INTRODUCTION

I. CONTROL OF OXIDATIVE METABOLISM IN BRAIN

Brain requires a constant supply of oxidative substrates to support its activities. A characteristic feature of the carbohydrate metabolism in brain is the low carbohydrate store of the organ (Strang and Bachelard, 1971). The levels of intracellular glucose are also low (Lowry and Passonneau, 1964) and brain has little fuel reserves as carbohydrate. It therefore relies on using rapidly, glucose brought to it in the blood stream.

Neural tissues are aerobic and require glucose as the major substrate for energy metabolism (McIlwain and Bachelard, 1971; Siesjo, 1978). However, ketone bodies also form excellent substrates for brain (Patel et al., 1975; 1981; Roeder et al., 1984; Edmond et al., 1987). Although brain has the capacity to utilise ketone bodies much more efficiently than glucose (Sykes et al., 1986; Cordozo et al., 1986; Edmond et al., 1987) transport of ketone bodies to brain is the rate limiting step in their oxidation (Ruderman et al., 1974; Hawkins and Biebuyck, 1980). Therefore, glucose is the major substrate for cerebral energy metabolism under normal conditions. Although the intracellular content of glucose is the same in various
brain regions (Hawkins et al., 1979), the rate of glucose metabolism varies greatly (Sokoloff et al., 1977; Sokoloff, 1983). Since the intracellular level of glucose is low and the potential for glycolysis is very large, a control at the rate of glucose entry is considered to be the rate limiting step in glucose oxidation. This control is dependent on the level of plasma glucose and the level of glucose oxidation which is regulated by the intracellular redox states of the pyridine nucleotides and the concentration of key intermediary metabolites (McIlwain and Bachelard, 1971).

1. **Glucose transport into the brain:** Glucose is transported across the endothelial cells of the brain capillary by a Na⁺ and energy independent process, which is not regulated by insulin (Bachelard, 1983). Typical Michaelis-Menton kinetics for this transport with a \( K_m \) of 5-9 mM has been observed (Lund-Anderson, 1979). The glucose transport across the cerebral capillary walls are thought to be of low affinity (Pardridge and Oldendorf, 1975; Betz et al., 1979) and is primarily regulated by the plasma glucose concentration.

Diamond and Fishman (1973) showed the presence of a high affinity \( (K_m 0.25 \text{ mM}) \) system of glucose transport across the synaptosomal membranes. This was subsequently confirmed by Baquer et al. (1975). This process has been
shown to be insulin sensitive (Bachelard, 1980) and insulin at low intracellular glucose concentrations may promote its transport across the neuronal membranes. There is much evidence that glucose is transported more readily into the neurons than into the glial cells (Keller et al., 1975). In contrast to the synaptosomes, the cultured glioma and the astrocytes exhibit a low affinity system of glucose uptake (Cummins et al., 1979).

The pathways through which glucose is metabolised are the glycolytic route and tricarboxylic acid (TCA) cycle, the pentose phosphate pathway and the glutamate-GABA pathway. An integration of these pathways in brain is achieved through regulation and control of the enzyme activities by the concentration of key intermediary metabolites.

2. Glycolytic pathway: Under normal conditions, about 90% of glucose is metabolised by the glycolytic route and the TCA cycle (Wilson, 1983). The rate of glycolysis in the brain is controlled by the first step in glucose utilization i.e. phosphorylation, by the enzyme hexokinase. Although the potential activity of hexokinase is very high (Lowry and Passonneau, 1964) the rate of glucose phosphorylation proceeds only at 3-5% of the rate at which brain hexokinase is potentially capable (McIlwain and Bachelard, 1971; Hawkins and Mans, 1983). This is due to the inhibition of
the enzyme by its products D-glucose-6-phosphate (Ellison et al., 1974; Casazza and Fromm, 1976) and by ADP (Purich and Fromm, 1971). The control of the enzyme activity is also achieved by partitioning between the soluble and bound forms which differ in their kinetic properties (Knull et al., 1974; Wilson, 1978).

The second regulatory enzyme of the glycolytic pathway is phosphofructokinase, which is subject to regulation by the intermediates of glycolysis and the TCA cycle (Uyeda, 1979). Phosphofructokinase is also regulated by enzyme–enzyme interactions (Uyeda and Luby, 1974) and by phosphorylation/dephosphorylation mechanisms (Kagimoto and Uyeda, 1980; Furuya and Uyeda, 1980). Pyruvate kinase is another regulatory enzyme (Rolleston and Newsholme, 1967) regulated by its substrates and products (Schwark et al., 1971; Nicholas and Bachelard, 1974) and by phosphorylation/dephosphorylation mechanisms (Engstrom, 1978; Nieto and Castano, 1980).

3. **Tricarboxylic Acid (TCA) cycle**: The oxidation of pyruvate via the TCA cycle provides energy in the form of ATP. A secondary function of the pathway is the provision of carbon skeletons for the synthesis of amino acids like glutamate, GABA and aspartate and also citrate which is the major source of cytosolic acetyl CoA. The flux generating
step of the Krebs cycle is the pyruvate dehydrogenase reaction. Various intermediates regulate the activity of the enzyme by influencing the phosphorylation/dephosphorylation rates of the enzyme protein (Severson et al., 1974; Jope and Blass, 1975) which determines the rate of flux through the pathway. Another regulatory step in the Krebs cycle is at the level of the enzyme 3-oxoglutarate dehydrogenase which directs the 3-oxoglutarate metabolism either towards the synthesis of GABA or glutamine or further oxidation in the citric acid cycle. The enzyme is regulated by Ca\(^{2+}\), NADH/NAD\(^+\) and succinyl CoA/CoA-SH ratios (McCormack and Denton, 1981).

Flux through the glycolysis and TCA cycle normally shows a stoichiometric relationship, although the rate of glycolysis slightly exceeds that of the Krebs cycle under normal conditions (Hawkins and Mans, 1983).

4. **Glutamate-GABA pathway**: The presence of the glutamate-GABA pathway is unique to the brain tissue. GABA is synthesised by the decarboxylation of glutamate by the enzyme glutamate decarboxylase. Glutamate is synthesised from \(\alpha\)-ketoglutarate and it is estimated that approximately 10-40\% of the substrates metabolised through the Krebs cycle may be channelled via the glutamate-GABA shunt (McIlwain and
Glucose is also metabolised by the hexose monophosphate pathway, though it accounts for only a small proportion of the overall glucose utilization in brain. However, this pathway has been implicated in a variety of functions, essential for normal functioning of the brain. The significance of this pathway in brain is considered in detail in the following section.

II. FUNCTIONAL SIGNIFICANCE OF THE HEXOSE MONOPHOSPHATE SHUNT IN BRAIN

The hexose monophosphate (HMP) shunt or the pentose phosphate pathway (PPP) comprises of a series of reactions in which the two \( \text{NADP}^+ \)-dependent dehydrogenases namely, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) convert hexose-6-phosphates to pentose phosphates (the oxidative branch), followed by a sequence of reactions that interconverts pentose and hexose phosphates (the non-oxidative branch). The non-oxidative segment comprises of a series of rearrangements and transfers that produces 3,4 and 7 carbon sugar phosphates as intermediates. These reactions are essentially reversible and may operate in either direction. The oxidative branch is considered to be irreversible (Wood,
1986), although the reversibility of the first enzyme of this segment has been demonstrated (Horecker et al., 1953; Beutler and Kuhl, 1986).

The main function of the HMP shunt is the provision of pentose phosphates for nucleic acid synthesis and NADPH for reductive biosynthesis (Appel and Parrot, 1970; Eggleston and Krebs, 1974; Baquer et al., 1975). The activity of the HMP shunt is high in adipose tissues, lactating mammary glands, endocrine organs and liver, and lower in erythrocytes, skeletal muscles and in adult brain (Wood, 1986).

1. **Regulation of the HMP shunt**: A comparison of the equilibrium constant and mass action ratios has shown that glucose-6-phosphate dehydrogenase may be the control point in the oxidative segment of the pathway (Greenbaum et al., 1971). Transketolase has been identified as the rate limiting enzyme of the non-oxidative segment (Novello and McLean, 1968), although a reconsideration of this assumption suggests that transaldolase may also play this role (Wood, 1972).

