CHAPTER I

INTRODUCTION

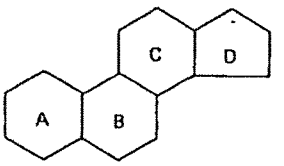
The capacity to respond to stress is one of the most basic mechanism in mammals, and the secretion of glucocorticoids by the adrenal cortex is a central feature of this response. They have many widespread effects within the body and, many of these effects are in common, protection of the organism from its own rapid response to stress. Thus effects of glucocorticoid is to reduce inflammation, maintain elevated blood glucose, promote fluid excretion *etc.* These effects although can be regarded as damping effects on response to stress, can lead to serious problems if glucocorticoid levels remain elevated. Extensive progress has been made to elucidate the molecular action of corticosteroids but is still incomplete. This chapter reviews the literature concerning glucocorticoids and highlights the problems remaining unsolved.

Structure and Synthesis:

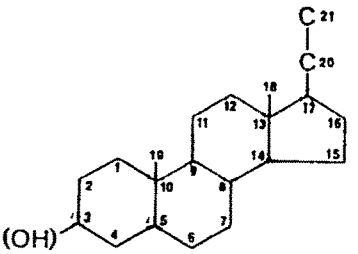
Corticosteroid structure resembles the group of terpenes. They may be considered as derivatives of fused, reduced ring system - cyclopentanoperhydrophenathrene nucleus, having three cyclohexane ring in the phenathrene arrangement and one cyclopentane ring at the terminal. Cortisol/corticosterone and cortisone (glucocorticoids) are two major adrenal steroids produced by the zona fasiculata of adrenal cortex as shown in fig. 1.1.

Regulation of Corticosterone Synthesis:

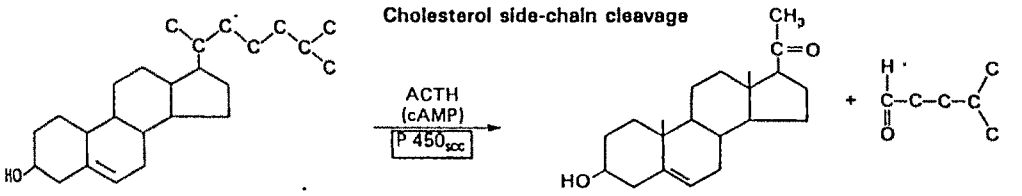
The hypophyseal ACTH regulates the secretory activity of adrenal cortex. ACTH increases the rate of synthesis by stimulating the conversion of cholesterol to pregnenolone,the rate limiting process. The process of stimulation by ACTH involves various aspects such as increased choles-



Cyclopentanoperhydrophenanthrene nucleus



Numbering of the carbon atoms Asymmetric carbons are shaded



Cholesterol

Pregnenolone + isocaproaldehyde

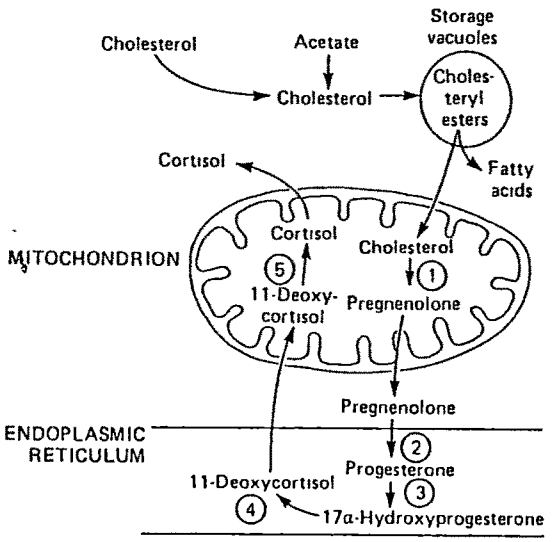


Fig.1.1. Structure and synthesis of glucocorticoids in the Adrenal Cortex. 1-*20,22 Desmolase complex*.2-*3β Hydroxy steroid hydrogenase isomerase*.3-*17αHydroxylase*.4-*21Hydroxylase*.5-*11βHydroxylase*. [4,5]

terol availability in the mitochondria for side chain cleavage (Mahaffe *et al.*, 1974), stimulating the formation of cholesterol in the gland by activating cholesterol esterase, apparently by phosphorylation (Pittman and Steinberg, 1977) and also by increasing plasma lipoprotein uptake so as to increase cholesterol levels (Gwynne *et al.*, 1976).

The secretion of ACTH is regulated by the balance of neural excitatory and inhibitory effects. The neural excitatory effect is influenced by corticotropin releasing factor (CRF) (Yates and Maran, 1979) and arginine vasopressin (Rivier and Vale, 1983). CRF is synthesized by paraventricular neurons located in the hypothalamic paraventricular nucleus in the rat which project into median eminence to release CRF into hypophyseal portal vessels (Plotsky, 1985) and is monitored by the glucocorticoid in circulation and control ACTH secretion. Recently age related decrease is observed in the density of CRF receptor in brain and pituitary gland (Heroux *et al.*, 1991). Variety of neural influence originating both from within the organism or environment may control CRF secretion (Hamamura *et al.*, 1984; Saphier and Feldman, 1987). For example, a wide variety of unrelated stimulus such as trauma, drugs, chemicals *etc* initiate the secretion of CRF from the nerve ending of the median eminence which may culminate in the secretion of glucocorticoid by ACTH from adenohypophysis. The rate of secretion of ACTH is related inversely to circulating level of corticosteroids. Increased rate of removal of these steroids by tissues lowers their blood level leading to increased ACTH secretion. Likewise elevated blood levels of the same depress ACTH secretion by a feed back mechanism. The precise locus of glucocorticoid feedback, however, is not clear. A

feedback control on the CRF secretion at the hypothalamic level has been envisaged with the predominantly inhibitory effects of glucocorticoids upon hypothalamic neurons (Feldman and Sarne, 1970; Mandelbroad *et al.*, 1974). More recently ionophorentically applied glucocorticoid hormone has been demonstrated to alter the electrical activity specifically of paraventricular neurons (Saphier and Feldman, 1988). Glucocorticoid has also shown to decrease the activity of mRNA for ACTH in the pituitary itself (Nakanishi *et al.*, 1977). Some of the recent developments are discussed in Plotsky (1991) and Eckland *et al.* 1991.

Thus secretion of glucocorticoid is under the control of ACTH secretion resulting from the control of nervous system and the negative feedback inhibition by circulating levels 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 levels, culminating in various alterations in both peripheral tissues and central nervous system. With the termination of these stress signals, elevated glucocorticoid level in blood exerts its feedback inhibition and basal blood glucocorticoid level is maintained.

High concentration of corticosterone in the foetal stage inhibit the ACTH secretion in response to stress such as hypotension and hypoxemia (McDonald *et al.*, 1990). In adult rats the ACTH secretion in adrenalectomized rats was inhibited by dexamethasone implanted at paraventricular nucleus and glucocorticoid at dorsal hippocampus (Kovacs and Makara, 1988). The exertion of feedback inhibition on the elevated glucocorticoid level is attained after the termination of signal and the regulation is mediated

through hippocampus (Moberg *et al.*, 1970). Recently it has been shown that the hypothalamus - pituitary-adrenal axis is also regulated by the free fatty acids (Widmaier *et al.*, 1992), nor-epinephrine (Leibowitz *et al.*, 1989) and prolonged treatment of synthetic glucocorticoid - dexamethasone (Calagero *et al.*, 1990).

Concentration of Glucocorticoid:

In humans, adrenal cortex generally secretes 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 tissue. The normal plasma concentration of cortisol and corticosterone in human is 240 µg and 80 µg respectively in dl of blood, and in rats it ranges from 4-30 µg/dl depending on the time of the day. Cortisol secretion normally follows a circadian pattern which attains peak level between 6 am and 9 am. It averages to about 16 µg/dl and attains minimum concentration between midnight and 2 am (Forsham, 1962; Carpenter and Bunmey, 1971). This circadial rhythm is notified with respect to early morning hours. In rats lowest corticosterone levels was seen in early morning hours while peak levels are reported at the onset of darkness or prior to darkness (Hellman *et al.*, 1970).

The levels of glucocorticoid in plasma vary with the age of the animal. The presence of corticosterone was detected on the 16th day of gestation. The maximal basal concentration 15-20 µg/dl is seen in the foetus and the same can be detected at 20 day postnatally. The corticosterone levels of circulation in animals is dependent on the circadian rhythm, stress condition and mode of stress (Allen and Kendall, 1967). As mentioned

earlier the responsiveness of stress in mammals is one of the basic instinct which results in secretion of glucocorticoid from the adrenal cortex. Beginning from the foetal period rats secrete corticosterone in response to variety of stressors. At this time the basal titers of corticosterone are very high (15 - 25 µg/dl) which reduces in the early postnatal period, beginning from postnatal day 2 and continued into the second week of life (1-3 µg/dl). The rats fail to or even weakly respond to variety of stressors in this period. This period of adrenocortical quiescense has been termed as **"Stress non responsive period"** (SNRP) with relative reactivity upon stress. After the second week of life the level of the plasma corticosterone begin to increase and reach the adult value at day 15 (10 - 15 µg /dl) (Martin *et al.*, 1977; Henning, 1978). At that time animals again respond to a wide range of stresses such as heat, electric shock, surgical invasion and cold stress which elevate the corticosterone levels to 200 - 400% which is similar to adult (Sapolsky and Meaney, 1986).

The neonatal rats also show less sensitivity to inhibitory effects of circulating corticosterone. Animals at younger ages showed no suppression of corticosterone titers in response to dexamethasone whereas 15 days of age or older showed prolonged suppression. This suggests that adrenocortical axis of the neonate is not only non responsive to the stimulatory effects of stress but also to the inhibitory effect on high levels of circulating corticosterone (Sapolsky and Meaney, 1986)(Table 1.1). The adaptive functions of the SNRP seem to be related to the influence of glucocorticoid on growth and development.

Table: 1.1

Reports describing the adrenocortical stress response in neonatal rats.

Stress	Age	Plasma corticosterone (µg/100ml)		
		Baseline	Stress	*Time
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	7	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

Taken from (Sapolsky and Meaney, 1986) * Period following the stressor.

Effects of Corticosterone:

As evident from the glucocorticoid levels in the blood during development and under various stress conditions it is obvious that a basal level is maintained with circadian phase and it increases several

fold over and above the basal levels in response to stress. Obviously this means that, glucocorticoids perhaps have two different functions. One at the basal levels which is known as permissive or normalizing effect and the other in response to a stressor. To what extent the basal levels of corticosteroid has a role in normal maintenance of the various body functions, have proved to be difficult to define and analyze, but can only be judged in the absence or deficiency of the same with subsequent replacement.

Stress can impinge on the organism and threaten homeostasis through tissue damage, metabolic and neural disturbance. Normal physiological defense system can restore the homeostasis by specifically intercalating with each stress. It was of view that glucocorticoid increase the resistance to stress by enhancing the defense mechanisms. Selye suggested that stress increases the need for sugar and sugar active glucocorticoid is essential for resistance to stress. The frequent appeal to the permissive enhancement of vascular and other response to catecholamine is an example for beneficial effects of glucocorticoid in the treatment of shock (Munck *et al.*, 1984).

The recent hypothesis put forward by Munck and colleagues (1984) is that (a) physiological functions of stress induces increase in glucocorticoid levels to protect not against the source of stress, but against the hormonal defense reaction that are activated by stress and (b) the glucocorticoid accomplishes this function by turning off the defense reaction thus preventing them from overshooting and themselves threatening homeostasis. The clearest example is of causing lymphtocytolysis by releasing performed antibodies and thereby enhancing the immune response to infection.

Irrespective of the basal or elevated levels, the generalized effects of glucocorticoids on various target tissues reported are as follows.

* Maintaining the normal blood sugar levels.
* Maintaining the normal arterial systemic blood pressure.
* Inhibition of inflammatory process.
* Thymolysis, lympholysis and immunosuppression.
* Resistance to many noxious stimulus.

All these effects are the consequences of catabolic and anabolic actions of the glucocorticoid hormone on the organism. The major effects are the metabolic action of glucocorticoid on the carbohydrate, lipid and protein distribution in the body,and are discussed below.

