CHAPTER - I

I N T R O D U C T I O N

INTRODUCTION

Life is a continuous adaptation to changes in internal and external conditions. Various vegetative functions of the body that are necessary for the normal metabolic processes which take place in the body's cells, as well as the adaptive changes to external or internal environment are regulated by homeostatic mechanisms. Two major systems, the nervous and the endocrines by their complex interaction bring about the homeostatic mechanisms. The neural processes involved in this include (a) stimulus detection, achieved by receptors which are sensitive to shifts in a particular body state away from its normal, and conduction of information to the Central Nervous System (CNS), (b) effectors, which respond to the sensory signals by producing an appropriate change in the body state i.e., a shift back toward its normal condition, (c) the response feedback to the CNS, a process called reafference, crucial for the termination of the response through effectors.
The integration and co-ordination of all these neural processes take place mainly at the CNS and are chiefly mediated by certain chemical signals called neurotransmitter viz., GABA, DA, NE, 5HT, etc.

The endocrine processes comprise of several internal glands called endocrine glands which secrete certain chemical signal molecules called hormones into the circulatory system. Largely, hormones in the circulatory system reach different tissues of the body and regulate many internal processes such as water balance, sexual functions and internal reactions to stress. The hormonal interactions and their circulatory levels in the blood are controlled meticulously by various factors which include CNS and certain endocrine glands themselves. A diagrammatic presentation of endocrine processes is given in Fig.1. The complex interaction of neural and endocrine processes during homeostatic mechanism often manifests itself in the expression of behaviour and its inter-relationship can best be expressed as

$$\text{Endocrine system} \rightleftharpoons \text{Nervous system} \rightleftharpoons \text{Behaviour}$$

For example, a drop in environmental temperature produces autonomic responses (involving certain adrenal secretions) such as constriction of blood vessels, which tend to conserve body heat. When the temperature drops, one may also do things that tend to preserve body heat, such as putting additional clothing or closing windows. One of the phenomena which illustrates this inter-relationship is the phenomena of lactation. In this the suckling stimulus to the nipple triggers the release of oxytocin which facilitates the ejection of milk and prolactin secretion which helps the mammary gland to replenish the supply of milk.

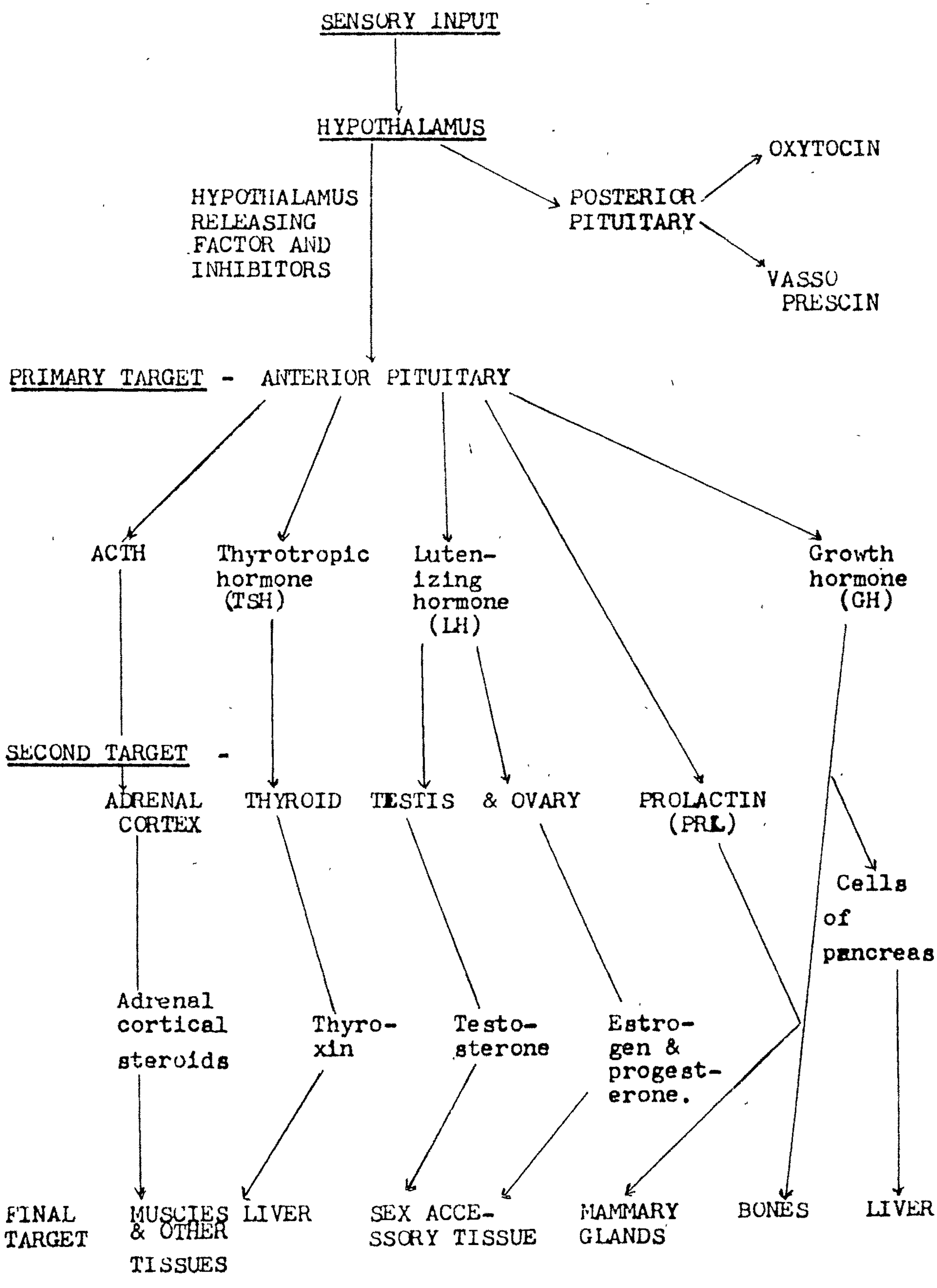


Fig. 1 A Diagrammatic Presentation of Endocrine Processes

Another phenomenon of behavioral control of anterior pituitary hormone secretion is the phenomenon of stress. Conditions associated with injury and surgical trauma, psychic stress of fear, novelty and joy can activate the release of ACTH which in turn stimulates the adrenal glucocorticoid secretion. These behavioral stimuli are mediated by complex neural pathways and can be modified readily by learning.

Thus during the process of homeostasis, hormones influence various aspects of brain chemistry. While doing so, hormones affect or facilitate certain pre-existing neuroendocrine pathways and/or behavioral patterns that are reversible when the hormone disappears,such effects are called activational effects. Hormones also affect the brain, with the effect lasting well beyond the time of presence of hormone itself. Such effects are called organizational effects. These effects could be brought about with the presence of hormone during specific phase of development critical for the establishment of neuroendocrine function and/or behaviour, which could normally be during the early period of brain development. One such hormone having profound effects on brain both during development and in adult stage is corticosteroid hormone.

1.1 Historical background : Corticosteroids are produced in the adrenal gland and its physiological significance began to be appreciated as early as 1855 as a consequence of the description by Addisson (1855) of the clinical syndrome resulting from destructive disease of the adrenal glands. Corticosteroids became subject of interest primarily because of two reasons (a) involvement in certain pathological conditions viz. Addison's disease and Cushing's syndrome (1932) and, (b) it's then suggested role of protecting the organism against stressors (Selye, 1946). Following observations highlight important milestone in corticosteroid research :-

(i) Reichsten and Shoppe (1943) isolated, crystallized and elucidated the structures of 28 steroids from the adrenal cortex which included corticosterone and cortisone.

(ii) Hench et al.(1949) demonstrated the anti-inflammatory effects of cortisone in rheumatoid arthritis for which Hench shared the nobel prize in medicine with Kendall and Reichstein in 1950.

(iii) Several reports were published on the importance of adrenocortical secretion in depletion of carbohydrate stores (Cori and Cori, 1927), breakdown of tissue protein during fasting (Long et al, 1940) and on the ACTH control of corticosterone secretion (Stone and Hector, 1954) through the intermediacy of cyclic adenosine 3',5'-monophosphate (Cyclic AMP) (Haynes et al, 1959).

(iv) Studies by Knox and colleagues, provided the clue that the biochemical action of glucocorticoid involves protein synthesis stimulation (Goldstein <u>et al</u>, 1962). The demonstration of certain specific proteins (receptors) that bind to glucocorticoid (Schaumberg and Bojesen 1968) further stimulated research at the molecular level in elucidating various glucocorticoid changes reported in the 1970 and 1980s.

1.2 <u>Structure</u> : Corticosteroids are a group of compounds bearing some structural resemblance to the terpenes. They may be considered as derivatives of a fused, reduced ring system, cyclopento(a)-phenanthrene, comprising three cyclohexane rings (A, B and C) in the non-linear or phenanthrene arrangement and a terminal cyclopentane ring. The structures and activities of some of the corticosteroids are given in the Fig.2.

Cortisol and cortisone are the two major adrenal steroids produced by the zona fasciculata of adrenal cortex. These have got 21 carbon atoms including a two carbon side chain at the 17th carbon atom. The important structural characteristics of these compounds include the system of conjugated double bonds at carbon atoms 3, 4 and 5, the so called α,β-unsaturated ketone, a hydroxyl substituent at position 11 and the state of oxidation of the side chain carbon atoms 20 and 21. In the case of cortisol, an extra hydroxyl group at 17th carbon atom is present.

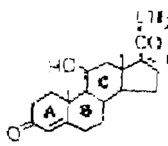
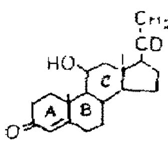
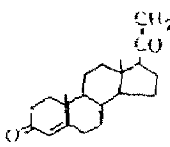
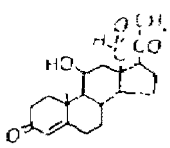
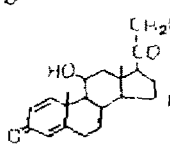
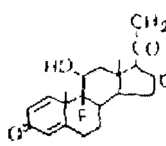
		Mineralo corticoid activity	Glucocorticoid activity	Anti-inflammatory activity	Anti-haemolytic activity
	Cortisol	1	1	1	1
	Corticosterone	2	0.2	0.3	10
	11-deoxycortisol (Reichstein's S) *Cortexolone*	0	0	—	10
	Aldosterone	400	0.1	—	0
	*Prednisolone*	0.6	4	3	1-2
	Dexamethasone	0.2	30	100	5-10

Fig. 2 Structure and Activities of some Corticosteroids

The biological activities of corticosteroids has been known to vary with the slight variation in its cyclopentane ring structure (Fig.2). The 4,5 double bond and the 3-ketone are both necessary for typical glucocorticoid activity. Introduction of a 1,2 double bond, as in prednisolone and dexamethasone (synthetic glucocorticoids) enhances the carbohydrate regulating potency. In addition, predinolone is metabolized more slowly than cortisol. The presence of an oxygen function at C11 is indispensible for anti-inflammatory and carbohydrate-regulating activity (Haynes and Murad, 1980). It is evident from these structure-activity relationship that the form of hormone used for the treatment is of utmost importance while looking for any specific biological response and that the synthetic hormone dexamethasone possesses enhanced biological activity in comparison with the corticosterone.

1.3 <u>Biosynthesis of corticosteroids*:</u> Corticosterone is produced by adrenal cortex with cholesterol as its obligatory precursor, obtained to a large extent from exogenous sources. Since corticosterone is not stored in adrenal tissue, rate of its biosynthesis is tantamount to the rate of secretion. Fig.3 shows the biosynthetic pathway of corticosterone and cortisol.

The cholesterol molecule contains 27 carbon atoms and one hydroxyl group at position 3. It's conversion to

* Hereafter, corticosteroids refer to corticosterone and is used interchangebly with glucocorticoids.