The activity of the HMP shunt has been shown to be regulated by the demand for NADPH synthesis and by the
cellular requirements of pentose phosphates (Baquer et al., 1975; 1977; Larrabee, 1987). The ribose-5-phosphate for RNA synthesis is produced not only by the oxidative branch but also by the non-oxidative segment of the pathway which starts with fructose-6-phosphate and is independent of NADP⁺.

The oxidative segment of the pentose phosphate pathway is controlled by the rate of reoxidation of NADPH (Appel and Parrot, 1970; Eggleston and Krebs, 1974; Baquer et al., 1975; 1977; Kather et al., 1972). NADPH is known to be a potent inhibitor of G-6-PD and 6-PGD (Sapag-Hagar et al., 1973; Procsal and Holten, 1972; Fabregat et al., 1985) which is competitive with NADP⁺. The activity of the HMP shunt, therefore, depends upon the NADPH/NADP⁺ ratio and the flux through the HMP shunt at any time is dependent upon the rate of NADPH producing and utilising pathways. It is significant that the actual potential capacity of the NADPH producing pathways is several fold higher than the NADPH utilising pathways (Andres et al., 1980; Larrabee, 1987). The pathway responds to variations in the ratio of NADPH/NADP⁺ of total nucleotides with a correlation index of 0.999 (Fabregat et al., 1985). This affords an excellent short term regulation, providing a rapid response to the biosynthetic needs of the tissues.
2. **Distribution of the pathway in brain**: The activity of the HMP shunt as measured by the activity of the two dehydrogenases, namely, G-6-PD and 6-PGD were found to be highest in a group of nuclei in the brain stem region of rats especially nucleus coeruleus and raphe nuclei. Other regions of high activity were the molecular layer of cerebellum, the anterior horn and dorsal columns of the spinal cord (Kato and Lowry, 1973; Kauffman, 1972). The pathway is active in all the cell types of brain. In vitro studies on the cultured glial and neuronal cells have shown a high utilisation of glucose by the pentose phosphate pathway in all cell types, with glial cells accounting for higher utilisation than the neuronal cells (Larrabee, 1982; Sykes et al. 1986; Lopez-Cordozo et al., 1986; Edmond et al., 1987).

All the enzymes of the pathway are located in the soluble portion of the cell and the pathway is considered to be located in the cytoplasm. However, all the six enzymes of the pentose phosphate pathway have been detected in the large particle fraction from different rat tissues (Bagdasarian and Hulanicka, 1965; Baquer and McLean, 1972; Shatton et al., 1971). It is significant that brain contains the highest proportion of particulate enzymes among the tissues studied so far (Baquer and McLean, 1972; Baquer et al., 1975).
3. **Significance of the HMP shunt in brain**: The contribution of HMP shunt to overall glucose metabolism in brain has been a subject of controversy. Gaitonde and Evans (1982) have shown that glucose is utilised at a rate of 16.5 n mol/min/g brain tissue which is approximately 2.26% of the overall rate of 730 n mol/min/g tissue. This agrees with the value of 1-3% reported by others (Hostetler and Landau, 1967; Baquer et al., 1975). Considerably higher values of 5-8% and 21% were reported by Hostetler et al. (1970) and Moss (1964) respectively. However, the experimental protocols used by these authors have been criticised (Sacks, 1983).

Although the contribution of HMP shunt to overall glucose metabolism in brain is small, the effect of its inhibition are far reaching. This is amply demonstrated in experiments using the neurotoxic antimetabolite, 6-amino-nicotinamide (6-AN). 6-Amino-nicotinamide after administration, is converted in vivo to 6-amino analogue of NADP⁺(6-ANADP) which is a potent inhibitor of 6-PGD and causes a blockade of the HMP pathway in brain (Herken et al., 1969; Kolbe et al., 1976; Hothersall et al., 1981b). The other metabolic changes include a decrease in the utilisation of glucose by 16% (Gaitonde and Evans, 1982), decrease in the content of glutamate and GABA (Bielicki and Kreiglstein.
1976; Gaitonde et al., 1981) and tissue catecholamines (Jansson et al., 1977). The reason for the decrease in glucose utilisation by the glycolytic pathway is believed to be due to the inhibition of phosphoglucone isomerase by 6-phosphogluconate which accumulates in the brain of 6-AN treated rats (Lange et al., 1970; Kauffman and Johnson, 1974; Hothersall et al., 1981b). But this assumption has also been questioned (Gaitonde et al., 1983). These metabolic changes are also accompanied by neurological disorders, such as impaired sighting responses, anorexia, unsteadiness of gait and irreversible paralysis (Herken et al., 1969; Gaitonde et al., 1983). Although the effect of this anti-metabolite on the blockade of the pathway is well established, whether the accompanying disorders are due to the primary or secondary effect of the blockade is not well understood. However, these studies have served to emphasise the vital role of the HMP pathway in brain tissue.

The maximum potential activity of the HMP pathway determined with the use of artificial electron acceptor, phenazine methosulphate is about 80-fold higher than the normal value (Baquer et al., 1977; Hothersall et al., 1979). In the brain of newborn animals, the pathway was found to be obligatorily coupled to lipid synthesis during myelination (Burt and Wenger, 1961; Guerra et al., 1967; Larrabee,
An inhibition of the de novo synthesis of fatty acids and cholesterol was observed in cultured glial cells, when the HMP pathway was blocked by 6-AN administration (Sykes et al., 1986). The maximum excess potential changed from 30 fold in younger animals to 80 fold in the adult (Baquer et al., 1977). This changing role of the HMP pathway has been implicated in a variety of brain functions, including neurotransmitter metabolism (Appel and Parrot, 1970; Tabakoff et al., 1974; Baquer et al., 1975; 1977), maintenance of the membrane sulphhydril groups in the reduced state (Hotta and Seventko, 1968) and protection of neuronal membranes from peroxidative damage (Baquer et al., 1975; Hothersall et al., 1982; Zubairu et al., 1983).

Activation of the HMP shunt by neurotransmitters was first demonstrated by Appel and Parrot (1970). The biogenic aldehydes derived from the oxidation of monoamines by the activity of monoamine oxidase require NADPH for their further metabolism by aldehyde reductase (Tabakoff et al., 1974). This activation is secondary to the NADPH utilising system, where the reoxidation of NADPH increases the flux through the pathway.

A further link was proposed by Baquer et al. (1975) and Hothersall et al. (1982) suggesting that NADPH produced by the HMP shunt also serves an additional function of
protecting the membranes from peroxidative damage due to the appearance of the monoamine degradation product, H$_2$O$_2$. The link between the HMP pathway, monoamine metabolism and peroxide detoxification is shown in Fig. A. The vital role of the HMP pathway in synaptic endings is substantiated by the following findings:

Detection of all the component enzymes and intermediates of the pathway in synaptosomes (Kauffman and Harkonen, 1977).

Activation of the pathway by catecholamine neurotransmitters in cerebral cortical fragments (Barondes et al., 1961) and in synaptosomes (Appel and Parrot, 1970; Baquer et al., 1975).

Inhibition of monoamine oxidase by pargyline blocks the stimulation of HMP pathway by monoamines but not by the products of MAO (Baquer et al., 1977).

Inhibition of aldehyde reductase by barbiturates and 5-HIAA reduces the stimulation of the HMP shunt (Tabakoff et al., 1974).

Enhancement of the HMP shunt activity in brain slices by electrical stimulation (Kimura et al., 1974).

Inhibition of the HMP shunt by imipramine, an antidepressant (Kimura et al., 1974).
A mechanism linking the oxidative segment of the hexosemonophosphate pathway to the monoamine oxidation and $\text{H}_2\text{O}_2$ detoxification system in brain (Zubairu et al.; 1983).
Inhibition of NADPH-glutathione reductase by low concentrations of Zn$^{2+}$ partially blocks the neurotransmitter induced stimulation of the HMP pathway (Hothersall et al., 1982); and

Oxidised glutathione can replace neurotransmitters as a stimulant of HMP pathway in synaptosomes (Hothersall et al., 1982).