Carbohydrate metabolism:

The blood sugar as well as glycogen level are maintained by glucocorticoid. In liver, the major action of glucocorticoid is gluconeogenesis for maintaining the blood sugar level. Glucocorticoid can mobilize the amino acids from extra hepatic tissues such as skin, muscle to liver and convert amino acids as a precursor for glucose and glycogen production. Glucocorticoid regulate the gluconeogenesis in liver *de novo* by inducing many enzymes in the pathway. The gluconeogenetic enzymes such as phosphoenol pyruvate kinase (PEPCK), fructose 1, 6 - bisphosphatase, glucose 6-phosphatase are induced within hours after glucocorticoid treatment. In mitochondria, the conversion of pyruvate to oxaloacetate by pyruvate carboxylase is a matter of minutes indicating the rapid effects on glucose synthesis (Adams and Haynes, 1969). Glucocorticoid cause deposition of glyco-

gen in liver of normal rats by regulating the glycogen synthase activity by phosphatase mediated dephosphorylation and inactivation by phosphorylation by kinase A (Mermann and Segel, 1969). In thymocytes, glucocorticoid decrease fructose 2, 6 - bisphosphate and lactate content (Aurioles and Sobrino, 1991).

Glucocorticoid are known to influence the glucose uptake by tissues such as muscle and adipose tissues as well as in some brain regions studied *in vitro* and *in vivo* where basal glucose uptake is decreased. For example, glucocorticoid inhibits glucose transport in hippocampal cells both *in vivo* and *in vitro* . This inhibition is found to be region specific as well as steroid specific (Kadekaro *et al.*, 1988; Horner *et al.*, 1990; Virgin *et al.*, 1991). However, its effects are not always on the normal uptake but it prevents only the stimulation induced glucose uptake. For example 3T3-L1 cells grown in culture will have same level of glucose uptake even in the presence or absence of dexamethasone. When these cells, however, are exposed to H_2O_2 which increases glucose uptake in normal but not in treated with dexamethasone (Nelson and Murray, 1987; Murray and Nelson, 1987). The mechanism of H_2O_2 stimulated glucose uptake is through its regulatory action on protein kinase C and sphingomyelin (Nelson, 1990). The glucocorticoid seem to affect the activity of protein kinase C and sphingomyelin synthesis which reveals that the action is not at the level of membrane but at the carrier protein binding site. Similarly concanavalin A stimulated glucose uptake in fibroblast cells is reduced by dexamethasone (Nelson and Murray, 1989). Glucocorticoid seem to decrease number of glucose transporter protein which appears in the membrane fractions (Horner

et al., 1987) and there by decrease the glucose uptake.

Protein and Amino acid metabolism:

It is well known that prolonged treatment of glucocorticoid resulted in muscle wasting and reduced growth rate. It may promote protein synthesis or protein catabolism in a wide range of other tissues as well as. Fasting adrenalectomized rats showed a decrease in nitrogen excretion and restoring urinary nitrogen by glucocorticoid replacement (Long *et al.*, 1940). Glucocorticoid treatment inhibits protein synthesis in muscle, lymphoid and other tissues with reduced amino acid uptake (Manchester, 1970). Some of the recent studies indicates dexamethasone reduces the transport of glutamine in rat skeletal muscle (Hundal *et al.*, 1991). Corticosterone alters the protein turnover through adrenergic receptor modulation in skeletal muscle (Yang and McElligot, 1989) and in rats altered the caracass protein content (Brown *et al.*, 1992).

In contrast to muscle, liver protein synthesis is enhanced with corticosterone treatment. Treatment enhanced amino acid nitrogen levels in fed rats while in fasting rats increase is observed only in few amino acids (Bass *et al.*, 1963; Weber *et al.*, 1965). In liver, several enzymes associated with amino acid metabolism have been shown to be induced by glucocorticoid such as tryptophan pyrolase,tyrosine transaminase *etc* (Segel and Kim, 1963; Kenney, 1970). Acute treatment of corticosterone decreases the muscle protein synthesis while it is the opposite in liver of rats and is sensitive to insulin (Southorn *et al.*, 1990). Chronic administration of corticosterone increases the calbindin mRNA and protein but adrenalectomy reversed it in brain (Icopino and Christakos, 1990).

Lipid metabolism:

Amongst the three, carbohydrate, protein and lipid metabolism, the latter seems to be the most diversely affected by glucocorticoid and each tissue and cells in body seem to respond differentially. The overall effects appears to be increased lipolysis and mobilization of adipose fat to the liver where lipogenesis is favoured. The increased lipolysis in adipose is associated with decrease in lipoprotein lipase activity and increased hormone sensitive lipase activity at the genetic level (Ong *et al.*, 1992), associated with this is the decreased glycolysis and increased gluconeogenesis in adipose tissue. On the other hand lipid metabolism in liver is enhanced by increasing triacylglycerol synthesis as judged by increased ^{3}H glycerol incorporation (Kaur *et al.*, 1989), increased activity of phosphatidate phosphohydrolase in liver cells. Corticosteroids inhibit fatty acid synthesis in HeLa cells and lymphocyte (Delloroco and Melnykovych, 1970). Cholesterol synthesis was decreased in HeLa cells with glucocorticoid treatment (Melnykovych *et al.*, 1976; Ramachandran *et al.*, 1978; Johnston *et al.*, 1980). Dexamethasone inhibits the acetate incorporation into lipids in lymphoma cell lines and indicate that GC induced changes in lipids are receptor mediated (Melnykovych *et al.*, 1992). In lungs, they are known to enhance the synthesis of lecithin involved in the constitution of primary surfactant (Torday *et al.*, 1975). Dexamethasone treatment to fibroblast cell line cause an increase in sphingomyelin content and also induce the enzyme in the synthetic pathway (Nelson and Murray, 1982). Sulfogalactoceramide synthesis was induced by hydrocortisone in oligodentroglial cell lines (Dawson and Kernes, 1978; 1979). Lipoprotein lipase activity or its gene

expression was decreased in corticosterone treatment and adrenalectomy in adipose tissue (Baggens *et al.*, 1987; Ong *et al.*, 1992). Chronic stress and treatment increase lipoprotein lipase activity and increased fat deposition in mesentric area of rats (Rebuffe-Scrive *et al.*, 1992).

Dexamethasone treatment showed a change in the phospholipid concentration in different tissues and liver mitochondria (Kaur *et al.*, 1989). Glucocorticoid can inhibit aryl sulfatase A enzyme in the brain cell culture (Stephens and Pieringer, 1984) while the neutral sphingomyelinase increased in fibroblast cell lines (Nelson, 1990a). Glucocorticoid can modulate protein kinase C which might catalyze phospholipase A_2 in basophilic cells (Zor *et al.*, 1990), while phosphatidyl inositol specific phospholipase C is regulated by phosphorylation - dephosphorylation by glucocorticoid in basophilic cell lines (Her *et al.*, 1991).

Genomic action of glucocorticoid:

Extensive studies have contributed to the natural mode of action of glucocorticoid in different tissues. The hormone molecule being lipid soluble gain free entry into cell and form a hormone - receptor complex in the target cell and are translocated to the nucleus. Thus regulating transcription resulting in synthesis of specific protein that mediate the effects.

In excitable cells, long term signaling by the circulating hormone via this mechanism plays an important role in shaping up cell structure and functions. In nervous system, the effects of glucocorticoid is confined to a special type of cells having intracellular receptors. The effects of these receptors ranges from induction of neurotransmitter metabolism and neurotransmitter receptors to induce synaptic and dendritic structures. Other

steroids also produce long lasting and apparently genomic effects on neural tissues. Glucocorticoid regulate a number of enzymes and structural proteins throughout the brain and also has effects on neuronal survival and destruction (McEwen, 1991).

After hormone treatment to sensitive cells, RNA processing have been reported to be altered, increased rate of overall RNA synthesis are sometimes observed leading to proliferative growth. Thus glucocorticoid activate massive accumulation of specific mRNA by the transcriptional and post transcriptional process which regulate several enzymes in the metabolic pathways in the brain and other tissues (Watson *et al.*, 1987). The induction as well as inhibitory effect of glucocorticoid on different enzymes control the metabolism. One well known example of enzyme inhibition by glucocorticoid is that of phospholipase A_2, which acts on phospholipids. The mechanism through which its inhibition is shown by increased synthesis of protein named as macrocortin (recently called as lipocortin) which inhibit phospholipase A_2 (Blackwell *et al.*, 1980; Hirata *et al.*, 1980) and adrenalectomy decreased the mRNA of lipocortin (Viswanath *et al.*,1992). Similarly plasmalogen activator, a serine proteinase has been shown to be inhibited by glucocorticoid by inducing an inhibitory protein (Seifert and Geleherter, 1978). Glucocorticoid inhibits the induction of nitric oxide synthase in macrophages explaining its anti-inflammatory potency (Moncada and Palmer, 1991). Dexamethasone induces tyrosine aminotransferase and trytophan oxygenase activity in the primary culture of rat hepatocytes and which is inhibited by sphingosine denote the possible involvement of protein kinase C (Sharma *et al.*, 1990). Δ 5 desaturase enzyme, convert 20:3 to 20:4

fatty acid in liver and is inhibited by 11-deoxy corticosterone by inducing a protein of 25 KDa which subsequently inhibit the enzyme (Alaniz *et al.*, 1992). Endogenous GC induce cyclooxygenase in macrophages (Masferrer *et al.*, 1992). Thus genomic mode of action has an effect on cellular metabolism of glucocorticoid (fig. 1.2).

Non genomic mechanism of glucocorticoid:

Most of the glucocorticoid effects cannot be explained on the basis of genomic actions alone as evident from some of the effects which are very rapid and elicit a response in presence of potent protein synthesis inhibitors. Numerous reports on the non transcriptional mode of action of glucocorticoid and other steroid were extensively reviewed by several workers (Duval *et al.*, 1983; Schumater, 1990; McEwen, 1991). Rapid effects of glucocorticoid on excitable membrane may alter the characteristic of the neuronal membrane by intercalating with phospholipid bilayer. However, it is more likely that these rapid effects are due to the binding of specific membrane receptors. For example glucocorticoid treatment result in a predominance of transformed glucocorticoid receptors that bind to t-RNA which lead to ubiquitin pathway of protein degradation, which is non-lysosomal, ATP dependent and does not involve in any protein synthesis (Farber and Ciechanover, 1986). Non-genomic effects of steroids are summarized in table 1.2.

Recent findings of the rapid effect leads to pursuit of the idea of occurrence of membrane receptors. The question is whether the free hormone or even the one which is bound to circulation protein is active at the membrane level?. In this connection it has been demonstrated that corti-

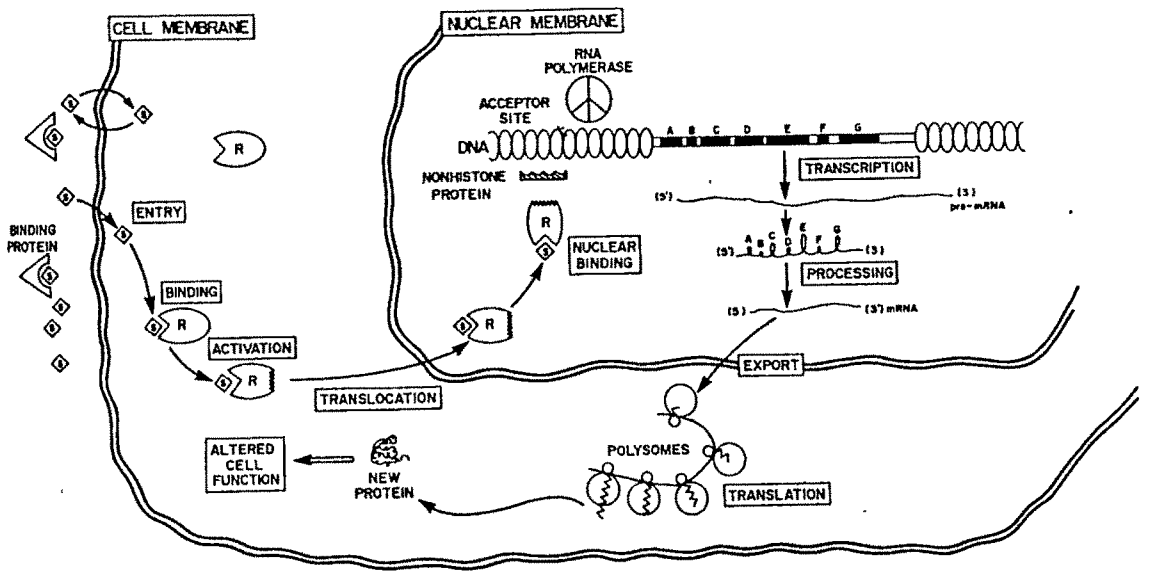


Fig. 1.2. Schematic representation of the molecular mechanism of steroid hormone action. S-Steroid hormone,R- Steroid hormone receptor. The letters A to G indicate intragenic spacers.
(Taken from Smith *et al.* 1983).

costerone bound to plasma carrier protein namely corticosteroid binding globulin (CBG) is able to bind to specific plasma membrane protein and activates second messenger system (Rosner, 1990). On the other hand the free steroid circulating can diffuse through the membrane and accumulation of steroid within neuronal membranes as a consequence of which can alter binding and gating characteristics of the neurotransmitter receptors and ionic channels within the membrane (Rosener, 1990; Ekinis, 1990). High affinity and low capacity binding of corticosterone to kidney plasma membrane has a Kd of 13nM and Bmax 616fmol/mg protein (Ibarrola *et al.*, 1991).