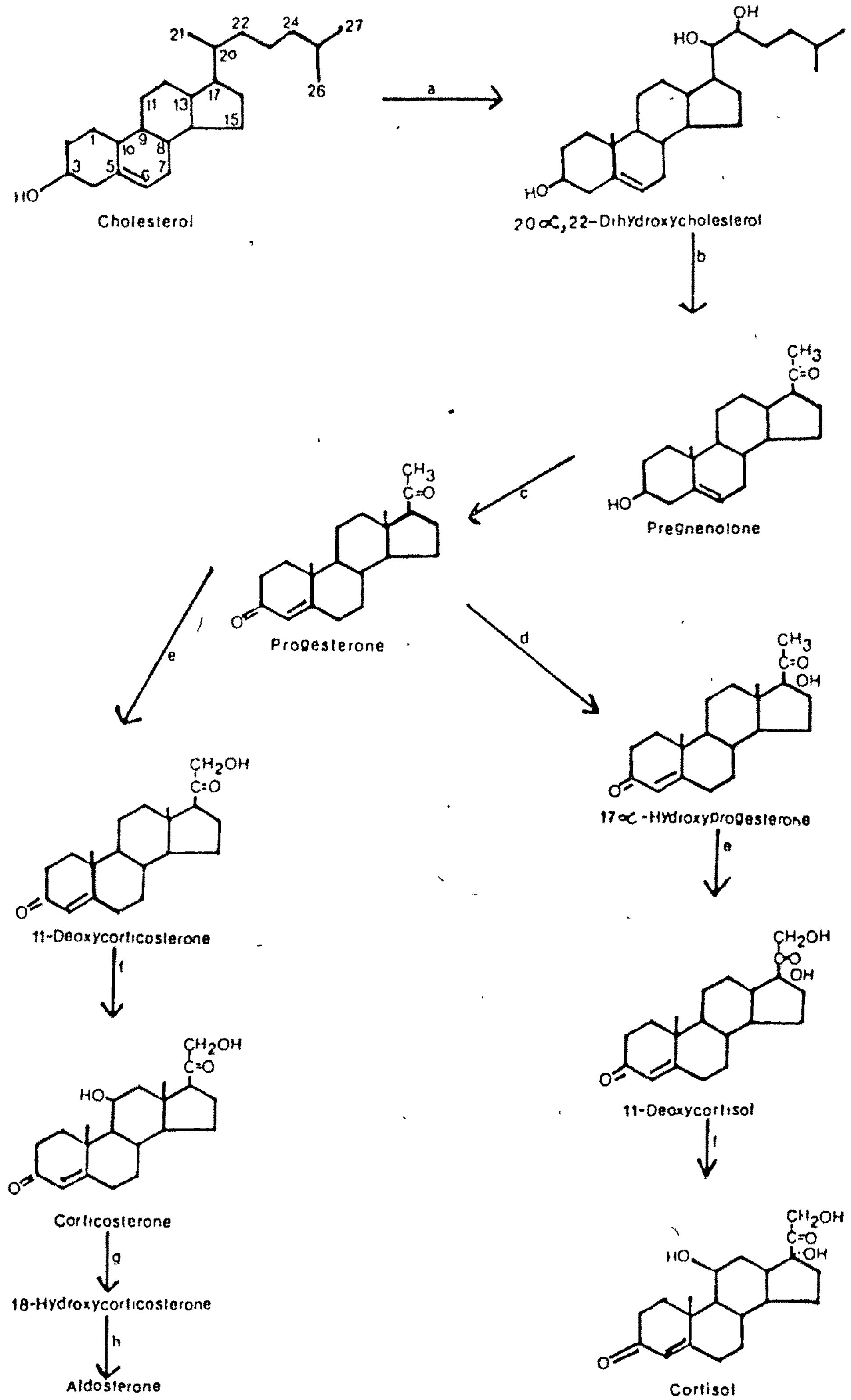


Fig. 3 The Biosynthetic Pathway of Corticosterone

a- Hydroxylases, b- Desmolase, c- 3β-OL-Dehydrogenase-Δ^5 - 3 Ketosteroid isomerase d- 17α-Hydroxylase, e- 21 Hydroxylase, f- 11β-Hydroxylase, g- 18 Hydroxylase, h- 18 Dehydrogenase corticosterone involves the removal of six carbon atoms from the side chain and introduction of three oxygen atoms. In mitochondria, the side chain of cholesterol is hydroxylated at C_{20} and C_{22} positions with the formation of 20 α, 22 - dihydroxycholesterol. Its conversion to pregnenolone is catalyzed by the enzyme desmolase and involves the cleavage of the bond between C_{20} and C_{22}. NADPH and oxygen are cofactors in all the three reactions. This is the most important step in corticosterone biosynthesis being the rate limiting step, and stimulated by cyclic AMP. ACTH stimulates c-AMP formation by activating adenylate cyclase in adrenal cortex cells leading to the regulation of corticosterone synthesis.

The next step involves the formation of progesterone from pregnenolone by the enzyme 3 β -OL-dehydrogenase Δ^5-3 ketosteroid isomerase which catalyzes the oxidation of 3 hydroxyl to a 3 ketogroup and isomerization of Δ^5 double bond to a Δ^4 double bond. Progesterone is in turn hydroxylated at 11 and 21 positions by hydroxylases present in mitochondria to form corticosterone. These adrenal steroid hydroxylating enzymes, specific for individual position of the steroid nucleus, are mixed function oxidases and utilize O_2, NADPH, adrenoxin and cytochrome P_{450} (White et al, 1973).

1.4 Regulation of corticosterone synthesis : The hypophyseal ACTH regulates the secretory activity of adrenal cortex. ACTH increases the rate of synthesis by stimulating the conversion of cholesterol to pregnenolone (side chain cleavage), a rate-limiting process. The process of stimulation by ACTH involves various aspects such as increased cholesterol availability in the mitochondria for side chain cleavage (Mahaffee et al, 1974), stimulating the formation of cholesterol in the gland by activating cholesterol esterase, apparently by phosphorylation of the enzyme (Beckett and Boyd, 1975; Pitman and Steinberg, 1977) and also by increasing the uptake of plasma lipoproteins (Gwynne et al, 1976) so as to increase cholesterol.

The secretion of ACTH in turn is regulated by the balance of neural excitatory and corticosteroid inhibitory effects. The neural excitatory influence involves certain factors called corticotropin releasing factor (CRF) (Yates and Maran, 1979) and arginine vasopressin (Rivier and Vale, 1983; Plotsky, 1985). CRF is synthesized by parvocellular neurons located in the hypothalamic paraventricular nucleus in the rat (Swanson et al, 1983) which project into median eminence to release CRF into hypophyseal portal vessels (Plotsky, 1985) and control ACTH secretion. The secretory rate of CRF is subjected to both positive and negative feedback controls. A variety of neural influences originating both from within the organism and its environment may control CRF secretion (Hamamura et al, 1984; Saphier and Feldman, 1987). For example,awide variety of unrelated stimuli like trauma, emotional stress, drugs, chemicals etc., initiate secretion of CRF by nerve endings in the posterior hypothalamus and median-eminence which may culminate in the secretion of corticosteroids via ACTH from adenohypophysis. The rate of secretion of ACTH is related inversely to circulating level of corticosteroid. Increased rate of removal of these steroids by tissues lowers their blood level leading to increased ACTH secretion. Likewise, elevated blood level of corticosteroid depress ACTH secretion by a feedback mechanism. The precise locus of corticosteroid feedback, however, is not clear. A feedback control on the CRF secretion at hypothalamic level has been envisaged with the predominantly inhibitory effects of glucocorticoids upon hypothalamic neurons (Feldman and Sarne, 1970; Mandelbrod et al, 1974). More recently iontophoretically applied glucocorticoid hormone has been demonstrated to alter the electrical activity specifically of paraventricular neurons (Saphier and Feldman, 1988). Glucocorticoids has also been shown to decrease the activity of mRNA for ACTH in the pituitary itself (Nakanishi et al, 1977).

Thus secretion of corticosterone is under the control of ACTH secretion resulting from the control of nervous system and the negative feedback inhibition by circulating corticosterone itself. Certain conditions such as stressful stimuli have been demonstrated to override the normal negative feedback control mechanisms leading to further elevation of blood corticosterone level, culminating in various alterations in both peripheral tissues and central nervous system. With the termination of the stress signal, elevated corticosterone level in blood exerts its feedback inhibition and the basal blood corticosterone level is maintained. This effect of negative feedback inhibition on adrenocortical activity has been suggested to be mediated by the hippocampus (Moberg et al, 1970).

1.5 Concentration of glucocorticoids :

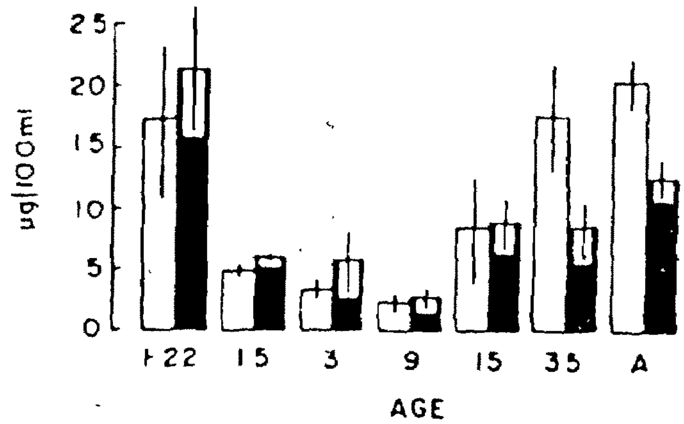
1.5.1. In adrenal cortex : In man, adrenal cortex normally secretes approximately 10-30 mg of cortisol and 2-4 mg of corticosterone per day. In adult rats, the main glucocorticoid, corticosterone concentration ranges from 9.6 - 14.6 µg/g.

1.5.2. In blood : Normal plasma concentration of cortisol and corticosterone in man is 240 µg and 80 µg respectively in 100 ml of blood. In rats, it ranges from 4-30 µg/100 ml depending on the time of the day. As mentioned earlier, the adrenal gland does not store corticosteroid and hence its secretory activity plays an important role in maintaining corticosteroid levels in circulating plasma.

Normal daily production of corticosteroids is about 25-35 mg, with cortisol representing 85-90% of the total in humans. Cortisol secretion normally follows a circadian pattern with peak plasma concentration averaging about 16 μg/100 ml between 6.00 am and 9.00 am and minimal concentrations of about 5 μg/100 ml between midnight and 2.00 am. (Carpenter and Bunney, 1971; Forsham, 1962). This circadian rhythm has been found to be characterized by episodic secretory bursts (an average of nine secretory episodes per day), which are intensified during the early morning hours (Hellman et al, 1970). In rats, the lowest level of blood corticosterone has been reported to be in the early morning hours and the highest, late in the day prior to or immediately after the onset of darkness. This phenomenon of diurnal rhythm has been shown to be light dependent. Such alteration in plasma corticosterone concentrations further alters the brain tissue concentrations of corticosterone (Butte et al, 1972) and further its binding to corticosterone-binding receptors in various brain regions (Stevens et al, 1973).

1.5.3. Corticosteroid levels during development : The plasma corticosteroid levels also vary with the age of the animal. Several studies have demonstrated the presence of corticosterone as early as 16 days of gestation in the rat. [illegible] In the foetus the maximal basal corticosterone level (15-25 µg/100 ml) has been shown to be around day 20 (Cf. Nathanielez, 1976). After birth, corticosterone titers decrease dramatically to about 1-3 µg/100 ml, especially over the first 6-8 h after birth. This low level of plasma corticosterone persists till around postnatal day 9 (Fig.4) after which it begins to gradually increase and approximate adult levels by day 15 (Sze et al, 1976; Henning, 1978; Sapolsky and Meaney, 1986).

1.5.4 Corticosterone levels during stress and stress non-responsive periods : As mentioned earlier, in adult rats plasma corticosterone level increases several fold in response to various stressors overriding the negative feedback effect of circulating corticosterone level on the pituitary as well as the hypothalamic neurons, including hippocampus. Following the removal of stress signal, plasma corticosterone returns to basal level. Such a response of increased plasma corticosterone to stressors has been reported to be present even at the late fetal period (Milkovic and Milkovic, 1972). However, in the rats, during the first two postnatal weeks starting from two days after birth stressors fail to elicite an increased plasma corticosterone and ACTH levels (Fig.4). This period of adrenocortical quiescence has been termed the 'stress non-responsive period' (SNRP) defined by the relative inactivity of the adrenocortical axis upon exposure to stress (Schapiro, 1968). During



Mean (± S.E.M.) corticosterone levels in male (■) and female (□) rats at various ages. F, fetal, A, adult (90-120 day

Stressor	Age	Plasma corticosterone (ug/100 ml)		
		Baseline	Stress	time (min)
Handling	2	3	4	30
Heat	2	3	4	30
Ether	2	3	6	30
Shock	1	18	22	15
Shock	4	11	14	15
Shock	9	3	9	15
Shock	21	8	24	15
Heat	1	18	28	15
Heat	4	11	16	15
Heat	9	7	8	15
Heat	21	8	22	15
Ether	5	< 1	2	15
Ether	9	< 1	2	15
Ether	11	< 1	3	15
Ether	15	2	12	15
Ether	25	4	42	15
Histamine	1	7	11	15
Histamine	3	4	7	15
Histamine	5	4	6	15
Histamine	9	4	4	15
Histamine	21	4	16	15
Ether	7 (males)	3	3	2.5
Ether	7 (females)	3	3	2.5
Ether	7 (males)	3	3	15
Ether	7 (females)	3	3	15
Histamine	2	7	20	25
Histamine	6	3	3	25
Histamine	10	2	4	25
Histamine	16	10	24	25

Fig. 4 **Plasma Corticosterone levels during development and under stress conditions** Taken from : Sapolsky and Meany (1986)

this period, the neonatal rat is not only less sensitive to the stimulatory effects of stress, but also to the inhibitory effects of circulating corticosterone i.e., unlike in adults, dexamethasone or corticosterone treatment does not suppress the corticosterone titres in 3-9 day old rats (Cf. Sapolsky and Meaney, 1986). It is interesting to note that such a state of non-suppression of adrenocortical activity to dexammethasone treatment has been reported under several psychiatric disorders and is rather used extensively as a diagnostic test in these conditions. In a recent review, Sapolsky and Meaney (1986) have analysed various experimental evidences with regard to the cause and consequences of SNRP and envisaged several factors that contribute to the existence of the SNRP viz., diminished concentrations of CRF and ACTH and reduced sensitivity to CRF at pituitary level. They further suggested that the exaggerated glucocorticoid negative-feedback on the pituitary and the resultant inhibition of responsiveness to CRF play a major role in mediating the SNRP. In addition, the low concentration of bioactive receptors for corticosterone in the hippocampus, involved in termination of stress response, is related to the delayed inhibitory phase of the stress response during SNRP. The consequences of SNRP and the decreased basal corticosterone level during this period has been hypothesized to be of profound importance for various changes that are taking place during the brain development (Bohn and Lauder,1978; Sapolsky and Meaney, 1986).

