CHAPTER III

RESULTS AND DISCUSSION

As mentioned earlier the objectives of the present studies were as follows.

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

(b) Do 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 and synaptosomal 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?.

In order to achieve these objectives following experiments were conducted.

Experiment Ia:

Changes in somatic growth, tissue weights and plasma Na+ levels with alteration in circulating levels of corticosterone:

As mentioned earlier,it would have been ideal to monitor circulating levels of corticosterone under various conditions at different ages,

however due to technical problems it was not possible to monitor the same. In the absence of the information on actual circulating levels of corticosterone, the parameters which have been selected are as sensitive index to alteration in circulating levels of corticosterone. These have been monitored in every experiment to ensure that the changes in these parameters reflect alteration in the circulating levels of corticosterone. The first few tables give the compiled information on these parameters.

Data on the body weight gain and tissue weight after corticosterone treatment at various ages are presented in table 3.1. It was observed that corticosterone treatment reduced the gain in body weight. This effect was significant at younger ages, i.e., at 10 and 20 day of age. At 40 day, average weight gain of treated animals was lower but was not statistically significant.

Corticosterone significantly increased the liver weight in all the age groups and a significant decrease in thymus weight. The whole brain weight was not significantly reduced although it weighed less at 20 days of age. A similar picture was obtained with steroid treatment other than corticosterone (Table 3.2).

Results obtained in adrenalectomized rats (Table 3.3, 3.4) showed a significant reduction in body weight, increase in brain weight and decrease in liver weight. The decrease in body weight was brought back to normal by corticosterone treatment. The plasma Na^+ levels showed a decrease in adrenalectomized rats which is consistent with reported study (Gallagher and Glaser, 1968). However, increase in brain weight was not reversed by corticosterone replacement of short term replacement (5 days), which is not suffi-

Table: 3.1.

Effect of corticosterone administration on body weight, brain weight, liver weight and thymus weight in at different ages in rats.

Group	Age (Days)	Gain in body weight(g)!	Liver weight (g)	Thymus weight (g)	Brain weight (g)
Cort.	10	2.9 ± 0.19***	0.55 ± 0.024*	nd	0.9 ± 0.015
Control		5.1 ± 0.24	0.47 ± 0.02		0.9 ± 0.021
Cort.	20	5.8 ± 0.31***	1.6 ± 0.07*	0.09 ± 0.03***	1.3 ± 0.040
Control		9.0 ± 0.49	1.4 ± 0.05	0.35 ± 0.01	1.4 ± 0.032
Cort.	40	11.4 ± 1.31	3.8 ± 0.14*		1.5 ± 0.021
Control		13.0 ± 1.50	3.3 ± 0.14	nd	1.5 ± 0.020

Number of observations are 11-15. Values are expressed in mean ± S.E. Cort- Corticosterone,

Levels of significance by Student's 't' test *P<0.05; *** P<0.001 from control. nd- not determined.

! Weight gain = Body weight at the time of decapitation - Body weight at the start of the treatment (3 days).

Table: 3.2.

Effect of Corticosterone, Dexamethasone and ACTH on body weight and brain weight of 20 day old rats.

Hormone	Gain in body weight(g)!	Brain weight (g)
Control	7.1 ± 0.63	1.25 ± 0.04
Cort.	5.3 ± 0.55*	1.21 ± 0.02
ACTH	6.0 ± 0.90	1.20 ± 0.02
Dex.	4.0 ± 1.25*	1.22 ± 0.02

Number of observations are 11-15. Values are expressed in mean ± S.E.

Levels of significance by Student's 't' test *P<0.05 from control.

Cort- Corticosterone,Dex-Dexamethasone, ACTH-Adrenocorticotropin hormone.

! Weight.gain = Body weight at the time of decapitation

- Body weight at the start of the treatment (3 days).

Table: 3.3.

Effect of Adrenalectomy and Cort. replacement on body weight and brain weight of rat after ADX at day 20.[1]

Duration	Groups	Gain in body weight(g)!	Brain weight (g)
15 days	ADX+H	60.4 ± 2.5@@@ (17)	1.6 ± 0.03 (17)
	ADX	38.9 ± 3.2*** (16)	1.6 ± 0.02** (15)
	SHAM	73.5 ± 2.0 (23)	1.5 ± 0.02 (20)
30 days	ADX+H	53.6 ± 5.9@@ (6)	1.6 ± 0.02@ (6)
	ADX	37.0 ± 4.4** (6)	1.7 ± 0.03** (6)
	SHAM	57.4 ± 2.5 (7)	1.6 ± 0.02 (7)

Number of observations are in parenthesis. Values are expressed in mean ± S.E. Level of significance by Student's't' test @ P<0.05; @@,** P<0.01; @@@,*** P<0.001 from ADX and SHAM respectively.

! Weight Gain = Body weight at the time of decapitation - Body weight at the time of surgery (20 day).

1= For 15 days duration - Replacement was given for last 5 days. For 30 days duration - Replacement was given for 29 days.

Dosage 10 mg/kg body weight.

Table: 3.4.

Effect of Adrenalectomy and Cort.replacement on body weight,brain weight, liver weight and plasma Na+ concentration in rat after ADX at 20 days of age.

	ADX+H	ADX	SHAM
Gain in body weight(g)!	60.4 ± 2.50@@@ (17)	38.9 ± 3.20*** (16)	73.5 ± 2.00 (23)
Brain weight(g)	1.6 ± 0.03 (17)	1.6 ± 0.02** (15)	1.5 ± 0.02 (20)
Liver weight(g)	3.7 ± 0.20@@@ (17)	2.7 ± 0.12*** (15)	4.0 ± 0.14 (20)
Plasma Na+ (mEq/dl)	17.4 ± 0.21@@@ (6)	15.5 ± 0.09*** (6)	17.2 ± 0.11 (6)

Number of observations are in parenthesis,Values are expressed in mean ± S.E.,mEq/dl= milliequivalent/100 ml of plasma.

Level of significance by Student's 't'test @@@, *** P<0.001; **P<0.01 from ADX and SHAM respectively.! Weight Gain=Body weight at the time of decapitation - Body weight at the time of surgery.

ADX-Adrenalectomy, ADX+H- Corticosterone replacement,

SHAM - Operated control. Replacement dosage 10 mg/kg body weight.

Duration (15 days),hormone replacement last 5 days.

cient enough to reverse the increase but the longer replacement did reverse the same. This decrease in body weight,decrease in liver weight, and increase in brain weight were concurrent with many reports (Devenport and Devenport, 1982; 1985; Gannon and McEwen, 1990; Meyer, 1983; 1987; Spencer *et al.*, 1991). Although the whole brain weight in corticosterone treatment for three days of both 10 and 20 day old was not significantly lower, some of the brain region weights were altered (Table 3.5). A decrease in weight of olfactory bulb, brain stem and hippocampus of rats at day 10 and decrease in cerebellum and striatum at day 20 of the hormone treated rats was observed. Although some regions had higher weight after adrenalectomy at day 40, they were not significant (Table 3.6).

It is evident from the data presented that both elevated as well as ADX show a decrease in body weight. This is consistent with several studies reported (Devenport and Devenport, 1982; 1985; Meyer, 1983; 1987). In the literature, with corticosterone treatment, the effects with age are less pronounced.

The changes in liver weight with elevated levels of corticosterone indicated an increase in liver weight. These effects again are more pronounced at younger ages. In contrast to elevated levels, ADX results showed significant decrease in liver weight. These effects are completely reversed by the hormone replacement indicating that depleted levels of corticosterone is responsible for such reduction in liver weight. Increase in liver weight with elevated levels of corticosterone has been reported not only from this laboratory (Jani *et al.*, 1991) but several groups have shown such an effect (Cotterrell *et al.*, 1972; Howard, 1973; Loeb,1975). A decrease in thymus

Table 3.5.

Effect of corticosterone administration on weights of different brain regions at 10 and 20 days of age.

Groups	Age (Days)	OB	CBL	BS	HC	STR
Cort.	10	0.07 ± 0.003*	0.18 ± 0.008	0.2 ± 0.005*	0.1 ± 0.002*	0.08 ± 0.004
Control		0.08 ± 0.003	0.19 ± 0.015	0.22± 0.006	0.12± 0.003	0.08 ± 0.003
Cort.	20	0.05 ± 0.002	0.17 ± 0.003*	0.15± 0.005	0.08± 0.002	0.05 ± 0.001*
Control		0.05 ± 0.002	0.18 ± 0.004	0.15± 0.003	0.08± 0.002	0.06 ± 0.001

Values are expressed in mean ± S.E. in g. Number of observations are 8-10. Level of significance by Student's 't' test *P<0.05 from control. For 10 day old,two rats were pooled/observation.

Table 3.6.

Effect of ADX & Cort. replacement on weights of diffetent brain regions after ADX at 40 days of age.

Groups	OB	CBL	BS	HC	STR
ADX+H	0.07 ± 0.006	0.22 ± 0.007	0.23 ± 0.010	0.10 ± 0.005	0.08 ± 0.002
ADX	0.08 ± 0.002	0.24 ± 0.009	0.21 ± 0.020	0.10 ± 0.003	0.08 ± 0.003
SHAM	0.07 ± 0.003	0.23 ± 0.015	0.24 ± 0.016	0.10 ± 0.002	0.07 ± 0.003

Number of observations are 7. Values are expressed in mean ± S.E as g., OB- Olfactory bulb, CBL- Cerebellum, BS- Brain stem, HC- Hippocampus, STR- Striatum. Cort- Corticosterone. ADX+H- Adrenalectomy and corticosterone replacement,ADX-Adrenalectomy, SHAM-Operated control. Replacement for last 5 days and duration for 15 days.

weight is one of the most significant and conspicuous effect of elevated levels of corticosterone and has been reported by others (Gannon and McEwen, 1990; Spencer *et al.*, 1991). This decrease in weight is a well known effect associated with diminished immune response.

It is well known that by complete removal of adrenal glands the salt balancing hormone -the mineralocorticoids are also lost. This results in imbalance of cations especially, a significant depletion of circulating levels of Na+ and hence ADX animals supplied with saline water. The data of plasma Na+ ions clearly indicates that the ADX is complete and the details are mentioned in methods and materials. The results in the present study are consistent with the reports of Gallagher and Glaser (1968).

With regard to brain weight the effects were observed at younger ages and they were dependent on the duration of treatment. The treatment given in the present study is relatively short (3 days). Although the gross brain weight shows a trend, a significant reduction in weight was not found. However, treatment for a longer duration (10 days) showed a significant decrease in brain weight (data not shown). Regional study didn't show any selective effect.

There is extensive literature showing that early treatment with glucocorticoid can substantially reduce brain weight and DNA synthesis, myelin deposition, behavioral alteration, protein/DNA ratio (Cotterrell *et al*, 1972; Gumbinas *et al.*, 1973; Howard, 1973; Kovacs, 1973; Loeb, 1975, Meyer, 1983; 1986; 1987; Carlos *et al.*, 1992). Further more, Devenport and Devenport (1982) have shown convincingly that loss of glucocorticoid rather than catecholamine or mineralocorticoid are responsible for enhancement of

brain growth after ADX at 25 days of age.

In adrenalectomy, the weight loss of animals had been attributed to mineralocorticoid deficiency (Devenport and Devenport, 1985) whereas higher brain weight in adrenalectomized rats has been exclusively due to corticosterone depletion (Meyer, 1987). The chief cause of weight loss in ADX rats is attributed to mineralocorticoid deficiency. This finding as mentioned by the authors is intriguing that aldosterone and deoxycorticosterone are rarely considered apart in their role in salt metabolism and associated body fluid dynamics (Devenport and Devenport, 1982). A variety of hormonal and physiological manipulations, for example, starvation or enrichment are known to simultaneously change brain and body growth, but only ADX has been shown to selectively influence the gross structure of the brain (Devenport, 1979; Devenport *et al.*, 1992). The ADX induced increase in brain weight has been attributed by the same authors to appear as a large permanent increment on tissue mass. Adrenalectomy caused an apparent stimulation of glial cell proliferation and myelinogenesis in animals less than 20 day old (Meyer and Fairman 1985). Yehuda *et al.* (1989) has already reported that ADX at postnatal day 11, caused an increase in cell proliferation in all brain regions despite differential schedules of neurogenesis and gliogenesis in different areas. In the present study animals were 19-20 days old when adrenalectomized. Animals younger than this age had very high mortality and it was not possible to keep them alive for a longer duration. Around 20 days of age the cell division is almost over and the increase in brain weight could partly be accounted for increase in cell constituents and myelin.

Mechanism of glucocorticoid action on brain cell proliferation is not fully understood. These hormones may cause premature arrest of division in proliferating population or lengthening the cell cycle. Glucocorticoid previously have been shown to increase the duration of either G1 or G2 phase in a number of non-neuronal cells (Fanger *et al.*, 1987). Direct hormonal effect on progenitor cell must be considered in the light of the ability of glucocorticoid to inhibit replication of glial cells (Beamont, 1987). It is also possible that glucocorticoid may alter the synthesis and action of various mitogenic factors (Herschman, 1986). Corticosterone interact with many endocrine factors such as growth hormone and thyroid hormone (Balazs and Richter, 1973) which are important for brain growth and development. This implies that more than one factor is involved in the change in brain weight. Meyer (1987) had reported that circulating T_4 levels was decreased in ADX rats but an increase in basal TRH stimulated thyrotropin secretions.

With regard to different regions it has been known that in normal condition postnatal cell division has been found in olfactory bulb in mice (Hinds, 1968) also considerable postnatal myelination where heavy neurogenesis occur (Agarwal *et al.*, 1970a). It has been reported that corticosterone inhibit postnatal cell proliferation in rat brain. This inhibition vary with respect to age and region (Balazs and Richter, 1973). In rat and mouse, besides postnatal glial cell formation, it involves selective increase in microneurons in regions such as olfactory bulb and hippocampus. These cells have been implicated in modifying functional organization of the developing neurons (Altman, 1970). The decrease in regional weight in

the present experiment suggest that corticosterone affect the cell proliferation. Cotterrell *et al.* (1972) had reported that even though body reduction was observed in cortisol treated rats, brain was less affected as in the case of cerebellum and cerebrum during the period of 13-20 days. In normal condition during the postnatal days, increase in cell number in cerebellum (65%) is much less than in cerebrum (400%). Daily treatment of cortisol cause a permanent deficit in cell number which account 20% in cerebrum while more than 30% in cerebellum. This explains the decrease in weight in cerebellum at 20 day.

From the above data it is fairly clear that methods used in this experiments are changing the steroid levels in experimental rats.
The first experiment was conducted on the whole brain tissue.

Experiment I b & c:

(b)Effects of corticosterone administration at different ages, of different steroids at 20 day and bilateral adrenalectomy on the concentration and ^{32}P incorporation in whole brain phospholipid fractions:.

At the initiation of these studies (1989), the literature indicated that corticosterone treatment or adrenalectomized rats seem to affect the myelin lipids, concentration and composition (Meyer and Fairman, 1985; Preston and McMorris, 1984). Similarly the enzymes associated with lipid metabolism such as GPDH, sulfotransferase, aryl sulfatase and CNPase vary with corticosteroid treatment and were studied in whole brain, cell lines or optic nerve (DeVellis and Inglish, 1968; Meyer *et al.*, 1982; Meyer and Czuprya, 1984; Meyer, 1983).

Initially the aim was to concentrate on polyphosphoinositides metab-

olism. Polyphosphoinositides are extremely labile and significantly decrease in post mortem condition (Eichberg and Hauser, 1967) and therefore rapid freezing technique by dropping the brain in liquid nitrogen is used. Since rapid freezing technique does not permit regional studies, especially regions such as hippocampus and striatum *etc.* the earlier experiments were restricted to whole brain to identify the gross lipid changes.

Experiment Ib:

Effect of Corticosterone administration:

The three day corticosterone administration at 40 mg/kg body weight, S.C. at different ages and whole brain was studied.

Ontogenic pattern of various fractions of phospholipids in the whole brain indicates an increase in concentration of all the fractions with age. However, the rate of increment seem to differ for various fractions. Majority of the phospholipids show greater increase between 10 and 20 compared to 20 and 40, whereas few such as phosphatidyl inositol-4,5-bisphosphate (PIP_2) and sphingomyelin (SM) seem to increase considerably even after 20 days of age (Table 3.7). The ontogenic pattern of various phospholipids are consistent with previous studies reported from this department and by others. (Eichberg and Hauser, 1967; Horrocks and Sharma, 1982; Uma and Ramakrishnan, 1983a; Lalitha *et al.*, 1988).

As can be seen from table 3.8,3.9. that corticosterone administration at any age did not alter the concentration of phosphoinositides in the whole brain. On the other hand, ^{32}P incorporation was higher in PIP_2 and PIP at 10 and 20 day ages in experimental animals compared to control whereas at day 40 these effects were not significant. However with regard

Table 3.7.

Concentration of phospholipids in developing rat brain.

Age(days)	10	20	40
		µg/g tissue	
PIP_2	161 ± 12.7	147 ± 7.8	247 ± 12
PIP	93 ± 7.9	129 ± 3.9	209 ± 26
		mg/g tissue	
PI	2.2 ± 0.13	1.5 ± 0.19	2.1 ± 0.31
PS	2.5 ± 0.23	4.5 ± 0.32	3.8 ± 0.38
SM	1.8 ± 0.13	2.2 ± 0.24	3.5 ± 0.27
PC	6.2 ± 0.30	14.6 ± 0.42	19.8 ± 0.50
PE	2.4 ± 0.36	13.7 ± 0.60	19.8 ± 0.91

Values are expressed in mean ± S.E. Number of observations are 6-8.