Table: 1.2.

Examples of local or *in vitro* steroid effects on cell surface events and properties.

Binding of steroid to membranes.

Neural membranes: corticosterone (100nM);
estradiol,testosterone,progesterone (10nM).
Liver membranes: dexamethasone(400nM).
Pitutary membranes: estradiol(0.04nM).
Liver,endometrium: estradiol immobilized (1nM).
Neural membranes: progesterone immobilized (30nM).

Effects on membrane binding of drug.

$GABA_A$ receptor: progesterone metabolites (20 -100nM).
Sigma receptor: progesterone (300nM).
Dopamine receptor: 2OH-estradiol(10μM).
α_1-adrenoreceptor: 2OH-estradiol(30nM).
5-HT receptor: estradiol(10nM-1μM).

Effects on Ca^{2+} entry/mobilization.

Oocyte maturation: progesterone (3μM).
Sperm acrosome reaction: progesterone (0.1- 1nM).
Endometrial cells; estradiol(1nM).
Pitutary cells Ca^{2+} currents: estradiol(1nM).

Rapid steroid effects on neurosecretion.

LHRH release: pregnanolone (0.03nM).
Dopamine release: progesterone (1nM).
Acetylcholine release: progesterone (300nM).
CRF secretion inhibition:anesthetic steroids

Non genomic effects on neural activity.

Preoptic-septal neurons: estradiol (ionotophoresis).
Medial amygdala neurons: estradiol (1nM).
Application to paraventricular neurons #
Response to $GABA_A$ receptors: alphaxalone (0.1- 30μM).
GABA receptors related excitation and inhibition: corticosteroid !!
Chloride channels opening time and frequency:corticosteroid**
Response to glycine: progesterone (20μM).
Rapid eletrophysiological effects:estradiol,hippocampus!
Glutamate response: progesterone (pressure),estradiol (pressure).

Effective concentration is indicated within parentheses,where relevant;pressure and iontophoretic are ways of applying steroid locally, where the final concentration is difficult to estimate.
LHRH-luteinizing hormone releasing hormone.! Wong and Moss, 1991,
!! Owen *et al.*,1986;** Lambert *et al.*, 1987.
!* Jones *et al.*, 1976;# Saphier and Feldman, 1988.
Taken from McEwen (1991).

Zuo and Yi-Zabg (1992) had shown glucocorticoid membrane binding sites in synaptic membrane from porcine brain regions with Kd 200nM/L and Bmax 486 fmol/mg protein. In hepatic membrane high affinity binding sites are located with a Kd of 44nM and capacity 7.3 pmol/mg protein with non competent binding to corticosterone and cortisol (Maitra *et al.*,1993). Recent studies showed that glucocorticoid binding to neuronal membrane of the amphibian brain strengthens the idea of membrane receptors and also that G-protein is modulating the membrane receptor effects of steroids (Orchnick *et al.*, 1991; 1992). Immunocytochemical studies showed that 145KDa protein present in leukemia cells from human is a GC receptor (Gometchu *et al.*, 1993).

To summarize one could say that the diversity in effects of glucocorticoids is reduced predominantly by the acceptor site on the genome as well as the differentiated state of the cell. It also plays an important role in the final outcome of the glucocorticoid action.

Glucocorticoid Receptor - Molecular outlook:

Glucocorticoid receptor consist of a single polypeptide species found in the cytoplasm and was therefore cytoplasmic. The glucocorticoid enter the target cell predominantly by passive diffusion, although facilitated transport has been described (Harrison *et al.*, 1974). The binding of the glucocorticoid to the receptor protein is an equilibrium reaction. Glucocorticoid receptor (GR) is shown to undergo an apparently irreversible process termed activation and inactivation (Holbrook *et al.*, 1984). The translocation of GR into nucleus (Higgins *et al.*, 1973) and the acquisition of the ability of the complex to bind to DNA (Simons, 1977). The biological effect of the glucocorticoid results active transcription and translation (Rousseau, 1984; Evans, 1988). The glucocorticoid binding receptor protein has been purified from various species and has been extensively studied for identifying the steroid and DNA binding region. Different analysis of the GR revealed that it is a single polypeptide species with molecular weight 90-100 KDa (Wrange *et al.*, 1984). Variety of proteolytic treatment shows that at least three functional domain exists within the receptor protein (Gigure *et al.*, 1986). The glucocorticoid binding domain has been identified and found to be localized at the C - terminal (Gustafsson *et al.*, 1987). The DNA binding domain have high amount of cysteine, arginine and lysine corresponding to 440 - 505 amino acid within GR. It has

got a high degree of homology with v-erb A oncogene (Weinberger *et al.*, 1985). This protein contain a repeating structure containing cysteine and histidine and having Zn^{2+} for DNA binding finger. Recently three dimensional structures of the DNA binding domain of GR suggests that a 71 residue protein fragment contain two Zn^{2+} finger domain and specific amino acid residue of the DNA binding domain involved a protein - DNA and protein - protein interaction (Hard *et al.*, 1990). The domains are of globular fold and glucocorticoid binds to DNA when the domain dimerizes and places the subunit adjacent to major groove of the DNA. This gives the target sequence and separation of subunit recognition surface realizing target sequence identity (Luisi *et al.*, 1991). The third domain known as acidic transactivation or enhancement region is found to be within the N-terminal domain, 20-600 amino acid conserved sequence between DNA binding and hormone binding region (Godowski *et al.*, 1988). They are essential for full transcriptional enhancer activity of the receptor and can increase activity of other transcriptional activator coupled with DNA binding region (Frankel and Kim, 1991).

The binding domain differ with respect to amino acids and with different hormones but they have common hormone response element (HRE) to which activated complex bind with high affinity. This HRE of GR and mineralocorticoid receptor (MR) have different effects with 25 nucleotide composite element having low affinity glucocorticoid responsive element (GRE) and AP1, transcription factor binding site. An activated bound GR can block transcription factor enhanced transcription from composite element but MR cannot (Funder, 1993). Both MR and GR have novel mechanism by which tran-

scription is controlled by modulating inactive antagonist competing receptor binding site in response element. Composite elements also identified in other GC responsive genes such as alphafetaprotein gene and gene encoding for phosphoenol pyruvate kinase (Pearse and Yamamoto, 1993).

Glucocorticoid receptor subtypes:

Two different cytosolic receptors for adrenal steroids have been described by receptor binding studies (Reul and DeKloet, 1985). The two adrenal steroid receptor types differ in their distribution and affinities. Type I adrenal steroid receptor also referred to as mineralocorticoid receptors are widely distributed in kidney and are found in other peripheral tissues. Type II adrenal steroids receptors are evenly distributed throughout central and peripheral tissues and high concentrations are found in spleen and thymus (Miller *et al.*, 1990).

Regulation:

The magnitude of biological response to adrenal steroid is the amount of corticoid - receptor complex that is bound to the cell nuclear chromatin. This binding capacity rely upon the concentration of hormone and number of receptors. The receptor site number is subjected to short term and long term regulation. The pH, ionic strength, inhibition and phosphorylation and dephosphorylation are considered as short term regulation. Long term regulation has been found during the life span, a consequence of environmental, endocrine and neural manipulation.

Glucocorticoid receptor is predominantly cytoplasmic and present in large oligomer by non covalent association of a dimeric or monomeric protein with 90KDa heat shock protein (Hsp90) (Aurichito, 1989). Hormone

binding to unligated receptors results in formation of nonactivated hormone receptor complexes. Under physiological condition these hormone immediately transforms into activated complex and rapidly binds to nuclei. Activated complex in association with Hsp70 has the ability to bind to DNA after the conformational changes of the complex accompanied by dissociation of oligomeric complex (Scherrer *et al.*, 1990). The function of receptor in normal cells are regulated by a hormone and ATP dependent phosphorylation and dephosphorylation cycle which regulate the activity and hormone binding capacity (Mendel *et al.*, 1986; 1990). Hormone binding capacity was increased by cGMP and decreased by cAMP and are dependent on ATP, divalent cation and particulate fractions (Orti *et al.*, 1992). Epinephrine treatment to human placental slices which stimulated cAMP production also inhibited the hormone binding (Heller *et al.*, 1986) whereas the same was increased in lymphoma (Gruol *et al.*, 1986). Protein kinase C activators and inhibitors have been reported to affect function of GR (Miller *et al.*, 1985). Protein kinase C activators inhibit glucocorticoid dependent transcription in NIH-3T3 cells (Vacca *et al.*,1989) while it enhances in other cells (Kido *et al.*, 1987). In WEH - 17 cells GR phosphorylation is not affected by pro-calcium dependent intrinsic kinase activity. Autophosphorylation of threonine residue and Mg^{2+} dependent serine/threonine kinase which phosphorylated the GR shows that receptors are lacking intrinsic kinase activity (Singh and Moudgil, 1984; Perisic *et al.*, 1987). *In vitro* studies showed that phosphorylation of GR is at the N-terminal domain by protein kinase A or proline directed serine kinase (Mazer *et al.*, 1990; Orti *et al.*, 1992).

Post-transcriptional modification such as glycosylation and acylation either at N-terminal or C-terminal of GR cause different patterns of phosphorylation (Cidlowski and Richon, 1984; Danze *et al.*,1987; Orti *et al.*, 1989a). Phosphorylation helps receptor transformation between nucleus and cytosol (Hamilton and Defranco, 1989). Hyperphosphorylation is induced by glucocorticoid agonist from 70% to 3-4 fold at N-terminal domain of the GR (Orti *et al.*, 1989b; Hoeck *et al.*, 1989; Hoeck and Groner, 1990). The location of phosphorylation at serine residue and minor levels in threonine and tyrosine residues (Bodwell *et al.*, 1991) are found to be at N-terminal domain. Some reports shows that dephosphorylation of GR result in activation (Reker *et al.*, 1987) but *in vitro* study revealed that dephosphorylated GR lacked the ability to bind to DNA (Dallman *et al.*, 1988). Recently proposed cyclic model of GR phosphorylation - dephosphorylation has suggested that hsp90 is involved in activated and inactivated complexes (Orti *et al.*, 1992). Many null receptors in ATP depleted cells and phosphatase treated receptors lack hormone binding affinity due to loss of hsp90 (Bresnick *et al.*, 1989). Phosphorylation increases the binding of DNA to many transcriptional factors and increased in transcriptional activity (Danielsen *et al.*, 1987). Other than phosphorylation, the binding of glucocorticoid to its receptor is regulated by the degree of unsaturation and chain length of non esterified fatty acid (Vallette *et al.*, 1991). Gottlicher *et al.* (1992) had shown that fatty acid can also activate glucocorticoid receptor.