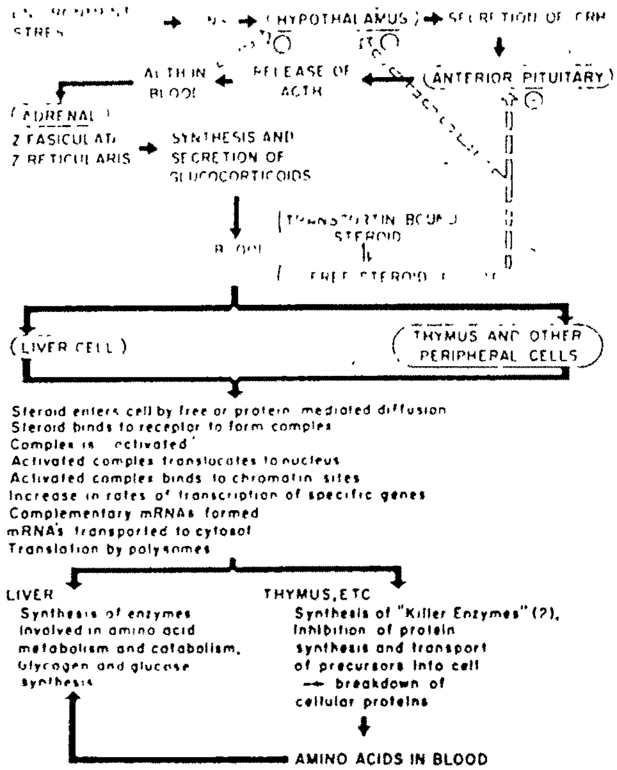
1.5.5. In target tissues : A variety of tissues in the body concentrate corticosterone which include the CNS. The liver and the thymus are the major corticosterone concentrating areas. In the CNS uptake of corticosterone from circulation varies amongst different regions and in specific cells. Studies utilizing autoradiographic technique by injecting (^{3}H)-corticosterone have suggested a wide range of intensity in labelling of different regions. It has been suggested that such differences in apparent 'uptake' affinities reflect differences in functional responses, which has been formulated as the 'principle of differential hormone uptake and response threshold'. Accordingly, under certain physiological conditions, and normal corticosteroid level, only certain tissues or cells with high nuclear uptake may be stimulated, while other target cells may not respond. Under increased corticosterone level cells with even lower uptake affinity may respond thus resulting in the regulation of endocrine feedback and behaviour which involves the brain, pituitary and peripheral endocrine organs (Stumpf and Sar, 1976).

In short, corticosterone concentration in blood varies depending on circadian rhythm, age and also on the physiological state of the animal. The stress nonresponsiveness associated with a very low basal corticosterone during the first two postnatal weeks seem to be important for the developing tissues, especially the CNS, which occurs during the same period. The alterations in the circulating level of plasma corticosterone during development could also lead to altered tissue levels resulting into varied effects of corticosterone in different target tissues. Such alterations in the circulating corticosterone are presumed to reflect the varying needs of the organism for corticosterone.

1.6 <u>Functions/effects of corticosterone</u> : Corticosterone exerts widespread influence on various target tissues (Fig.5). The overall result of corticosterone action at the whole animal level of organization include

(a) maintenance of normal blood sugar level,
(b) maintenance of normal arterial systemic blood pressure,
(c) inhibition of inflammatory response,
(d) eosinopenia, thymolysis, lympholysis and immunosuppression,
(e) support of resistance to many noxius stimuli.

Underlying these effects of glucocorticoids are certain metabolic actions. The major effect of glucocorticoids upon the metabolism of the organism as a whole is in the redistribution of proteins, carbohydrates and lipids in the body. A brief account of glucocorticoid effects on these aspects follows here a



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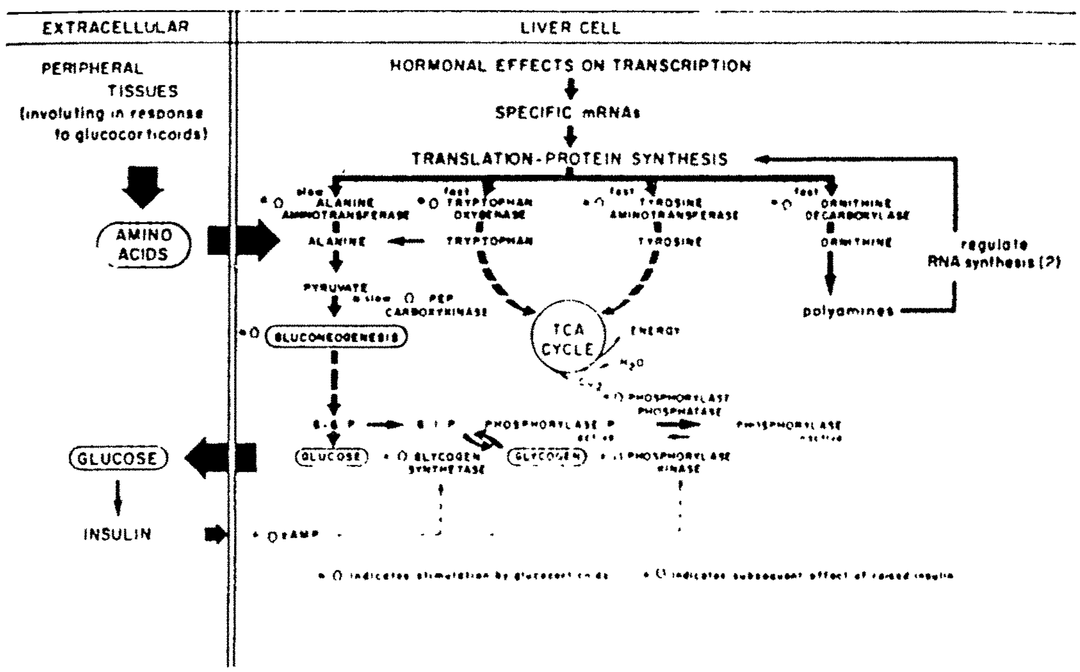

Fig. 5 *Overview of Glucocorticoid Action*

a) Sequence of events culminating in tissue specific responses to envirotmental stress

b) Metabolic effects in the glucocorticoid stimulated liver cells
Taken from : Cake and Litwack (1975)

1.6.1 <u>Carbohydrate metabolism</u> : Glucocorticoids maintain blood sugar and liver glycogen content through several means. One of its major action is promoting the gluconeogenesis in the liver by both peripheral and hepatic actions. From extrahepatic tissues such as muscle, skin, etc., glucocorticoids mobilise amino acids and funnel it to liver through circulation where they serve as substrates for enzymes involved in the production of glucose and glycogen. In the liver the glucocorticoids induce *de novo* synthesis of a number of enzymes involved in gluconeogenesis.
For example, the hepatic enzyme phosphoenolpyruvate carboxykinase (PEPCK), Fructose-1,6-diphosphatase and glucose-6-phosphatase which catalyse reactions of glucose synthesis, are increased in concentration.
However, induction of these enzymes require a matter of hours and cannot account for the earliest effects of the hormones on gluconeogenesis. More rapid effects of glucocorticoids are apparent on hepatic mitochondria, such that they carboxylate pyruvate to form oxaloacetate at an accelerated rate (Adam and Haynes, 1969).
This is the first reaction in the synthesis of glucose from pyruvate catalysed by pyruvate kinase. *In vivo* corticosteroid administration causes a large increase in PEPCK, an enzyme known to have important regulatory action in gluconeogenesis. Several *in vitro* experiments using rat liver perfusion with adrenal

manipulations have demonstrated that both glucagon and corticosteroids are necessary for the enhanced gluconeogenesis and suggest a permissive effect of corticosteroids in this process (Eisenstein et al, 1966, 1968). Thus irrespective of the clarity about the mechanism it is well established that corticosteroids enhance gluconeogenesis.

Glycogen synthesis has also been shown to be influenced by corticosteroids causing deposition of glycogen in the liver of normal and adrenalectomized animals. Corticosteroids enhance glycogen synthetase activity by a phosphatase-mediated dephosphorylation and inactivation occuring via a kinase-catalysed rephosphorylation (Mersmann and Segal, 1969). This effect of deposition of glycogen in the liver found after treatment with corticosteroids is thought to be atleast, in part, secondary to the rise in plasma insulin concentration elicited by the elevated plasma glucose (Kreutner and Goldberg, 1967).

In addition to glucocorticoid effects on gluconeogenesis and glycogen synthesis, utilization of glucose by various tissues have been shown to be affected. Corticosteroids both in vivo and in vitro inhibit glucose uptake by peripheral tissues including muscle, adipose and lymphoid tissues. This action occurs primarily at a transport site. Very recently Munck and associates have shown that in fibroblast cells, treatment with corticosteroid decreases the number of glucose transporter protein which appears in the intracellular membrane fractions (Horner et al, 1987) and thereby decrease glucose uptake.

1.6.2 Protein and aminoacid metabolism : Corticosteroids promote protein catabolism and interfere with protein synthesis in majority of the tissues. Adrenal gland removal from fasting rats result in a prompt reduction of nitrogen excretion and decline of blood glucose and liver glycogen which following corticosteroid replacement restores urinary nitrogen to previous levels (Long et al, 1940). Glucocorticoid treatment markedly inhibits protein synthesis in muscle, lymphoid and other tissues with accompanying reduced aminoacid uptake. Since protein degradation in these tissues is continuing, aminoacids leave these structures resulting in wasting of soft tissues and osteoporosis (Manchester, 1970).

In contrast to muscle, liver protein synthesis increased with corticosterone treatment. In fed rats injection of corticosteroid produced a rapid increase of the free amino nitrogen levels in liver (Weber et al, 1965). In fasting rats elevation of only selected amino acids occurred with cortisol injection, thus indicating that the nutritional state of the animal influences the hormone response (Bass et al, 1963). Deposition of protein in liver following adrenal steroid treatment is affected by the amount of energy provided by diet with greater protein synthesis occurring when the calorie content of the diet is high.

Several enzymes associated with the amino acid metabolism have been shown to be induced in liver by corticosteroids. Prominent amongst the enzymes induced are tryptophan pyrrolase, tyrosine transaminase (Kenney, 1970) and elevation of glutamic-alanine transaminase (GAT) levels (Segal and Kim, 1963).

1.6.3 Lipid metabolism : Glucocorticoids favour the mobilization of fatty acids from adipose tissue to liver, where the metabolism of free fatty acids may lead to the appearance of products that inhibit glycolytic enzymes and favour gluconeogenesis (Weber, 1968). The lipid mobilization is mainly exerted by controlling the rate of triglyceride hydrolysis in adipose tissue and occurs only in the presence of both growth hormone and corticosteroids. However, neither of the hormones alone stimulate fatty acid release (Fain et al, 1965). Corticosteroids also increase sensitivity of fat cells to the lipolytic actions of epinephrine mediated through cyclic AMP-mediated activation of lipase.

The effect of corticosteroids on lipid synthesis appears to vary according to the target cell studied. It inhibits fatty acid synthesis in HeLa cells and lymphocyte (Dellorco and Melnykovych, 1970; Seillan et al., 1981), whereas in lungs they are known to enhance the synthesis of lecithins involved in the constitution of the pulmonary surfactant (Torday _et al_, 1975; Oldenberg and Van Golde, 1977). Hays and Hill (1960) have shown that in chronically cortisone treated rats, triglyceride synthesizing activity increased and the acyl CoA synthetase activity in hepatic microsomes increased by 30%. Nevertheless, the bulk of evidence available indicates that fat synthesis is inhibited by corticosteroids.

1.6.4 Role of corticosteroids during stress : The role of glucocorticoids during stress has been a subject of controversy ever since the traditional view put forward by Selye. According to this view, glucocorticoids increase resistance to stress by enhancing our defense mechanisms. Selye, in his 1946 review (Selye, 1946) suggested that stress increased the need for sugar and hence the sugar active glucocorticoids were essential for resistance to stress. Another example is the frequent appeal to the permissive enhancement of vascular and other responses to catecholamines (Ramey and Goldstein, 1957) in order to explain the beneficial effects of glucocorticoids in the treatment of shock. Another suggestion in support of this traditional view is that by causing lymphocytolysis, the glucocorticoids release preformed antibodies and thereby enhance the immune response to injection. The last suggestion was, however, rejected long ago. Besides, the role of glucocorticoids in increasing blood sugar level, inhibiting the peripheral tissue utilization excepting by skeletal muscles during stress and the redistribution of much needed sugars have all been thought to support it's role in increasing resistance to stress.