The existence of high potential activity and correspondingly high oxidative enzymes of the pathway (Hothersall et al., 1981a) suggests a role for neuronal protection mechanism against toxic effects of H$_2$O$_2$ and for the efficient removal of biogenic amines and their products. All these provide a compelling evidence for a close association between the HMP pathway and neuronal events connected with synaptic function.

III. EVALUATION OF THE ALTERNATIVE METABOLIC ROUTES BY THE USE OF DIFFERENTIALLY LABELLED [$^{14}$C] GLUCOSE

The use of [$^{14}$C] glucose labelled on different carbon atoms have been used to evaluate approximately the various alternative pathways of carbohydrate metabolism, namely, the glycolytic route and the TCA cycle, the pentose phosphate pathway and the glutamate-GABA pathway.
There are five important irreversible reactions that release $^{14}\text{CO}_2$ from labelled glucose. They have been used for the approximate evaluation of the alternative metabolic pathways. The fate of each carbon atom of glucose is illustrated for two different metabolic pathways, where glucose is converted to pyruvate and then through the TCA cycle (Fig. B) and where glucose is metabolised through the pentose phosphate pathway and then the pyruvate and the TCA cycle (Fig. C).

If the $^{14}\text{CO}_2$ yields from glucose molecules labelled on carbon atoms 1, 2, 3, 4 and 6 and from uniformly labelled glucose are obtained, then an approximation of the contribution of the various pathways can be made. The rate of $^{14}\text{CO}_2$ released at any particular time depends on the specific radioactivity of the precursor atom destined to form $^{14}\text{CO}_2$ and the flux through the decarboxylating reaction.

Hothersall et al. (1979; 1981b) described a system for the estimation of the flux of glucose through the alternative pathways using a wide range of specifically labelled glucose molecules. They are:

(i) The glycolytic pathway and pyruvate dehydrogenase reaction which release $^{14}\text{CO}_2$ from C-3 and C-4 of glucose.
FIG B: Fate of carbon atoms in various positions of glucose on metabolism by glycolysis and the TCA cycle. Fate of labelled carbons is shown in selected metabolites at key positions along the metabolic pathway. In TCA cycle, boxes indicate the next carbon to be released (Hawkins et al., 1985).
FIG C: Fate of carbon in various positions on glucose metabolism by the pentose phosphate and glycolytic pathways. It is assumed here that glucose is metabolised through the pentose phosphate pathway, then through glycolytic pathway to pyruvate and no recycling of the intermediary metabolites (e.g. fructose-6-phosphate) occurs (Hawkins et al., 1985).
(ii) The TCA cycle which in principle yields $^{14}\text{CO}_2$ from 1, 2, 5 and 6 of glucose in equal amounts.

(iii) The glutamate-GABA pathway where $^{14}\text{CO}_2$ from carbons 2 and 5 of glucose will be liberated at the isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase steps in the second turn of the TCA cycle, while carbons 1 and 6 will be retained in the glutamate formed from 2-oxoglutarate. The further decarboxylation of glutamate to yield GABA will also yield $^{14}\text{CO}_2$ preferentially from carbons 2 and 5 of glutamate, with carbons 1 and 6 being retained in the GABA moiety.

(iv) The pentose phosphate pathway where the flux of glucose through the 6-phosphogluconate dehydrogenase reaction releases C-1 of glucose in the first turn of the cycle and from C-2 in the second turn of the cycle.

(v) The glucuronate-xylulose route which yields $^{14}\text{CO}_2$ from C-6 of glucose. The activity of this pathway is very low in brain and hence not considered significant.

$^{14}\text{CO}_2$ obtained from C$_1$-C$_6$ gives the approximate activity of the pentose phosphate pathway and
$^{14}$CO$_2$ obtained from C$_2$-C$_6$ gives the approximate activity of the glutamate-GABA pathway.

Since the activity of the pentose phosphate pathway in brain is low, the recycling in the pathway as seen by the liberation of C-2 in the second turn of the cycle will be half of this already low rate, which is negligible under normal conditions.

The estimation of the various pathways by the above method is only an approximation, since a full evaluation would require a knowledge of the pool size, the specific activities of the various intermediates and the distribution of the pool sizes within the various cellular and subcellular compartments (Hothersall et al., 1979). The specific radioactivity of the labelled carbon also decreases by dilution, as the labelled compound passes through various intermediary metabolite pools. This is seen particularly in the exchange reactions of oxoacids and amino acids, which proceed at a higher velocity than the reactions of the TCA cycle. The labelled molecules are removed rapidly and are replaced by unlabelled molecules (Krebs, 1965; Balazs, 1969; Hawkins and Mans, 1983). This process lowers the specific radioactivity of the intermediary metabolites and retards $^{14}$CO$_2$ evolution.
This method of evaluation of the alternative routes is also subject to other limitations (Katz and Wood, 1963; Katz et al., 1966; Larrabee, 1978; 1987). This is particularly seen in the evaluation of the pentose phosphate pathway. Katz and Wood (1963) and Katz et al. (1966), introduced several models for the calculation of the relative contribution of the pentose cycle to glucose metabolism. An assumption underlying these methods was the complete isotope equilibration of glucose-6-phosphate and fructose-6-phosphate and the triose phosphates, which are in sufficient equilibrium under normal conditions. If the $^{14}\text{CO}_2$ yields from [1-$^{14}$C] glucose are twice or more than that from [6-$^{14}$C] glucose, then the errors which might arise from the incomplete isomerisation will be negligible and would still provide a fair estimation of the pentose cycle (Katz et al., 1966). Larrabee (1978; 1982) also provided a mathematical approach to calculate the various events of intermediary metabolism and the variations arising due to the differences in the extent of recycling in the pentose phosphate pathway, the partitioning of labelled glucose between the pentose cycle and glycolysis and between the pentose cycle and the TCA cycle.

In spite of the limitations stated above, this method provides a fair estimation of the relative contribution of the pathways of carbohydrate metabolism and has been applied
for studies both in vivo (Kamp et al., 1980; Sacks et al., 1983; Hawkins et al., 1985) and in vitro in normal (Hostetler et al., 1970) and in hormonal and drug induced conditions (Hothersall et al., 1981b; Sochor et al., 1984).

IV. NADP⁺-DEPENDENT DEHYDROGENASES IN BRAIN

1. Glucose-6-phosphate dehydrogenase: Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate : NADP⁺ 1-oxidoreductase EC 1.1.1.49) is the first enzyme of the oxidative segment of the pentose phosphate pathway. It catalyses the reaction:

\[ \text{D-glucose-6-phosphate} + \text{NADP}^+ \rightleftharpoons \text{D-glucono-6-lactone-6-phosphate} + \text{NADPH} \]

Although the reaction catalysed by glucose-6-phosphate dehydrogenase (G-6-PD) is thermodynamically reversible, it is rendered ineffectively irreversible by the rapid hydrolysis of D-glucono-6-lactone-6-phosphate. In addition to spontaneous hydrolysis, a widely distributed lactonase ensures the irreversibility of G-6-PD catalysed reaction under physiological conditions, although the reversibility of the G-6-PD reaction has been demonstrated (Horecker and Smyrniotis, 1953; Beutler and Kuhl, 1986). This enzyme has been a subject of intensive study due in part to the great number of variant forms characterised in human populations.
(Luzzato and Testa, 1978). The gene for human G-6-PD is located on the X-chromosome. It has been cloned from human hepatoma Li-7 and completely sequenced (Takikawa et al., 1986). The G-6-PD mRNA levels measured in tissues like liver, brain and muscle are roughly proportional to the enzyme activity and the enzyme may be subject to specific transcriptional regulation (Battistuzzi et al., 1985).

There is extensive literature on the purification and properties of glucose-6-phosphate dehydrogenase from microbial sources and mammalian tissues. Among the mammalian tissues, the enzyme has been purified and well studied from rat liver (Holten, 1972), pig liver (Kanji et al., 1976) bovine erythrocytes (Shreve and Levy, 1977), human erythrocytes (Yoshida, 1966; Cohen and Rosemeyer, 1969) and human adrenal, liver, platelets and erythrocytes (Shreve and Levy, 1977).