PIP_2-Phosphatidylinositol-4,5-bisphosphate, PIP-Phosphatidylinositol-4-phosphate, PI-Phosphatidylinositol, PS-Phosphatidyl serine, SM-Sphingomyelin, PC-Phosphatidyl choline, PE-Phosphatidyl ethanolamine.

Table: 3.8.

Effect of Corticosterone administration on the concentration of phosphoinositides in rat brain.

Age(days)		10	20	40
PIP_2 µg/g tissue	Cort.	156 ± 14.1	134 ± 6.2	268 ± 12.3
	Control	161 ± 12.7	147 ± 7.8	247 ± 12.0
PIP µg/g ,,	Cort.	107 ± 8.9	116 ± 7.8	197 ± 9.1
	Control	93 ± 7.9	129 ± 3.9	209 ± 26
PI mg/g ,,	Cort.	2.0 ± 0.22	1.7 ± 0.10	2.5 ± 0.31
	Control	2.2 ± 0.13	1.5 ± 0.19	2.1 ± 0.31

Table: 3.9

Effect of Corticosterone administration the ^{32}P incorporation *in vivo* into phosphoinositides in rat brain.[1]

Age(days)		10	20	40
PIP_2	Cort.	3.1 ± 0.10**	10.8 ± 0.75**	10.4 ± 1.20
	Control	2.2 ± 0.20	8.0 ± 0.56	6.9 ± 1.10
PIP	Cort.	2.0 ± 0.02***	4.6 ± 0.37*	4.0 ± 0.48
	Control	1.7 ± 0.05	3.5 ± 0.21	2.7 ± 0.32
PI	Cort.	1.3 ± 0.08***	2.4 ± 0.10	21.7 ± 1.17**
	Control	2.4 ± 0.11	2.7 ± 0.27	12.4 ± 2.60

Number of observations are 6-8. 1= Values are expressed in mean ± S.E. as CPM x 10^3. Level of significance by Student's't'test

*P<0.05;**P<0.01;***P<0.001 from control.

to PI, significantly higher incorporation was at day 40, lower at day 10 and remained unaltered at 20 day. These results reflected the PI ⇌ PIP ⇌ PIP_2 exchange being affected emphasizing the increased turnover of these phospholipids with corticosterone treatment.

At 10 days of age corticosterone elevated the concentration of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) whereas other phospholipids were not changed (Table 3.10). At 20 days of age an opposite effect was observed with decreased concentration of phosphatidyl serine (PS) and phosphatidyl ethanolamine (PE) whereas no change was found in any of the phospholipids at 40 day. Thus response to corticosterone are different at various ages. It is likely that this effect on PE reflected myelination which was reported to be decreased by others with glucocorticoid administration (Preston and McMorris, 1984).

With regard to ^{32}P incorporation the maximum effects were found only on 20 days of age, when PS and PE had significantly higher incorporation in steroid treated animals while SM had lower incorporation. SM also had lower incorporation at day 10 whereas no other phospholipid fractions showed any significant effects. At 40 days of age none of the fractions showed any significant deviation. (Table 3.10, 3.11). The results of the concentration and incorporation study reveal that brain phospholipids are sensitive to corticosterone in an age specific manner and effects are not identical on all phospholipids.

No change in concentration of any of the phosphoinositides with a change in ^{32}P incorporation reveals alteration its turnover. At 10 and 20 days of age PI ⇌ PIP ⇌ PIP_2 interconversion seem to be the major pathway

Table: 3.10.

Effect of Corticosterone administration on the concentration of phospholipids in rat brain.

Age(days)		10	20	40
		mg/g tissue.		
PS	Cort.	1.9 ± 0.18	3.4 ± 0.22**	3.9 ± 0.16
	Control	2.5 ± 0.23	4.5 ± 0.32	3.8 ± 0.38
SM	Cort.	2.1 ± 0.16	2.4 ± 0.18	3.8 ± 0.42
	Control	1.8 ± 0.13	2.2 ± 0.24	3.5 ± 0.27
PC	Cort.	8.6 ± 0.40**	14.8 ± 0.59	17.2 ± 0.91
	Control	6.2 ± 0.30	14.6 ± 0.42	19.8 ± 0.50
PE	Cort.	4.6 ± 0.31***	11.2 ± 0.46*	16.5 ± 1.31
	Control	2.4 ± 0.36	13.7 ± 0.60	19.8 ± 0.91

Number of observations are 6-8. Values are expressed in mean ± S.E. Level of significance by Student's`t'test *P<0.05; **P<0.01;***P<0.001 from control.

Table: 3.11.

Effect of Corticosterone administration on the ^{32}P incorporation *in vivo* into phospholipids in rat brain.

Age(days)		10	20	40
		CPM/g tissue		
PS	Cort.	3.7 ± 0.29	10.8 ± 0.80*	17.5 ± 1.90
	Control	3.3 ± 0.69	8.4 ± 0.60	14.4 ± 1.30
SM	Cort.	0.6 ± 0.12***	3.1 ± 0.16**	16.9 ± 1.20
	Control	1.5 ± 0.12	4.4 ± 0.27	20.7 ± 3.00
PC	Cort.	4.0 ± 0.64	15.5 ± 0.87	57.5 ± 6.80
	Control	3.5 ± 0.91	14.5 ± 1.20	64.7 ± 6.00
PE	Cort.	2.7 ± 0.38	8.3 ± 0.33***	28.6 ± 3.50
	Control	2.0 ± 0.23	5.6 ± 0.37	27.6 ± 2.90

Number of observations are 6-8. Values are expressed in mean ± S.E. as CPM x 10^3.Level of significance by Student's't'test *P<0.05; **P<0.01; ***P<0.001 from control.

getting affected, possibly due to enzyme phosphomonoesterase present in the membranes. At 40 days of age, incorporation in PI was significantly high but the concentration was not changed. This led to the conclusion that there is an increased turnover of phosphoinositides. Since ^{32}P incorporation would mean PL-C action, it appears that the activity of this enzyme is increased in response to corticosterone administration. The PL-C is regulated by phosphorylation-dephosphorylation and in this connection PI specific PL-C was found to be affected by glucocorticoid in basophilic cells (Her *et al.*, 1991).

Significantly low concentration and higher rate of incorporation certainly indicate increased turnover for PS. Since PE is largely present in the myelin (Horrocks and Sharma, 1982) and this being a rapid phase of myelination (Sun and Sun, 1976), at day 20 , decrease PE concentration indicates decrease in myelination. Such effects of myelin deposition affected with corticosterone treatment were observed by others (Preston and McMorris, 1984; Meyer and Fairman, 1985; Gumbinas *et al*, 1973).

The next experiment was carried out to check the steroid specificity. The glucocorticoid synthetic analog-dexamethasone,ACTH - which elevates endogeneous glucocorticoid levels and testosterone- a gonadal hormone were used to compare the effect. The result (Table 3.12) obtained conveys that dexamethasone as well as ACTH had similar effects which were observed with corticosterone administration. Interestingly testosterone also had similar effects on the concentration. Since the levels of PS and PE are significantly lower in all the groups and the final picture emerges as altered PE levels with corticosterone (Table 3.12).

Table: 3.12.

Effect of Corticosterone, Dexamethasone, ACTH and Testosterone on the concentration of phospholipids in 20 day old rat brain.

Phospholipid Fractions	Control	Cort.	Dex.	ACTH	Testo.
		µg/g tissue			
PIP_2	201 ± 9.7	189 ± 5.1	182 ± 12.4	179 ± 20.1	199 ± 27.9
PIP	141 ± 10.1	157 ± 3.2	158 ± 8.5	136 ± 8.5	158 ± 14.7
		mg/g tissue			
PI	1.5 ± 0.18	1.7 ± 0.10	1.3 ± 0.12	1.9 ± 0.16	2.1 ± 0.30
PS	4.5 ± 0.32	3.4 ± 0.22**	2.3 ± 0.18***	2.3 ± 0.21***	1.9 ± 0.16***
SM	2.2 ± 0.24	2.4 ± 0.18	2.1 ± 0.20	2.3 ± 0.21	1.8 ± 0.14
PC	14.6 ± 0.42	14.8 ± 0.59	15.6 ± 0.38	12.9 ± 0.86	15.9 ± 0.50
PE	13.7 ± 0.6	11.2 ± 0.46*	11.5 ± 0.64*	11.2 ± 0.97	10.7 ± 0.4**

Number of observations are 7-9. Values are expressed in mean ± S.E. Levels of significance by Student's 't' test *P<0.05;**P<0.01;***P<0.001 from control.Cort- Corticosterone, Dex-Dexamethasone, Testo-Testosterone, ACTH-Adrenocorticotropin hormone.

With regard to ^{32}P incorporation again PIP_2 and PIP both showed significantly higher incorporation with no apparent change in concentration indicating increased turnover of these phospholipids. PE showed increased incorporation with corticosterone and to some extent in ACTH treated rats, while with dexamethasone and testosterone, the effects were not significant (Table 3.13). It has been reported that acute treatment of ACTH increased the polyphosphoinositides (PIP and PIP_2) incorporation in adrenal cortex (Farese *et al.*,1979). Gispen *et al.* (1985) and had shown that ACTH decrease the phosphorylation of B-50, a brain protein and altered the brain polyphosphoinositide turnover. ACTH can activate the protein kinase which phosphorylate the membrane protein B-50. This phosphoprotein is assumed to act as a regulator for plasma membrane bound phosphatidyl inositol 4- phosphate kinase (PIP Kinase)(Jolles *et al.*, 1980). The results obtained in the present study indicate that these effects are mediated either through ACTH or corticosterone.

PS and PE are the only two phospholipids which seem to be decreased. As mentioned earlier PE is present in higher concentration in white matter. Therefore myelin formation or oligodendrocyte formation is a major target for hormone action. GPDH, a glial enzyme known to be induced by corticosterone is also localized in oligodendroglial cells (LeVielle *et al.*, 1980). This perhaps indicates an effect on non neuronal cells, which are also known to have receptors for corticosterone (Meyer and McEwen, 1982). The earlier studies using ^{14}C glucose incorporation also had shown that the incorporation in PE and PC were significantly lowered in hippocampus and striatum (Bhargave *et al.*, 1991) but, the concentration was not

Table: 3.13.

Effect of Corticosterone,Dexamethasone,ACTH and Testosterone on the ^{32}P incorporation into phospholipids in 20 day old rat brain.

Phospholipid Fractions	Control	Cort.	Dex.	ACTH	Testo.
			CPM/g tissue		
PIP_2	2.8 ± 0.29	5.3 ± 0.46***	7.6 ± 0.8***	6.5 ± 0.46***	4.5 ± 0.4**
PIP	2.5 ± 0.32	3.8 ± 0.27**	4.5 ± 0.29***	3.2 ± 0.14*	2.9 ± 0.20
PI	2.7 ± 0.25	2.4 ± 0.11	2.4 ± 0.25	3.0 ± 0.25	1.6 ± 0.20
PS	8.4 ± 0.67	10.8 ± 0.83*	7.2 ± 0.36	7.0 ± 0.51	7.1 ± 0.60
SM	4.4 ± 0.27	3.1 ± 0.16**	3.4 ± 0.47	3.8 ± 0.44	5.2 ± 0.40
PC	14.5 ± 1.23	15.5 ± 0.87	16.2 ± 0.41	14.9 ± 1.1	14.6 ± 2.20
PE	5.7 ± 0.37	8.3 ± 0.33***	6.5 ± 0.46	7.0 ± 0.50*	5.8 ± 0.20

Number of observations are 7-9. Values are expressed in mean ± S.E. as CPM x 10^3.Levels of significance by Student's't' test *P<0.05;**P<0.01;***P<0.001 from control.

measured at that time.

To summarize, this study on whole brain revealed changes in the phospholipid concentration and ^{32}P incorporation *in vivo*. Younger animals seem to be more sensitive than the older ones- a fact which has been noticed earlier. The administration of dexamethasone, ACTH and even testosterone all seems to have similar effects irrespective of the type of steroids; however testosterone differed with respect to ^{32}P incorporation. ^{32}P incorporation *in vivo* is an index of exchange and /fresh synthesis of phospholipids, especially through the role of phospholipase C. It appears that steroids seem to affect phospholipase C acting specifically on all phospholipids and for polyphosphoinositides there may be an additional enzyme getting affected.

Experiment Ic:

Effect of Adrenalectomy:

Results on adrenalectomized animals are given in Table 3.14 to 3.17. It appears that 15 days after adrenalectomy the concentration of any of the phospholipid fractions were not different from sham operated control except the PIP concentration which seem to be significantly lower than the sham animals. The decrease was not reversed by hormone replacement for 5 days (ADX+H group). Radioactive studies showed a decrease in ^{32}P incorporation in PIP_2, PC and SM in ADX rats compared to sham control and this decrease in incorporation is reversed by corticosterone replacement to ADX rats. The incorporation of PI increased while that of PS decreased in adrenalectomized rats with corticosterone replacement.

The results on the adrenalectomized animals are quite interesting

Table: 3.14.

Effect of Adrenalectomy and Cort.replacement on concentration of phosphoinositides in rat brain after ADX.

	ADX+H	ADX	SHAM
PIP_2 µg/g tissue	211 ± 10.8 (9)	200 ± 9.5 (15)	238 ± 16.2 (19)
PIP µg/g ,,	136 ± 6.2 (12)	155 ± 7.7*** (15)	218 ± 16.3 (14)
PI mg/g ,,	2.4 ± 0.19 (17)	3.3 ± 0.32 (12)	2.8 ± 0.40 (14)

Table: 3.15.

Effect of Adrenalectomy and Cort. replacement on ^{32}P incorporation *in vivo* into phosphoinositides in rat brain after ADX.

	ADX+H	ADX	SHAM
		CPM/g tissue	
PIP_2	4.2 ± 0.60 (10)	3.1 ± 0.19*** (9)	6.2 ± 0.57 (17)
PIP	3.9 ± 0.40 (10)	3.9 ± 0.33 (11)	3.8 ± 0.15 (15)
PI	4.9 ± 0.30@@ (10)	3.8 ± 0.28 (12)	3.5 ± 0.30 (13)

Number of observations are in parenthesis.Values as expressed in mean ± S.E. as CPM $x10^3$.Level of significance by Student's 't'test @@ P<0.01; *** P<0.001 from ADX and SHAM respectively.

Age of ADX-20 days old, Duration-15 days,Replacement -last 5 days.

Table: 3.16.

Effect of Adrenalectomy and Cort. replacement on concentration of phospholipids & cholesterol in rat brain after ADX at day 20.

	ADX+H	ADX	SHAM
	mg/g tissue		
PS	5.4 ± 0.38 (16)	4.5 ± 0.50 (13)	3.5 ± 0.40 (13)
SM	3.8 ± 0.19 (15)	4.0 ± 0.30 (12)	4.5 ± 0.33 (14)
PC	18.4 ± 1.14 (13)	17.8 ± 1.31 (12)	18.1 ± 1.00 (12)
PE	19.9 ± 1.20 (9)	21.8 ± 1.16 (8)	23.7 ± 1.00 (7)
Total Phospholipid	34.9 ± 1.20 (16)	32.7 ± 3.70 (14)	35.6 ± 1.30 (17)
Cholesterol	13.3 ± 0.54 (10)	14.2 ± 0.37 (10)	14.1 ± 0.70 (10)

Table: 3.17.

Effect of Adrenalectomy and Cort. replacement on ^{32}P incorporation *in vivo* into phospholipids in rat brain after ADX at day 20.

	ADX+H	ADX	SHAM
	CPM/g tissue		
PS	3.3 ± 0.23@@@ (13)	4.9 ± 0.35 (14)	5.4 ± 0.55 (17)
SM	3.4 ± 0.30@ (9)	2.6 ± 0.18** (14)	3.8 ± 0.29 (13)
PC	9.1 ± 0.53@@ (9)	7.0 ± 0.43* (8)	8.6 ± 0.52 (11)
PE	4.1 ± 0.33 (12)	4.6 ± 0.43 (14)	4.5 ± 0.48 (15)

Number of observations are in parenthesis. Values as expressed in mean ± S.E. as CPM $x10^3$.

Level of significance by Student's 't'test @,* P<0.05;@@,** P<0.01; @@@ P<0.001 from ADX and SHAM respectively.

because in spite of the increase in gross weight of the whole brain,and possibly an increase in myelin, the concentration of all the phospholipid fractions were similar to sham operated control. If this is true, it would mean that there may be overall increase in the amount without affecting the quality.

With regard to effects of corticosterone depletion and replaced it is evident that after the age of 20 the susceptibility to corticosterone reduces as judged from the concentration of phospholipid fractions. The rate of ^{32}P incorporation was significantly altered in specific phospholipid fractions, mainly polyphosphoinositides. The incorporation was significantly low. This effect is opposite to what was observed with corticosterone administration. On the other hand, SM had decreased incorporation under both conditions and effects on rest of the phospholipids such as PE, PC and PS are variable. Since the study is carried out on the whole brain these effects could be either a myelin or non myelin or both fractions of the phospholipids.