Glucocorticoid receptors in brain:

It was known for many years that brain is one of the target of

glucocorticoid action in response to altering stimuli resulting in behavioural change. First demonstration of adrenal steroid receptors in the brain came from the uptake studies of ^{3}H corticosterone to adrenalectomized rats and was revealed that corticosterone was retained for more number of hours in nuclear fraction of hippocampus and to a lesser extent in septum and amygdala (McEwen *et al.*, 1968; Knizley, 1972), which led to the conclusion that genomic site of action involve nuclei (McEwen and Wallch 1973; McEwen *et al.*, 1976). This opens to the question that both corticosterone as well as aldosterone have different receptors or a single one ?. Later on the presence of glucocorticoid binding macromolecule in cytosol from the rat brain and was demonstrated subsequently showed that it is proteineous in nature with distinct properties (McEwen *et al.*, 1972). McEwen and his workers ascribed a second type of putative receptors in hippocampus - **"corticosteroid preferring site"** and have high affinity for corticosterone than dexamethasone and was established that such sites are indeed for high affinity GR (DeKloet *et al.*, 1975; 1982; 1983; 1990). Thus rat brain contain two receptors system, Type I- corticosterone preferring sites and the other classical Type II glucocorticoid receptor (Reul and DeKloet, 1985). Type I resembles kidney mineralocorticoid receptors (MR) (Krozowski and Funder, 1983) which is expressed in brain as type I mineralocorticoid as well as Type I corticosterone preferring sites (McEwen *et al.*, 1986a; DeKloet and Reul, 1987). Type I MR is thought to mediate mineralocorticoid effects of aldosterone on behavioural effects (Magarinos *et al.*, 1986).

Type I corticosterone preferring receptors are located in the limbic structure (Reul *et al.*, 1987a) and to a lesser extent in hypothala-

mus and cortical brain regions. The binding affinity is labile having high affinity to corticosterone (apparent affinity Kd_2) at 0°C is 0.5 - 1 nmol/L and aldosterone Kd_2 at 0°C is 0.75 -1.5 nmol/L while it shows lesser affinity to synthetic glucocorticoid dexamethasone (DeKloet *et al.*, 1975; Reul and DeKloet, 1985). Type I receptors have stringent corticosterone specificity in responsiveness to corticosterone in a different manner (Micheau *et al.*, 1985) while aldosterone act as a antagonist (DeKloet *et al.*, 1983). Type II glucocorticoid receptor is widely located in brain and are found both in neurons and glial cells (Fuxe *et al.*, 1985 a,b; Meyer and McEwen, 1982). They have high affinity to synthetic glucocorticoid 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) which resembles glucocorticoid receptor in liver (Velduis *et al.*, 1982). The distribution of type I and type II in some brain regions are given in fig. 1.3. As evident there is a regional variability in type I and type II receptors distribution. Hippocampus having the highest number and cerebellum the lowest. Similar pattern is seen with regard to type I receptors which are also highest in hippocampus and almost absent in cerebellum where type II seem predominate.

Recent studies on receptor distribution using immunoreactive methods and mRNA studies have indicated more precise distribution of type I and type II receptor (VanEekelen *et al.*, 1991) and reviewed by Ahima *et al.* 1992. Earlier it was assumed that glial cells express only type II receptors, however study of Chou *et al.* 1991 on glial cells culture indicates the presence of type I receptors on astrocytes as well. According to immunoreactive studies most glial cells express type II but type I are present only

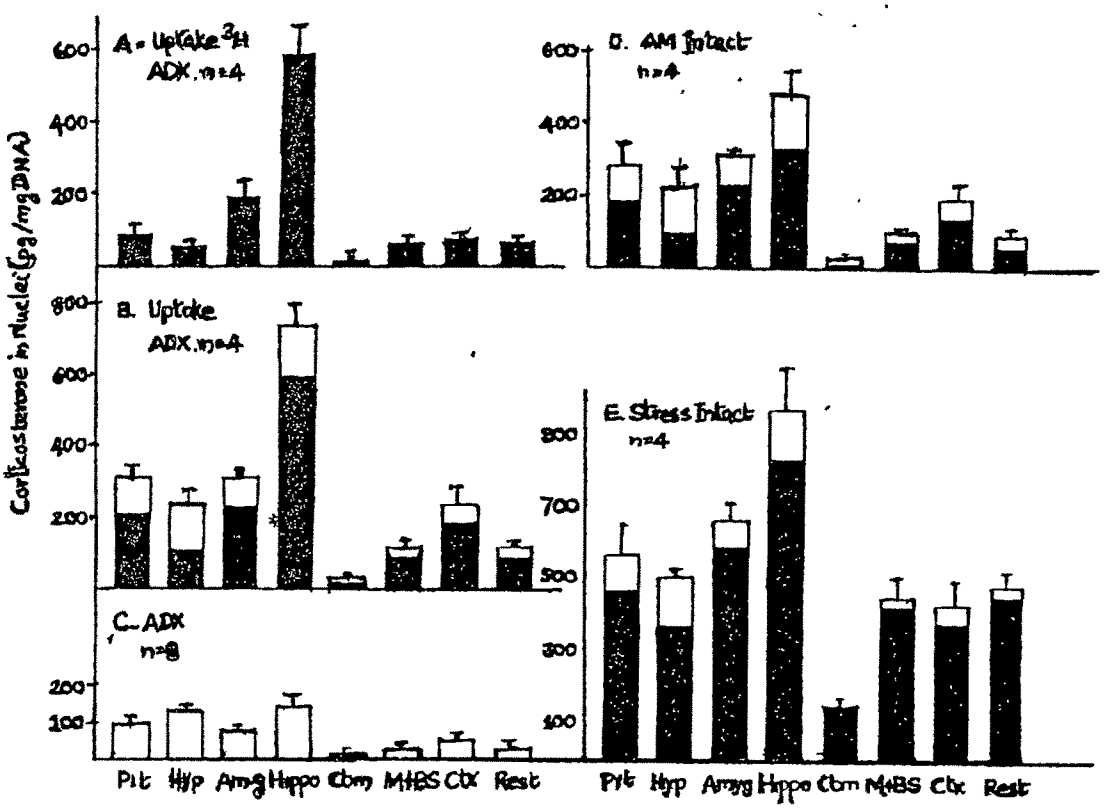


Fig.1.3. Cell nuclear corticosterone levels measured in rat brain regions and pituitary. n- number experiment on pooled tissue for five to six rats.
Pit,pituitary;*Hyp*,hypothalamus;*Amyg*,amygdala;
Hippo,hippocampus; *Cbm*, cerebellum;
M+BS, midbrain +brain stem;
Ctx,cerebral cortex;*Rest*, remaining brain tissue.
Open bar total radioimmunoassayable material;
Black bar corticosterone level with ADX value substracted.
A-Uptake of ^{3}H corticosterone,
B- Unlabled corticosterone
C- ADX rats,D- Intact rats, E- After ether stress
(Taken from McEwen, 1982)

in few fibers, e.g., corpus callosum, spinal trigeminal tract and funiculi of spinal cord (Ahima *et al.*, 1992).

Functions of receptors in brain:

The widespread distribution of type I and type II receptors in CNS mediate most of the diverse effects of corticosterone in neurons and glial function. Glucocorticoid regulate synthesis and transport of many products which are regulated by gene expression by modulating the glucocorticoid responsive element (Chao and McEwen, 1990; Evans and Azzira, 1989). Since type I and type II receptors differ in their affinity for corticosterone, it has been suggested that the glucocorticoid related functions associated with basal levels (permissive action) is mediated through type I receptors, whereas when the glucocorticoid levels are increased during stress, the action mediated by glucocorticoid is supposed to be through type II.

Protein-protein interaction also occur between type II corticosterone receptor component and transcription factor AP1 (Diamond *et al.*, 1990; Mohr and Schmitz, 1991). Tubulin, microtubule associated with hsp90 which involve the transport of activated type II receptor from the cytoplasm to nucleus (Akner *et al.*, 1990). Glucocorticoid have post translational action such as type II receptor mRNA level to stabilize the transcript (Dong *et al.*, 1988), activated type II receptor bind to t-RNA (Ali and Vedeckij, 1987), small nuclear RNA (Rossini *et al.*, 1989). Cytoplasmic type II receptors are in close association with GABA synthetic machinery and GABA transport protein (Sloviter and Nilaver, 1987).

A close association with type II receptor and protein kinase C in brain reflects GC affecting second messenger system. Protein kinase C

amplifies some of the enzyme activity by glucocorticoids and was found to be inhibited by protein kinase C inhibitors (Kido *et al.*, 1986; 1987; Sharma *et al.*, 1990; Sharma, 1991). Excessive type II receptor cause an elevation in the GPDH and glutamine synthase activity in obese rats (Langley and York, 1990). Angiotensin II, regulate the electrolyte balance in mammals and induced drinking habits due to the specific GR type II but not by the MR type I in the brain (Sumners *et al.*, 1991). Type I receptor activation is necessary to protect the adenalectomy induced cell death in dentate gyrus of hippocampus (Woolley *et al.*, 1991). From this recent reports it is becoming evident that a lot more information is yet to come.

Receptor regulation in brain:

In brain type I and type II receptors are under regulatory process. After stress negative feed back response to the occupancy and the sensitivity of receptor is lowered by dexamethasone. Repeated stress causes a down regulation of the cytosolic corticosterone receptors in hippocampus but not in other regions (McEwen, 1982; Meaney *et al.*, 1985; Sapolsky and McEwen, 1985). Receptor binding values ranged from 220 to 550 fmol/mg protein in hippocampal cytosol and changes with environmental influences. *In vitro* uptake of ^{3}H corticosterone in hippocampus varies with circadian rhythm (Valeri *et al.*, 1980). Negative regulation of glucocorticoid receptors by corticosterone revealed a biphasic increase of hippocampal receptors. Adrenalectomy upregulated while repeated stress down regulated the glucocorticoid receptor in both frontal cortex and amygdala (Tornello *et al.*, 1982; Meaney and Aitken, 1985; Sapolsky and McEwen, 1985). Dexamethasone treatment instead of corticosterone showed no significant down

regulation of the cytosol receptor capacity in hippocampus, although there is a decrease in receptor level in pituitary, amygdala hypothalamus (McEwen *et al.*, 1986a). Neuropeptides play a role in receptor regulation in the hippocampus. Physiological concentration of corticosterone down regulates the type I and type II receptor in brain regions and also in pituitary (Spencer *et al.*,1991).

Glucocorticoid receptors during development show that receptor capacity for glucocorticoids vary with the brain region and with development. On postnatal day one, rats have lower number of receptor site and the capacity increases four fold and gradually reaches adult values around 4 weeks of age (Clayton *et al.*, 1977) and receptors in neurons appear after a few days of the final cell division of dentate gyrus and pyramidal cells of hippocampus. (Altman and Beyer, 1975). In hippocampus, at postnatal day 15 there is an increase in labeling in the dentate gyrus and decrease in Ammon's horn for type II receptor (Olpe and McEwen, 1976). GR of unspecified subtype is present in limbic brain and higher level in developing cerebellum in late foetal stages in the rat and undergo developmental decline (Pavlick and Buresova, 1984; Meaney *et al.*, 1985). Pituitary CR and GR differ in their ontogenic pattern, GR do not change from day 1 to adult level whereas CR appear only after day 6 and reaches adult values after 4 weeks while CBG molecule detectable at day 10 rise rapidly to adult level at 4 weeks of age (Sakly and Koch, 1981). Circadian rhythamicity appear gradually and reaches the adult value after 3 weeks of age (Ader, 1968). Receptors that bind to ^{3}H corticosterone *in vivo* and *in vitro* decline with age and is pronounced in hippocampus (Sapolsky *et al.*, 1984).

Glucocorticoid and brain growth:

Mention has been made regarding the "stress non-responsive period" in the early postnatal age in rats. This has been speculated to be a natural process of protecting deleterious effects of glucocorticoid on brain maturation. It is therefore found that if administered exogeneously glucocorticoid would have deleterious effect. As early as in 1954, Field showed that neonatal cortisone administration inhibits development of rodent brain (Field, 1954). Later on several studies have demonstrated inhibition of brain growth especially in cerebellum and cerebrum of rats (Cotterrell *et al.*, 1972; Kovacs, 1973; Howard, 1974). This inhibition has been associated with decreased DNA content (cell number) (Schapiro *et al.*, 1968; Cotterrell *et al.*, 1972), increase postnatal cell death (Burdman *et al.*, 1975; Anderson and Schanberg, 1975) and also antiproliferative action by inhibiting DNA synthesising enzymes such as DNA polymerase and thymidine kinase and thymidylate synthase in chick embryo (Tesoriere *et al.*, 1989).

Bohn and Launder (1978; 1979) have demonstrated a permanent reduction in number of interneurons derived from external granular layer of the cerebellum. However the investigators failed to demonstrate a permanent deficit in hippocampal microneurons of the animal treated with hydrocortisone in an early age. Thus hippocampus seem to have greater plasticity and efficient recovery from glucocorticoid effects compared to cerebellum.