Another school of thought on the role of glucocorticoids during stress has been lead by Munck and his colleagues (1984). Glucocorticoid functions in stress, in their own words - ....." (i) the physiological function of stress-induced increases in glucocorticoid levels is to protect not against the source of stress itself, but against the normal defense reactions that are activated by stress and (ii) glucocorticoids accomplish this function by turning off those defense reactions, thus preventing them from overshooting and themselves threatening homeostasis". In a comprehensive review regarding the glucocorticoid functions in stress, they have argued that several cellular defense related processes are activated with stressful stimuli. These cellular defense related responses are the activation of immune processes viz., secretion of immune interferons, macrophage activating factors and natural killer stimulating activities, and mediators of allergic and inflammatory reactions such as leukotrienes, prostaglandins and thromboxanes in addition to histamines. Besides this, there are normal processes of tissue remodelling, initiated by plasminogen activator and metabolic readjustments, needed in terms of several hormones such as insulin, glucagon and catecholamines in carbohydrate metabolism. The enhanced glucocorticoid level during stress, they have argued, inhibits the already activated above mentioned cellular mediators and inhibits secretion of insulin while it enhances that of glucagon and epinephrine resulting into prolonged effects of the latter two hormones in increasing blood glucose level. This influence is assumed to be sufficiently delayed in relation to the initial stress stimulus to allow the appropriate defense mechanisms to be activated. Most glucocorticoid effects already have a built-in delay of hours in their time of onset with a subtle control of timing as in the case of the immunosuppressive actions(Munck et al, 1984). These authors also believe that the high levels of glucocorticoids that exert regulatory functions are necessary to temporarily occupy most glucocorticoid receptors in the body and influence maximally the cellular processes involved in defense mechanisms. Thus from the foregone review of literature it is evident that the changes in glucocorticoid during normal and stress period are very crucial for the homeostasis, the glucocorticoid effects being very wide ranging in peripheral tissues and could lead to deleterious effects on the organism, if present in abnormal levels.

1.7.1 Mechanism of glucocorticoid action : From intensive studies on the mechanisms of a variety of glucocorticoid effects, there has emerged the general picture of primary actions (Fig. 6). The hormone molecules, being lipid soluble gain entry freely into all cells, form hormone-receptor complexes in their target cells that are translocated to the nucleus; there, apparently by regulating transcription, result in synthesis of specific proteins that mediate the effects.

With the advent of several molecular biological techniques,the above mentioned scheme of glucocorticoid mechanism of action has become clearer at molecular level. In this regard, glucocorticoid receptors (GR) have been characterised in some detail, the $\sim$ 87-KD hormone-binding protein has been purified to near homogeneity (Wrange et al, 1979, 1984), monoclonal antibodies have been produced and complementary DNAs (cDNAs) have been isolated and sequenced from the rat, human and mouse (Miesfeld et al, 1984; Hollenberg et al, 1985). In rat this receptor is of 795 amino acid residues with a 86-amino acid segment important for eliciting glucocorticoid effect (Miesfeld et al, 1987). Biochemical studies implied that the receptor carries separate functional domains for hormone and DNA binding (Carlstedt-Duke et al, 1982). When complexed with glucocorticoid, it binds to specific DNA sequences that are called glucocorticoid

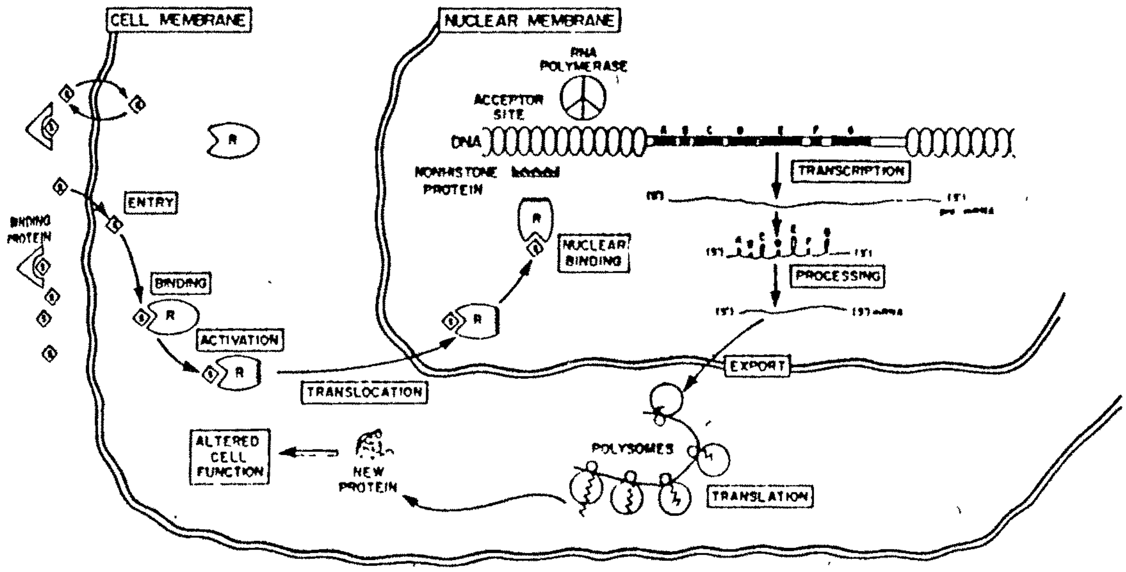

Fig. 6 *Mechanism of Action of Steroid Hormone*

S-steroid; R-receptor

dependent transcriptional enhancers. On the well studied mouse mammary tumor virus genome, there are five such binding regions (one up-stream and four down-stream). In vitro, binding of the hormone receptor complex occurs independently to these sites. In vivo, these receptor binding sequences confer stimulation by glucocorticoid when fused in either orientation to another promoter (a DNA sequence involved in binding of RNA polymerase to initiate transcription whose expression is not normally hormone regulated). This suggest that the type of response to a given steroid is determined by the acceptor site and not by the receptor (Green and Chambon, 1987). It also depends on the differentiation state of the cell (King, 1987), receptor modifying factors (Housley et al, 1984) and stage of the cell cycle (Cidlowski and Cidlowski, 1982). Several alterations in the chromatin structure of the hormone-activated gene(s) can be detected. Firstly, general DNAase I sensitivity increases upon hormone addition and persists long after hormone withdrawal. Secondly, discrete DNAase-I-hypersensitive regions develop near the sites of hormone-receptor bindings; these disappear when hormone is removed and ultimately lead to the transcriptional enhancement of specific proteins.

Mechanisms acting at other levels of gene expression are the selective stabilization of hormone-stimulated transcripts against cytoplasmic degradation.

For instance, in Xenopus laevis, the half-life of vitellogenin mRNA is about 3 weeks in the presence of the inducing hormone esterogen; it drops to 16 hrs after estrogen withdrawl. Such selective mRNA stabilization has also been observed for a number of other genes that are stimulated by steroid hormones. In addition, after hormone treatment of sensitive cells, RNA processing has been reported to be altered, and increased rates of overall RNA synthesis are sometimes observed leading to proliferative growth. Thus glucocorticoids achieve massive accumulation of specific mRNAs by concerted manipulation of both transcriptional and post-transcriptional processes (Watson _et al_, 1987).

Induction and inhibition of enzymes : The transcriptional products thus formed, may regulate several key enzymes involved in metabolic pathways that are induced by glucocorticoids, thus mediating its effect. Reports on the inhibitory effect of glucocorticoids on certain enzymes are meagre with respect to the mechanism involved therein. One of the well known example of enzyme inhibition by glucocorticoids is that of phospholipase-A_2. The molecular mechanism through which its inhibition is facilitated involves glucocorticoid receptors and protein synthesis. A certain protein is synthesized with glucocorticoid treatment which acts as inhibitor for phospholipase A_2 (Blackwell _et al_, 1980; Hirata _et al_, 1980). Similarly, plasminogen activator, a serine proteinase has been shown to be inhibited by glucocorticoids by induction of an inhibitory protein (Seifert and Geleherter, 1978).

1.7.2. <u>Non-genomic mechanisms of action</u> : All the earlier mentioned glucocorticoid effects, however, cannot be explained on the basis of genomic effects alone as evidenced by the very short time period required for eliciting a response and by using transcriptional and translational inhibitors, e.g., the lymphocytolytic effects of glucocorticoids. There are numerous reports of non-genomic effects of glucocorticoids, as well as of other steroid hormones (Robins and Schimke, 1978; Mc Knight and Palmiter, 1979). For example, as mentioned earlier, the GR binding to tRNA might alter the efficiency of translation and this could modulate protein synthesis. Very recently, it has been shown that the glucocorticoid treatment results in a predominance of a transformed glucocorticoid receptor that binds to t-RNA (Ali and Vedeckis, 1987) which could lead to its participation in ubiquitin pathway of protein degradation. The mechanism involved in non-lysosomal, ATP-dependent, ubiquitin mediated protein degradation, specifically requires t-RNA for the attachment of ubiquitin to proteins (Farber and Ciechanover, 1986). The ubiquitinization of proteins serve as a tag, leading to degradation of ubiquitinized proteins. Thus glucocorticoids could affect the expression of hormonally regulated proteins by modulating ubiquitinization and subsequent degradation of specific proteins or by modulating it's own stability. In fact, glucocorticoid hormone treatment has been shown to stimulate protein catabolism associated with muscle wasting.

In the brain, several glucocorticoid effects have been attributed to non-genomic mediation, for example, the rapid neuronotropic effects of glucocorticoids such as alterations of neuronal excitability and inhibition of secretion of corticotropin releasing hormone (CRF) from the hypothalamus. Furthermore, steroids have been used as anesthetic agent since long which could depress the brain activity even within minutes. These effects of glucocorticoids on brain occur within seconds to minutes which preclude the genomic mediation. In vitro incubation of hypothalamus in the presence of Ach and corticosterone showed an inhibition of CRF release and suggests a fast feed-back action of corticosterone (Jones et al, 1976). Electrophysiological studies by iontophoretically applied corticosterone to specific neurones in paraventricular nucleus have demonstrated selective inhibition or excitation of neurons within 30-50 seconds in a dose related manner (Saphier and Feldman, 1988). The rapid effects of corticosterone on brain neurons have been localized at various aspects of membrane. The excitation or inhibition of brain neurons by corticosteroids has been suggested to be occurring via $GABA_A$ receptors. Corticosterone or endogenous metabolites of cortisol increased the muscimol binding affinity of $GABA_A$ receptors (Owen et al, 1986), affected the activity of chloride channel linked to $GABA_A$ receptors by increasing the chloride ion channel opening time or its frequency (Lambert et al, 1987). Higher steroid concentrations directly activate the transmembrane current as shown by increased chloride ion flux in rat brain vesicles (Majewska et al, 1986). Thus glucocorticoids could exhibit non-genomic effects directly at membrane level and on the receptor molecule complexes.

In short, glucocorticoids exhibit wide ranging effects on various tissues by acting mainly as transcriptional enhancers, although not all of its effects could be accounted for by this mechanism. The specificity of hormone action is rendered predominantly by the acceptor sites on the genome as well as by the differentiation state of the cell. Furthermore, the non-genomic mechanism of glucocorticoid action could also be of considerable importance at cellular level.

1.8 Glucocorticoid receptors in brain :-

1.8.1 Types of receptors : It has been known since long that brain acts as a target organ for glucocorticoids and responds by way of alterations in certain behavioural changes. Several studies in the later part of 1960's showed that there exists an association between the corticosteroids and brain cytosol fractions (DeVenuto and Chader, 1966; Bottoms and Goetsch, 1967). In 1969, McEwen et al., demonstrated a biochemical evidence that corticosteroids are differentially recognised by certain neural systems, by injecting (^{3}H)-corticosterone into adrenalectomised (ADX) rats and then measuring hormone uptake and retention by specific regions, such as the hippocampus and septum. Grosser and his colleagues first demonstrated the presence of corticosteroid-binding macromolecules in soluble fractions prepared from brain (Grosser et al, 1971) which was subsequently shown to be proteinaceous in nature with distinct properties from those of serum corticosteroid-binding proteins (McEwen et al, 1972). Since then several reports have appeared describing the differential localisation in brain regions of these receptors, their biochemical characteristics and functional implications. Ten years ago, Bruce McEwen and his colleagues described a second type of putative glucocorticoid receptor in rat hippocampus, called 'Corticosterone-preferring sites" since they have a higher affinity for corticosterone than dexamethasone (De Kloet et al, 1975). Subsequently, work from Ron de Kloet's laboratory has established that such sites are indeed physiological high-affinity glucocorticoid receptors (De Kloet et al, 1982, 1983, 1986b). It is now well established that the rat brain contains two receptor systems for corticosterone; the type-I corticosterone preferring receptor and the classical type-II glucocorticoid receptor (Reul and De Kloet, 1985). The type-I receptor physicochemically resembles the kidney mineralocorticoid receptor (Krozowski and Funder, 1983) and is phenotypically expressed in the brain in two subtypes, the type-I mineralocorticoid receptor and the type-I corticosterone preferring sites, as shown by radioligand binding assays and functional experiments (De Kloet et al, 1986a; McEwen et al, 1986a; De Kloet and Reul, 1987).

The type-I mineralocorticoid receptor is thought to mediate the mineralocorticoid effect of aldosterone on behaviour driven by salt apetite, which is presumably effectuated in circumventricular structures (Magarinos et al, 1986).

The type-I corticosterone-preferring receptor resides mainly in neurons of limbic structures, e.g. the hippocampus and dorso-lateral septum. This receptor system displays highest affinity for corticosterone (apparent affinity (K^{d}_{2}) at 0°C is 0.5-1 nmol/l) and aldosterone (K^{d}_{2} at 0°C is 0.75-1.5 nmol/l) while it exhibits very low affinity for the synthetic glucocorticoid dexamethasone (De Kloet et al, 1975; Veldhuis et al, 1982; Reul and De Kloet, 1985). The type-I corticosterone preferring receptor shows stringent specificity in responsiveness to corticosterone in steroid-dependent aspects of behaviour (Micheau et al,1985) and serotonergic neurotransmission (De Kloet et al, 1982) while aldosterone acts as an antagonist (De Kloet et al,1983).