(i) **Kinetic properties:** The enzymes from mammalian sources catalyse an ordered sequential mechanism in which NADP⁺ binds first and is released last e.g. the enzyme from human platelets (Kosow, 1974), rat liver (Thompson et al., 1976) and pig liver (Kanji et al., 1976). Equilibrium dialysis studies have shown that approximately 2 moles of NADP⁺ bind per subunit and D-glucose-6-phosphate does not bind to the
free form of the enzyme (Holten et al., 1976). D-glucose-6-phosphate in the natural substrate for G-6-PD which gives the highest \( V_{\text{max}} \) and the lowest \( K_m \) for the enzyme. Most of the mammalian G-6-PDs prefer NADP\(^+\) and can utilise NAD\(^+\) only at very high unphysiological concentrations. This makes NADP\(^+\) the natural coenzyme for the enzyme reaction in vivo.

The kinetic constants for G-6-PDs from a wide variety of sources were summarised by Levy (1979). The enzyme has low \( K_m \) for NADP\(^+\) and \( K_i \) for NADPH and in vivo, where the ratio of NADPH/NADP\(^+\) is very high in most of the tissues, an almost total inhibition of G-6-PD can be expected. Therefore, in normal in vivo conditions, Eggleston and Krebs (1974) postulated that this enzyme in liver is almost 98\% inhibited. The inhibition of G-6-PD by NADPH is competitive with respect to NADP\(^+\) and the regulation of the enzyme activities by NADPH/NADP\(^+\) ratio therefore provides a fine control of the enzyme reaction and the HMP pathway in many tissues.

Besides NADPH, the enzyme is also regulated by other metabolites, the most important being ATP. ATP inhibition of numerous G-6-PDs have been reported (Yugari and Matsuda, 1967; Greenhouse et al., 1969; Levy, 1979). This provides an important regulatory feature of G-6-PDs in many pathological states where the energy metabolism is altered.
significantly. The enzyme is also inhibited by ADP (Beitner and Naor, 1972; Levy, 1979) and long chain acyl CoAs (Taketa and Pogell, 1966; Levy, 1979) and steroids (Raineri and Levy, 1970). Eggleston and Krebs (1974) have shown that oxidised glutathione and AMP can de-inhibit the G-6-PD reaction but its physiological significance is not clear.

(ii) **Multiple Molecular Forms of Glucose-6-phosphate Dehydrogenase**: Multiple molecular forms of G-6-PD in several mammalian tissues have been reported. The multiple enzyme forms include polymers of a single subunit differing in conformation and proteins derived from one polypeptide chain. In mammalian tissues, the multiple forms appear to be polymers of a single subunit (Hedrick and Smith, 1968; Levy, 1979).

Further investigations have shown that subunits of G-6-PD are composed of a single polypeptide chain and they are identical and catalytically inactive (Bonsignore et al., 1971). Two discrete polymeric states have been described (Cohen and Rosemeyer, 1969), namely the dimers and tetramers, both of which are associated with catalytic activity. The balance between the two active forms is shifted towards
tetramers at low ionic strength and pH (Bonsignore et al., 1970; 1971) and also by some divalent cations, while dissociation to dimers prevail at alkaline pH and high salt concentration (Cohen and Rosemeyer, 1969; Bonsignore et al., 1970; 1971).

Multiple forms of G-6-PD have been shown in mice liver (Hizi and Yagil, 1974) and in rat liver where forms differing with respect to both charge and molecular weight have been identified (Taketa and Watanabe, 1971; Schmukler, 1970). All the forms have been shown to be immunologically identical to one another and to other rat tissue G-6-PDs (Watanabe and Taketa, 1973).

Three forms of G-6-PD have been detected in mammary glands (Hilf et al., 1975, 1976). The relative proportion and amounts change during pregnancy and lactation (Hilf et al., 1975). In brain tissue, Pokrovskii and Korovnikov (1970) reported the presence of four isoenzyme forms of G-6-PD. Although it is agreed that the multiple molecular forms of G-6-PD are aggregates of identical subunits, the factors regulating the formation of different forms in vivo are not known.

(iii) Role of Glucose-6-phosphate Dehydrogenase in Brain:
Glucose-6-phosphate dehydrogenase has been implicated in diverse functions in brain tissue. The activity of G-6-PD
has been shown to be high in dorsal column (an area consisting of long myelinated fibres) which supports the concept that this enzyme serves a special function in lipogenesis in heavily myelinated tracts (McDougal et al., 1961; Luine and Kauffman, 1971). A 6-fold increase in G-6-PD activity has been found in the dorsal columns from day 1-15 after birth which coincides with the synthesis of cerebrosides (Dalal and Einstein, 1969). Similar studies by Burt and Wenger (1961) and Baquer et al. (1975) have shown that the activity of this enzyme is coupled to lipid synthesis.

The function of glucose-6-phosphate dehydrogenase and the oxidative segment of the pentose phosphate pathway of which the enzyme is a part of, has been highlighted in a previous section. The enzyme is thought to play a vital role in synaptic endings by providing reducing equivalents in the form of NADPH for neurotransmitter metabolism and peroxide detoxification (Appel and Parrot, 1970; Baquer et al., 1975; 1977; Zubairu et al., 1983). This is substantiated by the ready detection of G-6-PD in the synaptosomes (Kauffman and Harkonen, 1977). Thus glucose-6-phosphate dehydrogenase plays a crucial role by the virtue of its capacity to provide NADPH for various processes starting from myelination to other events connected with normal synaptic function.
(iv) **Hormonal Regulation**: Glucose-6-phosphate dehydrogenase in liver has been shown to be regulated through complex interactions of many hormones although the precise details of such interactions are not clear.

Activity of G-6-PD is increased by the injection of glucocorticoids (Bernadier et al., 1976; Wurdeman et al., 1978) and decreased by glucagon (Garcia and Holten, 1975). The effect of glucocorticoids in increasing G-6-PD activity was suggested to be due to their role in amplifying the effect of insulin on the enzyme (Kelley and Kletzien, 1984). Nakamura et al. (1982) demonstrated that an increase in protein synthesis accounted for the insulin induced increase in hepatic G-6-PD. The mechanism by which increase in protein synthesis is brought about is controversial. Sun and Holten (1978) suggested a modulation in the translational efficiency of mRNA encoding the enzyme, whereas other workers (Miksicek and Towle, 1982; Kletzien et al., 1985) have demonstrated a linear relationship between the rate of enzyme synthesis and mRNA level.

The activity of G-6-PD is also regulated by thyroid hormones (Glock and McLean, 1955; Miksicek and Towle, 1982). Miksicek and Towle (1982) suggested that thyroid hormones along with carbohydrate acted via an increase in enzyme synthesis.
A sex linked differential response of G-6-PD has been observed in a variety of hormonal manipulations. It has been observed that T₃ administration to the diabetic male rat could restore the level of enzyme activity to that of the normoglycemic animal, but it was much less effective in the female animal. In contrast, the administration of insulin to the normoglycemic animal increased the level of G-6-PD in the female rats but was without any effect on the male rats (Barton and Bailey, 1986). It is possible that diabetes indirectly affects sex hormone metabolism and sex linked effects, since the administration of T₃ to diabetic rats caused a restoration of the sex linked difference in G-6-PD levels.

No direct effect of thyroid hormones or insulin on brain glucose-6-phosphate dehydrogenase has been demonstrated. Schwark et al. (1972) showed that brain G-6-PD decreased in neonatally thyroidectomised rats which could be restored to normal by thyroid hormone administration. This effect of thyroid hormones was not shown to be present in the brain of the adult animals. Much remains to be learned about the effect of the hormones on glucose-6-phosphate dehydrogenase in brain tissue.

2. 6-Phosphogluconate Dehydrogenase: 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:NADP⁺ 2-oxidoreductase
(decarboxylating; EC 1.1.1.44) is the second dehydrogenase of the oxidative segment of the pentose phosphate pathway. It catalyses the reaction,

\[
\text{6-phospho-D-gluconate + NADP}^+ \rightleftharpoons \text{D-ribulose-5-phosphate + CO}_2 + \text{NADPH}
\]

Together with glucose-6-phosphate dehydrogenase, this enzyme is important for the provision of NADPH for reductive biosynthesis (Rognstad and Katz, 1979). The enzyme has been purified and studied in detail from several mammalian tissues including sheep liver (Villet and Dalziel, 1969; Dysen et al., 1973), rat liver (Procsal and Holten, 1972), pig liver (Toews et al., 1976), human erythrocytes (Pearse and Rosemeyer, 1974), rat brain (Sinicropi and Kauffman, 1979) and human brain (Weisz et al., 1985).