To summarize the studies on whole brain phosphoinositides metabolism ($PI+PIP+PIP_2$) reveal that as far as fractions of phosphoinositides are concerned at all ages as well as with ADX, the concentrations remain unaffected but turnover as judged by ^{32}P incorporation clearly indicate changes in the rates of incorporation. It increased with corticosterone treatment and decreased in depleted conditions. These inverse effects indicate a possibility that corticosterone levels regulate the PI metabolism. The effects are either on $PI \rightleftharpoons PIP \rightleftharpoons PIP_2$ exchange and/or phospholipase C mediated effects. In this connection, PI specific phospholipase C is known

to be inhibited in dexamethasone treated basophilic cells (Her *et al.*, 1991). Duman *et al.* (1986) has reported that basal levels of IPs change with chronic ACTH treatment. It is also likely that phosphomonoesterase as well as PI kinase which are membrane bound are also under the influence of corticosterone. The phospholipid subclasses such as PC and PE increase at day 10 whereas PS and PE decrease at 20 days. Lower concentration of these phospholipid subclasses at younger ages indicate that the enzymes involved in PS-> PE-> PC conversion or cytidyl transferase are under the influence of corticosterone in young ages but not as the age advances. In this connection PC synthesis is enhanced by corticosterone in lungs as it is an important component of the surfactant. On the other hand studies in liver carried out by Kaur *et al.* (1989) indicate an increase in turnover of the same in liver mitochondria with dexamethasone treatment. In the present study the ^{32}P incorporation was higher in PS and PE and lower in PC with ADX. This is consistent with the observations indicating that the pathway of PS-> PE-> PC is regulated by corticosterone.

It was interesting to note that the change in concentration of these phospholipid subclasses were similar not only with dexamethasone or ACTH but also with testosterone- a gonadal steroid, but corticosterone and testosterone differed with respect to rate of ^{32}P incorporation in these lipids, hence may have a different effect. These results gave an impetus that like other tissues, brain phospholipids are also regulated by circulating levels of corticosterone.

Experiment No. II:

Effects of corticosterone administration and adrenalectomy on the concentration of various lipids in different brain regions:

The studies on whole brain clearly indicated that corticosterone levels do play a role in the brain phospholipid concentration and or metabolism. However, these data in no way can be correlated on the functional aspect. Regional, cellular and subcellular fractions are important for the same. Since corticosterone effects would vary with receptor density and affinity it is likely that the effects on phospholipids metabolism could also vary with the same. The regional variability of receptors, affinity changes with development *etc.* has been discussed earlier. The experiment in this part deal with the regional variation in concentration of phospholipids and its turnover. The regions selected namely olfactory bulb, cerebellum, brain stem, hippocampus, striatum and cerebral cortex. The hippocampus, striatum and cerebral cortex have high steroid receptors whereas cerebellum and olfactory bulb have low steroid receptors. Brain stem in addition to good number of receptors it is a lipid rich region.

Experiment II a:

Effect of Corticosterone administration:

As can be seen from the tables 3.18 and 3.19 corticosterone treatment in 10 day old animals had significantly low cholesterol concentration only in olfactory bulb and brain stem whereas galactolipid remain unaltered. The various fractions of phospholipid in different regions showed different picture. Only two regions, olfactory bulb (OB) and brain stem (BS) showed significant change in the concentration of phospholipid while rest of the

Table: 3.18.

Effects of Corticosterone administration cholesterol and galactolipid concentration in 10 day old rat brain regions.

Regions	Groups	Cholesterol	Galactolipid
		mg/g tissue	
OB	Cort.	4.8 ± 0.18**	1.78 ± 0.35
	Control	5.9 ± 0.25	1.03 ± 0.11
CBL	Cort.	6.4 ± 0.37	1.32 ± 0.11
	Control	6.4 ± 0.33	1.27 ± 0.09
BS	Cort.	6.1 ± 0.25**	3.7 ± 0.15
	Control	9.1 ± 0.56	3.5 ± 0.23
HC	Cort.	5.5 ± 0.37	1.4 ± 0.08
	Control	5.9 ± 0.25	1.6 ± 0.15
STR	Cort.	5.5 ± 0.3	1.3 ± 0.12
	Control	5.8 ± 0.2	1.1 ± 0.06
CTX	Cort.	5.5 ± 0.4	1.4 ± 0.08
	Control	6.2 ± 0.3	1.5 ± 0.09

Values are expressed in mean ± S.E. Number of observations are 8-10.

Level of significance by Student's't' test **P<0.01 from control.

For each observation two rats were pooled.

Table: 3.19.

Effects of Corticosterone administration on the individual phospholipid concentration in 10 day old rat brain regions.

Regions	Groups	PI	PS	SM	PC	PE
				mg/g tissue		
OB	Cort.	3.5 ± 0.30	4.2 ± 0.20	4.2 ± 0.21*	12.1 ± 0.47	9.5 ± 0.50*
	Control	3.1 ± 0.30	3.4 ± 0.33	3.3 ± 0.25	10.9 ± 0.62	6.8 ± 0.40
CBL	Cort.	1.9 ± 0.23	2.1 ± 0.22	1.8 ± 0.32	10.1 ± 0.41	6.2 ± 0.34
	Control	1.8 ± 0.20	2.8 ± 0.15	2.5 ± 0.22	9.7 ± 0.33	6.0 ± 0.19
BS	Cort.	2.2 ± 0.25	2.3 ± 0.27	2.7 ± 0.24*	11.5 ± 0.36	6.8 ± 0.35*
	Control	2.1 ± 0.18	2.5 ± 0.20	1.9 ± 0.18	11.4 ± 0.50	8.0 ± 0.33
HC	Cort.	2.3 ± 0.30	2.4 ± 0.15	2.1 ± 0.12	11.2 ± 0.57	4.0 ± 0.25
	Control	2.0 ± 0.12	2.8 ± 0.20	1.7 ± 0.14	11.6 ± 0.71	4.0 ± 0.27
STR	Cort.	2.0 ± 0.18	2.8 ± 0.20	2.3 ± 0.30	12.2 ± 0.63	4.8 ± 0.42
	Control	2.2 ± 0.18	2.6 ± 0.15	2.7 ± 0.20	12.6 ± 0.40	5.6 ± 0.44
CTX	Cort.	1.8 ± 0.19	2.3 ± 0.27	1.6 ± 0.16	9.9 ± 0.34	4.1 ± 0.50
	Control	1.8 ± 0.27	1.8 ± 0.09	1.7 ± 0.14	10.4 ± 0.56	4.4 ± 0.23

Values are expressed in mean ± S.E. Number of observations are 8-10. Cort-Corticosterone.

Level of significance by Student's 't' test *P<0.05 from control. For each observation two rats were pooled.

regions didn't show any effect. SM was significantly increased in both the regions whereas PE was increased in OB but was decreased in BS. Thus at 10 days of age only brain stem and OB had significant changes in lipid concentration.

At 20 days of age (Table 3.20,3.21) cholesterol concentration was altered but effects varied. Cholesterol levels in the brain stem were lower whereas hippocampus, striatum and cortex had significantly higher cholesterol levels. With regard to individual phospholipid fractions, striatum showed maximum effects as PI, SM and PE were significantly elevated whereas in brain stem only SM was higher and PE was lower compared to controls. PS was elevated in hippocampus and PC and SM concentration increased in OB. The two regions namely cerebellum and cortex had no change in any of the fractions of phospholipid.

Experiment II b:

Effect of Adrenalectomy:

Studies were carried out in which rats were adrenalectomized at 40 days of age and decapitated after 15 days of post surgery period. Few of the adrenalectomized rats were given corticosterone replacement therapy for 5 days. The regions were studied as mentioned earlier.

The results are presented in table 3.22 and 3.23. A decrease in sphingomyelin concentration was observed in OB of ADX rats. Also the decrease is brought back to normal after hormone replacement. Cholesterol, another membrane component seems to decrease in hormone replacement group, although ADX 'per se' had no effect. Cerebellum showed no change in the individual phospholipids or cholesterol concentration (Table 3.22). In

Table: 3.20.

Effects of Corticosterone administration on cholesterol and galactolipid concentration in 20 day old rat brain regions.

Regions	Groups	Cholesterol	Galactolipid
		mg/g tissue	
OB	Cort.	13.8 ± 0.8	9.9 ± 0.5
	Control	13.5 ± 0.7	9.2 ± 0.4
CBL	Cort.	11.9 ± 0.5	8.4 ± 0.3
	Control	11.9 ± 0.5	8.2 ± 0.3
BS	Cort.	17.1 ± 0.7**	13.2 ± 0.7
	Control	21.3 ± 0.5	12.8 ± 0.8
HC	Cort.	11.5 ± 0.3**	5.6 ± 0.2
	Control	7.4 ± 0.9	5.3 ± 0.3
STR	Cort.	9.1 ± 0.2**	5.9 ± 0.1
	Control	7.9 ± 0.2	5.7 ± 0.1
CTX	Cort.	15.2 ± 0.8**	5.7 ± 0.3
	Control	12.2 ± 0.6	5.9 ± 0.7

Values are expressed in mean ± S.E in mg/g tissue. Number of observations are 8-10.

Level of significance by Student's 't' test **P<0.01 from control. Cort- Corticosterone.

Table: 3.21.

Effects of Corticosterone administration on the individual phospholipid concentration in 20 day old rat brain regions.

Regions	Groups	PI	PS	SM	PC	PE
				mg/g tissue		
OB	Cort.	3.9 ± 0.30	3.8 ± 0.44	4.8 ± 0.30**	16.1 ± 0.30*	6.8 ± 0.54
	Control	3.2 ± 0.20	3.8 ± 0.50	3.4 ± 0.32	14.0 ± 0.90	5.2 ± 0.90
CBL	Cort.	2.3 ± 0.15	2.3 ± 0.20	3.2 ± 0.20	15.4 ± 0.30	10.6 ± 0.80
	Control	2.2 ± 0.22	2.7 ± 0.20	2.8 ± 0.12	14.5 ± 0.30	10.1 ± 0.80
BS	Cort.	2.4 ± 0.20	3.9 ± 0.30	5.1 ± 0.30**	16.9 ± 0.60	12.6 ± 0.32**
	Control	2.3 ± 0.20	4.7 ± 0.64	3.8 ± 0.31	17.3 ± 0.60	14.3 ± 0.40
HC	Cort.	2.4 ± 0.23	4.4 ± 0.43*	3.9 ± 0.90	14.8 ± 0.70	6.5 ± 0.60
	Control	2.5 ± 0.18	3.1 ± 0.44	3.8 ± 0.32	13.7 ± 0.13	6.5 ± 1.0
STR	Cort.	5.2 ± 0.40*	4.7 ± 0.40	6.2 ± 0.30**	15.0 ± 0.70	7.3 ± 0.30**
	Control	4.3 ± 0.26	4.5 ± 0.30	5.2 ± 0.22	15.3 ± 1.2	5.7 ± 0.45
CTX	Cort.	3.5 ± 0.22	3.4 ± 0.23	3.7 ± 0.21	12.7 ± 0.60	6.1 ± 0.30
	Control	3.2 ± 0.20	3.1 ± 0.20	3.1 ± 0.20	10.7 ± 1.0	4.9 ± 0.50

Values are expressed in mean ± S.E. Number of observations are 8-10.

Level of significance by Student's't' test * $P<0.05$;** $P<0.01$ from control.

Table: 3.22.

Effects of Adrenalectomy and Cort. replacement on individual phospholipid concentration in rat brain regions.

Regions	Groups	PI	PS	SM	PC	PE
			mg/g tissue			
	ADX+H	2.5 ± 0.39	3.3 ± 0.62	4.4 ± 0.54	19.5 ± 1.93	11.8 ± 1.40
OB	ADX	2.4 ± 0.53	3.8 ± 0.55	3.2 ± 0.41*	14.2 ± 2.10	10.1 ± 0.13
	SHAM	2.6 ± 0.26	3.3 ± 0.33	4.4 ± 0.21	14.7 ± 1.40	9.7 ± 0.84
	ADX+H	1.9 ± 0.51	2.8 ± 0.31	2.8 ± 0.26	17.4 ± 1.55	10.9 ± 0.80
CBL	ADX	1.5 ± 0.15	3.1 ± 0.27	2.9 ± 0.24	15.7 ± 0.90	11.5 ± 0.55
	SHAM	1.1 ± 0.09	2.4 ± 0.30	3.4 ± 0.10	16.1 ± 0.65	12.0 ± 0.59
	ADX+H	2.0 ± 0.36	4.7 ± 0.50	5.2 ± 0.24	18.5 ± 1.80	22.1 ± 1.54
BS	ADX	1.7 ± 0.37	4.4 ± 0.40	4.1 ± 0.40	18.2 ± 1.04*	20.0 ± 1.13
	SHAM	1.4 ± 0.24	3.7 ± 0.60	4.6 ± 0.44	14.5 ± 0.98	19.0 ± 0.83
	ADX+H	2.5 ± 0.42	3.7 ± 0.60	5.1 ± 0.19	19.0 ± 1.11	13.9 ± 1.23
HC	ADX	2.7 ± 0.39	4.4 ± 0.33	4.8 ± 0.27	16.3 ± 1.62	12.0 ± 1.14
	SHAM	2.6 ± 0.24	3.6 ± 0.20	4.6 ± 0.82	15.9 ± 1.20	9.6 ± 1.02

table continued.....

	ADX+H	3.8 ± 0.60@@	3.7 ± 0.60	5.3 ± 0.81	18.5 ± 1.70	13.1 ± 1.10
STR	ADX	1.5 ± 0.29**	4.3 ± 0.81	4.7 ± 0.14	16.9 ± 1.52	13.2 ± 1.10
	SHAM	2.9 ± 0.31	4.5 ± 0.44	4.3 ± 0.70	16.8 ± 1.50	12.8 ± 0.32
	ADX+H	3.3 ± 0.40	3.7 ± 0.40	3.8 ± 0.31	15.3 ± 1.50	13.5 ± 0.90
CTX	ADX	2.7 ± 0.37	3.4 ± 0.34	3.2 ± 0.33	15.1 ± 0.60	11.9 ± 0.70
	SHAM	2.6 ± 0.30	3.2 ± 0.34	4.7 ± 1.00	14.8 ± 0.70	11.6 ± 0.73

Values are expressed in mean ± S.E. in mg/g tissue. Number of observations are 5.

Level of significance by Student's't' test @@,** P<0.01; * P<0.05 from ADX and SHAM respectively.

Adrenalectomized age - 40day old, Duration - 15 days; Replacement - last 5 days

Table 3.23.

Effects of Adrenalectomy and Cort. replacement on cholesterol concentration in rat brain regions.

Regions	ADX+H	ADX	SHAM
	mg/g tissue		
OB	10.6 ± 1.01@@	14.5 ± 0.41	13.7 ± 1.33
CBL	11.6 ± 0.72	12.8 ± 1.22	11.5 ± 0.76
BS	20.5 ± 0.23@@	26.5 ± 1.50*	21.7 ± 0.87
HC	17.2 ± 1.41	12.3 ± 2.20	13.2 ± 1.48
STR	15.5 ± 2.60	15.6 ± 2.30	13.9 ± 1.70
CTX	11.6 ± 0.51	12.5 ± 0.50	12.8 ± 1.40

Values are expressed in mean ± S.E. in mg/g tissue. Number of observations are 5.

Level of significance by Student's't' test @@,** P<0.01; * P<0.05 from ADX and SHAM respectively.

Adrenalectomized age- 40 days old; Duration-15 days; Replacement- last 5 days.

brain stem, the results disclose that adrenalectomy caused an increase in cholesterol and PC concentration. The other phospholipids were not affected (Table 3.22 and 3.23). This increase in cholesterol was reversed back to normal by replacement. Hippocampus showed no effect in any of the phospholipids either in ADX or corticosterone replaced group. An increasing trend was observed in PC, PE and PS concentration in ADX rats but they were not significant. A decrease in the PI concentration was observed in striatum of ADX rats and reversed by corticosterone replacement. None of the other phospholipids or cholesterol showed any changes in this region (Table 3.23). In cerebral cortex, no change in either individual phospholipid or cholesterol was noticed.

The results of the steroid treated, adrenalectomized and hormone replacement experiments are discussed taking following points into consideration- age, region, receptors and development, as all these factors are known to influence the parameters studied.

Taking all the results into consideration, olfactory bulb is sensitive to corticosterone treatment at 10 days of age. Cholesterol, PE and SM, were the three which significantly increased. At postnatal age 20, SM and PC concentration increased whereas PE and cholesterol were not affected. In contrast to younger age group, in adrenalectomized animals, there was significant decrease in SM concentration which seems to be reversed by hormone replacement. Although adrenalectomy did not alter cholesterol levels, the hormone replaced group showed significantly higher cholesterol levels. In this connection the ontogenic pattern of cell accretion in this region has been reported to be significantly extended as microneurons are developed

after 15 days of age (Hinds, 1968). Thus neurogenisis and gliogenesis are continued in this region beyond two weeks of age. Corticosterone is known to inhibit neurogenisis and favours gliogenesis (Bohn, 1980).