In this connection foetus which is exposed to high levels of circulating glucocorticoid as a result of maternal stress or due to therapeutic administration of synthetic steroids to prevent respiratory distress syndrome in premature would have greater deleterious effects. The study of

Carlos and his associates in 1991 and 1992 have demonstrated that the dose of glucocorticoid plays an important role. At lower doses, the brain is spared, however at higher doses the glucocorticoid diminishes normal response of the brain macromolecule synthesis when exposed to a stress. Thus a loss of adaptive response of reducing macromolecule synthesis is lost in animals when exposed to high glucocorticoid levels in the foetal or very early neonatal stage (Carlos *et al.*, 1991; 1992). Neonatal glucocorticoid treatment retards the development of cortical dendrite spines (Oda and Huttenlocher, 1974) and reduce brain gangliosides which are enriched in neuronal process (Horwitz and Schanberg, 1979) and suppress myelinogenesis in developing rat brain (Gumbinas *et al.*, 1973). Rats treated early in life with corticosterone or hydrocortisone show delay in maturation in sensory evoked potential (Salas and Schapiro, 1970) and glucose homeostasis (Taylor and Howard, 1971).

Its effects on growth seem to be linked with decreased release of growth hormone. Noguchi *et al.* (1982) have shown that hydrocortisone induced deficit can be completely suppressed by exogeneous supply of growth hormone as well as normal CNPase activity indicating normal myelinogenesis. Wehrenberg *et al.* (1990a; 1990b) have hypothesized that glucocorticoid may partially inhibit growth by increased somatostatin secretion.

There are not many studies on long term steroid administration in animals, gross brain weight or other biochemical parameters as not much effect has been speculated.

Since glucocorticoid are growth inhibitory in nature many investigators have been interested in studying the deficiency of the same on brain

growth, cell number (DNA content), thymidine incorporation and myelin content and composition.

Adrenalectomy at an early age alters the gross structure of rat brain. Even though there is a decrease in the body growth, the brain size is found to be increased (Devenport and Devenport 1982; 1985; Meyer 1983; 1986; 1987). This increase in brain size is attributed to increase cell proliferation denoted from the increase in thymidine kinase activity and DNA content (Yehuda *et al.*, 1989). Adrenalectomy at weaning age when prolonged for either 2 months or 5 months do change the brain size and recovered myelin content but the increment in both parameters between 2 months and 5 months is very low (Meyer and Fairman, 1985). This indicates that brain lipids are under direct influence of glucocorticoids. Myelin isolated from the adrenalectomized rats was found to be deficient in galactolipid and phospholipids but optic nerve CNPase activity was normal. The authors have concluded that the effects are not uniform and glucocorticoids do not affect all brain area equally (Meyer and Fairman, 1985). However, Preston and McMorris (1984) have reported contradictory results that showed that early adrenalectomy caused hypomyelination. The increase in brain weight is due to increased water content and not due to myelin deposition in rat brain. Adrenalectomy studies suggest that adrenal secretion may exert a tonic inhibitory effect on brain growth in normal developing rat. Similarly adrenalectomy of rats at the age of 25th day cause an increase in brain weight and reversed by replacement (Devenport and Devenport, 1982; 1985). Brain growth is independent of improvised diet and food intake (Devenport and Stith, 1992; Devenport *et al.*,1992).

No information regarding effects of adrenalectomy on GH levels or somatostatin release have been reported but one thing is obvious that the effects of glucocorticoid has a mechanism which is quite complex. Since somatic growth was reduced with adrenalectomy which is according to expected role of glucocorticoid it should show an enhanced growth. The retardation in somatic growth and increase in brain weight are opposite effects of glucocorticoids thus leading to the speculation that these effects are differentially regulated.

It is interesting to note that adrenalectomized animals do have higher brain weight but it does not go on increasing at the same rate, in fact, rate falls down in the same proportion as that of control and therefore the difference in sham and adrenalectomized brain weight is maintained more or less or rather slightly decreased. It is also interesting to note that effects are less pronounced in females than males. Thus perhaps even steroids other than glucocorticoid could also play a role in brain growth.

Glucocorticoid and hippocampus:

In addition to its role during the development glucocorticoids have been implicated in aging phenomenon. It has been widely documented that early exposure to high levels of glucocorticoids can have long term consequence (Sapolsky, 1985a;b) even normal aging is attributed to high basal levels of circulating glucocorticoids in aging animals (Sapolsky *et al.*, 1983; 1985). The feed back inhibition of the adrenocortical axis is also diminished considerably in aged rats as reported recently (Sapolsky, 1991; 1992). This would mean that aged animals are like young animals in the

most sensitive time period of SNRP. Similarly in aged rats due to lack of feed back control any stressful situation would keep glucocorticoid levels high for long period of time and a chronic stressful situation would keep the basal levels high. Consistent with this notion is several findings reported from the beginning of 80's. Two groups in this field have done pioneer work and are led by Landfield and McEwen.

Corticosterone at 10^{-9}M which is similar to basal levels seem to potentiate effects of toxins and high levels of the glucocorticoid can cause further damage. These effects of the hippocampal neurons has been implicated to diminished glucose uptake in the neurons resulting in cell death (Sapolsky, 1987). It appears from the recent studies of Sapolsky and his associates that hippocampal neuronal damage could not only be due to decreased glucose uptake in these cells but also to the diminished function of astrocytes for the uptake of glutamate excitatory neurotransmitter, since astrocytes themselves are energy depleted due to decreased glucose transport. The effects of primary and secondary culture showed this effects exclusively with corticosterone but not with other steroids (Virgin *et al.*, 1991).

Recent study by the same group demonstrated corticosterone (100nM) endangers the survival of hippocampal cultures. A period of substrate deprivation affect both neurons and astrocytes. Treatment reduces glucose uptake and glycogen content in astrocytes which might weaken the ability of these hippocampal cells to resist irreversible damage. *In vitro* study interpreted that direct effect may be sufficient to aggravate brain injury under conditions of cerebral energy depletion (Tombaugh *et al.*, 1992).

The GC exacerbation of hippocampal damage appears to involve activation of NMDA receptor. It was hypothesised that GC increase the excitatory amino acids and increase the amount of Ca^{2+} mobilization per unit NMDA receptor activation. Due to the energy depletion by GC exacerbate the NMDA cascade results in calcium mobilization and depolarization induced by. opening of voltage gated calcium channels (Armanini *et al.*, 1990). Very recently it was shown that GC receptor activation resulted in increased Ca^{2+} influx causing the aging process in brain and hippocampal neuronal cell loss (Kerr *et al.*, 1992).

The direct effect of elevated levels of corticosteroid could directly be on one or both corticosteroid receptors in brain. In the hippocampus glucocorticoid binding to type I receptor is with high affinity and to type II receptor with low affinity (McEwen, 1982). Type I GC receptor is largely occupied by basal level of endogeneous hormones whereas higher GC levels are required to occupy the type II receptor (Reul and Dekloet, 1985).

It appears that the selective effect on CA_3 pyramidal cells is due to alteration in the input from granule cells of dentate gyrus. It was observed that short term adrenalectomy 3- 7 days results in massive cell death of granule cells in dentate gyrus indicating that these cell require glucocorticoid for their survival (Woolley *et al.*, 1991).

It can be summarized that excess of glucocorticoid in circulation damages the hippocampal neurons and exacerbate the toxic insult to these neurons. Many mechanisms are responsible for the cell loss in hippocampus. However no conclusive efforts have been made for the exact cause of cell death. Recent studies have shown that type II receptor activation are re-

sponsible for cell death. Aldosterone as well as very low amount of corticosterone is necessary for the cell survival (Gould *et al.*, 1991).

Glucocorticoid and Neurotransmitter:

Apart from its role in the growth and cell survival, effects of glucocorticoids on brain is ultimately expressed in behaviour and neuroendocrine regulation. Glucocorticoid have been known to affect the neuronal as well as muscle excitability. It is also implicated in affecting mood, sleep, sensitivity to sensory stimuli and also play an important role in adaptation, however no significant effect on learning of active and passive avoidance or approach behaviour have been observed. Corticoids usually facilitate extinction of active avoidance behaviours (DeWeid, 1967; Kovacs and Telegdy, 1978).

The question of permissive effect and excess of steroids in behaviour had been looked in a different perspective with a special reference to the function of receptor subtypes. It has been stated that behavioural studies carried out with excess of glucocorticoid would involve the action of receptor type II which is not occupied by the circulating levels of glucocorticoids. The physiological (permissive) function of glucocorticoid is considered as a function of type I receptors which is studied by subjecting the animals to bilateral adrenalectomy with subsequent replacement of the glucocorticoid in a dose range not exceeding the physiological level. The summary of the effects of glucocorticoid on behaviour have been given in table 1.3.

Table: 1.3

Effect of Adrenalectomy and Hormone Replacement on Behaviour*

Behavioral paradigm	Species	Effect of adrenalectomy or adrenocortical insufficiency	Steroid specificity
Sensory perception of olfactory, auditory stimuli.	Man, rat	Detection increased, recognition decreased	Glucocorticoid
Mood and affect	Man	Disturbed	Glucocorticoid
Sleep	Man	REM sleep decreased, attenuation of	Glucocorticoid Glucocorticoid
	Rat	circadian variation decreased 3 h before light	Corticosterone
Feeding	Rat	Food intake reduced	Glucocorticoid
Drinking	Rat	Salt appetite increased	Mineralocorticoid Glucocorticoid
Motor activity	Rat	Running wheel activity reduced General activity increased (gross body movement)	Glucocorticoid
Stress - induced analgesia	Rat	Latency of tail - flick response reduced	Corticosterone
Aggression	Rat	Reduced	Glucocorticoid
Social interaction	Rat	Reduced	Corticosterone
Exploration of novel environment	Rat	Reduced	Corticosterone
Acquisition fear motivated (avoidance behaviour)	Rat	No effect	--
Intertrial responses of active avoidance	Rat	Increased	Glucocorticoid
Free operant avoidance, effect of post stimulation	Rat	Absent	Mineralocorticoid
Extinction of active avoidance behaviour	Rat	Delayed	Glucocorticoid
Extinction of food reinforced response	Rat	Facilitated	Corticosterone
Retention of passive avoidance	Rat	Facilitation	Glucocorticoid
Acquisition and retention passive of passive avoidance	Rat	With 6-OHDA lesions: impaired	Not tested
Forced extinction of passive avoidance	Rat	Impaired	Corticosterone
Consolidation of memory	Rat	Inhibition of drug or surgery-induced amnesia	Glucocorticoid

* Taken from Dekloet and Veldhuis (1985)

It is not therefore surprising to find large number of observations made regarding the effects of glucocorticoid on the neurotransmitter related parameters including Na+K+ATPase. In adult animals variable effects on the brain Na+K+ATPase activity have been reported in the literature. Sadasivudu *et al.* (1977) have reported one week injection of hydrocortisone increased the activity in cerebral cortex, cerebellum and brain stem. Huttenlocher and Amemiya (1978) showed that administration of methylprednisolone for 4 days to 24 day old rats increased the Na+K+ATPase activity in brain and also ACTH treatment increased the enzyme activity in young kitten cerebral cortex. There are several studies in young animals with corticosterone on Na+K+ATPase activity. Stastny *et al.*(1971) have reported that hydrocortisone treatment cause an increase in cerebral Na+K+ATPase in chick embryo. Braughler and Hall (1978) reported that acute treatment of methylpredisolone increased the Na+K+ATPase activity in rat spinal cord synaptosomes. Interestingly only one report with respect to adrenalectomy studies showed no change in the enzyme activity in brain homogenate (Gallagher and Glaser, 1968). *In vitro* addition of cortisol increased the Na+K+ATPase activity and alters the membrane fluidity in dog synaptosomal membranes (Deliconstantinos, 1985).

Number of observations have been made in adult animals but not in young ones for other parameters. There are limited studies with respect to glucocorticoid administration in young animals but not with adrenalectomy followed by hormone replacement at younger age. Studies carried out by Hanbauer *et al.* (1975) indicates that administration during the second week

of postnatal life elevates TH activity in locus coerulus which is blocked by two antiglucocorticoid, cortexone and progesterone. The same treatment has no effect in adults. Glucocorticoid play a role in the developmental increase in adrenomedullary PNMT and the maintenance of adult levels of enzyme activity but it is not essential for the initial expression of PNMT in adrenal chromaffin cells (Betito *et al.*, 1992). Dexamethasone treatment over 6- 13 days elevates hypothalamus and midbrain PNMT in neonatal rats (Moore and Phillipson, 1975). In rats, neonatal dexamethasone treatment suppresses development of sympathetic action on the heart and retards adrenomedullary development, possibly via suppression of ACTH release (Dekloet and Veldhuis, 1985).