(i) **Kinetic properties**: The enzyme from human brain shows a random order of binding of the substrates, 6-phosphogluconate (6-PGA) and NADP\(^+\) for the forward reaction (Weisz et al., 1985). Weisz et al. (1985) proposed a mechanism of action for brain 6-phosphogluconate dehydrogenase as shown in Fig. D. Both 6-phosphogluconate and NADP\(^+\) bind to the enzyme randomly. Ribulose-5-phosphate and NADPH are released in random order with NADPH being the final product released.
FIG D: A proposed mechanism for human brain 6-phosphogluconate dehydrogenase (Weisz et al., 1985).
The apparent $K_m$ values for the mammalian 6-PGD for 6-phosphogluconate and NADP$^+$ are in the range of 4-23 $\mu$M for NADP$^+$ and 13-71 $\mu$M for 6-phospho-D-gluconate. The purified enzyme from human brain has an apparent $K_m$ of 3.7 $\mu$m for NADP$^+$ and 41 $\mu$M for 6-phospho-D-gluconate (Weisz et al., 1985). Both 6-phospho-D-gluconate and NADP$^+$ offer considerable protection against sulphydryl blocking agents indicating the presence of thiol groups in the enzyme essential for catalytic activity (Silverberg and Dalziel, 1973).

(ii) Regulation of enzyme activity: The activity of 6-phospho gluconate dehydrogenase is regulated primarily by its products. NADPH is a powerful inhibitor of the enzyme in brain which is competitive with NADP$^+$ and non-competitive with 6-phospho-D-gluconate (Weisz et al., 1985). The dissociation constant for the E-NADP$^+$ complex is similar to that of the E-NADPH complex and hence the activity of the enzyme is regulated by the NADPH/NADP$^+$ ratio of the cell (Toews et al., 1976; Procsal and Holten, 1972). The enzyme is also inhibited by its other products D-ribulose-5-phosphate and CO$_2$ (Weisz et al., 1985).

(iii) Multiple molecular forms: The active form of the enzyme appears to be a dimer in mammary glands and human
erythrocytes, with a molecular weight of 1,04,000 (Betts and Meyer, 1975; Pearse and Rosemeyer, 1974). The molecular weight of the human brain enzyme has been reported to be 90,000 (Weisz et al., 1985). The subunit molecular weight has been shown to be 48,800 for the rat brain enzyme (Sinicropi and Kauffman, 1979) which is in general agreement with the values reported for the enzyme from other tissues, e.g. 47,000 for sheep liver enzyme (Silverberg and Dalziel, 1973) and 52,000 for rat liver (Procsal and Holten, 1972) and rabbit mammary gland enzymes (Betts and Meyer, 1975). The enzyme has been shown to aggregate into catalytically active isomers of various sizes although only one form of the enzyme was reported to be present in the homogenates of rat brain (Sinicropi and Kauffman, 1979). This is in contrast to the observations of Pokrovskii and Korovnikov (1970) who reported the presence of two molecular forms of the enzyme in brain tissue. Three isoenzymic forms of 6-phosphogluconate dehydrogenase have been detected in human erythrocytes (Pearse and Rosemeyer, 1974) whereas the enzyme appears to occur in a single isoenzymic form in rabbit mammary gland (Betts and Meyer, 1975). The significance of the existence of tissue specific molecular forms of 6-phosphogluconate dehydrogenase is not clear.

(iv) The significance of 6-phosphogluconate dehydrogenase in brain: The activity of this enzyme in brain is quite low
about 0.5 units/g tissue (Weisz et al., 1985; Baquer et al., 1988). A large proportion of the enzyme was found to occur in the large particle fraction from the brain tissue (Baquer and McLean, 1972; Baquer et al., 1975). The enzyme is widely distributed in wide variety of structures in the mammalian nervous system. While G-6-PD varies with the lipid content in various brain regions, an inverse relationship between lipid content and 6-PGD activity has been observed (McDougal et al., 1961; Luine and Kauffman, 1971). 6-PGD activity also closely correlates with that of the non-oxidative enzymes of the pentose phosphate pathway similar to that reported in rat liver (Novello et al., 1969). Sinicropi and Kauffman (1979) suggested that the coordinated control of the activities of 6-PGD and the non-oxidative enzymes of the PPP might be regulated at the gene level.

The oxidative segment of the pentose phosphate pathway comprising of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase has been implicated in a variety of brain functions, like neurotransmitter metabolism (Appel and Parrot, 1970; Tabakoff et al., 1974; Baquer et al., 1975), maintenance of glutathione in the reduced form (Hotta and Sevenko, 1968) and peroxide detoxification system in brain (Baquer et al., 1975; Zubairu et al., 1983).
All these functions are mediated by NADPH generated in the enzymatic reaction of G-6-PD and 6-PGD. The activity of 6-PGD has been shown to be increased with the onset of axonal sprouting and after axotomy of the superior cervical ganglion. This increase is suggested to be due to the function of the dehydrogenase in providing pentose phosphate sugars for nucleic acid synthesis and NADPH for lipid synthesis during axon regeneration. The increase in 6-PGD activity observed under these conditions is due to an increase in the steady state concentration of the enzyme (Sinicropi and Kauffman, 1979).

Although it is the activity of glucose-6-phosphate dehydrogenase which actually determines the flux through the decarboxylating reaction of 6-phosphogluconate dehydrogenase, this enzyme also serves as an important regulatory point in the HMP pathway. 6-Amino nicotinamide which forms 6-amino analogue of NADP⁺ (6A-NADP) in vivo after administration, is a potent inhibitor of 6-PGD (Herken et al., 1969; Kolbe et al., 1976). It is not clear whether the derangement in brain functions under such conditions is due to primary or secondary effect of the inhibition of the enzyme but nevertheless emphasises that the optimal activity of the enzyme is required for normal functioning of the brain.
(v) **Hormonal regulation**: The activity of 6-phosphogluconate dehydrogenase is subject to regulation under dietary and hormonal conditions. An increase in the enzyme activity was seen in starvation and subsequent feeding of high carbohydrate diet (Novello et al., 1969). Thyroid hormones increase the concentration of cellular mRNA for 6-PGD with a subsequent increase in enzyme synthesis (Miksicek and Towle, 1982; 1983; Hutchison and Holten, 1978). The pre-translational regulation of 6-PGD activity has been observed only in liver and not in other tissues like brain, heart, kidney, lung and spleen (Miksicek and Towle, 1983). In contrast to this effect, a decrease in the 6-PGD activity has been observed in the brain of neonatally thyroidectomised rats which could be restored by T3 administration (Schwark et al., 1972).

The activity of 6-PGD is also regulated by insulin. A decrease in the activity of this enzyme has been observed in the liver of diabetic rats (Das and Ganguly, 1982). Whether this increase is due to a direct effect of insulin on the enzyme or an indirect effect subject to alterations in the metabolic intermediates of the diabetic liver is not well understood.

3. **Malic Enzyme**: Malic enzyme or NADP⁺-malate dehydrogenase (malate : NADP⁺ oxidoreductase (oxaloacetate-
The enzyme has been well studied in several mammalian tissues like pigeon liver (Hsu and Lardy, 1967; Hsu, 1970), rat liver (Saito et al., 1971; Saito and Tomita, 1973), heart (Saito and Tomita, 1972) and muscle (Taroni et al., 1987), mouse erythrocytes (Shows et al., 1970) and rat brain (Salganicoff and Koepp, 1967).