A point which emerges from this study is that irrespective of age, the phospholipid which seems to be most sensitive is sphingomyelin at all ages and depletion or excess showed opposite effects. Cholesterol also seems to be influenced, because at 10 days of age as well as hormone replacement (excess) seem to show a similar effect indicating that excess steroid but not permissive level has any role in cholesterol levels. As mentioned earlier and emphasized again that the hormone replacement group is more like a hormone excess. Since the amount given and duration of treatment both are comparable to high dose of exogenous chronic treatment carried out by several other groups (Melby *et al.*, 1981; Patel *et al.*, 1983a; Dickinson *et al.*, 1985).

In the brain stem, corticosterone administration at both 10 and 20 postnatal ages, decrease in cholesterol and PE levels and increase in SM levels were found. With adrenalectomy, cholesterol levels significantly increased whereas with hormone replacement, the same parameter were significantly lowered. The only phospholipid which showed a significant change with adrenalectomy was PC. Thus in brain stem cholesterol seems to be the only parameter altered to all ages. Excess seem to decrease while permissive level tend to cause higher cholesterol concentration. Sphingomyelin and PE levels were influenced in younger age groups but not in adult ages. PC seemed to be increased by permissive levels i.e., after adrenalectomy but ADX+H was not brought back to normal.

It is known that brain stem matures earlier as judged by the myelination and DNA content (Howard, 1973). Decrease in cholesterol as well as PE concentration at both 10 and 20 days of age with corticosterone administration indicates a possible reduction in myelination. In this connection, HMGCoA reductase, the regulatory enzyme in the cholesterol biosynthesis, is known to inhibited by corticosterone treatment in HeLa cells and in other tissues (Melnykovych *et al.*, 1979; Johnson *et al.*, 1980). A reduction in myelinogenesis with glucocorticoid or treatment has been reported earlier (Casper *et al.*, 1967; Gumbinas *et al.*, 1973). SM concentration was elevated at both 10 and 20 days of age, whereas ADX didn't have any effect. This could be an age specific effects on the enzymes involved in SM synthesis namely PC- Ceramide choline phosphotransferase,an anabolic enzyme for sphingomyelin, which is known to be under the influence of glucocorticoid. Nelson and Murray (1982) had reported that dexamethasone treatment cause an increase in synthesis of sphingomyelin in 3T3-L1 cell lines, by enhancing the activity of PC- Ceramide choline phosphotransferase in 3T3-L1 cell lines by 83% and inhibited by cyclohexamide, . With ADX only PC level was significantly increased indicating permissive role on regulating PC levels in BS.

The next region which seemed to be responsive to manipulation of corticosterone is striatum, which although was not affected at 10 day postnatally had significantly higher levels of cholesterol at day 20. PI, SM and PE at day 20 and only PI at older ages, as ADX had significantly low levels while was higher in hormone replaced group. In striatum, glucocorticoid binding affinity starts to increase from 2nd week postnatally and

remain constant till 35 days of age. During this period exogenous glucocorticoid treatment induces many enzymes of dopaminergic systems in striatum (Markey *et al.*, 1982; Towle *et al.*, 1982). Highly immunoreactive type II receptor cells are present in the striatum during the end of postnatal period at around 20 day (Ahima *et al.*, 1992). During the development similar immunoreactive interneurons are observed on postnatal day 25 and 30 transiently (Ahima and Harlan, 1990). It is very clear from this study that PI metabolism in striatum is under the direct regulation of corticosterone, since elevated levels increased PI concentration at all ages and levels were lower with ADX. This would indicate that PI specific phospholipase C is under the influence of glucocorticoid as reported by Her *et al.*(1991).

The two most important regions, namely hippocampus and cortex are known to be rich in receptors and are the ones which showed least response to manipulation of corticosterone levels. The only age at which some effects were evident was at postnatal age 20, where cholesterol levels were significantly elevated with corticosterone treatment and there was significant increase in PS levels only in the hippocampus. Thus except at postnatal day 20, hippocampus is not responsive to either excess or permissive levels. This region is known for highest number of receptors type I as well as type II. The most plausible explanation could be that the transcription of the genes of the enzymes involved in lipid metabolism are perhaps not regulated by corticosterone or the transcription regulatory factor may act as an additional factors by influencing the gene transcription (Godowski and Picard, 1989). It is well established that corticosterone receptors in hippocampus are very sensitive to circulating levels of corticosterone and

clearly show up and down regulation with altered plasma corticosterone levels (McEwen *et al.*, 1986a), it is likely that through adjusting its receptor number it maintains the phospholipid levels. The most interesting aspect in this region is that somehow this strict regulation seems to be inoperative at around 20 days of age. At this age there is a significant increase in cholesterol and PS levels indicating that enzymes associated with these two lipid fractions are rendered responsive to corticosterone.

There is maximum neuronal population and comparatively low receptor number in cerebral cortex than hippocampus (McEwen *et al.*, 1986a). With regards to steroid receptor it was reported that the efficiency of receptors is evident only after 2nd week even though the receptors appear 3rd day postnatally (Ahima *et al.*, 1991). This explains why no changes were observed in 10 day old rats after corticosterone treatment. At day 20, the only parameter which showed an increase is cholesterol levels. Neither ADX nor ADX+H showed any effect on cholesterol. The changes in phospholipid concetration perhaps due to corticosterone induces the glial specific enzyme GPDH towards phospholipid biosynthesis in cortex of 20 day old rats. DeVellis and Inglish (1968) have shown that cortisol increases the GPDH activity of rat cerebrum. So one can think that cortex tends to protect the phospholipid levels from glucocorticoid effect. Considering the role of cortex, it is not surprising that this hormone has least influence on the phospholipids. It is one of the most extensively studied and responsive region with respect to glucocorticoid effect on NE sensitive cAMP effect. In this connection adult cortical glial cells become non responsive to glucocorticoids as the GPDH or GS can not be induced by corticosterone (Meyer,1986).

The effects cannot be explained solely on the basis of development as the cerebellum didn't show any effect at any age. No change in the cerebellum at day 10 may be due to its late cell proliferation peak. Altman (1970) described that development of cerebellum with respect to cell number responds to the recruitment of stem cells in the germinal sites of the cerebellum two weeks after the birth. This could be due to the complete lack of type II receptor at postnatal day 10. Ahima *et al.* (1992) had reported that type II receptors, specific for glucocorticoids, appear in cerebellum only at the postnatal day 15 and increased to adult level on day 20 in the Purkinjee cells in cerebellum. However even at 20 day or thereafter young adult (ADX+H) also did not show any effect. This perhaps indicates that the transcription regulating factors involved in phospholipid and cholesterol regulating enzymes are either low or are not responsive to low corticosterone levels since cerebellum is known to have low receptor number (Ahima and Harlan, 1990). Cerebellum is known to respond to the same dose of treatment by inducing glutamine synthetase as shown by Patel *et al.* (1983a) and Bhargava (1988).

From this study it appears that one of the most affected parameters is cholesterol in many regions in 10 day or 20 day old with corticosterone treatment or adrenalectomy. The brain stem needs a special mention as cholesterol levels decreased with corticosterone administration; the reverse was observed with ADX. As reported by Howard and Benjamins (1975) the decrease in cholesterol after corticosterone administration resulted in a decrease in myelin content, cholesterol being a marker for myelin. Brain stem has higher percentage of myelin (Norton and Poduloso, 1976). The de-

crease in cholesterol content can be attributed to decrease in myelin and was suggested that corticosterone directly inhibits glial cell division (Beaumont, 1987). Both astrocytes and oligodentroglial cells maintain a relationship to certain dendrite or axonal surface areas. When these areas are below normal proportion, multiplication of glia would be correspondingly limited and this in turn affects the lipid content (Howard and Benjamins, 1975). The decrease in cholesterol concentration may be due to the inhibition of HMGCoA reductase, the regulatory enzyme and /or HMGCoA synthase, another enzyme for cholesterol biosynthesis (Ramachandran *et al.*, 1978). Adrenalectomy showed an increase in cholesterol in brain stem which was similar to other studies. The increase in cholesterol concentration suggests that the inhibition of HMGCoA synthase enzyme by glucocorticoid is relieved after adrenalectomy (Ramachandran *et al.*,1978). Thus cholesterol synthesis in brain stem seems to be regulated in a fashion similar to what is reported in other tissues/cell cultures (Mitropoulos and Balasubramaniam, 1976; Melby *et al.*, 1981; Murray *et al.*, 1982).

In contrast, the cholesterol metabolism, rather synthesis, is regulated in a very complex fashion in other regions studied. Elevated levels of corticosterone only influenced in a age dependent fashion in selective regions,i.e., at 20 days of age there was a significant increase in the cholesterol concentration in HC, STR and CTX. This is opposite to known effects of glucocorticoid in other tissues as well as in BS which was observed in the present study. Lin and Snodgross (1982) have shown both *in vivo* and also in cultured liver cells, an increase in cholesterol level associated with an increased HMGCoA reductase activity depending on the

circadian cycle. So it could be possible that corticosterone itself or other factors are involved in the regulation of HMGCoA reductase. It would be likely that the vital role which cholesterol plays is to maintain the membrane fluidity (Kandutsch and Chen, 1977). The fluidity of the membrane is influenced by several factors including cholesterol to phospholipid ratio, phospholipid concentration and fatty acid composition of membrane lipids. Dexamethasone lowers the membrane fluidity by inhibiting the cholesterol synthesis (Johnson *et al.*, 1980). In addition to these enzymes mentioned above glucocorticoids inhibit aryl sulfatase A enzyme in glial cell culture (Stephens and Pieringer, 1984) while the neutral sphingomyelinase increased in fibroblast cell lines (Nelson, 1990). Glucocorticoid also modulates protein kinase C which might catalyze phospholipase A_2 in basophilic cells (Zor *et al.*, 1990). Phosphatidylinositol specific phospholipase C is regulated by glucocorticoid in basophilic cell lines (Her *et al.*, 1991). Thus like cholesterol other lipids such as SM, PI contents are also modulated by corticosterone.

Thus the regional studies on various fractions of lipids are quite contrary to the expectations. Regions having higher number of glucocorticoid are rigorously regulated than the regions such as olfactory bulb or brain stem, where age (developmental stage) seem to play a greater role than the receptor number. Region like cerebellum, neither the age nor the receptor number has any role to play in influencing phospholipids levels. This is not to say that cerebellum is not affected by glucocorticoid levels. In fact studies carried out in our department and elsewhere have shown that elevated levels of corticosterone can induce GS activity even

in adult stage (Patel *et al.*, 1983a; Bhargava, 1988) whereas GPDH is influenced only at younger age in cerebellum (Breen and DeVellis, 1974). So one tends to think that the regulation of phospholipids levels is different in various regions.

Experiment No. IIIa &b:

In Vitro ^{32}P incorporation into phospholipids of different brain region slices in corticosterone administered, bilateral adrenalectomized and in hormone replaced animals:

The phospholipids can modulate function of the membranes by two ways, one either by changing its concentration or by altering its turn over. Role of phospholipids in generating second messengers such as 1,2 DG and IP_3, eicosanoids, prostaglandins *etc.* are all well known examples of phospholipid turnover associated products.

The phospholipids have two active components, the fatty acid at the C-2 position and the base group. The turnover of these groups are under the control of activity of phospholipase A_2 and phospholipase C respectively. The role of glucocorticoid on phospholipase A_2 activity has been well documented in various cell types and in glial cell culture. The phospholipase A_2 activity is controlled by a protein (lipocortin) which inhibits the activity of phospholipase A_2. Glucocorticoid induces the synthesis of this protein (Hirata *et al.*, 1980) and thus inhibits the phospholipase A_2 mediated generation of second messengers. With regard to phospholipase C regulation by glucocorticoid, studies on basophilic cells showed that glucocorticoid inhibits the antigen induced PI breakdown and IP accumulation, however basal levels of IPs production was not changed (Her *et al.*,

1991). In contrast, Duman and his associates demonstrated that ACTH treatment for two weeks increased significantly the basal levels of IP production without any effect on NE stimulation in cortical slices. In addition to inositol phospholipids recently other phospholipids such as PC and SM also seem to have phospholipase C mediated function (Koval and Pagano, 1991; Qian and Drewes 1991). It was therefore thought to check whether the basal turnover of phospholipid is in any way affected by altering glucocorticoid levels.

As early as 1955 Hokkin, and his associate demonstrated that when pancreatic slices or cerebral cortical slices were stimulated with acetyl choline, the ^{32}P incorporation into various fractions of phospholipid registered a significant change. The incorporation was considered as reflection of turnover rate whereas ^{14}C acetate as a biosynthetic (Hokkin and Hokkin, 1955). Thus the ^{32}P incorporation *in vitro* on tissue slices have been used to measure phospholipid turnover. As reported by Hokkin and Hokkin (1955) the percentage incorporation in the present study also vary with lipid fractions, highest incorporation being in PI and PA, intermediate in PC, PS and PE and least in SM.

One of the advantages of *in vitro* study is that it overcomes the problem of blood brain barrier and can access more exactly the mode of action on the membranal lipids.

In the present study only three regions were taken for investigation such as cerebral cortex, brain stem and hippocampus. The details are discussed in the materials and methods.

The result obtained (Table 3.24 & Fig.3.1a,b,c) showed that three

Table: 3.24.

Effects of Corticosterone administration on ^{32}P incorporation into phospholipids at 20 day old rat brain region slices.

Regions	Groups	PI	PS	SM	PC	PE	PA
				CPM/mg protein			
CORTEX	Cort.	148 ± 55.6**	101 ± 11.3	12 ± 2.6*	181 ± 19.4	46.6 ± 5.2	107 ± 18
	Control	1153 ± 248	96 ± 15.5	24 ± 4.1	135 ± 26	54.2 ± 4.0	72.4 ± 11.6
BRAIN STEM	Cort.	323 ± 67*	170 ± 21	25 ± 4.0***	512 ± 40	147 ± 17	190 ± 44
	Control	944 ± 200	161 ± 24.5	113 ± 9.5	486 ± 51	190 ± 18	147 ± 48
HIPPOCAMPUS	Cort.	325 ± 43	133 ± 16.5	37 ± 2.5	359 ± 14.2	112 ± 5	87.6 ± 12.8
	Control	354 ± 63	166 ± 18.3	59.9 ± 6.9	344 ± 19	182 ± 37	169 ± 26.5

Values are expressed in mean ± S.E. in cpm/ mg protein. Number of observations are 6-7.

Levels of significance by Student's 't' test *P<0.05; **P<0.01; ***P<0.001 from control.

CHRONIC CORT. ON 32-P INCORPORATION INTO PHOSPHOLIPIDS IN 20 DAY OLD RAT

Fig 3.1a CORTICAL SLICES.

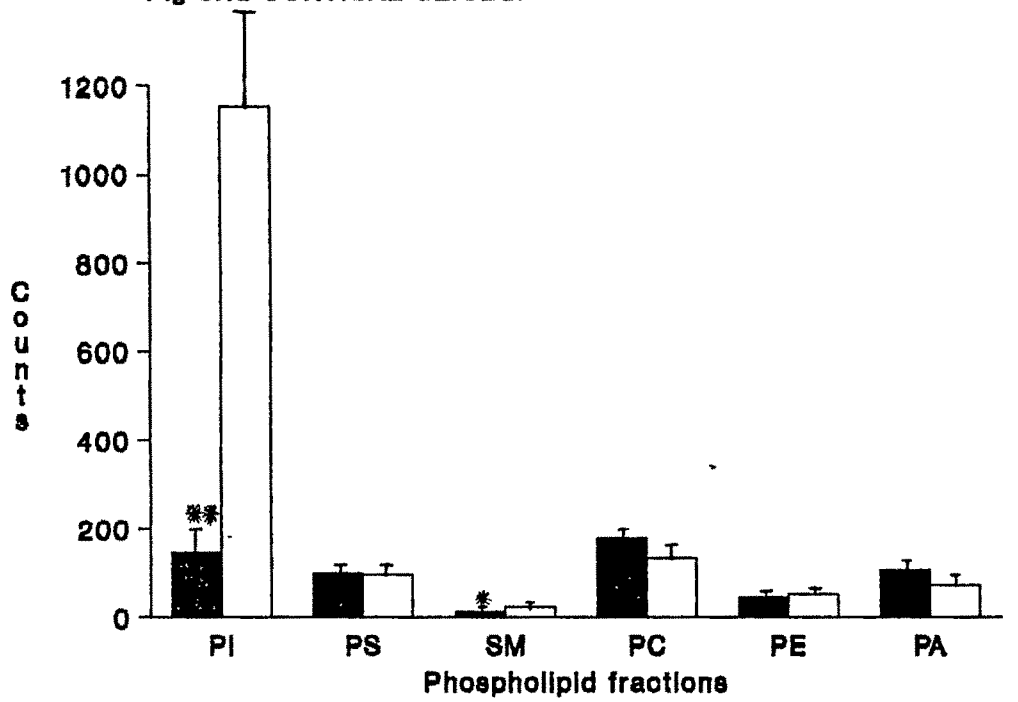


Fig 3.1b BRAIN STEM SLICES.