The type-II glucocorticoid receptors are widely localized in the brain and are found in both neurons and glial cells.(Reul and De Kloet, 1985; Fuxe et al,1985a,b). This receptor exhibits higher affinity for synthetic glucocorticoids, such as dexamethasone (Kd at 0°C is 1.5-2.0 nmol/l) than for corticosterone (Kd at 0°C is 2.5-5 nmol/l) and physicochemically resembles the glucocorticoid receptor found in peripheral tissues, specifically liver (Veldhuis et al, 1982; Reul and De Kloet, 1985).

The distinction between these receptors and corticosterone-binding globulin (CBG) became possible with the introduction of two synthetic glucocorticoids, RU 26988 and 28362. These steroids do not bind to CBG but bind with high affinity to type-II glucocorticoid receptor and not to type-I mineralocorticoid receptor (Philibert and Moguilewsky, 1983). RU 28362 can be used to measure brain type-II glucocorticoid receptor directly. Type-I corticosterone receptor bind with high affinity to corticosterone but with low affinity to dexamethasone. Dexamethasone does not bind to CBG and type-I mineralocorticoid receptors while binding with high affinity to only type-II glucocorticoid receptors.

1.8.2 Distribution of receptor types in brain : Microdissection of frozen brain regions revealed a considerable differentiation of type-I and type-II corticosterone receptors within the hippocampus and elsewhere in the brain. Type-I receptors predominated in the hippocampal region, specially in the subiculum, the pyramidal neurons and dentate granular neurons (Stumpf and Sar, 1976) while its density is much lower in other regions throughout the brain. In contrast type-II sites were found widely distributed throughout the brain. The greatest density of type-II sites are found in hippocampal pyramidal and granular neurons. In addition, they are also found in brain regions which are known to be involved in the integrative regulation of the stress response (the paraventricular nucleus, supraoptic nucleus and the nucleus arcuatus-median eminence complex as neuroendocrine centres), the noradrenergic cell groups A6 (locus coeruleus, arousal), A2 (nucleus tractus solitarii, centre of blood pressure regulation) and the serotonergic cell groups (raphe nuclei) (Fuxe <u>et al</u>, 1985a,b).

The CBG like binders are present in a large quantity in pituitary cells and to a smaller extent in the hippocampal septal complex (Koch <u>et al</u>, 1976; De Kloet <u>et al</u>, 1982). Unlike type-I corticosterone receptors, CBG-like binders lack the ability to transport the steroid to the cell nucleus (Alexis <u>et al</u>, 1983).

1.8.3 <u>Functional significance of different receptor subtypes</u> :
Corticosterone interacts with both type-I and type-II receptors, albeit to differing degrees; each receptor population likely subserves a different role in the brain. Based on behavioural and biochemical studies, it has been

postulated that the type-I sites are involved in a tonic activating influence of the hormone on brain function while type-II receptors mediate the feedback action of corticosterone (De Kloet and Reul, 1987). Recently, they have obtained certain evidence to this effect by studying the occupation of type-I and type-II receptors using the endogenous ligand at different times after adrenalectomy, the stress of a novel environment, injection of dexamethasone, and measuring the number of available sites of each receptor type with radiolabelled steroids.

Experiment with the relative occupancy of type-I and II receptors after adrenalectomy showed that these receptors became available in the soluble cell fraction for binding of the radiolabelled steroid at a strikingly different rate during the first 24 hr after adrenalectomy. Availability of type-I receptors increased maximally between 4-7 h while type-II remained relatively constant for first 24 h after adrenelectomy but increased there after (Reul et al,1987).

Studies on the relative occupation of type-I and II receptors after activation and suppression of the pituitary-adrenal system following the novelty stress and dexamethasone treatment respectively, demonstrated that occupation of type-II receptors occur parallel with the stress-induced rise with the subsequent decrease and the circadian variation of the plasma concentration of corticosterone. This suggests mediation of type-II receptors in feedback actions of corticosterone on stress-activated brain mechanisms and in circadian functioning with the steroid level acting as the rate limiting signal. Since the type-I receptor is always extensively occupied irrespective of total receptor sites, adrenalectomy and subsequent hormone replacement are necessary to explore its function suggesting its insensitiveness to plasma corticosterone changes. Behavioural and biochemical studies employing this experimental approach have revealed an important role of this receptor system (type-I) in limbic functions involved in interpretation of sensory information important for daily activities and in regulation of sleep related events (Micco et al, 1979; Bohus and De Kloet, 1981; Micheau et al, 1985; Jhanwar-Uniyal et al, 1986). Such effects were specific for corticosterone and found to be of a tonic activating nature. Therefore, it has been suggested that the corticosterone signal transmission may be modulated by type-I receptors by alteration in receptor capacities while in type-II receptors corticosterone level changes could rely the corticosterone signal.

With regard to the intracellular pool of CBG in the pituitary, probably it plays a major role in retarding corticosterone action on this structure. It's role,however, in some brain regions is not yet clear. In pituitary, CBG-like protein is thought to compete with the type-I and II receptors for binding to corticosterone and as a consequence less steroid is made available to the receptor for cell nuclear translocation (De Kloet et al, 1975: Koch et al, 1981). Since dexamethasone does not bind to CBG and bypasses the CBG-like binders, it could translocate into pituitary cell nuclei and effectively inhibit ACTH secretion by these cells (McEwen et al, 1986a), in fact much more effectively than corticosterone. The role of CBG-like binders in pituitary seems to be very important, more during the early period of development (SNRP) and is discussed later.

1.8.4 Regulation of glucocorticoid receptor system : Of the several factors that determine the magnitude of the biological response to glucocorticoid, one is the number of available receptor sites for the steroid. The number of receptor sites is subject to short-term and long-term regulation. Short-term regulation includes influences by changes in pH, ionic strength (Densmore et al, 1984), low molecular weight inhibitors, and by an internal phosphorylation/dephosphorylation cycle (De Kloet and Veldhius, 1984; Schmidt and Litwack, 1982).

Long-term regulation of receptor number has been found during the life span as a consequence of environmental, endocrine and neural manipulations. Unfortunately most of these studies were done earlier to the discrimination between type-I and type-II receptors became known. However, for the in vivo receptor binding, studies pertain mainly with the usage of (^{3}H)-corticosterone and hence to type-I receptors.

1.8.5 <u>Regulation/changes in receptor number during development</u> : Receptor capacity for corticoids in the hippocampus shows large changes during life span. Discrimination of receptor types with RU 26988 demonstrated presence of type-I and type-II receptors early in the rat's life (Sakly and Koch, 1981). <u>In vivo</u> (^{3}H) corticosterone nuclear uptake studies have demonstrated that in the limbic system of rat at late fetal period the receptor concentrations are comparable to those found in the adult. As with the adrenal cortex and plasma corticosterone levels, a regression occurs shortly after birth such that by postnatal day 3 receptor concentrations have declined to about 20% of those found in the adult (Turner, 1978; Meaney <u>et al</u>, 1985a,b). Corticosterone receptor concentrations rise readily in the brain after day 3, reaching adult levels by day 15. In other words, receptor concentration in brain parallel the plasma corticosterone levels both in magnitude and directions of change. A notable feature of this receptor development is that unlike in adult, elevations in corticosterone level does not decrease receptor concentration i.e., down-regulation is not observed during the early postnatal period (Meaney <u>et al</u>, 1985a,b). Similar decline in type-II receptors does not occur in the pituitary where receptor levels remain at adult-like values throughout postnatal development whereas type-I appears only at day 6 and reach adult levels after 4 weeks. Pituitary CBG-like protein, exhibits a developmental pattern similar to that of brain corticosterone receptors (Sakly and Koch, 1981, 1983). This alongwith the decreased circulating CBG titres may be responsible for the increased hyporesponsiveness of the pituitary-adrenal axis during the early postnatal period (SNRP) (Sapolsky and Meaney, 1986), wherein the feedback inhibition of even low levels of corticosteroid becomes exaggerated as less of corticosterone sequestration by CBG-like protein in pitutitary occurs. Autoradiography (Turner, 1978; Meaney _et al_, 1985b) showed that receptors in neurons follow by a few days the final cell divisions of dentate gyrus and pyramidal neurons (Altman and Bayer, 1975). Within the hippocampal formation, the dentate gyrus shows a developmental peak of labelling around postnatal day 15, whereas Ammons horn showed a gradual postnatal increase (Meaney _et al_, 1985b). The hippocampal cytosolic receptor sites measured _in vitro_ shows a similar trend of development while hypothalamic cytosol binding changed to a much smaller extent (Olpe and Mc Ewen, 1976). A notable exception to the developmental decline in corticosterone receptors as the fetus approaches term is that of cerebellum wherein the high level of type-II receptors disappear after postnatal day 10 (Pavlik and Buresova, 1984).

Receptor levels, as measured by *in vivo* (^{3}H)-corticosterone uptake in hippocampus and septum (probably mediated by type-I receptor) vary with social settings in mice and also during the circadian cycle (Valeri *et al*, 1978). Subordinate mice and mice subjected to crowding stress had reduced specific (^{3}H)-corticosterone uptake in the limbic structures (Valeri *et al*, 1978, 1980). In addition, subsequent studies showed that stress down-regulates the number of cytosol corticosterone-labelled receptors specifically in the hippocampus (Sapolsky *et al*, 1985) as also the exogenously administered corticosterone (Tornello *et al*, 1982). The extent of such down regulation, by repeated stress or corticosterone treatment, increases with increasing postnatal age, being low prior to weaning and gradually increase to adult levels (Meaney *et al*, 1985a,b). Besides, receptors that bind (^{3}H)-corticosterone *in vivo* and (^{3}H)-dexamethasone *in vitro* decline with age, the reduction most pronounced in hippocampal CA_3 neurons (Sapolsky *et al*, 1983). This decline accompanied by the neuronal cell loss (Sapolsky *et al*,1984a) appears to be associated with elevated corticosterone level. The information regarding the plasticity of adrenal steroid receptors in brain has been summarised here. Adrenalectomy results in a time-dependent elevation of receptor levels and stress or corticosteroid treatment results in a time-dependent depression of levels. This plasticity is especially evident in hippocampus when corticosterone treatment or stress is used and it presumably involves the type-I receptor system. However, when dexamethasone is used, pituitary, hypothalamic and amygdaloid receptors are depressed without a change in hippocampal receptors (Tornello et al, 1982; Sapolsky et al, 1984; Sapolsky and Mc Ewen, 1985). These results may point to changes or differential access of type-I and/or type-II receptor systems in these brain regions to corticosterone and dexamethasone and consequently differential neurochemical alterations with these treatments.

To summarise this section, discrete regions of rat brain possess corticosterone binding receptors (type-I and type-II) with different affinity. Limbic structures stand out with very high densities of these receptors. The development of these receptors in the brain almost parallels that of plasma corticosterone developmental pattern indicating a desirability by CNS for a decreased glucocorticoid access during its early postnatal development (implications of which is discussed later). The differential affinity for these receptor subtypes with respect to the corticosterone and dexamethasone may in itself result in varied response of developing CNS to these steroid treatments.

1.9 Development of CNS :

Several comprehensive reports on the development of CNS have appeared over the years. Of interest for the present study is the stages and factors that are crucial during CNS development. The structural and biochemical maturation of the nervous system is associated with a number of features which are time related. The timing of the occurrence of these changes varies in different brain regions and in different species. Their manifestation requires a favourable milieu during critical stages of development which otherwise will lead to the irretrievable losses in the functional outcome of the brain.

The critical stages of brain development include (a) neurogenesis, (b) post differentiation state, i.e., when neurons and glial cells form their interrelationship; neurons form synaptic contacts and glial cells modulate the neuronal microenvironment by participating in neuronal outgrowth, myelination, ionic regulation and synaptic expression. Glucocorticoid hormones appear to be a part of epigenetic factors which may exert influence at all these critical stages of brain development as is evident from the foregoing literature review.

1.9.1 Effect of glucocorticoids on rat brain during development :

A brief survey of the effect of corticosteroids on these aspects of developing brain and also on adult brain responses is presented herein. The major two approaches followed hitherto in this regard are - (i) corticosteroid administration and (ii) adrenalectomy followed by hormone replacement. In addition, studies with stress associated changes in brain parameters has also been followed.

1.9.1.1 Effect on brain growth and cell proliferation :

In the brain cell multiplication takes place mainly at certain sites, such as subependymal layer lining the forebrain ventricles and the external granular layer covering the surface of the cerebellum although there are also stem cells, probably glial cell precursors, which are dispersed throughout the brain (Balazs et al, 1975a,b). Extensive cell formation in the brain is restricted to about first three weeks after birth, although cell multiplication does not cease completely after that age; the external granular layer (EGL) disappears by about 24 days, but remnants of the subependymal layer are still present in the adult rat. In most of the species studied, neurogenesis during the early postnatal period leads to a selective increase in interneurons in certain brain regions such as the olfactory lobe, hippocampus and cerebellum. It seems that the formation occurs in a strictly chronological order, for example, in the rat cerebellum, the origin of the basket cells is at 2-6 days, that of the stellate cells at the end of the second week, whereas 50% of the granule cells are formed in the third week of life.