(i) **Intracellular distribution:** The distribution of malic enzyme in several animal species appears to be tissue specific (Brdickza and Pette, 1971; Frenkel, 1971; 1975). The enzyme appears to be present exclusively in the cytosol of rat liver, adipose tissue, and adrenal medulla (Frenkel, 1975), whereas a high proportion of malic enzyme activity has been detected in the mitochondrial fraction of brain, heart (Brdickza and Pette, 1971; Frenkel, 1975) and adrenal cortex (Simpson and Estabrook, 1969a; 1969b). Variants of mitochondrial enzyme with dual nucleotide specificity has been shown in various tissues like heart (Lin et al., 1975), intestinal mucosa (Nogel and Sauer, 1982), mouse hepatoma cells (Moredith and Lehninger, 1984) and skeletal muscles (Taroni et al., 1987).
Kinetic properties: Hsu (1970) postulated a general mechanism for the reaction of pigeon liver malic enzyme. According to the proposed mechanism, an enzyme bound oxaloacetate is required as an intermediate in the enzyme reaction which proceeds in a compulsory order sequence.

Differences in the kinetic properties between the mitochondrial and cytosolic malic enzymes have been demonstrated in bovine adrenal cortex (Simpson and Estabrook, 1969a), heart (Freedland, 1969) and bovine brain (Frenkel and Cobo-Frenkel, 1973; Frenkel, 1975). These two forms differ in their sensitivity towards dicarboxylic acids. Dicarboxylic acids like, L-aspartate, succinate or 2-mercaptosuccinate have no effect on cytosolic malic enzyme but increase the activity of the mitochondrial enzyme at low concentrations of malate, indicating the presence of an allosteric site in the mitochondrial enzyme. Frenkel (1975), suggested that the regulation of the mitochondrial malic enzyme by dicarboxylic acids, may revolve around the capability of the mitochondrial enzyme to oxidize the excessive dicarboxylic acids that might accumulate during high TCA cycle activity under some circumstances.

Significance of malic enzyme in brain: Luine and Kauffman (1971) showed that malic enzyme activity paralleled
changes in the lipid content in the anterior horn region which is rich in synaptic connections. They suggested a correlation between malic enzyme activity and lipid synthesis in the grey matters of brain regions. Frenkel (1973; 1975) suggested that the cytosolic and mitochondrial malic enzymes may have different roles to play in brain. The cytosolic malic enzyme may participate in a metabolic shuttle between cytosol and mitochondria. Reducing equivalents generated in the cytosol would then be utilised in the mitochondrial space by employing malate as a reduced substrate arising from the carboxylation of pyruvate by malic enzyme. The end product of the mitochondrial reactions would be pyruvate which could subsequently close the cycle by its return to cytosol. This pathway is identical to that described for the malic enzymes of the adrenal cortex by Simpson and Estabrook (1969a). Malic enzyme located in the mitochondria could also be used for the production of pyruvate inside the organelles themselves, whenever the supply of malate and other dicarboxylic acids are increased. The decarboxylation of malate to pyruvate would be favoured by the activating effects of succinate, aspartate and fumarate.

Another striking difference between the two isoenzymes from brain is found in their kinetic behaviour at low
concentrations of malate. While the cytosolic enzyme follows the Michaelis-Menten kinetics with this substrate, its mitochondrial counterpart shows a clear sigmoidicity under the same conditions (Frenkel, 1975). Besides differences in the kinetic properties, the cytosolic and mitochondrial malic enzymes from brain also differ in their electrophoretic and chromatographic properties (Frenkel, 1972). This shows that the two forms of the malic enzyme have a well defined role to play in the two metabolic compartments in brain.

(iv) Hormonal regulation: The influence of hormones on the activity of malic enzyme in several tissues has been a subject of investigation for many years. The regulation of malic enzyme activity by thyroid hormones has been investigated intensively. The increase in the enzyme activity in rat liver after thyroid hormone administration has been shown to be due to an increase in enzyme synthesis (Li et al., 1975) with a proportionate increase in the template activity of its mRNA (Towle et al., 1981). Although the malic enzyme gene is constitutively expressed, the hormonal control and induction are tissue specific. The only other nonhepatic tissues which show malic enzyme induction by thyroid hormones are kidney and heart, though the response is much lower than that in liver (Dozin et al.,
1985; 1986). Dozin et al. (1986) showed that T₃ increased the malic enzyme gene transcription in liver and heart and additionally caused a specific accumulation of malic enzyme mRNA in liver, presumably by altering the rate of degradation and processing of these RNA sequences in the nucleus and in the cytoplasm. The liver malic enzyme synthesis is directed by two distinct and functional cytoplasmic mRNAs which are regulated by T₃ to the same extent (Dozin et al., 1985). The lack of T₃ response in brain which has intermediate number of T₃ receptors argues that the nuclear binding might be necessary but not sufficient for hormonal regulation of malic enzyme (Dozin et al., 1985).

Malic enzyme activity in liver is also regulated by insulin. In diabetes, a decrease in the malic enzyme activity is seen which is reversed by the administration of insulin (Nepokroeff et al., 1974; Belfiore et al., 1974). Increase in the enzyme activity following insulin administration has been shown to be due to the increase in both the enzyme quantity as well as the specific activity, with an increase in the catalytic activity of each enzyme molecule (Thompson and Drake, 1982a, 1982b; Drake et al., 1984). A comparison of the processes controlling the increase in the hepatic malic enzyme activity in insulin
treated normal and diabetic animals have indicated the existence of two distinct regulatory processes. The increase in enzyme activity in the liver of normal rats after insulin administration, is due to an increase in the quantity of malic enzyme, whereas the increase in the malic enzyme activity in the liver of diabetic rats is due to an increase in both the enzyme quantity and specific activity (Drake et al., 1983).

A decrease in the activity of malic enzyme in the liver of diabetic rats could be restored to control level by T₃ administration (Sochor et al., 1987). It was suggested that the major factor in the diabetes induced decrease in the enzyme activities is the associated hypothyroidism and to a lesser extent the deficiency of insulin. The hormonal regulation of malic enzyme has not been well studied in the brain tissue. Preliminary studies by Murthy and Baquer (1983) have shown a decrease in malic enzyme activity in alloxan diabetes which is reversed by insulin administration. Though no direct effect of insulin and thyroid hormones on malic enzyme activity in brain has been reported, much has to be learned about the regulation of this enzyme in various pathophysiological conditions.
4. **NADP⁺-isocitrate dehydrogenase**: NADP⁺-isocitrate dehydrogenase (threo D₅-isocitrate : NADP⁺ oxidoreductase (decarboxylating, EC 1.1.1.42) catalyses the reaction:

\[ \text{threo D₅-isocitrate} + \text{NADP⁺} \rightarrow \text{2-oxoglutarate} + \text{CO₂} + \text{NADPH} \]

The enzyme has not been studied well and very little is known about its kinetic properties. It does not appear to be an allosteric enzyme (Lehninger, 1975). The enzyme is present in both the cytosolic and mitochondrial fractions of liver and brain. Nearly 70% of the enzyme activity in brain is located in the mitochondrial fraction (Salganicoff and Koepepe, 1968). A relatively higher activity of the enzyme has also been detected in the synaptosomes (Salganicoff and Koepepe, 1968; Rafalowska and Ksiezak, 1976).

The activity of NADP⁺-isocitrate dehydrogenase is high in both the grey and white matters of the brain tissue and no direct correlation with the lipid content of these regions has been suggested (Luine and Kauffman, 1971). However, a high activity of the enzyme in the spinal cord is suggested to be due to the participation of the enzyme in some reactions indirectly connected with lipid synthesis (Luine and Kauffman, 1971). This would involve a series of reactions converting α-ketoglutarate to acetyl CoA, with NADP⁺-isocitrate dehydrogenase required for the reductive
carboxylation reaction (Madsen et al., 1964). This is substantiated by the finding that small amounts of radioactive acetyl groups and lipid are produced from $^{14}$C glutamate in the developing rat brain (D'Adamo and D'Adamo, 1968). Rafalowska and Ksiežak (1976) suggested that the mitochondrial and cytosolic NADP$^+$-isocitrate dehydrogenases are two isoenzymic forms which participate in different functions in the two compartments. In brain mitochondria, citrate oxidation is dependent on the NADP$^+$-isocitrate dehydrogenase, bypassing the ATP citrate lyase enzyme. In synaptosomes and cytosol, citrate is oxidized with the participation of cytoplasmic NADP$^+$-ICDH and ATP-citrate lyase to yield acetyl CoA, which is utilized for the synthesis of fatty acids and acetylcholine.