Administration of glucocorticoid to normal intact developing rats early in life showed precocious induction of brain TPH (Sze *et al.*, 1976). The same group have shown that early adrenalectomy (on postnatal day 12) totally abolished the normal developmental rise of midbrain TPH activity which was restored by glucocorticoid replacement (Sze, 1981). Furthermore, adrenalectomy blocked the stimulation of mouse TPH produced by reserpine or ethanol (Sze *et al.*, 1976) agents that could stimulate pituitary -adrenal activity. Studies with respect to glucocorticoid regulation of enzyme DβH, COMT and MAO associated with catecholamine and indolamine metabolism during various stages of development are very scarce. Besides, except for a few studies such as the study from this department on bioamines showed that hormone decreases the content of tryptophan, 5-HT, 5HIAA in hypothalamus, striatum and hippocampus while NE content increases in hypothalamus (Bhargava, 1988), 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, in the peripheral nervous system (Iversen and Salt, 1970), there seems to be paucity of information on the important aspect of monoamine uptake and release processes during development especially at the regional level. 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).

The effect of glucocorticoid on enzymes of GABA and glutamate metabolism are very limited, only one study was carried out in this department. Also there are no reports on the effect of early adrenalectomy with respect to the same. The only enzyme which is studied to a certain extent is glutamine synthase, which is localized in astrocytes and will be discussed later. The results of GABA and glutamate related enzymes during development indicate that chronic corticosterone increased the activity of GAD and decreased the activity of GABA-T in hippocampus and striatum while hypothalamus showed a increased activity of GABA-T (Bhargava, 1988). Exposure of high and even physiological concentration of GC exacerbate the accumulation of glutamate and aspartate in the hippocampus after excitoxic seizures. GC can alter the regulation of the excitatory amino acids either before or after the seizure and these actions appear to rise from some of the hippocampal energy metabolism (Beherens *et al.*, 1992). There are several studies reported on permissive effects evaluated by adrenalectomy and subsequent replacement as well as excess of glucocorticoid either by injecting hormone or by inducing the stress. The interpretation on

these results are slightly complexed by the fact that most of the earlier biochemical studies is used as a high level of glucocorticoid supplementation during the replacement process without taking into consideration of type I and type II receptor mediated action. It can be very well observed from the table 1.4. which is a compilation and summary of the studies reported in the literature.

The effect with replacement studies indicate a reverse effect of adrenalectomy often and behave similar to excess hormone studies. In spite of these complexity one can see that the permissive and excess actions are opposite to each other indicating that glucocorticoids has significant diverse effects in the range of 30% to 60% above or below the basal values.

Table: 1.4.

Reports on the effects of corticosterone on neurotransmitter metabolism in adult rat brain

Neurotransmitters/ associated enzymes	Effects of			Brain region	Reference
	ADX	Hormone replacement	Excess		
Catecholamines:					
NE turn over	Increased	Reversed		Whole brain	Jovoy *et al.*, 1968.
	Increased	Reversed		Hypothalamus	Fuxe *et al.*, 1973.
NE synthesis			Decreased	Hypothalamus	Iuvone *et al.*, 1977.
Tyrosine level			Increased	Whole brain	Diez *et al.*, 1977.
			Decreased	Hypothalamus	Bhargava, 1988.
TH activity	No effect	No effect	Induced	Ganglia	Dailey & Batterbee,1982.
			Increased	Locus coerulus	Markey *et al.*, 1982.
				Locus coerulus	Melia *et al.*, 1992
	Increased		Decreased	Brain Stem	Lachuer *et al.*, 1992.
DβH activity	Decreased	Reversed	Increased	Hypothalamus	Shen & Ganong, 1976 a,b.
PNMT activity			Increased	Brain stem	Turner *et al.*, 1979.
			Increased	Hypothalamus	Moore & Philipson, 1975
Dopamine level			Increased	Frontal cortex	Rothchild *et al.*,1984.

Serotonin:					
5- HT content	Decreased	Reversed		Hippocampus ¦ Hypothalamus¦	Vanloon *et al.*, 1981.
5- HT turn over	Decreased	Reversed		Hippocampus	DeKloet *et al.*, 1982.
5- HT receptor density	Increased			Subicullum,DG ¦ raphe nucleus ¦	
	No change			Cortex, ventral¦ hippocampus ¦	DeKloet *et al.*, 1986.
Try-OH activity	Decreased	Reversed		Mid brain	Azmita & McEwen, 1969.
			Increased	Whole brain	Azmita & McEwen, 1974.
	Decreased			Whole brain	Vanloon *et al.*, 1981.
	Decreased		Increased	Frontal cortex¦ Brain stem ¦	Singh *et al.*, 1990.
Amino acids:					
Glutamine	Decreased	Reversed		Hippocampus ¦ Striatum ¦ Hypothalamus¦ Cerebellum ¦	Zonta *et al.*, 1992.
Glutamic acid	Decreased		Decreased	Cortex, Whole brain	Sutherland & Rickmaru,1964.
Aspartic acid	Decreased			Whole brain	Suhterland & Rickmaru,1964.
GDH			Decreased	Cortex	Sadasivadu *et al.*, 1977.
GAD			Increased	Striatum	Bhargava, 1988.
			Increased	Supraoptic ¦	
			Decreased	Amygdaloid ¦	
	No change		No change	Hippocampus¦	Acs *et al.*, 1980.
GABA uptake	Increased	Reversed		Hippocampus	Miller *et al.*, 1978.
GABA binding	Increased	Reversed		Mid brain¦ Striatum ¦	Kendall *et al.*, 1982.
GABA amino transferase	No change			Whole brain	Edwards & Rosseau, 1980.
Acetyl Choline:					
Choline acetyl transferase			Increased	Cerebellum	Bau & Vernadakis, 1982.
Acetylcholine esterase			Increased	Cerebellum	Vernadakis & Tamiras, 1967.
Choline uptake			Increased	Ganglia	Sze *et al.*, 1983.
				Putamen magnum	Ricker *et al.*, 1979.
	No change	No change			Vermes *et al.*, 1976.

Peptides:					
β- Endorphin	Decreased			Hypothalamus; Midbrain	Lee *et al.*, 1980.
VIP	Decreased	Reversed		Hippocampus	Rotztejn *et al.*, 1980.
Substance P	Increased			Ganglia	Smith *et al.*, 1991.
Somatostatin	Decreased	Reversed		Ganglia	Smith *et al.*, 1991.
Neuropeptide			Increased	Striatum	Chao and McEwen, 1990.
Neuropeptide Y	Decreased	Reversed		Whole brain	Stanley *et al.*, 1989.

This raises the possibility that glucocorticoid can only partially regulate the gene expression.

Glucocorticoid and Neuroglial cells:

Glial cell differentiation is extensively modulated by corticosteroid by regulating two important glial enzymes such as glutamine synthetase (GS) and glycerol 3 -phosphate dehydrogenase (GPDH) and glial fibrillary acidic protein (GFAP).

Glutamine synthatase catalyzes the formation of glutamine and ammonia detoxification and storage for subsequent release. In rat brain, primarily glial origin is located in astrocytes of white matter (Norenberg, 1979). Studies from others as well as our department have shown that glutamine synthatase activity is found to be increased by corticosterone in cerebellum, olfactory bulb and forebrain (Patel *et al.*, 1983a), cerebellum, hypothalamus and hippocampus as well(Bhargava, 1988). The soluble NAD linked glycero -3- phosphate dehydrogenase (GPDH), another important glial enzyme has been studied extensively with regard to corticosterone. This enzyme is primarily associated with lipid biosynthesis in the CNS. Relatively high level of GPDH activity have been found in bulk isolated bovine oligodentroglial cells (Cammer *et al.*, 1982), but lower activity was report-

ed in rat brain neurons and astrocytes. Leveille and coworkers (1980) found a strict localization of immunoreative GPDH in rat cerebellum. Therefore it can be reasonably concluded that in the brain GPDH is found primarily in oligodentroglial cells but present to a smaller degree in Bergman's glia cells, astrocytes, neurons (Fisher *et al.*, 1981).

Glial fibrilliary acidic protein (GFAP) is a 50 KDa intermediate filament protein of astrocyte. Its content increases several fold in brain during any sort of injury (Brock and O'Callaghan, 1987), aging (Landfield *et al.*, 1981) and different neurodegenarative disease (Morrison and Rosenberg, 1983). Other than the above mentioned enzymes, 2, 3'cyclic nucleotide- 3 - phosphodiesterase (CNPase) activity was reported to be modulated by glucocorticoid. CNP is a membrane bound enzyme enriched in oligodentroglia cells and myelin rich fractions (Sims and Caranegeric, 1978). The detail of the effect of glucocorticoid on these glial cell parameters are summarized in table 1.5.

From these studies it is evident that glial cell enzymes are induced after excess levels of glucocorticoid in the circulation. Glutamine synthase activity seem to be induced almost in all the brain region after the elevation of circulating glucocorticoid. However there are few studies only with respect to the permissive action of glucocorticoid on this enzymes in rat brain either in adult or young animals. The induction of GS activity by glucocorticoid were studied only in young rats but not in the adult rats. The other glial enzyme GPDH was studied extensively in all

Table: 1.5.

Reports on the effect of glucocorticoid on brain glial cells

Parameters	Effects of: ADX	Hormone replacement	Excess	Brain region	Reference
GS			Increased	Whole brain	Piddington and Moscona, 1967.
			Increased	Retina	Rief-Lehrer and Amos, 1968.
			Induced	Primary neural cultures	Juurlink *et al.*, 1981.
				Hypothalamic cells	Vaccaro *et al.*, 1979.
				C6 glioma cells	Hallernmeyer *et al.*, 1981.
				Neuroblastoma cells	Rief- Lehrer, 1971.
			Increased	Cerebellum	Patel *et al.*, 1983a.
				Forebrain	
				Olfactory bulb	
			Increased	Cerebellum	Bhargava, 1988.
				Hypothalamus	
				Hippocampus	
	Decreased	Reversed		Cerebellum	Zonta *et al.*, 1992.
				Hippocampus	
				Hypothalamus	
				Striatum	
	Decreased	Reversed		Hippocampus	O'Callaghan *et al.*, 1991.
	Decreased	No change		Cortex	
GPDH	Decreased	Reversed		Cerebrum	DeVellis andInglish, 1968.
				Cerebellum	
			Increased	Foetal whole brain	Breen and DeVellis, 1974;1975.
			Increased	Cerebral culture	
			Increased	Oligodentroglial	McCarthy and DeVellis, 1980.
			Increased	Glial cells	Kumar *et al.*, 1989.
		Increased		Hippocampus	Meyer *et al.*, 1979.
	Decreased			Cerebrum	Meyer, 1983.
	Decreased			Whole brain	Preston and McMorris, 1984.
GFAP			Increased	Whole brain	Scheff *et al.*, 1980.
			Decreased	Hippocampus	
				Cortex	
				Cerebellum	
				Midbrain	O'Callaghan *et al.*, 1991.
	Increased	Reversed		Hippocampus	O'Callaghan *et al.*, 1989.
	Increased	Reversed		Cortex	
				Striatum	
				Midbrain	O'Callaghan *et al.*, 1991.

GFAPmRNA			Increased	Astrocyte	Nichols *et al.*, 1988.
	Increased	Reversed		Hippocampus Cortex	Nichols *et al.*, 1990.
			Decreased	Cerebrum	Tsuneishi *et al.*, 1991.
Other glial ezymes					
CNPase	No change			Whole brain Optic nerve	Meyer and Fairman, 1985.
			Increased	C6 glioma cells	Wazıri and Sahu, 1980.
Galactosyl sulfotransferase					
	Increased		Decreased	Oligodentro cell lines	Dawson and Kernes, 1978.
Arylsulfatase A			Decreased	Primary cell culture	Stephens and Pieringer,1984.