(a) Corticosterone administration : Corticosterone can exert a negative effect on brain growth and cell proliferation. The most general effect of neonatal glucocorticoid treatment on rodent brain is a long lasting decrease in tissue weight in the cerebrum and cerebellum (Cotterell et al, 1972; Kovacs, 1973; Howard, 1974). These brain weight decreases are accompanied by significant reductions in DNA content suggesting a lesser cell number with treatment. The biochemical studies of ornithine decarboxylase (Slotkin et al, 1983) and thymidine kinase activity and thymidine incorporation into DNA (Burdman et al, 1975) indicate a general suppression of cell proliferation.

In order to examine more specifically the influence of neonatal glucocorticoid administration at postnatal days (P_1-P_7) on developing neuronal populations, Bohn and Lauder (1978) observed a temporary inhibition of cell proliferation in the EGL of the rat cerebellum and also on granule cell formation in the dentate gyrus (Bohn, 1980). Subsequently, administration of hydrocortisone on P_7-P_{18} days have been shown to affect EGL development more profoundly than earlier administration suggesting an age dependent specificity in hormonal sensitivity. Several studies have also shown that neonatal glucocorticoid administration inhibits glial cell proliferation in the subependymal layer of the developing rat brain (Bohn, 1979) and in the optic nerve (Bohn and Friedrich, 1982). Cessation of the hormone treatment has been shown to exhibit a compensatory cell proliferation, although not to a complete extent.

Glucocorticoids can alter brain growth in different ways besides inhibiting cell proliferation. For example, the neonatal glucocorticoid administration retards the development of cortical dendritic spines (Schapiro et al, 1973), retards neuronal processes as measured by decreased ganglioside levels (Howard and Benjamin, 1975; Horowitz and Schanberg, 1979) suggesting an interference in synapse formation; a decrease in myelination in pyramidal tract (Gumbinas et al, 1973) and in optic fibre (Bohn and Freidrich, 1982).

Studies employing organ and organotypic culture systems on the effect of glucocorticoids have shown that the migration rate of glial cells from explants was markedly higher than that of control cultures (Hanaway, 1967) and also increased adhesion of cells to growth substrates in culture (Ballard and Tomkins, 1970). These results could imply that glucocorticoid may influence neural growth by their action on glial cell function also.

(b) Adrenalectomy studies : Studies on the early adrenalectomy on rat brain development have shown substantial increases in both cerebral and cerebellar weight, brain protein, DNA content, [illegible] a corticosterone reversible cell proliferation (Meyer, 1983) and also increased cerebral myelin (Meyer and Fairman, 1985). These studies suggest that adrenal secretions may exert a tonic inhibitory effect on brain growth in the normal developing animal. Such an effect is not restricted to early periods of postnatal development, since brain growth in rats is stimulated even in rats adrenalectomized on day 25 of life (Devenport and Devenport, 1983).

1.9.1.2 <u>Effect on myelination</u> : Myelination can be measured by isolating myelin by differential centrifugation or by certain relatively specific constituents of myelin. It is generally agreed that cerebrosides and sulfatides are good markers for myelin; cholesterol, which is also used as an index, is less specific. The rapid phase of myelination begins in the rat brain during the second week after birth and continues for another 3-4 weeks, although a slow rate of myelin deposition can be detected upto six months. Due to the heterogenity with respect to various neurochemicals in different regions of the brain, the development of myelination may proceed at various rates in these regions. Information in this regard has been very scarce and is available mainly on gross regions such cerebrum, cerebellum and brain stem.

Studies on the effect of glucocorticoid administration on myelination in rat brain regions is very meagre and has been discussed in greater length in the results and discussion section. Briefly, an earlier study by Casper et al. (1967) showed a higher cerebroside concentration in the spinal cord, but not in the cerebellum, of the cortisol treated animals. In a later study, Granich and Timiras (1971) examined the effects of cortisol on brain lipid patterns in chick embryos and young chicks. They found that in embryos,cortisol treatment produced a significant increase over control values in total lipid content relative to brain weight. Similarly, the percentage of total sphingolipid and of cerebroside in brain lipid extracts from treated embryos were greater than in controls. Although, contradicting a more recent report by Meyer and Fairman (1985) with adrenalectomy, these studies indicate that the process of myelination appears to be affected by glucocorticoid treatment. Further, Dawson and Kernes (1979) using oligodendroglioma clonal lines obtained evidence that cortisol may be involved in the process of myelination via their influence on glial cells. In view of the fact that several glial enzymes such as glycerol-3-phosphate dehydrogenase (GPDH) localised mainly in oligodendroglia, galactosylceramide sulfotransferase related to sulfatide accumulation in brain have been induced by glucocorticoid (De Vellis and Inglish, 1973; Dawson and Kernes, 1978), the former in an age specific manner, it would be of interest to investigate further the age dependent effects of corticosterone on myelin associated features in the developing brain regions.

1.10 Effect of glucocorticoids on neurotransmitter differenentiation and development :

On a biochemical level neuronal differentiation is often assessed in terms of neurotransmitter expression by a given cell population. Several well established neurotransmitters such as catecholamines (dopamine (DA) and norepinephrine (NE)), indoleamines (5-hydroxytryptamine), gamma aminobutyric acid (GABA) and glutamic acid have been discussed both with respect to their development and response to glucocorticoids in the ensuing portion.

1.10.1 Catecholamines : Studies have demonstrated certain role of glucocorticoids in the expression of catecholaminergic enzymes, their developmental increments and also of possible role in survival of such cells in the nervous system. These studies pertain mainly to enzymes such as Phenylethanolamine-N-methyltransferase (PNMT), Tyrosine hydroxylase (TH), Dopamine β-hydroxylase (DBH), and Tryptophan hydroxylase (TPH) involved in the synthesis of catecholamines and 5HT.

(a) Phenylethanolamine N-methyl transferase (PNMT) :

PNMT catalyzes the formation of epinephrine (E) from NE in the adrenal medulla, extra-adrenal chromaffin tissue, some small intensely fluorescent (SIF) cells in sympathetic ganglia and a few neuronal cell groups in the CNS. Depletion of glucocorticoids indirectly by hypophysectomy decreased the adrenal PNMT activity and E synthesis which could be reversed by ACTH or dexamethasone treatment. This led to the suggestion that PNMT activity in the rat adrenal medulla is normally stimulated by endogenous glucocorticoid (Wurtman, 1966). Later work established that hypophysectomy leads to a glucocorticoid-reversible increase in the proteolytic breakdown of PNMT (Ciaranello, 1978). S-adenosyl methionine (SAM), a cofactor of the enzyme that serves as a methyl donor stabilizes PNMT in intact animals, apparently due to glucocorticoids which increase tissue SAM concentration.

Glucocorticoids play a role in the developmental increase in adrenomedullary PNMT (Teitelman et al, 1982) and the maintenance of adult levels of enzyme activity (Wurtman, 1966) but is not essential for the initial expression of PNMT in adrenal chromaffin cells (Teitelman et al, 1982).

Glucocorticoids could also exert a major influence on peripheral catecholamine-tissues by preventing the normal postnatal disappearance/death of certain cells. A case in point is that of SIF cells in the developing sympathetic ganglia (Superior Cervical Ganglion, SCG) which primarily secrete DA or NE in adult rats. SIF cells numbering around 200-300 in a newborn rat increase in number (600-800) after the 2nd postnatal week (Eranko and Soinila, 1981). Beginning at embryonic day 17 (E17) and extending to the end of 2nd postnatal week, these cells express PNMT activity transiently (Bohn et al, 1982). Glucocorticoid administration to newborn rats increased PNMT expression associated with increased SIF cells (Eranko and Eranko, 1972; Ciaranello et al, 1973) whose effect disappeared rapidly after cessation of treatment (Ciaranello et al, 1973). This could either be due to death of this cell population, diminished expression of catecholaminergic characteristic, or loss of glucocorticoid responsivity (Bohn, 1983). Another example of preventing certain cell death is that of extra-adrenal chromaffin tissues - glucocorticoid treatment prevents the normal postnatal involution of extra-adrenal chromaffin tissue (para-aortic chromaffin body) in rats in a dose and time dependent manner. This led to the suggestion that normal degeneration of extra-adrenal chromaffin tissue in the rat is due to a decline in circulating corticosteroid concentration during the early postnatal period (Lempinen, 1964). These results, in comparison with possible tonic inhibition of cell proliferation suggest a differential, but yet selective, effect of glucocorticoids on various tissues during development.

The ability of glucocorticoids to influence the postnatal development of SIF cells and ganglionic PNMT activity is restricted to a narrow time span. Neither effect can be observed in adult rats or even in animals <1 week of age (Ciaranello et al, 1973; Bohn et al, 1982).

(b) Tyrosine hydroxylase :

Tyrosine hydroxylase catalyzes the conversion of tyrosine to DOPA, a rate limiting step in the synthesis of neurotransmitters DA and NE. Glucocorticoids modulate TH activity both in peripheral as well as CNS tissues. Studies by Hánbauer and colleagues provided tentative evidence that endogenous glucocorticoids might have a modulating influence on the inductive process. They reported that dexamethasone administration elevated TH activity in SCG of adult rats which could be blocked by pretreatment with cortexolone, a glucocorticoid antagonist (Hanbauer et al, 1975). Several additional factors have been shown to play important role in glucocorticoid mediated induction of TH, apparently by trans-synaptic induction requiring the post-synaptic nicotinic receptor in sympathetic ganglia of adult rats (Thoenen, 1970; Otten and Thoenen, 1976a). Such induction of TH in the SCG was correlated with the circadian rhythm as being maximal during the afternoon in SCG whereas in adrenal medulla during the morning (Otten and Theonen, 1975). TH induction in the rat SCG by NGF has been shown to be potentiated by glucocorticoids suggesting a permissive action of glucocorticoids independent of trans-synaptic induction discussed above (Otten and Theonen, 1976b). Such modulatory effects of glucocorticoids have also been demonstrated in the developing CNS regions. Diez et al. found increased whole brain TH activity following repeated hydrocortisone (HC) administration to intact mice beginning on postnatal day 7 (Diez et al, 1977), an effect subsequently shown to be localized to locus ceruleus but not the substantia nigra and occurred only at 7-13 days of age (Markey et al, 1982). In adults, only hypothalamus showed a selective increase in TH with repeated corticosterone treatment (Dunn et al, 1978). Adrenalectomy study showed that TH activity declined in the median-eminence that could be reversed by dexamethasone replacement (Kizer et al, 1974) indicating that even in certain brain regions TH activity is modulated by glucocorticoids, at least during specific period of development.

(c) Dopamine β- hydroxylase (DBH) :

The enzyme that catalyzes the formation of NE is DBH which has been shown to be stimulated in hypothalamus with high dose of corticosterone treatment (100 mg/kg) and decreased with adrenalectomy in hypothalamus and brain stem (Shen and Ganong, 1976a, b).

(d) Tryptophan hydroxylase (TPH) :

The rate-limiting step in the formation of 5HT is catalyzed by the enzyme TPH. The relationship between adrenal steroids and TPH has been examined primarily with respect to the effects of adrenal manipulations on brain enzyme activity in rats and mice. As in the case of TH, glucocorticoid seems to play a modulatory role in the normal developmental role as well as enzyme induction of TPH in the rat brain. Sze and his associates have examined the role of glucocorticoids in the normal development of neural enzymes (TPH, TH) and showed that early adrenalectomy (on postnatal day 12) totally abolished the normal developmental rise of midbrain TPH activity which was restored by glucocorticoid replacements (Sze, 1981). Furthermore, adrenalectomy blocked the stimulation of mouse brain TPH produced by reserpine (Sze et al, 1976) or ethanol (Sze and Neckers, 1974), agents that could stimulate pituitary-adrenal activity.

Administration of glucocorticoid to normal intact developing rats early in life showed a precocious induction of brain TPH (Sze et al, 1976; Yuwiler et al, 1978) and this response seems to be confined to second postnatal week only (Sze et al, 1980). Midbrain TPH activity, however, showed rapid increase with high-dose of corticosterone even in adult intact rats (Azmitia and McEwen, 1974). These studies thus, suggest that like TH, TPH activity is also under modulation by glucocorticoid in specific brain regions atleast during certain period of development. Studies with respect to glucocorticoid regulation of enzymes DBH, COMT and MAO associated with catecholamine and indoleamine metabolism during various stages of development are very scarce. Besides, except for a few studies such as on the inhibition of low affinity NE uptake in cerebellar explants from 16 day old chick embryo by cortisol (Vernadakis,1974), and extraneuronal uptake of NE, uptake$_2$, in the peripheral nervous system (Iversen and Salt, 1970), there seem to be paucity of information on the important aspect of monoamine uptake and release processes during development. Another notable exception to this is the study by Slotkin and associates who have studied the development of NE uptake and release processes in the whole brain with dexamethasone treatment and showed a decrease in uptake (Slotkin et al, 1983).

1.10.2 Studies on glucocorticoid effect on GABA enzyme system during development : GABA, an inhibitory neurotransmitter in mammalian central nervous system is synthesized, metabolised and distributed differentially in various brain regions.