Kauffman et al. (1974) showed a high activity of NADP$^+$-ICDH and the other NADP$^+$-dependent dehydrogenases in certain catecholamine containing neurons like nucleus coeruleus and in superior cervical ganglion. They suggested that the NADPH generated by the enzymatic reaction may be utilised for the reduction of tetrahydrobiopterin cofactor of tyrosine hydroxylase, which is involved in catecholamine biosynthesis and also for the maintenance of the non-protein sulphydryl groups in the reduced form in the catecholamine containing neurons of these regions. The activity of NADP$^+$-
ICDH is the highest among all the NADP⁺-dependent dehydrogenases in brain and it also has the lowest $K_m$ for NADP⁺ (Luine and Kauffman, 1971). In brain, where the ratio of NADPH/NADP⁺ is high (Veech et al., 1973), this finding therefore suggests an important role for NADP⁺-ICDH in various brain functions.

Baquer et al. (1976) and Sochor et al. (1987) suggested that this enzyme was not under hormonal control, since no change in the enzyme activity was observed in liver during thyroid hormone deficiency. Much has to be learned about the role and regulation of NADP⁺-ICDH in hormonal conditions.

V. HORMONAL REGULATION

1. THYROID HORMONES: There is extensive literature on the effect of thyroid hormones on the growth and development of the central nervous system (Tata, 1966; Cocks et al., 1970; Sterling and Lazarus, 1977). The thyroid hormones are mainly secreted as thyroxine ($T_4$) and as triiodothyronine ($T_3$). Although $T_4$ is known to stimulate growth and development and increase the level of certain proteins, the hormonal effects are generally attributable to $T_3$ which is about 5 times greater than that of $T_4$ and all the apparent $T_4$ activity could be accounted for by its conversion to $T_3$.
(Chopra et al., 1978). Accordingly, T₄ has come to be regarded as a prehormone with little intrinsic nuclear activity by itself (Surks and Oppenheimer, 1978). Small activity of T₄ has been observed even in the absence of T₄-T₃ conversion (Samuels et al., 1974). The difference in the magnitude of biological activity between T₄ and T₃ might be explained by the differences in the binding of the T₃ or T₄-receptor complex to DNA. Whilst a major fraction of sites bind T₄-receptor complex tightly, a substantial proportion have little affinity to this hormone when compared to that of the T₃-receptor complex (Wilson et al., 1984).

(i) **Molecular mechanism of action**: Studies in various tissues have demonstrated the presence of high affinity low capacity T₃ binding sites (Oppenheimer et al., 1972; 1983). The thyroid hormone receptor is a nuclear protein, present in the nucleus in the absence of the hormone (Ferreira et al., 1988). It has been identified as the protein product of the proto-oncogene c-erb-A (Sap et al., 1986; Weinberger et al., 1986). Binding of its natural ligand T₃ activates the receptor, thereby modulating the expression of genes under thyroid hormone control. This augments gene expression which is seen by the generalized increase in the content of mRNA, as well as disproportionate increase in specific RNA sequences (Oppenheimer, 1983). T₃ has been
shown to increase the cellular level of specific RNAs including mRNA encoding growth hormone in the pituitary (Martial et al., 1977) and mRNA coding for malic enzyme and α-2-euglobulin in liver (Towle et al., 1980). The induction of malic enzyme synthesis by thyroid hormones has been shown to be highly tissue specific and involves regulation at both the transcriptional and post-transcriptional levels (Dozin et al., 1985; 1986).

Thyroid hormones also stimulate RNA synthesis by isolated neuronal nuclei of the neonatal rat brain cortex, with an increase in the poly(A) polymerase activity and the content of poly A containing mRNAs (Lindholm, 1984). A gradient of free T₃ concentration from plasma or cytosol to nucleus has been observed in some tissues and the possibility of a T₃ concentrating mechanism in the nuclear membrane has been suggested (Oppenheimer and Schwartz, 1985). This ratio has been found to be higher in brain than in other tissues (Ferreira et al., 1988).

Evidences indicate that thyroid hormones exert more direct extranuclear effects on certain membrane related processes which are prompt in onset and are independent of RNA and protein synthesis. These effects are not clear but are thought to be due to the binding of thyroid hormones to specific plasma membrane receptors and may involve, a Ca²⁺-
dependent stimulation of adenylate cyclase (Segal and Ingbar, 1986).

Extra nuclear $T_3$ binding sites have been identified in synaptosomes and mitochondria (Mashio et al., 1983; Sterling et al., 1978). $T_3$ has been shown to be selectively concentrated in the synaptic endings (Dratman et al., 1976). The synaptosomal binding sites have been localized predominantly in the synaptosome membrane and two distinct binding sites have been identified (Mashio et al., 1983). This suggests a possible direct action of $T_3$ on synaptic function.

(ii) Ontogenesis of thyroid hormone receptors and brain development: Thyroid hormones exert profound effect on the development of the central nervous system (Nunez, 1984; Legrand, 1986). In rat brain, $T_3$ receptors appear on day 13 of fetal life, 4 days before fetal thyroid gland begins to function (Castillo et al., 1985). It attains 30-50% of adult levels during the phase of neuroblast proliferation in the last 2 or 3 days before birth with a subsequent increase in the receptor concentration in the neonatal period (Castillo et al., 1985; Luo et al., 1986; Schwartz and Oppenheimer, 1978). During the same period, a high saturation of the nuclear receptors has been observed in brain compared to the other tissues (Ferreira et al., 1988).
In humans, the appearance of the receptor in the 1-16th week is coincident with the period of neuroblast proliferation (Bernal and Pekonen, 1984). Taken together, these observations suggest that thyroid hormones influence brain neuroblast development and or differentiation through actions mediated by binding to the nuclear receptor. A deficiency of thyroid hormones during brain development might therefore be responsible for the neurological disorders seen in cretinism.

The nuclear T₃ receptors in brain are unevenly distributed. They are highest in the cerebral cortex and lowest in the cerebellum (Schwartz and Oppenheimer, 1978), and 5-10 fold higher in neuronal nuclei than in the glial cell nuclei (Yokota et al., 1986). The synaptosomal T₃ binding sites increase with age and attain a maximum level in the young adult (Mashio et al., 1982), in contrast to mitochondrial T₃ binding sites which can be detected only upto 12 days in neonatal rats (Sterling et al., 1978).

Although the profound effects of thyroid hormones on the developing brain is well documented, its role in the adult brain is not very clear. Adult brain has been considered as a T₃ unresponsive tissue, since it fails to respond to T₃ with a characteristic increase in O₂.
consumption (Reiss et al., 1956), inspite of having intermediate number of T₃ receptors. But the effect of thyroid hormones on the monoamine metabolism (Engstrom et al., 1974) and its influence on some enzyme systems in adult brain including that of monoamine oxidase and Na⁺K⁺ATPase (Kim et al., 1979; Mayanil, 1983) suggests that O₂ consumption cannot be considered as an appropriate parameter for thyroid hormone effect on brain. Besides, the increased synaptosomal binding of T₃ observed in the brain of the adult animal than that of the neonatal animal implies a definite role for thyroid hormones on some events connected with neuronal function in the adult brain.