GS - Glutamine synthetase, GPDH - Glycerol - 3 -phosphate dehydrogenase.
GFAP - Glial fibrillary acidic protein.

types of brain neuroglial cells and different brain regions. This enzyme is induced by glucocorticoid in brain with high level of glucocorticoid the like case of stress condition. On the other hand permissive effect of glucocorticoid attained by adrenalectomy leads to decrease in the GPDH activity in adult rats as well as young rats. On the other hand long term effects of adrenalectomy on the GPDH activity in rat brain is still obscure. GFAP, protein associated with glial cells modulated by glucocorticoid by increases the levels while adrenalectomy reverse the effect. Other glial enzymes associated with myelin were seen to be influenced by both permissive as well as stress effects. This leads to the inference that the permissive as well as elevated levels of glucocorticoid act opposingly for the glial cell metabolism.

Glucocorticoid and Second messenger system:

The possible involvement of pituitary adrenal axis in mood disorder was speculated as early as 1972 (Exton *et al.*, 1972). It began with the possible correlation in the way that most of the psychoactive drug act through alteration in adrenergic pathways and led to the revision catecholamine hypothesis of affective disorder. It was therefore thought that there has to be some alteration in endogeneous factor that may result in affective disorders. Adrenal steroids were thought to be the possible factors since in peripheral tissues such as liver and adipose tissues glucocorticoid can alter the responsiveness of the adenylate cyclase to NE stimulation (Ros *et al.*, 1989).

First report on the altered response of adenylate cyclase activation in the brain was by Mobely and Sulser (1980 a,b). Two weeks following bilateral adrenalectomy the NA induced cAMP accumulation was significantly higher in slices from the rat frontal cortex as compared to the levels in sham operated control. Dose response curve comparing response in tissue from adrenalectomized and sham operated animals suggested that this change in sensitivity to NA is due to a shift in the maximum responsiveness of the system. The replacement therapy of corticosterone, 10 mg/kg body weight for 5 days prior to killing completely abolished this response indicating the permissive role of corticosterone on regulating NE stimulated second messenger levels. The results on the brain cortical slices were different from the peripheral system in the sense that, in liver, the enhanced cAMP response after adrenalectomy is associated with an increase in number of β - adrenergic receptors whereas in cortex it was found to be not associat-

ed with any change in the β - adrenergic receptors (Harrelson and McEwen, 1987).

These results made the investigators to look into greater details of the mechanism. Several studies has been reported and some are summarized in table 1.6. It is obvious that alteration in cAMP levels can only be observed when the tissue is stimulated with some adrenergic or other ligands. These effects were opposite in adrenalectomized and with excess of hormone treatment or by inducing stress.

The change in cAMP levels is not associated with corresponding change in activity of either adenylate cyclase or phosphodiesterase (Mobely and Sulser, 1980a) nor the β - receptor number - the Bmax or the affinity. Thus the changes in the NE activation of adenylate cyclase which are induced by adrenalectomy must be restricted to the non - β component of the response. Since α - adrenergic receptor agonist are known to inhibit the increase in cAMP elicited by NE in brain, it was thought that increase in cAMP could be due to decreased inhibitory effects mediated by α component, however adrenalectomy did not cause any significant changes in the number of either α_1 or α_2 NE receptor. Stone (1979) had shown that stress has opposite effects to adrenalectomy on NE stimulated cAMP. They also failed to demonstrate any effects on β - receptor number or in the activity of adenylate cyclase or phosphodiesterase. It was postulated by Mobely *et al.* (1983) that corticosteroid may regulate the NE sensitive adenylate cyclase by affecting the efficiency of coupling of the non - β - population of NE receptor to adenylate cyclase.

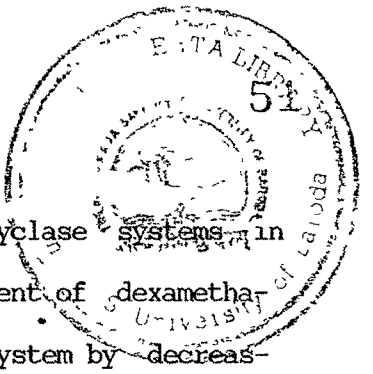
Table: 1.6.

Some reports on the effect of glucocorticoid on cAMP second messenger system

Reference	Parameters	Adrenalectomy		Adrenalectomy + Hormone-Replacement		Excess		Regions	Dosage/treatment
		Basal	Stimulated	Basal	Stimulated	Basal	Stimulated		
Mobley and Sulser (1980a)	cAMP	No change	Increased (NE 100μM)	No change	Decreased (NE 100μM)			Cortex	Cort-10mg/kg b.wt. for 5 days. 15 days after ADX.
Mobley *et al.* (1983)	AC,PDE	No change		No change				Cortex	Cort-10mg/kg b.wt. for 5 days. 15 days after ADX.
Duman *et al.* (1986)	cAMP					No change	Decreased (NE,Iso+PNE, Iso+baclofen)	Cortex	Cort-5mg/kg b.wt. for 14 days.
Harrelson *et al.* (1987)	cAMP	No change	Increased (VIP 50nM,Iso)	No change	Decreased			Hippo. Septum, Amygdala	Dex-25% pellet & Cort- 5 mg/kg b.wt. for 6 days.
Duman *et al.* (1989)	cAMP					No change	Increased (NE,Iso,VIP, 2-Chladeno.)	Cortex	Dex- 4mg/kg b.wt. for 33 days
Gannon and McEwen (1990)	cAMP					No change	Decreased (NE 100μM)	Cortex Hipp.	Cort-50mg/kg b.wt for 21 days. Stress for 21 days
	AC					No change	No change (Forskolin)	Cortex Hipp.	,,
	CaM -AC					No change	Decreased (300nM CaM)	Cortex	,,
Cell lines									
Forray and Richelson (1985)	cAMP						Increased (1μM PGE_1)	Neuro blastoma	Dex-(1μM) 24 hours Cort(10μM) 24 hours.
	AC					Increased	Increased (Forskolin)		Dex-(1μM) 24 hours
	PDE					No change			,,

Balmforth *et al.* (1989)	cAMP	No change	Decreased (10μM DA)	Astro cytoma	Dex-(1μM) for 2 days
	AC	No change	Decreased (10μM DA)		,,
			Increased (10μM PGE_1)		

The NE stimulated alteration in cAMP is not only modulated by corticosterone alone but other neuromodulators such as GABA itself or $GABA_B$ agonist - baclofen, 2-chloro adenosine, histamine and VIP also influence the cAMP levels in response to NE indicating that there is a mechanism which is perhaps common to all modulators (Duman *et al.*, 1986, 1989, Harrelson *et al.*, 1987). Since extracellular calcium is required for the augmenting action, Enna and his associates (Duman *et al.*, 1986) explore the possible role of phospholipase A_2 and phospholipase C and inositol phosphates levels. They demonstrated that chronic corticosterone treatment for 14 days decreased the cAMP levels in response to NE and isoproterenol/ baclofen or isoproterenol/6- fluro norepinephrine combinations without affecting the β - adrenergic response. Since corticosterone is known to reduce the phospholipase A_2 activity in many tissues (Blackwell *et al.*, 1980) it was assumed that the Ca^{2+} dependency and involvement of PL-A_2 are the factors mediating the NE stimulated rise in cAMP levels. Since PL-A_2 releases arachidonic acid which in turn generates prostaglandin to mediate the action, the same group showed that hormone induced cAMP is not associated with any change in IP levels. However the basal levels itself are altered by hormone treatment indicating that a change in phospholipid composition or enzymes associated with phospholipid metabolism might have changed. The authors have stated that arachidonic acid or some other product of phospholipid metabolism may



influence neurotransmitter receptor - coupled adenylate cyclase systems in brain. The same group has shown that a long term treatment of dexamethasone has a bimodel effect on the adrenergic receptor system by decreasing its sensitivity to certain types of stimulation. It was suggested that long term administration glucocorticoid modified some post receptor component such as phosphodiesterase, guanine nucleotide binding protein or adenylate cyclase by making the system more sensitive to activators. However authors failed to see any effect on above mentioned parameters and indicated a possible change in membrane parameters (Duman *et al.*, 1989).

McEwen and associates studied limbic region instead of cortex and demonstrated that the effects are neurotransmitter and region specific. In hippocampus, VIP and isoproterenol activation leads to increase in cAMP accumulation after adrenalectomy and decreases after dexamethasone treatment whereas histamine dependent activation declines after adrenalectomy and is restored by dexamethasone indicating the neurotransmitter specific effects (Harrelson *et al.*, 1987). Similarly amongst hippocampus, septum, amygdala, olfactory bulb and frontal cortex, the most sensitive to adrenalectomy related effects are on hippocampus whereas frontal cortex and septum were relatively less sensitive. Their study have not been able to demonstrate any specific mechanism through which this effect is mediated. Gannon and McEwen (1990) have implicated decrease in calmodulin dependent adenylate cyclase and functional coupling and compartmentilization. This was observed only in parietal cortex but not in hippocampus. Thus suggesting differential coupling of α - adrenergic receptors to cAMP generating systems in brain regions.

Since α_1 adrenergic receptors are known to be coupled with phospholipase C which generates IP_3 and DG production, attempts were made to investigate alteration in IP_3 levels by altering glucocorticoid levels. Neither ADX nor metapirone (11-β- hydroxylase inhibitor) treatment significantly affected the accumulation of inositol phosphates. However at all points the levels of the same were higher in ADX rats compared to controls. Earlier study of Duman *et al*, (1986) showed a higher basal production of inositol phosphate with chronic ACTH treatment. Thus phosphoinositides although may not directly be involved in glucocorticoid receptor mediated action but seem to be affected by alteration in glucocorticoid levels. For example in vasular smooth muscle of rats dexamethasone enhances noradrenaline-induced inositol phosphates production (Liu *et al.*, 1992).

Since receptor mediated generation of cAMP is mediated through G - proteins and in adipocytes adrenalectomy was found to decrease steady state levels of α and β subunit of the adenylate cyclase regulating subunit G_s (Ros *et al.*, 1989). Few studies on G - proteins demonstrated that glucocorticoids play an important role in maintaining the levels of $G_{s\alpha}$ and $G_{i\alpha}$ units. Up regulation of $G_{s\alpha}$ with no change in $G_{s\beta}$ and down regulation of $G_{i\alpha}$ with no change in $G_{i\beta}$, thus serves to alter effector system and both of these are linked to regulate cAMP levels (Saito *et al.*, 1989).

Szetendrei and Fekete (1990) attempted to identify the mechanism of glucocorticoid modulation of α_2 - receptor mechanism using ^3H antagonist - agonist and ^3H agonist - antagonist displacement experiment to identify different regulatory protein- nucleotide associated forms of receptor. The change in the GTP sensitivity suggesting that the nucleotide regulating

system may be involved in the action of adrenal steroids on central α_2-receptor modulation.

Although the role of ADP ribosylation factor in regulating G-protein in *in vivo* is not clear the study of Duman and his associates indicate that ARF1 and ARF2 expression is under the control of glucocorticoid. After one week of corticosterone treatment 1.8 and 3.7 Kb ARF mRNA increased 30 to 40% over the basal levels and ADX had an opposite effect. Thus G- proteins and their modulation seem to be under the control of glucocorticoids (Duman *et al.*, 1990).

Few studies have been carried out on cell lines or cell culture to identify the basic functions at the cellular levels. Interestingly not only neuroblastoma cell lines but astrocytoma cell clone D384 was also found to respond to exposure to corticosterone for different time period. The study on neuroblastoma indicates that long term treatment to the cell clone with glucocorticoid produced an enhancement of PGE_1 mediated cAMP formation which seem to exert its effect on cAMP levels by enhancing adenylate cyclase activity (Forray and Richelson, 1985). Since astrocytoma clone D384 expresses β - adrenergic and PGE_1 receptor it appears that dexamethasone increases the above mentioned receptor number whereas D_1 receptor number decreases. These effects seem to be mediated by inducing the synthesis of proteins which regulate the cAMP levels. This alterations is evident from the decrease and increase of adenylate cyclase activity respectively. These results suggests that glucocorticoid affect step (s) of the hormone - sensitive adenylate cyclase system that occur prior to the coupling of G_s to the catalytic subunit (Balmforth *et al.*, 1989). In Ros cell lines it has

been shown that dexamethasone treatment enhanced β- adrenergic receptor number and G_s regulatory G protein associated with cAMP system (Rodan and Rodan, 1986). It is obvious that the studies carried on *in situ* animals using ADX or corticosterone treatment paradigm indicated no change in β receptor number. On the other hand studies on cell lines with or without exposure to glucocorticoids invariably indicates changes in receptor number. Thus differential response observed makes difficult to extrapolate result from *in vitro*/cell lines to the *in situ* condition.