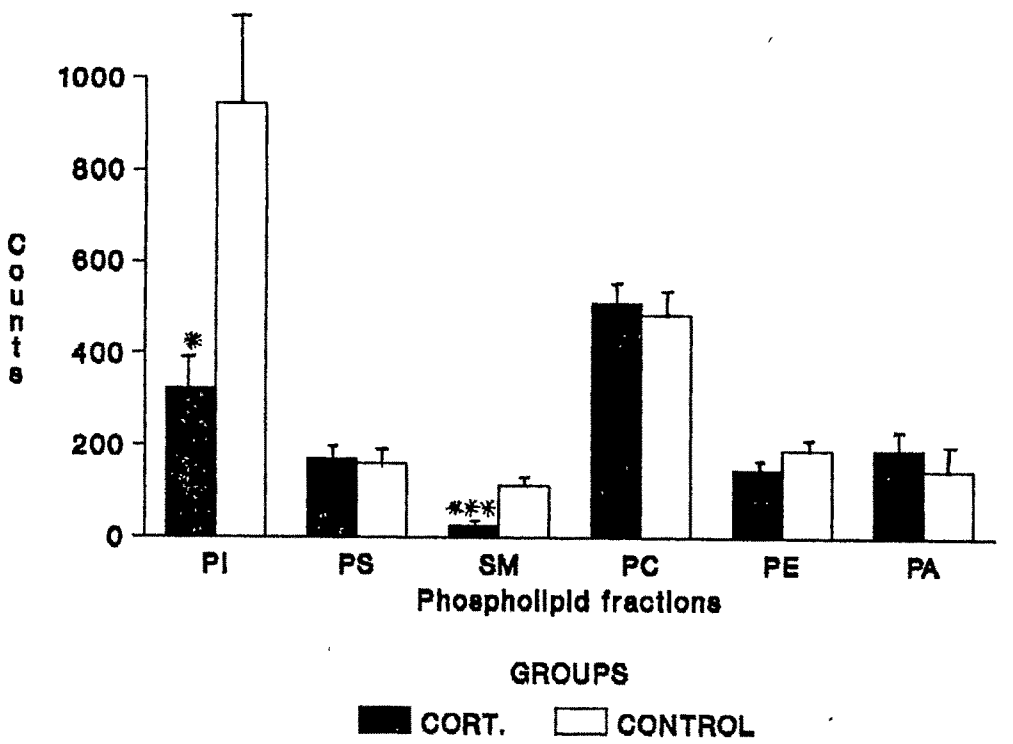
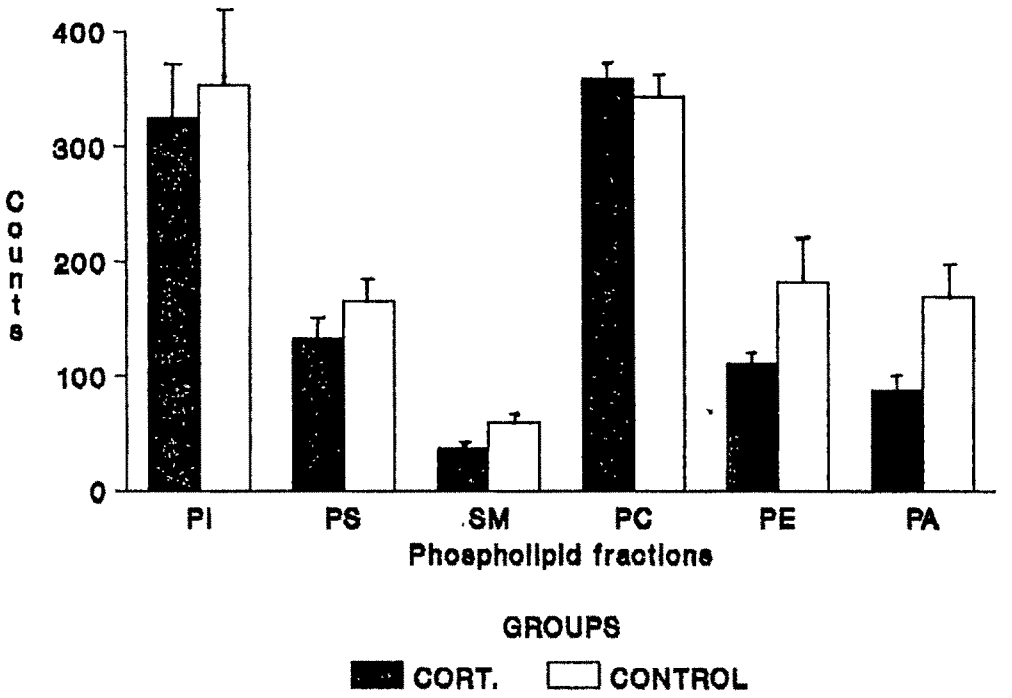


Fig 3.1c HIPPOCAMPAL SLICES.



Values are expressed in mean ± S.E. in cpm/ mg protein. Number of observations are 6-7. Cort- Corticosterone Levels of significance by Student's 't' test *P<0.05; **P<0.01 from control. PI-Phosphatidyl inositol, PS-Phosphatidyl serine, SM-Sphingomyelin, PC-Phosphatidyl choline,PE-Phosphatidyl ethanolamine, PA-Phosphatidic acid.

day corticosterone treatment decrease the *in vitro* ^{32}P incorporation in PI and SM in cerebral cortical slices as well as in brain stem slices but not in hippocampus. The study on polyphosphoinositides showed again an increase incorporation in cerebral cortical slices (Table 3.25). On the other hand results obtained on different duration of ADX are quite variable. 5 days after adrenalectomy the PI initially had a low ^{32}P incorporation which increased on day 15 and declined again with longer duration. Thus incorporation in PI showed two trends. With longer duration PS and SM also showed lower incorporation, however with steroid replaced group this effect was not reversed. The metapirone treatment for 5 days resembles the effect of adrenalectomy observed after 15 days post surgery. In addition to PI, SM increases while PC showed lower incorporation. All the results are presented in tables 3.24- 3.29 and fig. 3.1-3.5)

Thus one can see that the ^{32}P incorporation on tissue slices were variable. The results on ^{32}P incorporation in control slices are expressed in CPM/mg protein to avoid any difference in the amount taken similarly. The pattern in control were also quite variable within the groups in specific experiments remained constant. Hence one can compare the relative incorporation rather than absolute radioactivity.

It was seen that the concentration of any of the phospholipids were not affected in cortex under these conditions. Thus the decrease in ^{32}P incorporation in PI is not due to altered concentration but due to the turnover of PI being low with corticosterone treatment and higher with permissive level. With longer duration of ADX, both SM and PS turnover rate seem to be decreased.

Table: 3.25.

Effect of Corticosterone administration on ^{32}P incorporation into polyphosphoinositides in cortical slices.

Age (days)		10	20
		CPM x 10^3/tissue	
PIP_2	Cort.	5.79 ± 0.44	2.92 ± 0.16**
	Control	7.79 ± 0.85	2.36 ± 0.10
PIP	Cort.	13.39 ± 1.73	5.91 ± 0.39*
	Control	10.92 ± 0.80	4.90 ± 0.20

Values are expressed in mean ± S.E. in cpm x 10^3/g tissue.

Number of observations are 7-10.

Levels of significance by Student's 't' test *P<0.05 ; **P<0.01 from control.

Table: 3.26.

Effect of Adrenalectomy and Cort. replacement on ^{32}P incorporation into phospholipids in rat brain region slices.

Regions	Group	PI	PS	SM	PC	PE	PA
				CPM/mg protein			
CORTEX	ADX+H	62.7 ± 10.3	78.4 ± 8.2	52.2 ± 8.4	97.4 ± 12.7@	65.1 ± 7.5	86.6 ± 16.2
	ADX	52.4 ± 5.0*	49.8 ± 7.8	50.4 ± 8.3	166 ± 21.3	46.4 ± 6.5	69.7 ± 8.9**
	SHAM	108 ± 21.7	110 ± 16.5	71.0 ± 7.7	137 ± 19.7	102.5 ± 23.7	129 ± 9.3
BRAIN STEM	ADX+H	152 ± 17.9	113 ± 19.5	100 ± 28	225 ± 57	138.0 ± 42@	125 ± 19@
	ADX	314 ± 34	157 ± 31	126 ± 15	224 ± 18	307 ± 59*	366 ± 55*
	SHAM	246 ± 38	160 ± 25	116 ± 14	239 ± 52	135 ± 16	166 ± 42

Values are expressed in mean ± S.E in cpm/mg protein. Number of observations are 6.

Level of significance by Student's't' test @,* P<0.05, @@,**P< 0.01, from ADX and SHAM respectively.

Adrenalectomized age-20 days old;Duration -5 days; Replacement- 4 days.

ADRENALECTOMY & CORT.REPLACEMENT- 5 DAYS ON 32-P INCORPORATION INTO PHOSPHOLIPIDS

Fig.3.2a CORTICAL SLICES.

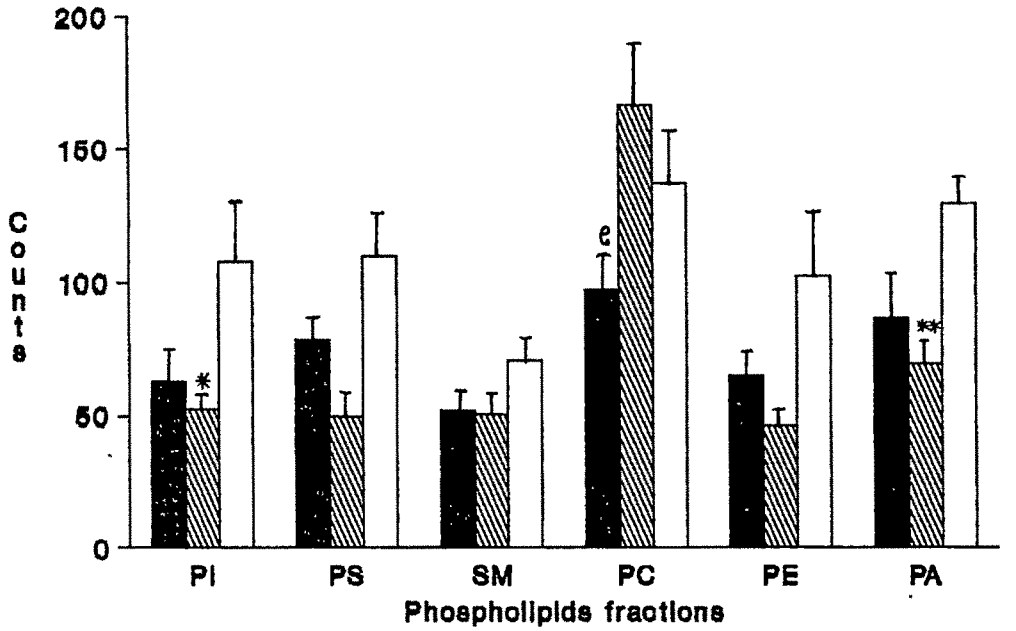
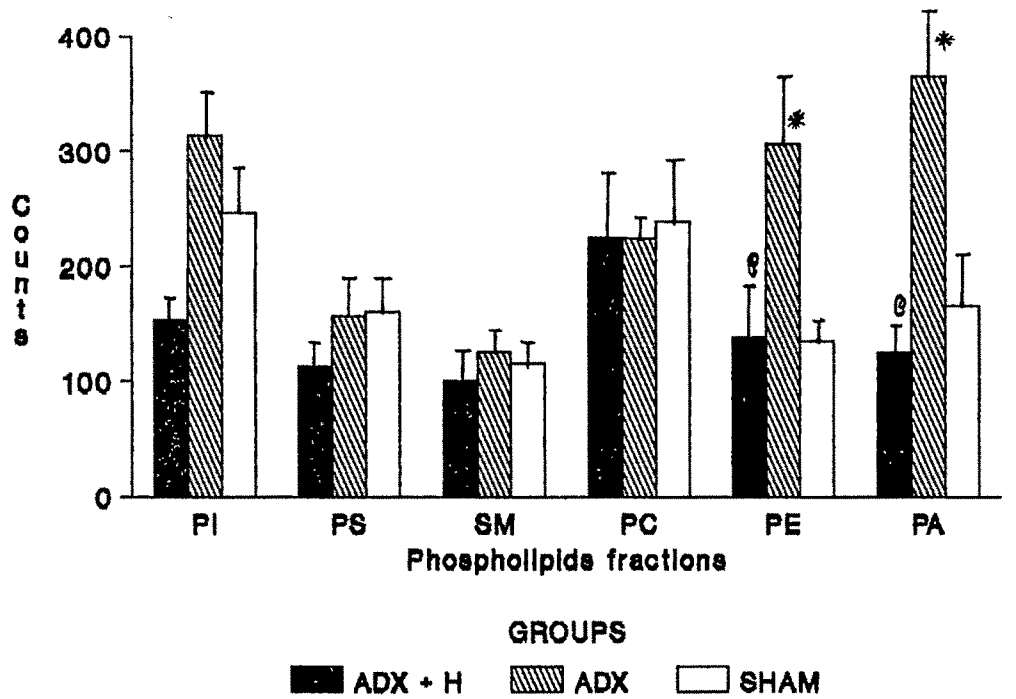


Fig.3.2b BRAIN STEM SLICES



Values are expressed in mean ± S.E in cpm/mg protein. Number of observations are 6. Level of significance by Students't' test @,* P<0.05; @@,**P< 0.01 from ADX and SHAM respectively. PI-Phosphatidyl inositol, PS-Phosphatidyl serine, SM-Sphingomyelin, PC-Phosphatidyl choline,PE-Phosphatidyl ethanolamine.PA-Phosphatidic acid. ADX+H- Adrenalectomy and corticosterone replaced, ADX- Adrenalectomy, SHAM- Operated control.

Table: 3.27.

Effect of Adrenalectomy on ^{32}P incorporation into phospholipids in rat brain region slices.

Regions	Group	PI	PS	SM	PC	PE	PA
		CPM x 10^3/g tissue					
CORTEX	ADX	31.8 ± 5.7*	16.7 ± 2.78	3.68 ± 3.9	32.8 ± 3.9	5.8 ± 0.23	30.2 ± 5.23
	SHAM	13.6 ± 0.9	10.9 ± 1.90	6.50 ± 1.3	26.9 ± 3.2	5.2 ± 0.38	28.6 ± 4.30
BRAIN STEM	ADX	36.6 ± 8.9	16.5 ± 3.8	10.20 ± 2.7	28.4 ± 5.0**	5.3 ± 0.60**	25.0 ± 2.30
	SHAM	22.4 ± 7.3	13.9 ± 3.2	6.90 ± 1.2	58.4 ± 5.4	12.2 ± 0.07	33.6 ± 2.90
HIPPOCAMPUS	ADX	15.2 ± 1.8	11.8 ± 0.9*	3.50 ± 0.34	42.8 ± 7.7	7.5 ± 1.15	22.4 ± 4.70
	SHAM	19.5 ± 3.0	7.4 ± 0.9	2.60 ± 0.34	33.9 ± 8.3	7.7 ± 1.70	24.1 ± 7.40

Values are expressed in mean ± S.E. in cpm x 10^3/ gm tissue. Number of observations are 5-7.

Level of significance by Student's't' test * P<0.05; **P< 0.01 from SHAM.

Adrenalectomized age -20 days old; Duration - 15 days.

ADRENALECTOMY (15 DAYS)ON 32-P INCORPORATION INTO PHOSPHOLIPIDS

Fig.3.3a CORTICAL SLICES.

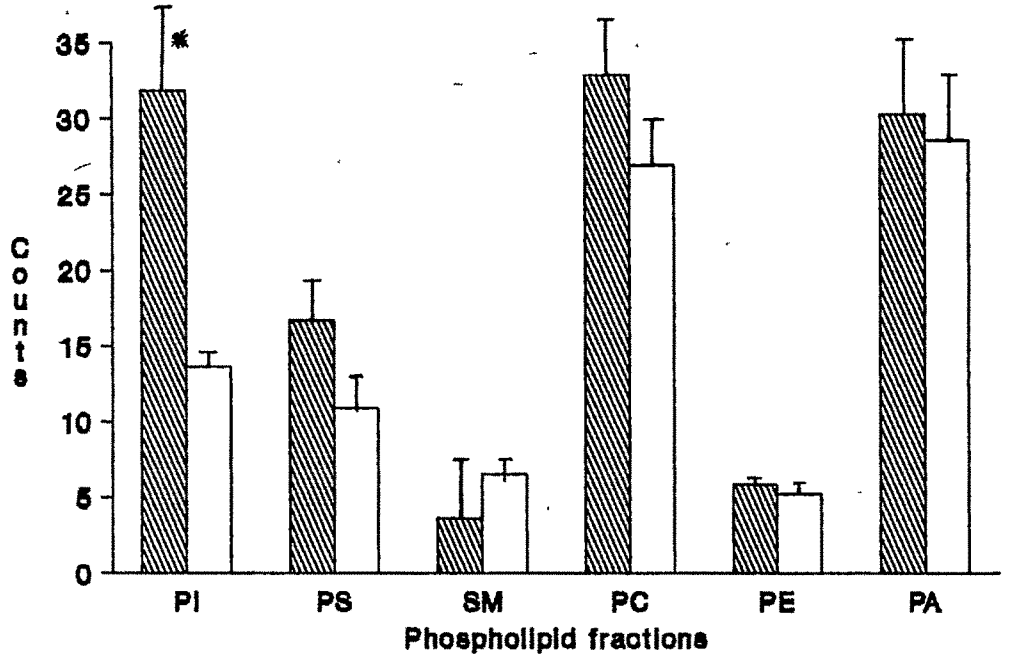


Fig.3.3b BRAIN STEM SLICES.