2. INSULIN

Despite many years of intense biochemical research, the precise mechanism by which insulin elicits the varied physiological responses is not clear. It stimulates the uptake of glucose and amino acids, increases the synthesis of enzymes including glycogen synthase and enhances the synthesis of protein, RNA and DNA (Jacobs and Cuatrecasas, 1981; Kahn, 1979; 1985; Baskin et al., 1987). The role of insulin in brain has been a subject of special interest. The brain was hitherto considered to be an insulin insensitive organ. But the discovery of insulin and insulin receptors in brain have stimulated the research to determine the precise role of insulin in brain function.
Origin of insulin in brain: The finding of insulin and insulin receptors in brain (Havrankova et al., 1978; Rosenzweig et al., 1980) led to an intense speculation on the possible origin of insulin in brain. Two points of view have emerged regarding its cellular origin: that, (1) it is synthesised by the brain cells, or (2) it is taken up from the plasma. Some studies have indicated a neural origin of insulin, where pro-insulin like immunoreactivity has been detected in cultures of fetal mouse cells (Birch et al., 1984). This assumption has also been questioned. Insulin in brain was shown to be entirely of pancreatic origin (Gidding et al., 1985) and evidence for both immunoreactive proinsulin in brain extracts and insulin mRNA production in brain is negative (Seldon et al., 1986). Several lines of evidences suggest that insulin is taken up from plasma across the blood brain barrier (Woods et al., 1985). Increases in the concentration of insulin in the cerebrospinal fluid has been observed after intravenous insulin infusion (Wallum et al., 1987). Transport of insulin through the insulin receptors present in small brain microvessels have been observed (Pardridge et al., 1985). Thus, insulin is transported across the blood brain barrier into the brain, where it enters the neural tissue directly or is taken up into the neural tissue from the cerebrospinal fluid.
(ii) **Insulin receptor**: The basic structure of the insulin receptor is that of a heterotetramer glycoprotein. The two distinct subunits of the insulin receptor are specialised to perform the major functions of the receptor. The $\alpha$-subunit has a molecular weight of 135,000 and is the insulin binding subunit. The $\beta$-subunit has a molecular weight of 95,000 and appears to be a transmembrane protein. The $\beta$-subunit autophosphorylates on tyrosine after binding insulin with subsequent activation of its exogenous tyrosine kinase activity (Ulrich et al., 1985; Kahn, 1985). Both the subunits of the receptor are glycoproteins with complex carbohydrate side chains (Hedo et al., 1981). The carbohydrate residues have been implicated in insulin binding (Jacobs and Cuatrecasas, 1981). Although multiple forms of the receptor have been proposed, the most commonly accepted model is the $\alpha_2\beta_2$ structure, where $\alpha$ and $\beta$ subunits form disulphide linked oligomers (Kahn, 1985). Tyrosine kinase activity intrinsic to the $\beta$ subunit has been shown to be important in transmembrane signalling. Elimination of the enzyme activity either through selective mutation of the gene encoding the insulin receptor (Ellis et al., 1986), or by blocking this activity by specific antibodies (Morgan et al., 1986) significantly reduces many of the cellular actions of insulin.
Consistent with the idea that insulin may have a unique function in brain, is the finding that the structure of the brain insulin receptor and that of the peripheral tissues differ (Yip et al., 1980; Heidenreich et al., 1983; Hendricks et al., 1984). A difference in the molecular weight of the α-subunit of the brain insulin receptors and that of the peripheral tissues have been observed, which has been attributed to the differences in the glycosylation of the brain α-subunit (Heidenreich et al., 1983; Heidenreich, 1985). In brain, two forms of insulin receptors have been shown (Roth et al., 1986; Grunberger et al., 1986), one of them, the same size as the insulin receptors in the peripheral tissues detected in the fetal brain and the other of a lower molecular weight reported in the adult brain (Roth et al., 1986). The change in the insulin receptors from a high molecular weight form to a low molecular weight form during brain development is due to specific loss of sialic acid residues. This desialylation of brain insulin receptors have been shown to facilitate their expression. It is significant that this process occurs during the period of tremendous brain growth and differentiation (Brennan, 1988).

There is maximum localisation of insulin receptors in the regions with high dendritic arborisation, which receive
Rich synaptic input e.g. the plexiform layers of olfactory bulb and the molecular layer of cerebellum. The density of the insulin receptors is also high in choroid plexus, hypothalamus and hippocampus (Werther et al., 1988). Studies by Van Houten et al. (1986) have shown the presence of high density insulin binding sites in the axons and axon terminals of the hypothalamus. This suggests that insulin may act through the synaptic mechanisms to influence hypothalamic circuits regulating energy balance and hypophyseal functions.

(iii) **Insulin-receptor interactions**: Upon binding to the membranes, insulin induces changes in both the receptor conformation and aggregation state of the receptors. Aggregation of receptors including patch and cap formation has been observed (Schlessinger et al., 1980). The mechanism responsible for this aggregation is not known. The kinetics of insulin-receptor binding are quite complex. A negative co-operativity in insulin binding to its receptors has been observed. This is mainly due to site-site interactions among the binding sites which lowers the affinity for insulin (Gu et al., 1988). Insulin itself is the most important factor which regulates the number and affinity of its receptors. Exposure of cells both in vivo and in vitro to high concentrations of insulin results in a
decrease in the number of receptors. This process is termed as down regulation. Insulin stimulates glucose uptake and down regulation of its receptors in glial cells whereas these effects are not present in the neuronal cells (Boyd and Raizada, 1983; Clarke et al., 1984; Boyd et al., 1985). Internalisation of the hormone-receptor complex has been observed and it is the major step in the receptor endocytosis (Goldfine et al., 1979; Carpentier et al., 1986).

(iv) Molecular mechanism of action: Insulin initiates a number of early events which are important in the cellular action of the hormone. Although the exact role of the tyrosine kinase activity of the insulin receptor in insulin action is unknown, several factors suggest that the activation of the kinase may be the initial event in insulin action. There are at least two evidences to support this assumption. The activity of the enzyme is in the intracellular domain of the receptor and is stimulated by direct binding of insulin to the receptor. Second, the kinetics of receptor phosphorylation/dephosphorylation are consistent with the time course of insulin action. How the activation of tyrosine kinase results in signal transduction is uncertain, but indicates that these kinases phosphorylate one or more endogenous substrates like proteins with kinase
or phosphatase activity, leading to a cascade of phosphorylation/dephosphorylation reactions (Kahn, 1985). Seals and Czech (1981) proposed that the binding of insulin to its receptor activates a membrane structure, resulting in the release of small peptide fragments which mediate insulin action. The activity of pyruvate dehydrogenase has been shown to be modulated by such a soluble mediator (Seals and Jarret, 1980; Seals and Czech, 1981). Besides this, a large number of mediators or second messengers have been implicated in signal transduction process. The probable candidates include, cAMP, cGMP (Kahn, 1979), \( \text{H}_2\text{O}_2 \), \( \text{Mg}^{2+} \), membrane hyperpolarisation, phospholipids and \( \text{Ca}^{2+} \) (Kahn, 1985; Sacks and McDonald, 1988). There is substantial evidence for a supporting role of \( \text{Ca}^{2+} \) and calmodulin in the mechanism of insulin action (McDonald et al., 1986).

Insulin has been shown to phosphorylate calmodulin and, \( \text{Ca}^{2+} \) and calmodulin have been suggested to participate in the early cellular mechanism of insulin action (Graves et al., 1986; Colca et al., 1987; Sacks and McDonald, 1988).

Despite intensive research in this field for many years, the precise mechanism by which insulin exerts its manifold effects has not been elucidated.

(v) **Insulin in brain**: Insulin has been shown to elicit diverse physiological, developmental and behavioural
Insulin stimulates enzyme activity, synthesis of protein, RNA and DNA in cultured neurons and glial cells (Clarke et al., 1985). An increase in the binding of insulin to its receptors has been observed during brain growth and development in the rat (Kappy and Raizada, 1983). This has been shown to be due to an increase in the number of binding sites during this period (Brennan, 1988). Studies on the role of insulin in synaptic function have suggested an important role for insulin as a neuromodulator. Insulin has been shown to promote electrical coupling between sympathetic neurons in culture (Kessler et al., 1984; Wolinsky et al., 1985) and modulate the uptake of monoamines (Boyd et al., 1985), neurotransmitter amino acids (Rhoads et al., 1984) and increase the turnover and release of catecholamines from brain cells (Sauter et al., 1983). Release of insulin from neuronal cells has been observed under depolarisation conditions, which also makes insulin a likely candidate for a role as a neurotransmitter (Clarke et al., 1986).

The role of insulin in the regulation of the carbohydrate metabolism is well known. Pal and Bessman (1988) have recently found an inverse relationship between the
anabolic effects of insulin and particulate bound hexokinase activity in the developing brain. Changes in the concentrations of key intermediary metabolites in the brain of diabetic animals (Thurston et al., 1975; Ruderman et al., 1974) suggests a role for insulin in the regulation of carbohydrate metabolism in brain. The role of insulin in the nervous system is still open to question and much remains to be learned about this problem.