To summarize the various results on secondary messenger system, it is fairly established that glucocorticoids do not influence the basal levels of cAMP but exaggerate the NT stimulate cAMP levels with adrenalectomy and suppress the cAMP rise with excess of steroid levels indicating a membrane involved phenomenon. Although no conclusive evidence has yet come, one is inclined to believe that the glucocorticoid action is influencing neurotransmitter - nucleotide protein interaction and or regulation and modulation of nucleotide binding protein of various types (such as $G_{s\alpha}$, $G_{1\alpha}$,ARF *etc.*). Most of the studies do not reveal change in neurotransmitter receptor number or change in the property but few have observed change in number of receptors without affecting kinetics (Mobely and Sulser 1980a; Ros *et al.*, 1989) especially in cell lines.

Neurotransmitter - receptor and G - protein interaction is strictly a membrane associated phenomenon and it will not be surprising if any of the membrane parameter is altered in response to glucocorticoids. One possible factor could be the alteration in phospholipids compostion or fatty acid composition of these phospholipids.

In fact Duman and his group as well as others have suggested that phospholipid changes in membranes might be one of the possible factor in modulating neurotransmitter stimulated second messenger levels (Duman *et al.*, 1986; Gannon and McEwen, 1990). The α_2 adrenoceptor binding affinity was reported to be lost in cerebral cortical membranes after 10 days of hydrocortisone and this change was attributed to membranal changes (Szetendrei and Fekete, 1990).

Glucocorticoid effects on phospholipids and membranes:

From the studies on tissues other than the nervous system, it is evident that phospholipid composition of a variety of membranes can considerably affect metabolic process. Studies have demonstrated that effects of corticosterone influence the phospholipid composition in a number of tissues and subcellular fractions of glucocorticoid responsive cells and is extensively reviewed by Nelson (1980). It is seen that corticosterone affect the oxidative metabolism of rat brain mitochondria and brain mitochondrial oxygen utilization (Bottoms and Goetsch, 1968; Roosevelt *et al.*, 1973). It has been established that glucocorticoid has effect on phospholipid biosynthesis in foetal lung (Farrel and Zachman, 1973). Intrauterine injection of corticosterone into rabbit cause production of dipalmitoyl phosphatidyl choline, a pulmonary surfactant, by stimulating the activity of cytidyl transferase and lysolecithin acyl transferase (Rooney *et al.*, 1976). Adrenalectomy and corticosterone treatment changed the oxygen utilization and superoxide anion in lung microsomes (Nelson and Rhuman-Wennhold, 1975). Cytochrome P450 hydroxylation having lipid requirement is influenced by corticosterone (Rhuman-Wennhold and Nelson, 1978). In lung micro-

somes, the free radical anion increased after cortisol treatment (Nelson and Rhuman-Wennhold, 1979). Corticosteroids also reported to inhibit superoxide production in leukocyte by inhibiting pyrimidine nucleotide oxidase (Rossi *et al.*, 1976). Administration of dexamethasone *in vivo* decrease phosphatidyl choline while sphingomyelin and phosphatidyl ethanolamine increase in leukocytes (Nelson, 1980).

The glucose transport in fat cells were inhibited by dexamethasone which indicated an increase in sphingomyelin content (Murray *et al.*, 1982). H_2O_2 as well as protein kinase C induced glucose transport were inhibited by dexamethasone in many cell types (Murray and Nelson, 1987; Nelson and Murray, 1987; Nelson and Murray, 1989; Nelson 1990b). Effects of glucocorticoid on the transport of glucose and amino acids by the alteration of phospholipid or cholesterol are well documented (Czech, 1976; Murray and Nelson, 1987). It has been suggested that glucose transport through the membrane may involve Na^+K^+ATPase and/sodium linked sugar transport. Changes in the membrane lipids could affect the movement of carrier through membrane transport lipid-protein interaction (Harrison and Lunt, 1975). As demonstrated in the case of erythrocyte membrane, glucocorticoid effects the fludity of the membrane and has specific effect on the association of protein interaction with altered lipids (Borochov *et al.*, 1977). Study on liver microsomes demonstrated that adrenalectomy for 7-14 days significantly decreased the phosphatidyl choline concentration in ADX rats. Instead of reversing cortisol replacement there was a further decrease in the level of phosphatidyl choline while phosphatidyl ethanolamine and sphingomyelin which were not affected by adx and was found to be low in cortisol replaced

group. Fatty acid composition of phosphatidyl choline such as palmitic acid, oleic acid, linoleic acid increased while arachidonic acid and stearic acid decreased in adrenalectomized and cortisol treated animals. The replacement effects seem to be the excess of corticosterone rather than the permissive effect (Melby *et al.*, 1981).

There are many studies that show corticosteroid administration or adrenalectomy influence the receptor activity (Marshall *et al.*, 1978). Many studies have demonstrated that effect of high levels of corticosterone stabilizes the lysosomes or other membranes. This is due to high levels of corticosteroids which cause deformity of the lipid bilayer which is similar to the action of cholesterol intercalation with fatty acid chain of the phospholipids in the membrane (Weissmann and Thomas, 1964). Goodman and his associates were first to report of a biochemical evidence of membrane bound enzyme activities. Aldosterone treatment increased both lipid synthesis and the turnover of membrane phospholipid fatty acids within 30 minutes (Goodman *et al.*, 1971;1975). Subsequent studies showed using ^{14}C labeled and ^{3}H fatty acids as precursor that aldosterone stimulates specifically the elongation and desaturation of oleic acid. Treatment of prednisolone in tumor cell lines caused an increase in the ^{3}H acetate incorporation into phosphatidyl choline and phosphatidy serine while a decrease in cardiolipin, phosphatidyl ethanolamine and cholesterol ester (Melnykovych *et al.*, 1976). Glucocorticoids act on the membrane enzyme such as acyl transferase and phospholipases both of which require lipids for their action. This perhaps is due to altering sphingomyelin and phosphatidyl choline (Kito *et al.*, 1978; Brindley *et al.*, 1979). Glucocorticoids are known to regulate number

of enzymes in lungs for surfactant synthesis such as choline phosphate cytidyltransferase and lysolecithin transferase. Dexamethasone treatment showed a change in the phospholipid composition as well as ^{14}C acetate incorporation in different tissues of rat and hepatic mitochondria (Kaur *et al.*, 1989). Glucocorticoid can inhibit aryl sulfatase A enzyme in brain cell culture (Stephens and Pieringer, 1984) while the neutral sphingomyelinase increased in fibroblast cell lines (Nelson, 1990a).

Glucocorticoid can modulate protein kinase C which influences the phospholipase A_2 activity in basophilic cells (Zor *et al.*, 1990). Phosphatidylinositol specific phospholipase C is regulated by phosphorylation - dephosphorylation by glucocorticoid in basophillic cell lines (Her *et al.*,1991). Reports suggest that lecithin enhancement in foetal lung culture with cortisol is post transcriptional regulation and results in increase in fatty acid synthase activity, acetyl CoA carboxylase and ATP citrate lyase activity (Battenburg and Elfring, 1992). Both glucocorticoid and mineralocorticoid decrease the $\Delta 5$ desaturase enzyme, which convert eicosatrienoic acid to eicosatetraenoic acid in liver (Alaniz *et al.*, 1992).

Adrenalectomy alters the lipid metabolism in membranes such as erythrocytes and mitochondria with changes in the levels of phosphatidyl ethanolamine, phosphatidyl choline and cholesterol and these disturbance were reversed by vitamin K and vitamin E (Linder, 1988). Cortisone administration reduce both free fatty acid and triglyceride in adipose tissue (Nazir *et al.*,1988).

Some reports have shown an alteration in neurotransmitter receptor either in the brain region or in cell lines. Few studies have reported

difference in receptor number and alteration in protein synthesis. Forray and Richelson (1985) showed in neuroblastoma cell line, glucocorticoid addition increases the potentiation of cAMP formation by PGE_1. This increase in cAMP has been attributed to the glucocorticoid action at the catalytic site of adenylate cyclase. One possible factor for such an effect could be alteration in phospholipid composition or fatty acids incorporated in phospholipid. It is known that phosphatidyl inositol and phosphatidyl serine are required for the responsiveness of NE stimulated adenylate cyclase activity (Rodbell *et al.*, 1971a; Levey, 1971). These phospholipid helps the coupling of regulatory site to the catalytic site. After hormone binding and dissociation of the binding site, an alteration in conformation of the catalytic site occur which results in activation of the enzyme. In the absence of phospholipid this conformational change does not take place and hence activation does not follow hormone binding (Klein and Levey, 1971a).

Thus like all other tissues brain phospholipids also may be influenced by glucocorticoid and change in phospholipid composition or fatty acid composition of the same can influence receptor coupled cAMP generation. Studies by Massari and colleagues indicated that phosphatidyl serine is the most active component for inducing dopamine stimulated adenylate cyclase activity while phosphatidyl ethanolamine and lyso PE are less active (Benvegnu *et al.*, 1978). There is a possibility that phosphatidyl inositol turnover and cAMP systems are interrelated. Activation of protein kinase C by 1,2-DG after the action of phospholipase C on PI, resulted phosphorylation of G_s inturn increases the activity or facilitates its interaction

with the catalytic unit of adenylate cyclase. Alternatively C kinase might catalyses phosphorylation of G_i resulting in diminishing capacity to inhibit adenylate cyclase, thereby increasing the responsiveness of the cyclase system when it is activated through G_s. The latter hypothesis is supported by the findings in brain membrane (Enna and Karbon, 1987).

It is also known from the reconstitution experiments that coupling of β - adrenergic receptor and G_s or G_s-C takes place only when both components are present in a phospholipid bilayer. A combination of phosphatidyl ethanolamine : phosphatidyl serine (3:2 w/w) was the most effective (Levikzki, 1988). The association of G- protein with the membrane is essential to ensure critical conformation position for the action on the effecter and signal transduction. This association is achieved by either myristoylation or isoprenylation which results in attachment of α subunit to the membrane for its action (Spiegel *et al.*, 1991).

Thus it is evident that glucocorticoid does play a role in lipid metabolism in general and phospholipids in particular. all these studies have been on tissue/ cell other than nervous tissue. It is surprising that although several groups have implicated membrane lipids as causative factor in altering membrane associated phenomenon. On literature search it was found that there is paucity of information regarding role of glucocorticoid on brain lipids although few studies have been reported with regard to myelin (Preston and McMorris, 1984; Meyer and Fairman, 1985). Since phospholipids concentration or alteration in fatty acids composition could alter several membrane linked enzymes, receptors, coupling of G proteins etc. The effects of glucocorticoid in second messenger generation could also be

influenced indirectly by phospholipids.

Keeping the above mentioned points the objective of the present study was to investigate the following points.

(a) Do circulating glucocorticoid play any role in maintaing brain phospholipids or their turnover and is it age related?.

(b) Do the these effects of glucocorticoid relate to the type and distribution of their receptors in different brain regions?.

(c) Do permissive (basal) and excess of glucocorticoid have opposite effects on phospholipid levels or its turnover?.

(d) If there are any changes in phospholipids, whether these are associated with any of the membrane linked phenomenon such as fluidity and enzymes such as Na+K+ATPase, 5'Nucleotidase and acetylcholine esterase or phospholipid fatty acid composition?.

(e) Do the physiological alteration in circulating glucocorticoids (for example- ageing), also influence membrane bound enzymes. If so, can these changes be modulated by altering the circulating levels of glucocorticoids?.

The parameters included the total and subfractions of phospholipids, cholesterol and galactolipids in the whole brain and different regions namely olfactory bulb, cerebellum, brain stem, hippocampus, striatum and cortex.

^{32}P studies were carried out *in vivo* and *in vitro* in the subclasses of phospholipids in whole brain and regions mentioned above.

Fatty acid composition of the phospholipids in the synaptosomes from the cerebral cortex and membrane fluidity of the same.

Enzymes namely Na+K+ATPase, 5'nucleotidase and acetylcholine esterase in membrane preparation from different regions and fatty acid composition in synaptosomal fractions from cerebral cortex.

Histological studies on adult and aged hippocampal tissue of control and adrenalectomized animals.