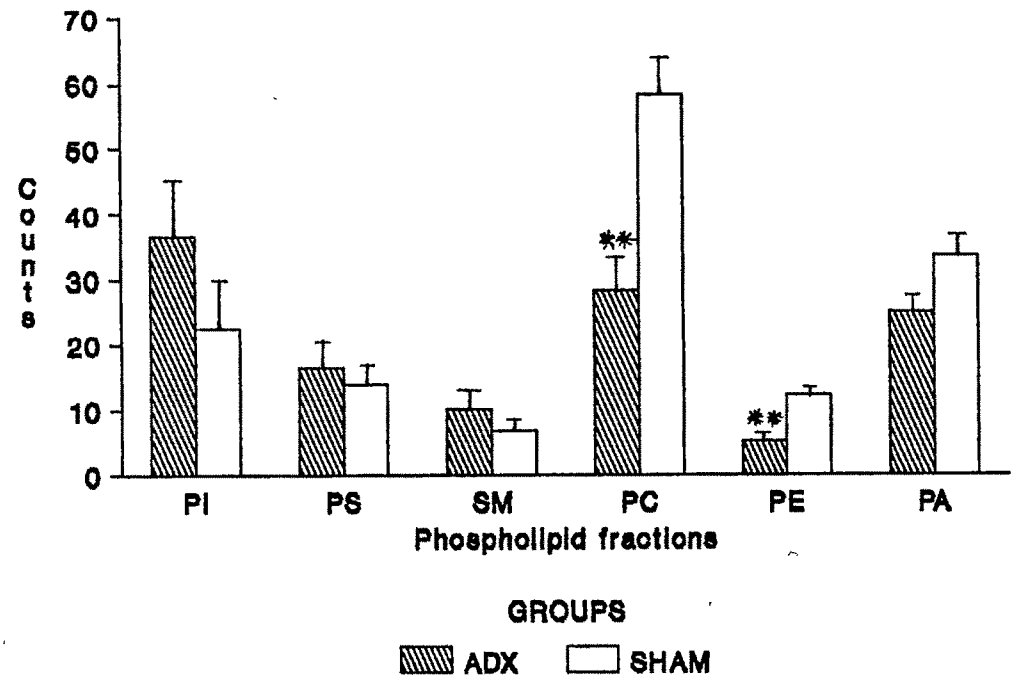
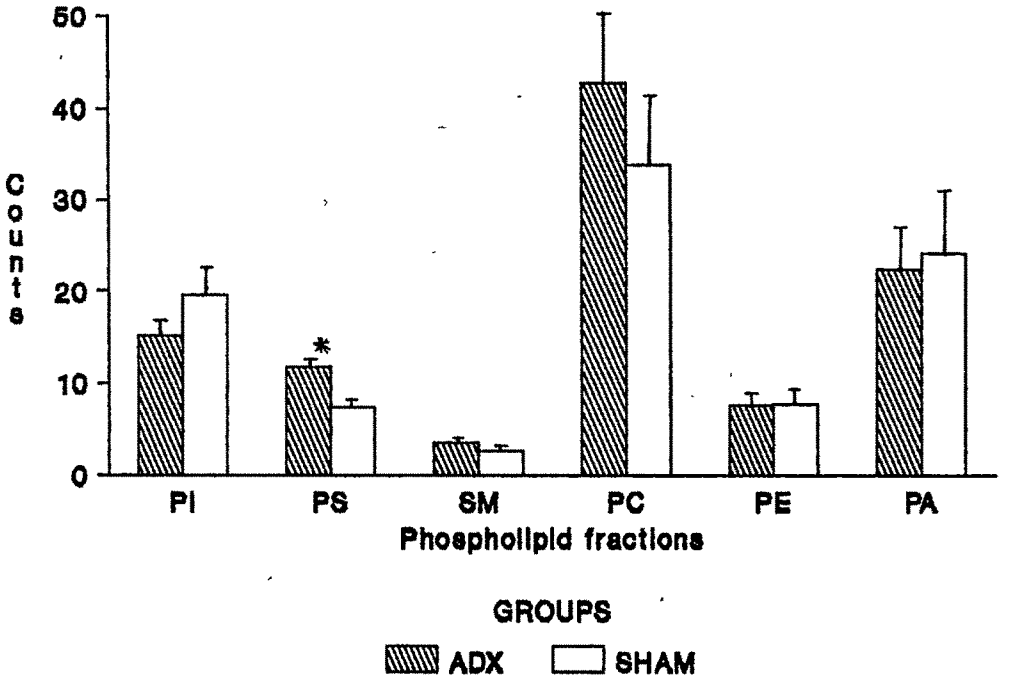


Fig 3.3c HIPPOCAMPAL SLICES.



Values are expressed in mean ± S.E in cpm x 10^3/ g tissue.
Number of observations are 5-7. Level of significance by
Student's't' test * P<0.05; ** P< 0.01 from SHAM.
PI-Phosphatidyl inositol,PS-Phosphatidyl serine,SM-Sphingomyelin,
PC-Phosphatidyl choline,PE-Phosphatidyl ethanolamine,
PA-Phosphatidic acid. ADX- Adrenalectomy, SHAM- Operated control.

Table: 3.28.

Effect of Adrenalectomy and Cort. replacement on ^{32}P incorporation into phospholipids in rat brain region slices.

Regions	Group	PI	PS	SM	PC	PE	PA
				CPM/mg protein			
CORTEX	ADX+H	1582 ± 375@	580 ± 210	40 ± 8.0	440 ± 49.6	97 ± 15	289 ± 26
	ADX	526 ± 202**	128 ± 24**	45 ± 8.2*	391 ± 42.5	91 ± 12	237 ± 36
	SHAM	1741 ± 277	552 ± 107	118 ± 23	397 ± 43	133 ± 28	390 ± 52
BRAIN STEM	ADX+H	2705 ± 825	352 ± 61	57 ± 10.5@@	769 ± 65	172 ± 12.5	378 ± 42
	ADX	2450 ± 416	483 ± 37	156 ± 18.4	651 ± 69	156 ± 28.3	311 ± 53
	SHAM	2056 ± 281	527 ± 109	110 ± 25.2	697 ± 57	220 ± 62.7	376 ± 56
HIPPOCAMPUS	ADX+H	730 ± 131	318 ± 38.9	86.1 ± 22.8	465 ± 84.4	99.9 ± 8.97	271 ± 79.6
	ADX	782 ± 127	380 ± 97.0	114 ± 11.0	458 ± 34.7	126 ± 12	319 ± 40.8
	SHAM	770 ± 102	462 ± 60.4	242 ± 66.0	706 ± 115	192 ± 27	367 ± 84.4

Values are expressed in mean ± S.E in cpm/ mg protein. Number of observations are 6.

Level of significance by Student's 't' test @,* P<0.05; @@,** P< 0.01 from ADX and SHAM respectively.

Adrenalectomized age -20 days old; Duration -30 days; Replacement- 29 days.

ADRENALECTOMY & CORT.REPLACEMENT-30DAYS
ON 32-P INCORPORATION INTO PHOSPHOLIPIDS

Fig.3.4a CORTICAL SLICES.

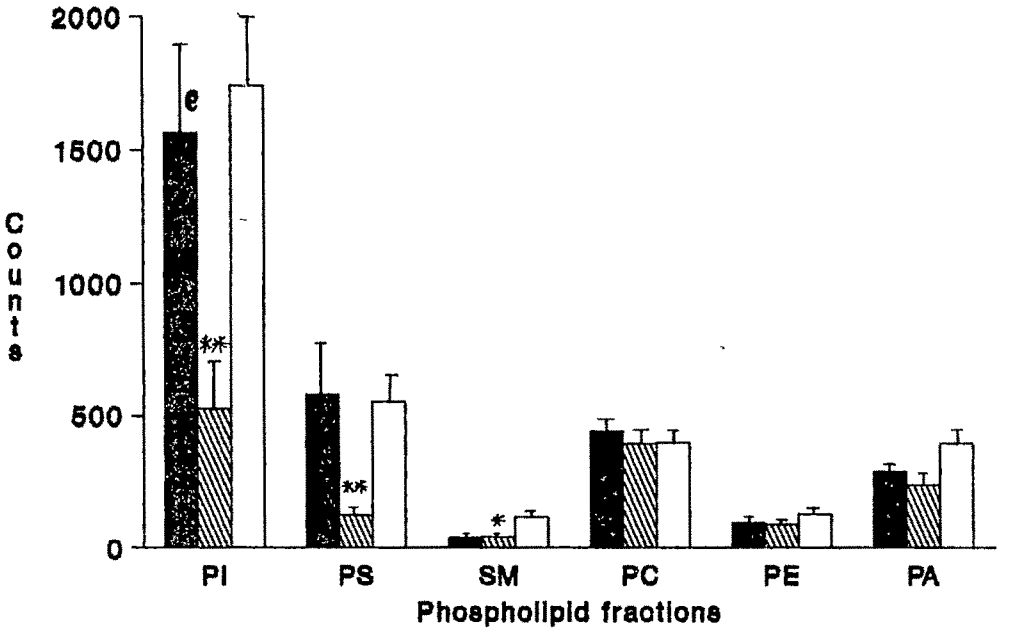


Fig.3.4b BRAIN STEM SLICES.

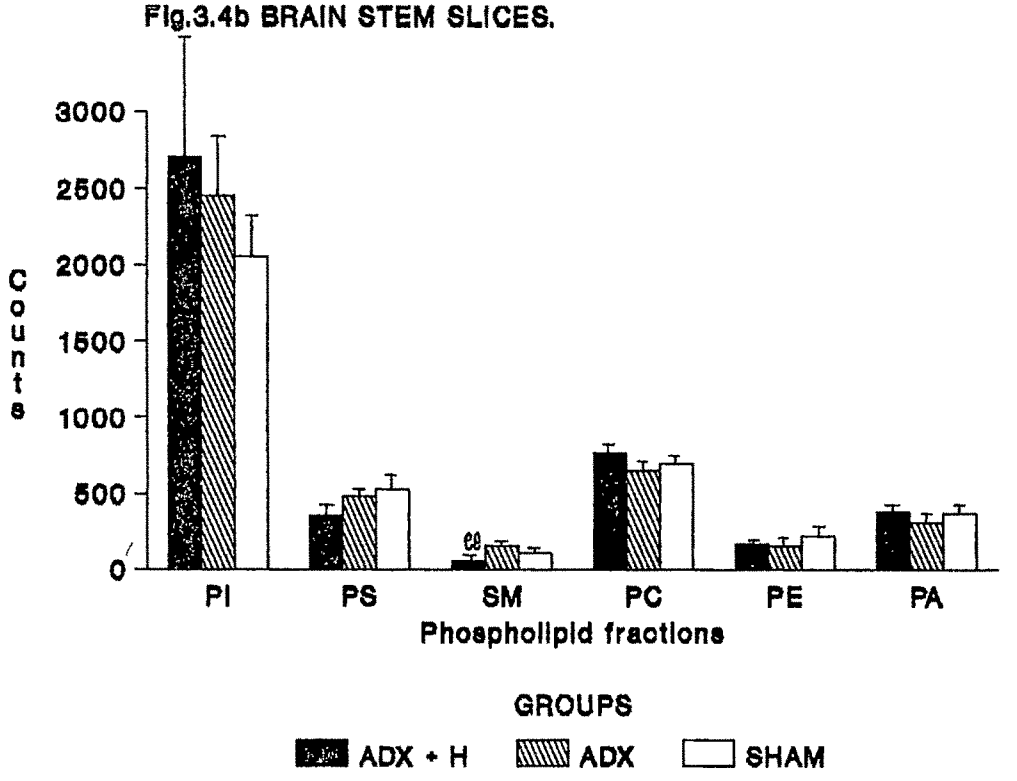
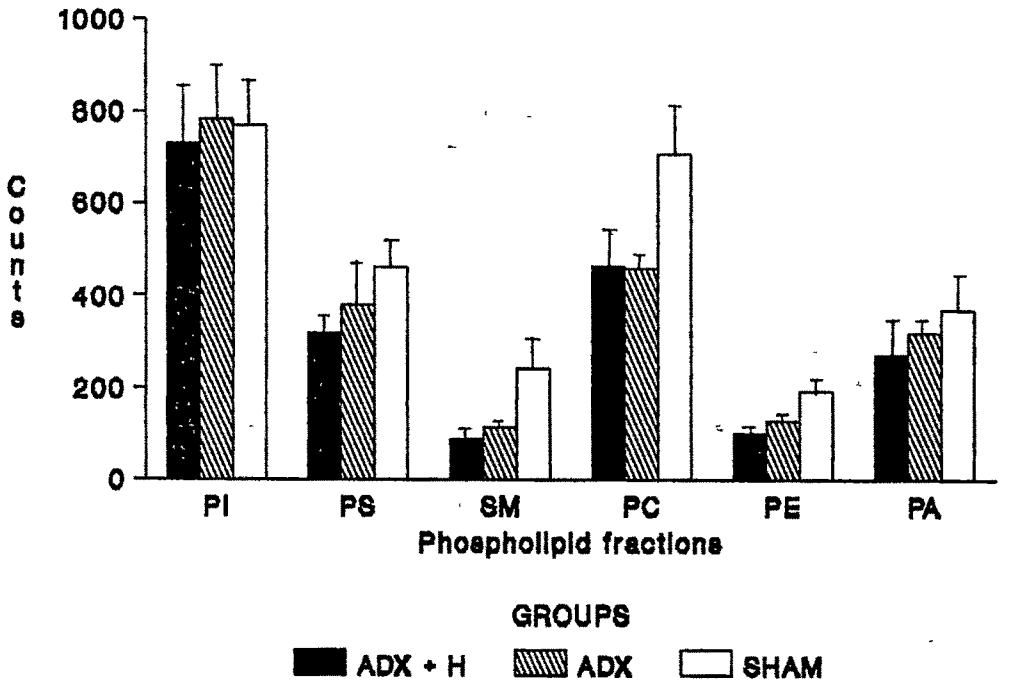


Fig.3.4c HIPPOCAMPAL SLICES.



Values are expressed in mean ± S.E in cpm/ mg protein. Number of observations are 6. Level of significance by Student's't' test @, * P<0.05; @@,**P< 0.01 from ADX and SHAM respectively. PI-Phosphatidyl inositol, PS-Phosphatidyl serine, SM-Sphingomyelin,PC-Phosphatidyl choline, PA-Phosphatidic acid, PE-Phosphatidyl ethanolamine. ADX+H- Adrenalectomy and corticosterone replaced, ADX- Adrenalectomy, SHAM- Operated control.

Table: 3.29.

Effect of Metapirone treatment and Cort. replacement on ^{32}P incorporation into phospholipids in rat brain region slices.

Regions	Group	PI	PS	SM	PC	PE	PA
				CPM/mg protein			
	MET+H	36.6 ± 5.9	23.2 ± 3.1	12.3 ± 2.7@@	73.1 ± 8.3@@	39.3 ± 4.2	58.1 ± 2.1
CORTEX	MET	172 ± 28**	47.3 ± 12.5	38.5 ± 7.0*	118 ± 9.8	33.5 ± 6.9	71.8 ± 7.4
	VEHICLE	39 ± 4.7	21.4 ± 3.9	19.3 ± 2.4	102.6 ± 12.6	45.9 ± 1.5	42.5 ± 5.5
	MET+H	209.8 ± 34.2	81.5 ± 7.0	53.8 ± 12.9	240 ± 48	63.8 ± 3.6	53.3 ± 8.0
BRAIN STEM	MET	173 ± 25.7	102 ± 14.3	41.9 ± 5.9	345 ± 85	86.4 ± 28.9	75.6 ± 23.1*
	VEHICLE	194 ± 20	97 ± 16.4	48.4 ± 9.1	355 ± 52.1	98.0 ± 16.5	57.6 ± 15.0

Values are expressed in mean ± S.E as cpm/ mg protein. Number of observations are 5- 6.

Level of significance by Student's't' test * P<0.05; @@,**P< 0.01 from MET and VEHICLE respectively.

MET- Metapirone injected, MET+ H- Metapirone and corticosterone replaced, VEHICLE- Injected control.

METAPIRONE INJECTION & CORT.REPLACEMENT ON 32P-INCORPORATION INTO PHOSPHOLIPIDS

Fig.3.5a CORTICAL SLICES.

