christensen, 1974), and more lithium was found in the soluble (post-mitochondrial) fraction than in the pellet (crude-mitochondrial) fraction (De Feudis, 1972). So the higher lithium concentrations due to accumulation in the particulate and soluble fractions might have caused the enzyme activity increases in these fractions.

It was clearly shown in the previous section that phosphofructokinase was affected in the lithium treated rats. The effect in some parts of the brain is quite different and opposite to that seen in the
other parts. As a result the normal regional distribution of the enzyme was altered. It is to be noted that of all regions of the brain, only cerebellum showed any increase in the enzyme activity both in the whole homogenate and the cytosolic fraction.

The cytosolic fractions of all the regions, except the cerebral hemispheres, showed a definite increase in the PFK activity. It may be improbable to speculate that these increases are due to higher lithium concentrations in the cytosolic fractions because, maintenance of a higher concentration of free Li+ in cytoplasm is unlikely (De Feudis, 1972).

While the present studies clearly indicate that lithium does affect PFK activity in the brain tissue, Agar et al. (1975) could not demonstrate any effect of either 0.2 mM or 2 mM lithium on PFK activity in red blood cells. The variations in these studies indicate, as mentioned elsewhere, that lithium might act differently on different types of the same enzyme.

Pyruvate kinase is inhibited by lithium in almost all subcellular fractions of all the regions of the brain and thus the effect being more consistent on this enzyme than hexokinase and phosphofructokinase.
Inhibition of PK activity was also reported by several other investigators, though rabbit muscle PK was used in their studies (Birch et al., 1974; O'Brien et al., 1977; Birch, 1978). Very recently inhibition of purified rat brain pyruvate kinase by lithium was shown (Birch et al., 1979).

It was already mentioned that the mitochondrial hexokinase activity was elevated to a considerable degree in the cerebrum, cerebellum and midbrain whereas the activity was decreased in the medulla after the lithium treatment. But the mitochondrial pyruvate kinase was found inhibited by lithium in all the four brain regions, the reduction of the activity being more striking. Similarly, lithium exerted quite different effects on the activities of cytosolic PFK and cytosolic PK. The activity of the cytosolic PFK was increased greatly in all the regions of the brain except in the cerebrum, where the decrease of the activity is very less. Whereas the cytosolic PK activity was increased only in the cerebrum and midbrain, the other two regions—cerebellum and medulla are showing decreased activity. All these effects in different subcellular fractions, which are quite different
from one another, caution that any single parameter like differential distribution of lithium in the subcellular fractions, if at all observed, may not explain completely the observed variations.

The quite diversified effects of lithium on the three glycolytic enzymes studied, which are all magnesium dependent, indicate that each one of these enzymes may be differently sensitive to lithium in different regions of the brain. These variations may be attributed in part, to the variations in the distribution of lithium and other electrolytes such as Na⁺, K⁺ and Mg²⁺, which occur after lithium treatment.

Several investigators demonstrated a dis-similar pattern of distribution of lithium after Li-treatment (Ebadi et al., 1974; Edelfors, 1975). Generally, lithium content was found higher in the cerebrum than in midbrain, cerebellum and medulla oblongata, the last one being the region with the lowest lithium content. These regional variations may be either due to different rates of penetration of lithium into different parts of the brain or selective extrusion of lithium from an area, and/or may be due to the varied rates of accumulation of
lithium throughout the brain (Edelfors, et al., 1974). Some of the effects of lithium on the enzyme activities in different regions of the brain, as observed in the present study, parallel with the regional variations of lithium. For example, in medulla oblongata, the changes observed in the total activities of hexokinase, phosphofructokinase and pyruvate kinase in lithium treated rats are less severe when compared with the changes in other parts of the brain. This effect may be because of lower lithium content in this region.

Since all the three enzymes are magnesium dependent, any change in the regional distribution of magnesium in the brain caused by lithium may also have a certain role in the occurrence of different changes in the enzyme activities in different regions of the brain. In fact, magnesium content of different regions of the brain was found decreased differentially after lithium treatment (Bond et al., 1975). Similarly, brain sodium was also decreased in different regions of the brain. An exact comparison of these electrolyte changes and the enzyme activity changes in each and every region of the brain is not possible because of the variations in
the brain dissection techniques; for example, in the present study hypothalamus is not separated from the rest of midbrain; whereas, Bond et al. (1975) dealt with the hypothalamus and midbrain minus hypothalamus separately. Moreover, to draw any corollary between the differential effects of lithium on the enzyme activities and the brain regional distribution of lithium, magnesium, sodium etc., right at this juncture involves uncertainty mainly because:

i) methods of lithium treatment varied from one study to another; and

ii) dosage schedule of the drug and the periods of treatment were also different in different reports.

From the results of the present work it may be concluded that lithium in therapeutic dosage (1 mmol/Kg body weight) may alter the carbohydrate metabolism by modifying the enzyme activities in the brains of 'normal' rats (i.e., rats not treated with any of the mood changing drugs which either induce hyperactivity or depression). It may also be suggested that not all the regions of brain would respond similarly to the lithium treatment. However, to get
a clear picture of the brain metabolic pathways in the presence of lithium, some more investigations on the changes in the substrate levels are warranted. The present investigation enables us to conclude that if the lithium dose is in the physiological range hexokinase activity is not significantly (statistically) altered. However, the other two key enzymes of the glucose metabolism, phosphofructokinase and pyruvate kinase do not appear to be unaltered. For this precise reason, it is suggested that more work is warranted before the 'harmless' nature of lithium as therapeutic agent is established firmly.