The effect of glucocorticoid treatment on development of the enzymes GAD, GABA-T, and GDH in the rat brain regions are not reported so far. Similarly there are no reports on the effect of early adrenalectomy with respect to the above mentioned enzyme activities. The only enzyme of GABA and glutamate system studied to a certain extent is glutamine synthetase (GS), which catalyzes the formation of glutamine from glutamic acid and ammonia. Its major functions in brain include ammonia detoxification and the synthesis of a storage form of glutamate for subsequent release and reutilization by glutaminergic and GABAergic neurons. It is present mainly in the glial cells of the rat brain and retina. Pioneering studies by Moscana and Piddington (1966, 1967) showed that during early embryonic development of chick neural retina, hydrocortisone (HC) administration induced GS activity precociously. Similar effects were also demonstrated in organ culture system of chick embryo retina (Reif Lehrer, 1968a,b) and also mammalian neural tissue cultures (Vaccaro et al, 1979; Hallermayer et al, 1981). In vivo studies have dealt with the normal ontogeny and hormonal responsivity of GS in gross brain regions like cerebellum, forebrain and olfactory bulbs. Injection of 40 mg/kg corticosterone to intact rats for 3 days beginning either on day 8, 17 or 87 showed that GS activity increased in the cerebellum at all time points, whereas hormone was only effective in altering forebrain or olfactory bulb GS in the younger animals suggesting an age dependent, region specific effect of corticosteroid on the induction of GS activity. This study also showed that the increased GS activity is associated with increased enzyme protein synthesis that could be decreased by inhibitors of protein synthesis (Patel et al, 1983b). Studies with adrenalectomy and hormone replacement on GS activity in rat brain regions could possibly clarify endogenous corticosterone role in the regulation of GS development. Patel and associates further showed that increase in GS activity with glucocorticoid treatment varies in different regions, possibly due to the varied biochemical make up of glial cell populations (Patel <u>et al</u>, 1985). Therefore, a detailed study on the development of GS activity in discrete regions like hippocampus, striatum and hypothalamus with corticosterone treatment needs to be done.

1.11 <u>Effect of glucocorticoids on neurotransmitters in adult organism</u> : Besides the effect on development of neurotransmitters as manifested by changes in the specific neurotransmitter synthesizing enzymes, glucocorticoids can also influence neurotransmitter function in a variety of other ways, including changes in turnover, precursor availability, receptor binding etc. A brief account of such effects are discussed herein, mainly with respect to the classical approach that has been followed in order to elucidate the glucocorticoid role in these aspects (Table-1). The approach of exogenous glucocorticoid administration and stress associated changes have been discussed later.

1.11.1 <u>Catecholamines</u> : Studies on the role of endogenous glucocorticoids on brain NE have suggested a possible tonic inhibition of the activity of brain noradrenergic systems. These studies include adrenalectomised adult rats, that showed increased NE turnover measured in whole brain (Javoy <u>et al</u>, 1968) or in specific regions such as the

TABLE-1 : EFFECT OF ADRENALECTOMY AND HORMONE REPLACEMENT ON BRAIN CHEMISTRY.

Biochemical parameter	Effect of ADX	Hormone replacement: Effect	Steroid	Dose/duration	Brain region
Catecholamines					
NE turnover	Increased	Decreased	B	Endogenous[b]	Whole brain
	Increased	Decreased	F	25 mg/kg, 2 d.[b]	Whole brain
TH activity	Decreased	Increased	DEX	0.3 mg/kg, 7 d.[b]	Median eminence
DBH activity	Decreased	Increased	B	100 mg/kg, 4 h[a]	Hypothalamus
MAO activity	Increased	Decreased	DEX	2 x 30 ug/kg, 10 d[b]	Hypothalamus
Sensitivity of NE-receptor-coupled adenylate cyclase	Increased	Decreased	B	10 mg/kg, 5 d.[b]	Frontal cortex
B_{max} of -adrenergic receptor.	Increased	Decreased	B	1 mg/kg, 12 h[a], 7 d.[b]	Hippocampus/dorsal NE bundle lesion
Serotonin					
TR-OH activity	Decreased	Increased	B	0.4-3 mg/kg, 4 h[a], 5 d.[b]	Midbrain
5-HT content	Decreased	Increased	B	1 mg/kg, 30-60 min[a]	Hypothalamus
	Decreased	Increased	B	1 mg/kg, 15 min[a]	Mesencephalon, amygdala, hypothalamus, hippocampus, septum.
5-HT uptake and release	Decreased	Increased	B	1 mg/kg, 30 min[a]	Hypothalamus
Developmental rise TR-OH activity	Blocked	Increased	B	5 mg/kg, 3 d.[b]	Whole brain, neonate,
Reserpine-induced rise TR-OH activity	Blocked	Increased	B	20 mg/kg, 6 d.[b]	Whole brain, adult
Try content	Decreased	Increased	B	10 mg/kg, 3-7 d.[b]	Brain stem
Tr-OH activity, 5HT content	Decreased	Increased	B	10 mg/kg, 3-7 d.[b]	Brain stem
5-HIAA content	Increased	Decreased	B	10 mg/kg, 3-7 d.[b]	Brain stem
5-HT turnover	Decreased	Increased	B	2 x 0.3-0.4 mg/kg, 2 h[a], 3. d.[b]	Hypothalamus, brain stem
	Decreased	Increased	DEX	0.25 mg/kg, 1 h[a]	Hypothalamus, brain stem
5-HT turnover	Decreased	Increased	B	0.3 mg/kg, 1 h[a]	Hippocampus, raphe area
	Decreased	Ineffective	DEX	0.3 mg/kg, 1 h[a]	Hippocampus, raphe area
GABA					
GABA uptake	Increased	Decreased	B	Solid implant, 4-10 d.[b]	Hippocampal synaptosomes
GABA receptor binding	Increased	Decreased	B	5 mg/kg, 24 h[a]	Midbrain, striatum
Miscellaneous					
GPDH activity	Decreased	Increased	F	2-3 mg/kg, 13-14 d.[b]	Brain stem, cerebellum.
VIP content	Decreased	Increased	B	160 ug/ml in drinking water, 14 d.[b]	Dorsal hippocampus
	Decreased	Increased	DEX	173 ug/ml in drinking water, 10 d.[b].	Dorsal hippocampus
Angiotensin content	Decreased	Increased	B	1 mg/kg. 4.d.[b]	Preoptic are, anterior hypothalamus, periaqueductal gray, area postrema.
Neurophysin and vasopressin content in terminals	Increased	Decreased	B	Solid implant, 14 d.[b]	Median eminence.

a - Acute injection; b - Chronic treatment; the dose of steroid given was injected daily.

B - Corticosterone, F - Cortisol, DEX - Dexamethasone.

(Taken from DeKloet and Veldhu 1984)

hypothalamus observed long after adrenalectomy. Replacement therapy with either corticosterone or HC completely eliminated the increased turnover (Fuxe et al, 1973). Studies with adrenal intact mice administered corticosterone (Ivuone et al, 1977) showed an increased NE synthesis rate. This discrepancy may perhaps be due to species differences (i.e., mice vs rats) or due to the manipulation of endocrine status.

With regard to NE uptake studies, adrenalectomy does not seem to affect hypothalamic NE uptake (Vermes et al, 1976) although a low affinity extraneuronal uptake system has been shown to be inhibited by low micromolar concentrations of corticosterone in sympathetically innervated tissues (Iversen, 1973).

Very little is known concerning glucocorticoid effects on central dopamine systems. Fuxe et al. (1973) using histochemical fluorescence technique, have shown that both corticosterone and dexamethasone produce a dose-related fall in dopamine in the striatum of adrenalectomized rats.

Studies on brain 5-HT concentrations with adrenalectomy followed by hormone replacement showed either no effect (Fuxe et al, 1973; McKenne et al, 1966; Shah et al, 1968) or a glucocorticoid-reversible reduction in this neurotransmitter (Telegdy and Vermes, 1975; Rastogi and Singhal, 1978). Based on the studies of short term effect of adrenalectomy on 5HT turnover rate in various regions of the rat brain, Van Loon et al.(1981a,b) proposed that glucocorticoid withdrawal reduced

5HT turnover in the hypothalamus, hippocampus and brain stem. Rapid reductions in hippocampal 5HT turnover after adrenalectomy have also been reported (De Kloet _et al_, 1982, 1983) which showed stringent steroid specificity in the reversal of this effect i.e., corticosterone replacement, but not dexamethasone or aldosterone suggesting corticosterone specific type-I receptor involvement. In agrement with decreased 5HT turnover with adrenalectomy, acute hormone administration in intact animals showed a stimulation of 5HT turnover (Millard _et al_, 1972; Neckers and Sze, 1975) associated with an increased tryptophan transport into serotonergic nerve endings (Neckers and Sze, 1975).

The only study on 5HT turnover with respect to long term adrenalectomy showed an increased 5HT turnover in the hypothalamic median eminence and nucleus centralis superior, 10 days after adrenalectomy which was not reversible by corticosterone replacement (Long _et al_, 1983). These studies thus suggest that glucocorticoids in adult organisms seem to play a regulatory role with 5HT turnover, atleast with respect to short term effects which are apparently a corticosterone and region specific.

As with NE, reports on the effect of endogenous glucocorticoid on 5HT uptake is conflicting. Adrenalectomy decreased hypothalamic synaptosomal 5HT uptake by ~ 30% and also increased synaptosomal 5HT release. A single dose of corticosterone replacement (1 mg/kg) 30 min before death prevented the decreased 5HT uptake (Vermes et al, 1976). A subsequent study (Tukiainen, 1981) found only a marginal change in 5HT uptake with adrenalectomy in rats which was not corticosterone reversible.

1.11.2 GABA : Adrenalectomy showed no changes in GAD and GABA-T activity in the rat whole brain (Edwards and Rousseau, 1980) and in rat hippocampus (Meyer et al, 1979) suggesting that adrenal steroids do not appear to regulate these enzyme activities, atleast in adult whole brain. Studies, however, are necessary to find out such possibility in discrete brain regions at various stages of development.

Glucocorticoids may influence other GABA associated processes such as GABA uptake, receptor binding, alterations in activation of chloride-channel associated with GABA and also on the benzodiazepine binding. Adrenalectomy induced increase in the Vmax of GABA uptake into synaptosomes prepared from rat hippocampus but not from cerebellum or frontal cortex. This increase was reversed by corticosterone (CS) replacement for 6-10 days (Miller et al, 1978a).

Adrenalectomy led to CS-reversible increase in GABA binding to membranes prepared from corpus striatum and midbrain, whereas no effects were observed in hippocampus, hypothalamus, cerebral cortex, cerebellum or brain stem

(Kendall et al, 1982). Adrenalectomy failed to alter GABA concentration or high-affinity GABA uptake in either of these brain areas.

Adrenal manipulations exert effects at several sites on the GABA-benzodiazepine complex with associated alterations in GABAergic neuronal function. The $GABA_A$ receptor complex consists of allosterically linked GABA and benzodiazepine binding sites and the GABA site in turn is coupled to a chloride channel (Haefely et al, 1985). Adrenal steroids may affect several aspects of this complex. Adrenalectomy in rats reduced muscimol-a GABA agonist, binding by 30-50% in cerebral cortex, cerebellum, thalamus and hippocampal crude synaptosomal membranes. In contrast muscimol binding increased in the hypothalamus. Nanomolar concentrations of corticosterone, but not dexamethasone, enhanced the adrenalectomy induced decrease in muscimol binding, apparently by enhancing the affinity of GABA receptors (Majewska et al, 1985). This study demonstrated that glucocorticoids directly modulate GABA agonist binding to rat brain membranes in a region specific manner.

Recent reports indicate substantial increase in benzodiazepine receptor binding in several brain regions in adrenalectomized rats and enhanced effects of GABA on benzodiazepine binding in several regions (DeSouza et al, 1986;

Goeders *et al*, 1986); glucocorticoid replacement reversed most of these alterations. Studies on brain membranes *in vitro* indicated that metabolites of deoxycorticosterone affected binding of the benzodiazepine receptor at the chloride ionophore as well as promoted chloride flux in brain vesicles (Majewska *et al*, 1986). Furthermore, *in vivo* study on adrenalectomized mouse brain,benzodiazepine receptor binding also increased in cortex, hypothalamus and hippocampus,1 week after adrenalectomy which returned binding to control values only with physiological but not lower doses of corticosterone and deoxycorticosterone replacement. Such reversal in binding did not occur with aldosterone and dihydroprogesterone suggesting the steroid modulation of benzodiazepine receptor binding *in vivo* is perhaps via the type-I corticosterone preferring sites (Miller *et al*, 1988). These studies thus suggest that glucocorticoid could regulate GABA benzodiazepine receptor function either by genomic (involving type-I corticosterone preferring receptor) or directly on the membrane associated changes as evidenced by a suppression of the chloride conductance, probably reducing the number of functional GABA receptor-ionophore complexes with prednisolone addition to primary afferent neurons in bull frog spinal ganglia, using intracellular and voltage-clamp techniques (Ariyoshi and Akasu,1987).

1.12 Other glucocorticoid effects on brain :

1.12.1 Glucocorticoid toxicity in the hippocampus :

Chronic glucocorticoid exposure, whether a result of prolonged stress or pathological glucocorticoid hypersecretion, can be profoundly deleterious largely due to the catabolic nature of the hormone's actions (Krieger, 1982; Munck *et al*, 1984). Among their injurious effects glucocorticoids can damage neurons of the hippocampus. Glucocorticoids also have been implicated in the normative loss of hippocampal neurons during senescence, as basal titers of glucocorticoids rise with age in the rat (Landfield *et al*, 1981; DeKosky *et al*, 1984) and mid-age adrenalectomy prevents such neuron loss (Landfield *et al*, 1981).