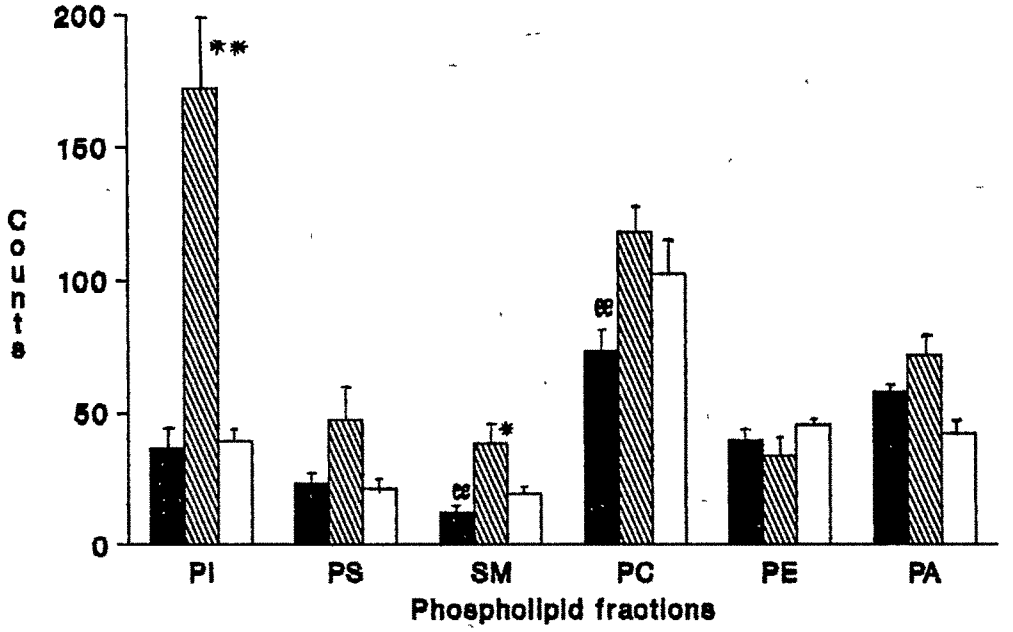
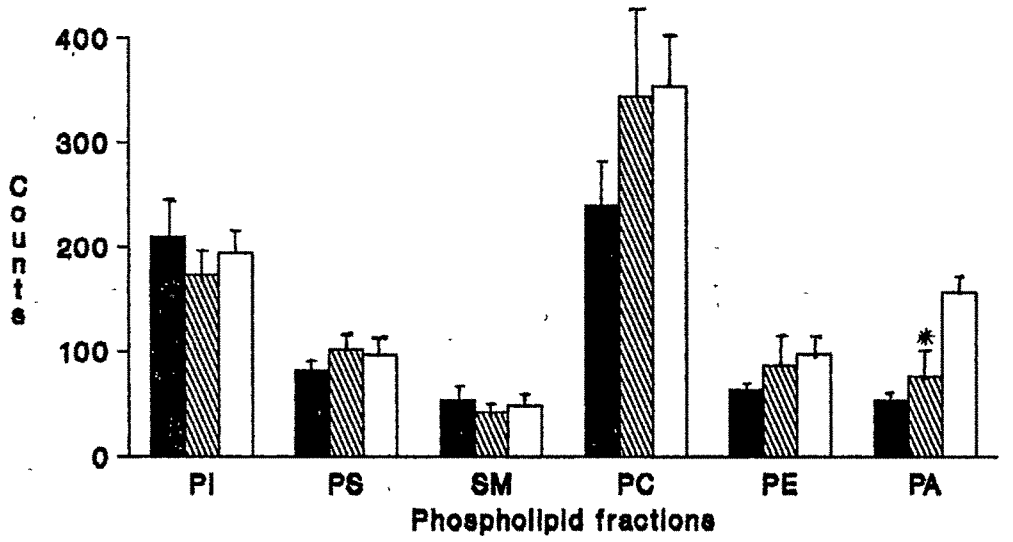


Fig.3.5b BRAIN STEM SLICES.



GROUPS

MET + H MET VEHICLE

Values are expressed in mean ± S.E as cpm/ mg protein. Number of observations are 5- 6. Level of significance by Students't' test * P<0.05; @@,**P< 0.01 from MET and VEHICLE respectively. MET- Metapirone injected, MET+ H- Metapirone and corticosterone replaced, VEHICLE- Injected control. PI-Phosphatidyl inositol, PS-Phosphatidyl serine, SM-Sphingomyelin, PC-Phosphatidyl choline,PE-Phosphatidyl ethanolamine,PA-Phosphatidic acid.

With regard to hippocampus although the corticosterone treatment in 20 day old animals had significantly elevated PS, the incorporation was not different from control. On the other hand, in ADX rats for 15 days showed no change in the concentration but ^{32}P incorporation into PS was significantly increased. Therefore it appears that PS turnover is significantly altered by glucocorticoid, lower turnover with excess and higher turnover with permissive levels. With longer duration of adrenalectomy the permissive effects seem to be abolished and the system tries to come back to normal.

In brain stem, ^{32}P incorporation into PI was significantly low in corticosterone treated rats whereas in ADX rats (15 days) the ^{32}P incorporation was significantly low in PC and PE but not in PI. These results with no change in the concentration of PI or PE and increase in PC indicates that lower incorporation in PC and PE in ADX is due to decrease in the levels of these phospholipids. With excess treatment the SM concentration was higher but the incorporation was lower. Thus it can be concluded that PI and SM turnover are altered with corticosterone treatment. Again with longer duration of ADX, the effects were completely abolished.

When these results are combined with those obtained on the concentration of phospholipids in different regions, the following points can be noted.

(A) The results do not agree with receptor levels nor their type.

(B) The phospholipid metabolism is an extremely dynamic process and several factors seem to regulate/influence the metabolism of the same. The dynamism can be observed from the fact that elevated levels of corticosterone only

affect at younger ages, and ADX of different durations show different effects on the rate of incorporation of ^{32}P. They more or less overcome the effects with longer duration of ADX. Only cerebral cortex seems to become more sensitive with prolonged ADX. The bimodel responses have been observed in cAMP levels with different duration of dexamethasone treatment (Duman *et al.*, 1989).

(C) The STR seems to be unique in the sense that at younger ages, elevated levels of corticosterone show relatively greater effects. The possible reason could be that striatal nuclei seem to show greater retention of corticosterone, perhaps enhance gene expression.

(D) Of all the regions studied BS lipid metabolism seems to be regulated in a manner very similar to what has been observed in the peripheral component, whereas rest of the regions show differential effects.

(E) Since concentration of PI in the cortex was not affected and changes in the rate of ^{32}P incorporation was observed at all ages with elevated or depleted status emphasizing PL-C mediated effects, PI metabolism in the cortex is a major factor.

Experiment III c:

Chronic corticosterone and Fatty acid composition in synaptosomes:

The next step after the phospholipids concentration and ^{32}P incorporation studies the other possible factor which may be modified by the glucocorticoid is fatty acid composition of phospholipid classes. This was considered the most important component of the membrane which likely to change as well as has great influence on the membrane fluidity.

The effects of glucocorticoids on fatty acid composition of various

membranes are discussed in the introduction. It is clear that such studies on non-neuronal tissue has indicated a clear effect on the fatty acid composition of various phospholipids due to an effect on the desaturase, fatty acid synthase and phospholipase A_2 inhibition *etc.* Unfortunately the availability of gas chromatography for fatty acid analysis was a major limiting factor for the present study. The permission was obtained from a private institute where such facility was available for a limited number of sample and the time. Therefore results obtained in the present study were only carried out once and needed further conformation. However the experiment was conducted with utmost care to avoid the oxidation of fatty acid by using N_2 gas and BHT (Butylated hydroxy toluene), an antioxidant. The details of the methods are described elsewhere.

The standard fatty acid and typical profile of fatty acids are given in fig. S. The comparison of the fatty acid composition of the various phospholipid class obtained from 20 day control animals with that of reported is given in table 3.30. As can be seen from the same, the profile in the present study was comparable with regard to most of the fatty acid composition.

As can be seen from the results of the fatty acid profile of control and experimental (Table 3.31) 16:0 and 18:0 were both present in PI and PS whereas in PC and PE the 16:0 fatty acid only predominated and 18:1 was present instead of 18:0. With regard to unsaturated fatty acid, 20:4 predominated in PI and PE whereas long chain fatty acids (22:4,22:6) were present in PC and PS. The presence of such high levels of unsaturated fatty acids are found to be in accordance with the functions of synaptosomal

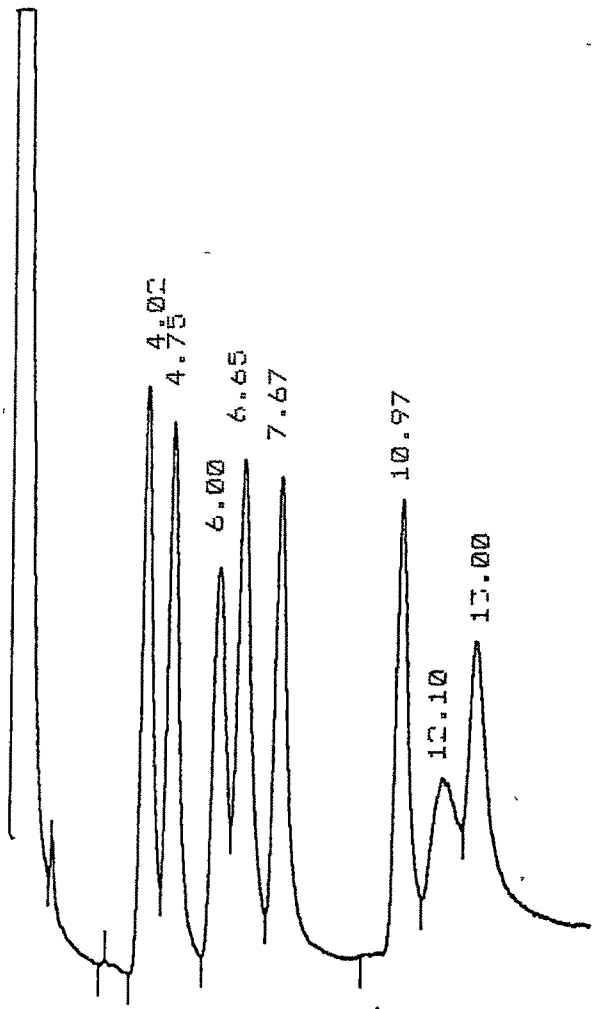


Fig. S. Standard fatty acid profile

(R.T - 4.02) 16:0 - Palmitic acid, (4.75)
16:1 palmitoelic acid
(6.00) 18:0- Stearic acid,(6.65) 18:1 - Oleic acid,
(7.67) 18:2- Linoleic acid,(10.97) 20:4 - Arachidonic acid,
(12.10)22:4- Docosatetraenoic acid,
(13.00)22:6- Docosahexaenoic acid.

Table: 3.30.

Comparison of present and reported values of fatty acid composition from synaptosomes and synaptic plasma membrane.*

Fatty acids		PI	PS	PC	PE
16:0	Present	20.9	16.5	34.3	9.8
	Reported*	10	0.5	57.2	5.9
18:0	Present	35.3	42.9	---	---
	Reported*	38.4	48.6	11.8	29.3
18:1	Present	---	---	26.0	21.9
	Reported*	8.5	7.6	22.1	7.8
20:4	Present	43.8	---	---	38.7
	Reported*	36.9	2.0	4.3	14.6
22:4	Present	---	5.2	8.9	---
	Reported*	---	3.9	0.2	7.3
22:6	Present	---	34.1	35.2	30.2
	Reported*	4.7	34.1	2.7	32.4

* Taken from Breckenridge *et al.* (1972) and expressed in percentage of total fatty acid. 16:0 - Palmitic acid, 18:0- Stearic acid, 18:1 - Oleic acid, 20:4 - Arachidonic acid, 22:4 - Docosatetraenoic acid, 22:6- Docosahexaenoic acid.

Table: 3.31.

Effect of Corticosterone administration on fatty acid composition of phospholipids in synaptosomes isolated from cerebral cortex.

Fatty acids		PI	PS	PC	PE
16:0	Cort.	6.6 ± 0.55***	9.7 ± 0.48*	15.0 ± 3.35**	6.1 ± 1.13
	Control	20.9 ± 1.29	16.5 ± 2.29	34.3 ± 3.32	9.8 ± 1.70
18:0	Cort.	11.7 ± 0.45***	43.6 ± 0.53	---	---
	Control	35.3 ± 1.52	42.9 ± 1.59	---	---
18:1	Cort.	---	---	31.5 ± 1.45	11.9 ± 1.50*
	Control	---	---	26.0 ± 3.15	21.9 ± 2.77
20:4	Cort.	74.3 ± 3.72***	---	---	58.7 ± 3.21**
	Control	43.8 ± 1.31	---	---	38.7 ± 2.40
22:4	Cort.	---	4.8 ± 0.61	6.2 ± 1.28	---
	Control	---	5.2 ± 0.32	8.9 ± 0.72	---
22:6	Cort.	---	43.0 ± 1.27*	47.7 ± 4.50	26.2 ± 3.90
	Control.	---	34.1 ± 2.53	35.2 ± 5.73	30.2 ± 3.35

Values are expressed in mean ± S. E. in percentage.

Levels of significance by Student's 't' test * P<0.05; ** P<0.01; *** P<.001 from control. 16:0 - Palmitic acid, 18:0- Stearic acid, 18:1 - Oleic acid, 20:4 - Arachidonic acid, 22:4 - Docosatetraenoic acid, 22:6- Docosahexaenoic acid. Number of observations are 5.

membranes.

The corticosterone administration seem to have generalized trend of decreasing in unsaturated fatty acids in practically all phospholipid fractions, however PE has very little of saturated fatty acid and high of mono and polyunsaturated fatty acids.

The decrease in saturated fatty acids is associated with increased unsaturated fatty acid 20:4. This increase was seen in PI and PE where 20:4 is the predominant unsaturated fatty acid whereas 22:6 significantly increased in PS.

As mentioned earlier there are not many reports on the fatty acid profile of the different membranes with corticosterone. However, a systematic study on urinary bladder membranes with aldosterone treatment has been reported as early as in 1971 and 1975 (Goodman *et al.*, 1971; 1975). These authors have also observed an alteration in the U-^{14}C glucose and 2-^{14}C acetate incorporation in the lipid within 20 minutes after aldosterone injection. The increase in incorporation of radioactive label was seen at 2nd position of phospholipids. Interestingly the effects were not specific to a specific class. It was observed that the fatty acid incorporated was greater in unsaturated rather saturated fatty acid after 6 hour especially long chain polyunsaturated fatty acids were increased. This show that aldosterone in the toad urinary bladder alters the fatty acid metabolism of membrane phospholipids (Goodman *et al.*, 1971). Subsequent study showed that aldosterone stimulates deacylation-reacylation cycle and although fatty acid utilization is increased, aldosterone specifically enhances elongation and desaturation of oleic acid (Goodman *et al.*, 1975).

In contrast to the observations on toad bladder, study on liver microsomes of adrenalectomized and hormone replaced animals carried out by Nelson and his colleagues (Melby *et al.*, 1981) have indicated that hepatic microsomal membrane fatty acid composition is altered due to changes in the plasma levels of glucocorticoids. The results indicated that a significant decrease in PC of adrenalectomized rats whereas in cortisol replacement group decreased further the PC levels. On the other hand although adrenalectomy `per se' didn't show any change in PE, PS and SM, the adrenalectomy-cortisol replaced group had decreased PE and SM. Since the amount injected was 5 mg cortisol for 2 days and 1 mg cortisol succinate 1 hour before killing. These results in cortisol replacement groups suggest an excess of glucocorticoid. Further, the fatty acid profile of various classes PC and PI+PS, indicated a decrease in 18:0 and increase in arachidonic acid of PC in ADX group and 16:0 in PS+PI of the same group. In the case of monounsaturated (18:1) and polyunsaturated fatty acids (18:2, 20:4) ADX group had a shift from monounsaturated (18:1) to polyunsaturated (20:4) in PC. With cortisol replaced group there was a decrease in polyunsaturated (20:4) to monounsaturated (18:1) fatty acids. Thus even liver microsomes indicated that circulating glucocorticoid levels influence desaturase enzymes responsible for the synthesis of PUFA (Melby *et al.*, 1981). This was found to be in consistent with reports on the effect of corticosterone administration on fatty acid profile of hepatic mitochondria from our laboratory. It was observed that corticosterone increases proportion of saturated fatty acids whereas PUFA were significantly reduced (Krishnakumar, 1993). A recent report by Alaniz *et al.* (1992) also indicates that corti-

costerone and aldosterone depresses the activity of enzyme converting 20:3 to 20:4. However, the mechanism differs with glucocorticoid and aldosterone. The former acts through induction of a protein which inhibits this conversion, similar to lipocortin action on phospholipase A_2. Thus the effect on various tissues seem to vary but it is obvious from all these studies that the fatty acids are the targets of glucocorticoid action.

In the present study the synaptosomal membranes had increased unsaturated fatty acids. This is similar to what was observed in toad bladder with aldosterone. Therefore one can speculate that even brain desaturases are equally responsive to corticosterone levels. The present finding of increased PUFA would mean that either there is an increase in the desaturase activity and/ or a decreased release of PUFA due to known inhibition of phospholipase A_2 by glucocorticoid. Elevated levels of corticosterone suppress the phospholipase A_2 activity at the basal level and it is likely that the normal turnover of fatty acid at the C-2 position is affected (Hirata *et al.*, 1980). It is well known that PI,PS and PC have 20:4 fatty acid invariably at the C-2 position. The concentration of PI was not affected but the turnover as judged by ^{32}P incorporation was significantly increased in this condition. This would explain the greater proportion of PUFA in PI. Thus it can be concluded that the present study indicates a change in fatty acid composition of the membrane which would alter its fluidity.