Even physiological concentrations of glucocorticoids can induce a state of vulnerability in these neurons since a variety of insults that damage the hippocampus have their potencies attenuated in adrenalectomized rats and exacerbated in rats with glucocorticoid concentrations elevated to the upper physiological range. These insults include hypoxic ischemia, microinfusion of the excitotoxin kianic acid (KA) or of the antimetabolite 3-acetylpyridine (3-AP) (Sapolsky,1987).

Glucocorticoids endanger hippocampal neurons by impairing their energy metabolism directly (Sapolsky, 1986b), rather than secondarily to metabolic effects elsewhere in the body, as the synergy between glucocorticoids and the above mentioned metabolic insults to neuronal loss occur in vitro with culture of dispersed fetal hippocampal neurons (Sapolsky et al, 1988). Supplementation of brain energy fuels such as glucose, mannose, fructose, or the ketone beta-hydroxy-butyrate reduced hippocampal damage induced by co-administration of glucocorticoids and either of the two, KA or 3-AP (Sapolsky, 1986b). The reduction in damage was less effective with glucose (whose utilization is non-competitively inhibited by glucocorticoids) and fructose (which does not readily penetrate the blood-brain barrier). These findings draw attention to the energy fuels, glucose and ketone bodies, utilization during early period of brain development which may be altered with elevated glucocorticoid levels under prolonged stress or pathological conditions. Conversely, the decreased glucocorticoid level and transient adrenal quiescence during first two postnatal weeks may suggest an important regulatory role of glucocorticoid by way of metabolic changes with respect to brain energy fuels.

1.12.2 Glucose and ketone bodies as fuel for developing brain :

Brain is dependent on glucose as energy substrate, given their extremely limited capacity for glycogen storage, as well as the limited number of energy sources that can penetrate the blood-brain barrier (Siesjo, 1978). During early period of development glucose utilization is favoured in the hexose monophosphate shunt (HMP) that produce two useful products for biosynthetic purposes, NADPH and riboses, utilized for lipogenesis and protein synthesis in myelinogenesis and nucleotide synthesis in cell division processes. Glucose,thus is used primarily to support activities such as the HMP shunt that cannot be supported by fat or its metabolites. Glucose uptake to rat brain _in vivo_ increases steadily from birth to three weeks of age where rates were close to adult value and the stage at which many of the glycolytic enzymes reached their maximal, adult activities (Cf. McIlwain and Bachelard,1985).

Many recent studies have led to the recognition that metabolites, other than glucose, can serve as fuel to meet the energy requirements of the developing central nervous system. Two lines of evidence contributed to this consensus. Studies on the development of enzymatic capacity for the utilization of ketone bodies (Fig. 7) showed an enhanced potential for the mitochondrial utilization of acetoacetate and D(-)-3-hydroxybutyrate by developing rat brain (Klee and Sokoloff, 1967; Page _et al_, 1971; Patel, 1979; Booth _et al_, 1980). In contrast the activity of the pyruvate dehydrogenase complex, the enzyme which controls the flow of glucose derived carbon to mitochondrial Acetyl-CoA, has a distinctive developmental profile,

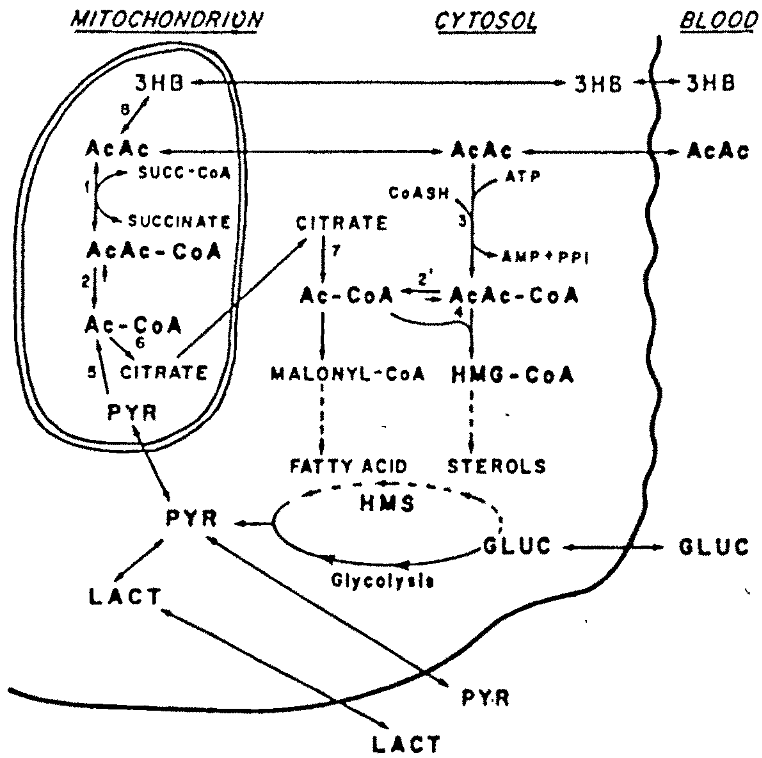

Fig 7 **Ketone body metabolism in the brain.**

AcAc from the blood can be used in the brain in the mitochondroin by the action of enzyme (number 1 in figure), 3-oxoacid-CoA transferase, then by the enzyme (number 2) mitochondrial acetoacetyl 1-CoA thiolase to give acetyl-CoA principally for use in energy production. 3HB from blood is converted to AcAc in the mitochondrion by the enzyme (number 8) D(-) 3-hydroxybutyrate dehydrogenase. AcAc can also be activated in the cytosolic compartment by enzyme (number 3), and its utilization for lipogenesis in the brain is similar to its utilization in the liver. Glucose can be converted to pyruvate by glycolysis (the Embden-Meyerhof pathway) and/or it can be used in the production of NADPH in support of lipogenesis and protein synthesis by the hexose monophosphate shunt. Carbon from pyruvate can be activated in the mitochondrion by the enzyme (number5) pyruvate dehydrogenase to provide acetyl-CoA. Citrate can be exported to the cytosolic compartment to provide acetyl-CoA by the action of the enzyme (number 7) citrate lyase. Developing brain aappears to convert pyruvate to lactate and export a substantial amount of both lactate and pyruvate. HMS, hexose monophosphate shut; GLUC, glucose; PYR, pyruvate; LACT, lactate.

it is delayed when compared to other enzymes associated with tricarboxylic acid cycle activity and develops linearly from a low prenatal level to adult levels by only 30 days after birth (Land et al, 1977). Studies by many investigators on metabolite utilization by developing brain either in vivo or in vitro have shown that acetoacetate and D-3-hydroxybutyrate are used by the developing brain (Williamson, 1982).

The interest in the metabolic capacity of developing brain to utilize ketone bodies was initiated by the in vitro studies of Itoh and Quastel (1970), who demonstrated that acetoacetate was a readily available substrate for respiration by brain slices. As much as 70% of the energy requirements of the developing rat brain could be credited to the oxidation of ketone bodies (Hawkins et al, 1971). In the interim many studies, particularly of rat and human, have shown the capacity to utilize both ketone bodies and glucose to meet its metabolic needs (Edmond, 1974; Patel et al, 1975; Patel and Owen, 1977; Yeh et al, 1977; Patel et al, 1981; Tildon et al, 1983) which include substrates for lipid synthesis. More recently Lopes-Cardozo et al.(1986) and Sykes et al. (1986a,b) have reported the utilization of ketone body substrates as lipid precursors and energy substrates in oligodendrocyte-enriched glial cultures from rat brain and in astrocytes and neurons in primary culture from the cerebral cortex of the mouse. It is evident from these reports that both glucose and ketone bodies serve as important substrates during the development of the brain.

Studies, however, on the availability and utilization of these substrates to developing brain under elevated glucocorticoid concentrations have not been reported so far. Only a few studies that are reported so far pertain chiefly to that of glucose uptake and utilization by brain regions with glucocorticoid treatment in adult rats. Adrenalectomy showed an increased glucose utilization while glucocorticoid administration to intact adult rats decreased, in a dose-dependent manner, the net uptake of glucose in certain brain regions such as hippocampus, striatum, hypothalamus and thalamus (Landgraf et al, 1978) as also in several peripheral tissues - thymus, adipose, hepatocytes and fibroblasts (Munck et al,1984). As mentioned earlier, the decreased uptake of glucose in fibroblasts has been shown to be due to translocation of glucose transporters from plasma membrane to intracellular membranes (Horner et al, 1987). Such a possibility with respect to brain regional inhibition of glucose uptake by glucocorticoid cannot be overlooked, while such studies are warranted, even more so, during the early period of brain development when the glucose uptake increase. With the important role played by ketone bodies in the developing brain, studies on the effect of adrenal manipulation of ketone body utilizing enzymes in neonatal period should also be interesting as a study with cortisol administration increased plasma ketone body level, atleast during the first 8 days of life (Aranguez et al, 1986).

From the foregoing review of literature several important observations could be made :-

(a) Glucocorticoids, secreted by adrenal cortex, play important role in the homeostasis of the organism. It's circulatory level being important, it varies during circadian cycle, development, stress and pathological conditions such as Cushing's syndrome and Addison's disease.

(b) It affects a wide range of processes in several peripheral tissues and the CNS both by genomic and non-genomic effects (includes receptor mediated non-genomic and membrane associated effects). Magnitude and direction of these effects vary depending on the structure of the glucocorticoids (e.g.,Corticosterone Vs. Dexamethasone) under question.

Briefly, glucocorticoid effect can be classified into two categories :

(i) regulatory effect of circulating glucocorticoids under normal conditions, e.g., possible tonic inhibition of cell proliferation during brain development. Such role can be normally elucidated by the classic approach of removal of endogenous hormone followed by its exogenous replacement.

(ii) effect of elevated circulating glucocorticoid under conditions of stress and pathological conditions e.g., redistribution of energy substrates, immunosuppression and certain behaviours associated with stress; have been studied by putting the animal to a specific challenge or stress and monitoring the neurochemical change. Such studies also include elevated glucocorticoid level by exogenous administration.

(c) CNS possesses two types of glucocorticoid receptor - type-I corticosterone preferring and type-II dexamethasone preferring receptors depending on the affinities of these steroids towards the receptors; in addition,corticoid-binding like protein is also present in certain brain areas. The distribution of these receptors in brain regions vary - with type-I more in limbic structures while type-II is distributed throughout the brain regions; former associated with neurons while the latter mainly in glial cells as well.

(d) Both the receptors in the brain as well as glucocorticoid circulatory levels decrease dramatically during first two postnatal weeks, associated with a relative quiescence of adrenal activity as well as stress related inhibitory phase feed back by hippocampus.

(e) Glucocorticoids besides affecting the development of brain,by inhibiting the cell proliferation, also affect possibly by alterations in the utilization of energy substrates, glucose and ketone bodies. Studies in this regard are very meagre.

(f) Glucocorticoids affect various aspects of neurotransmitter expression, development, uptake, release, receptor-binding etc., in brain regions. These effects, however, seem to be dependent on several factors such as timing of adrenal manipulation/alterations, dosage and the type of glucocorticoid used for either hormone replacement of adrenalectomised rats or intact rats.

(g) Studies on the development of GABA related enzymes during various stages of development of brain regions are lacking while a few studies with adrenalectomised rats on of these enzymes in adults have not shown any regulatory effects.

(h) Studies on the effect of adrenal manipulation on biogenic amines (DA, NE and 5HT) with respect to their content and turnover vary with the brain regions and are often contradictory, owing perhaps to different design of experiment which include time, dose and type of steroid hormone administration, in addition to the species and brain regions studied. Several studies have showed a possible role for glucocorticoid during the development of biogenic amine synthesizing enzymes such as TH and TPH by adrenalectomy; studies with exogenous glucocorticoid administration at different stages of these neurotransmitter development in various regions of the rat brain have not yet been reported. This assumes special significance in view of the association of change in biogenic amines with elevated corticoid levels during several pathological conditions as well as the therapeutic use of glucocorticoids under medical conditions.

In view of these observations, the present investigation aims at studying the effect of corticosterone administration on certain aspects of neurotransmitters in different regions of adrenal intact rat brain during various stages of development.

Glucocorticoid response of target cell, as mentioned earlier, depend on the acceptor site on the genome as also on several factors such as differentiation state of target cell, its hormonal mileu etc., - all of which have been known to vary with development. This could render the target tissue (brain regions, in the present case) to respond to elevated corticosterone level atleast during a specific period of development, which otherwise may or may not respond at adult stage. Furthermore, elevated corticosterone level during developmental stages of the brain could affect certain aspects non-genomically mediated with or without involving its receptors and also by direct effect on membrane.

In the presence of two types of receptors with varying affinity and accessibility to glucocorticoids having different structure, solubility and membrane permeability, the elevation of corticosterone level in circulation could make these receptor sites available for steroid hormone action even in the cells that do not have access to circulating glucocorticoid under normal conditions. Hence the present investigation aims at studying any such effect that may occur during the rat brain development, including SNRP, when elevated glucocorticoid level does not seem to be favourable for brain development. This could help in better understanding of the type of response a particular region could exhibit to elevated glucocorticoid level i.e., in terms of adaptive or detrimental with exposure during different developmental stages which may manifest itself at adult stage or under various pathological conditions.