Experiment IV a:

Effect of corticosterone administration and adrenalectomy on membrane bound enzymes:

It is well known that activities of some of the membrane bound enzymes have absolute requirement of phospholipids (Sandermann Jr., 1978). Some are extremely specific, for example mitochondrial F_oF_1 ATPase for cardiolipin (Daam, 1985). Other enzymes have requirement of one or more head groups or different fatty acid composition (Bernsohn and Spitz, 1974; Brivio-Hougland *et al.*, 1976). Depending on the alterations in the lipid composition the activities of these enzymes vary. Several reports in the literature have indicated such phospholipid requirement for Na+K+ATPase - a plasma membrane integral protein of all tissues including brain (Lee, 1992). Acidic phospholipids namely phosphatidyl inositol, phosphatidyl serine, and phosphatidyl glycerol have been implicated as required for Na+K+ATPase (Wheller *et al.*, 1975). The synaptosomal Na+K+ATPase activity is found to be significantly increased when intact synaptosomes were treated with phosphatidyl serine liposomes. The effects were specific for Na+K+ATPase as Mg^{++} ATPase and 5'nucleotidase were not altered. The authors attribute that the PS affects either `per se' or alters fluidity and/ or helps in expansion of plasma membranes through which ions and substrates pass more easily. A possibility of altered Ca++ ions is also suggested (Floreani *et al.*, 1981). The activity of Na+K+ATPase of the brain microsomal membranes has been shown to be inhibited by increasing chain length of fatty acids. Unsaturated fatty acids are more inhibitory in nature than their saturated counterparts (Ahmed and Thomas, 1971). The inhibitory effects were uncom-

petitive with respect to ATP, but competitive with K+ indicating that a possible change in the composition of phospholipid fatty acid could also influence the Na+K+ATPase activity (Ahmed and Thomas, 1971).

The *in vitro* study of addition of aliphatic alcohols of various chain length has indicated that these alcohols have a selective effect on synaptosomal Na+K+ATPase and not AchE. Since the inhibitory effect of the alcohols correlated with their lipid solubility, the authors have attributed these effects of alcohols to the lipid - alcohol interaction and have mentioned a possibility that ethanol acts on membrane bound Na+K+ATPase by occupying a hydrophobic site on the plasma membranes (Sun and Samorjaski, 1970).

The 5'nucleotidase is another plasma membrane integral enzyme whose active site is located out side surface of the cell (Meflah *et al.*, 1984). The enzyme catalyses the hydrolysis of phosphate esterified at 5'end of the ribose and deoxy ribose portions of nucleotide molecule. The activities of this enzyme is regulated by the sphingomyelin as well as phosphatidyl choline in the membrane (Merisko *et al.*, 1981). This enzyme is present in the synaptic membrane and is supposed to be involved in release of adenosine which is a neuromodulator or a putative neurotransmitter. The distribution of this enzyme however indicates multiple origins. Several reports indicates that this enzyme is equally distributed on the synaptic as well as myelin membranes (Cammer *et al.*, 1980). Besides, an appreciable amount is present in the cytosol, microsomes and mitochondria (Zimmermann, 1992).

Not many studies have been reported regarding the lipid requirement for the activity of this enzyme, especially with brain. The hepatic 5'

nucleotidase in the membrane seem to interact with sphingomyelin and phosphatidyl choline. The sphingomyelin influences the stability of the enzyme and PC influences the energy of activation (Merisko *et al.*, 1983). In another study, membranes from various tissues /cells namely kidney microsomes, rat liver slices and microsomes as well as lymphocytes indicated that treatment with phospholipase C results in disruption of specific interactions involving the phosphatidyl inositol molecule thus releasing 5' nucleotidase from the membranes. The percentage release varied in different tissues. Thus PI has been attributed to be an important phospholipid for holding this protein in the membrane (Low and Finean, 1978). A comparative study with Na+K+ATPase indicated that this enzyme activity was not influenced by incorporating PS in the synaptosomal preparation (Floreani *et al.*, 1981).

The role of fatty acids on 5'nucleotidase activity has been investigated by using essential fatty acid deficiency and subsequent replacement of n-3 or n-6 essential fatty acids in experimental animals. These studies indicate that decrease in liver membrane linoleate (n-3 series) significantly lowered 5' nucleotidase activity whereas total ATPase was higher, and a decrease in basal adenylate cyclase activity was seen (Brivio-Hougland *et al.*, 1976). A similar decrease in 5' nucleotidase was reported in brain homogenate of rats fed with fatty acid deficient diet (Bersohn and Spitz, 1974).

The third membrane protein of interest is acetylcholine esterase. This enzyme is a peripheral enzyme and also does not require any specific amino acid sequence for membrane spanning. The Arrhenius plot of this enzyme shows a clear discontinuity in the enzyme activity which implies a

possible influence of membrane lipids and other proteins. Interestingly this enzyme shows such a pattern from very early neonatal stage. On the other hand Na+K+ATPase shows such a pattern only after a certain stage of neonatal development (Gorgani and Mesami, 1979). The loss of AchE activity due to dissociation from cardiolipin has been demonstrated.

Previous results of the experiments on phospholipid composition and ^{32}P incorporation in phospholipid fractions have indicated that there are regional variation in both composition and ^{32}P incorporation. Also a limited study on fatty acid composition of cortex indicated a possible effect on proportion of saturated and unsaturated fatty acid. Therefore it was thought of interest to check various enzymes related to membranes. The choice was these three enzymes. The earlier studies carried out with the crude homogenate on Na+K+ATPase under similar conditions in three regions namely cerebellum, hippocampus and striatum indicated that Na+K+ATPase activity was decreased in these regions at 20 days of age after corticosterone administration for three days. These effects were also observed even with single injections. This indicated that at 20 days of age perhaps either the ionic concentration would be a factor as observed in the case of kidney plasma membrane or it could be because of direct binding of corticosterone to the membranes (Deliconstantinos, 1985; Orchinck *et al.*, 1991; Zuo and Yi-Zhang, 1992). To see if these effects are as a consequence of genomic action on the membrane phospholipids composition/ metabolism / protein or a culminative effect, it was thought to check the enzymes associated with membranes. To check this it is essential to work with isolated membranes. It was decided to prepare a crude membrane preparation

which is devoid of any metabolic factors as in the case of homogenate. Hence the crude membrane preparation was made as suggested by Smith *et al.* (1980), It was decided to make crude membranes of all the regions of interest to be studied. Additional studies were carried out with synaptosome and myelin membranes isolated from cerebral and cerebellar cortex to see whether the effects can be observed on these subcellular membrane fractions also.

As can be seen from the table 3.32 and fig. 3.6 a,b,c, with corticosterone treatment in 10 day old animals the activity of Na+K+ATPase was significantly elevated in the membrane preparation from hippocampus, striatum and cortex whereas activity in brain stem and cerebellum remained unaltered. 5' Nucleotidase activity elevated in all the regions except in the brain stem. In contrast to these two enzymes, AchE seemed to be least affected, as only striatum showed a significantly high activity.

In 20 day old animals, effects seemed to be opposite to what was observed at day 10. A trend of significantly low activity of both Na+K+ATPase and 5' Nucleotidase was observed (Table 3.33 and Fig. 3.7a,b) but the regional susceptibility varied. Activity of the former was not affected in the olfactory bulb and cortex whereas the latter was not affected in the hippocampus and cortex. Thus cortex seems to be less affected at 20 days, but from subcellular studies, both these enzymes are found to be decreased in corticosterone treated rat brain cortex as well as cerebellar synaptosomes (Table 3.34). Striatum showed difference in response- while a decreased Na+K+ATPase activity was seen, the 5'Nucleotidase activity showed an increase.

Results of the enzyme activities after adrenalectomy are presented in

Table: 3.32.

Effect of Corticosterone administration on membrane bound enzymes in 10 day old rat brain region membrane.

Enzyme	Group	CBL	BS	HC	STR	CTX
Na+K+ATPase	Cort.	3.7 ± 0.57	4.2 ± 0.62	4.3 ± 0.50*	5.9 ± 0.60***	4.9 ± 0.33**
	Control	3.8 ± 0.96	3.1 ± 0.42	2.7 ± 0.52	2.1 ± 0.33	2.7 ± 0.50
5'Nucleotidase	Cort.	1.7 ± 0.14**	0.6 ± 0.06	0.6 ± 0.05***	0.6 ± 0.03***	0.5 ± 0.03**
	Control	1.0 ± 0.08	0.7 ± 0.03	0.3 ± 0.03	0.2 ± 0.03	0.3 ± 0.04
Acetylcholine esterase	Cort.	2.6 ± 0.33	6.0 ± 0.15	2.5 ± 0.14	9.5 ± 0.56**	2.8 ± 0.08
	Control	2.8 ± 0.31	4.9 ± 0.56	2.6 ± 0.20	6.6 ± 0.36	2.1 ± 0.22

Values are expressed in mean ± S.E in enzyme unit. Number of observations are 6. Level of significance by Student's't' test *P<0.05; **P< 0.01;***P<0.001 from control. For each observation two rats were pooled.

Enzyme unit- μmoles of pi liberated/mg protein/ hour for both Na+K+ATPase & 5'Nucleotidase.

μmoles of substrate hydrolysed/ mg protein/ hour for acetylcholine esterase.

CHRONIC CORT. TREATMENT ON ENZYMES IN 10 DAY OLD RAT BRAIN REGIONS.

Fig.3.6a Na+K+ATPase.

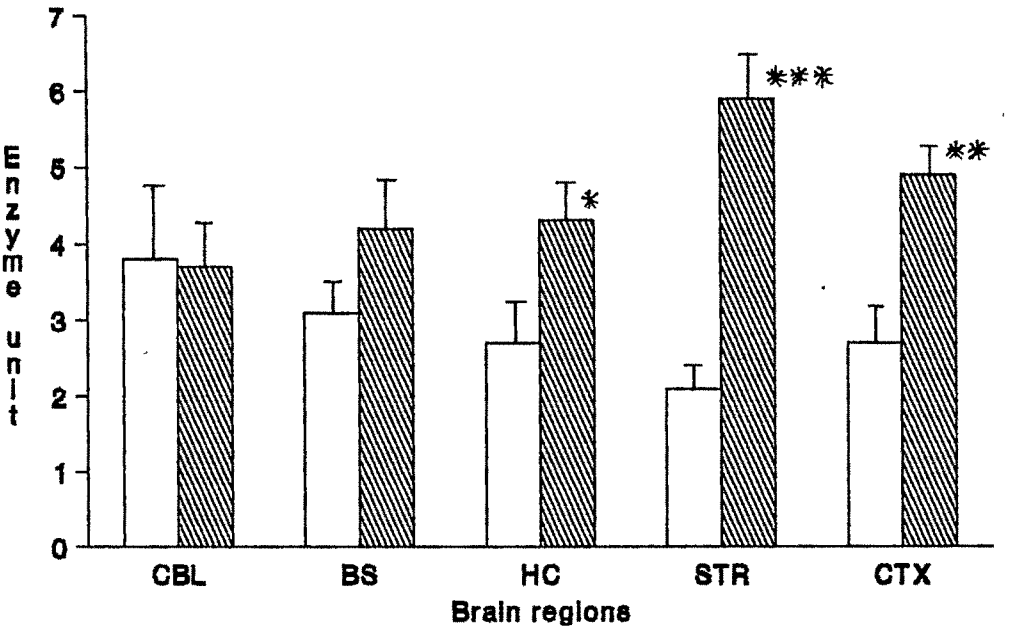


Fig.3.6b 5'Nucleotidase.

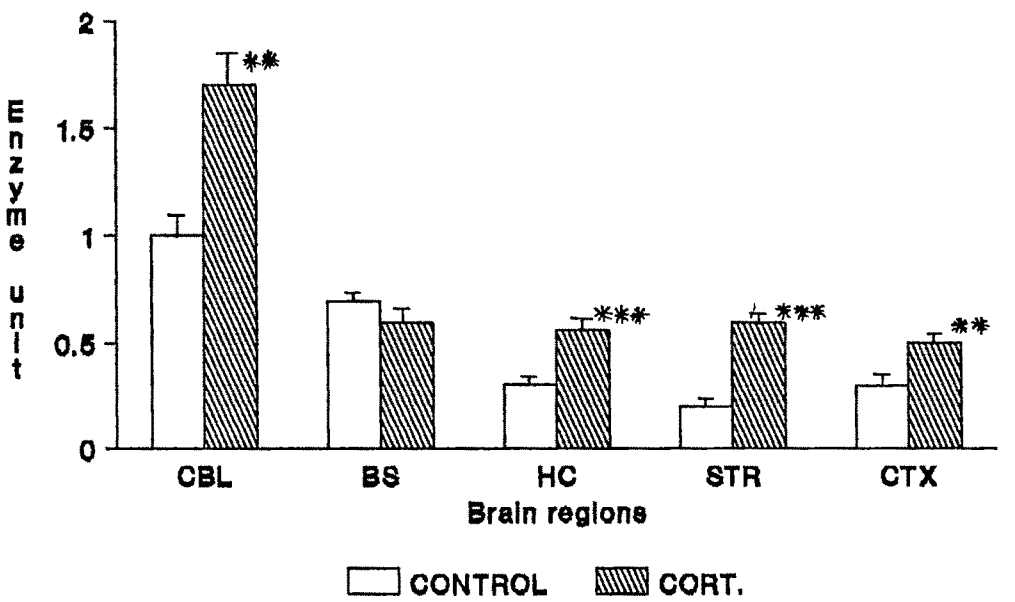
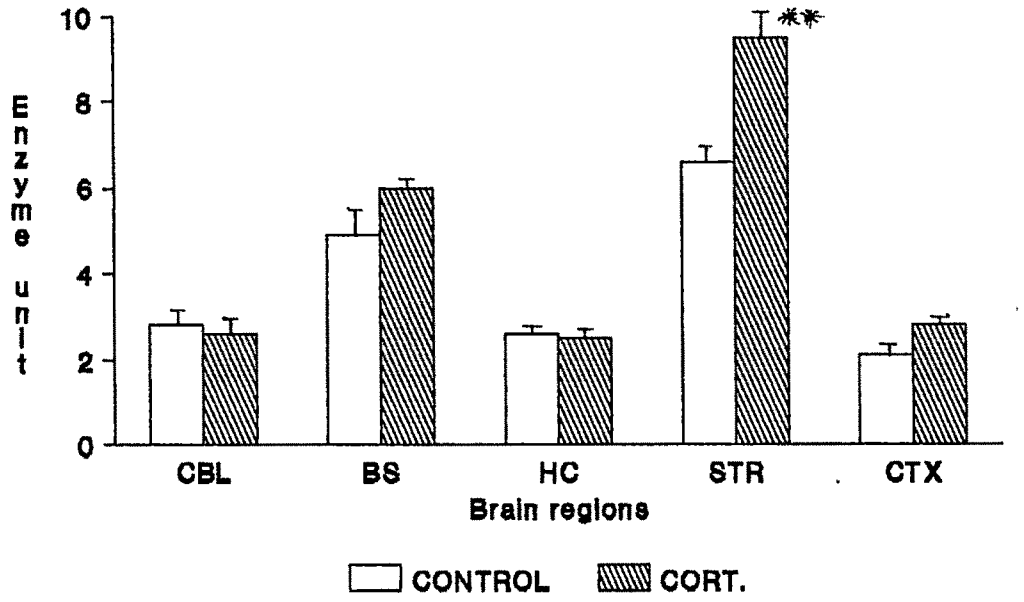


Fig.3.6c Acetylcholine esterase.



Values are expressed in mean ± S.E in enzyme unit.

Number of observations are 6. Level of significance by Student's 't' test *P<0.05; **P< 0.01;***P<0.001 from control.

For each observation two rats were pooled. Cort.- Corticosterone.

Enzyme unit- µmoles of pi liberated/mg protein/ hour for both Na+K+ATPase & 5'Nucleotidase.

µmoles of substrate hydrolysed/ mg protein/ hour for AchE.

CBL- Cerebellum, BS- Brain stem, HC- Hippocampus, STR- Striatum and CTX- Cortex.

Table: 3.33.

Effect of Corticosterone on treatment

membrane bound enzymes in 20 day old rat brain region membrane.

Enzyme	Group	OB	CBL	BS	HC	STR	CTX
Na+K+ATPase	Cort.	4.2 ± 0.21	6.0 ± 0.46***	4.2 ± 0.45**	4.1 ± 0.41***	4.1 ± 0.19***	3.0 ± 0.25
	Control	5.9 ± 1.26	12.7 ± 0.55	8.1 ± 0.65	10.2 ± 0.99	6.2 ± 0.29	2.3 ± 0.12
5'Nucleotidase	Cort.	0.9 ± 0.08*	1.3 ± 0.11*	0.6 ± 0.08	0.8 ± 0.06	0.8 ± 0.06**	0.5 ± 0.07
	Control	1.6 ± 0.32	2.4 ± 0.47	0.7 ± 0.06	0.7 ± 0.10	0.6 ± 0.04	0.6 ± 0.08

Values are expressed in mean ± S.E in enzyme unit. Number of observations are 6.

Level of significance by Student's't' test *P<0.05; **P< 0.01; ***P<0.001 from control.

Enzyme unit- µmoles of pi liberated/mg protein/ hour.

CHRONIC CORT.TREATMENT ON ENZYMES IN 20 DAY OLD RAT BRAIN REGIONS.

Fig.3.7a Na+K+ATPase.

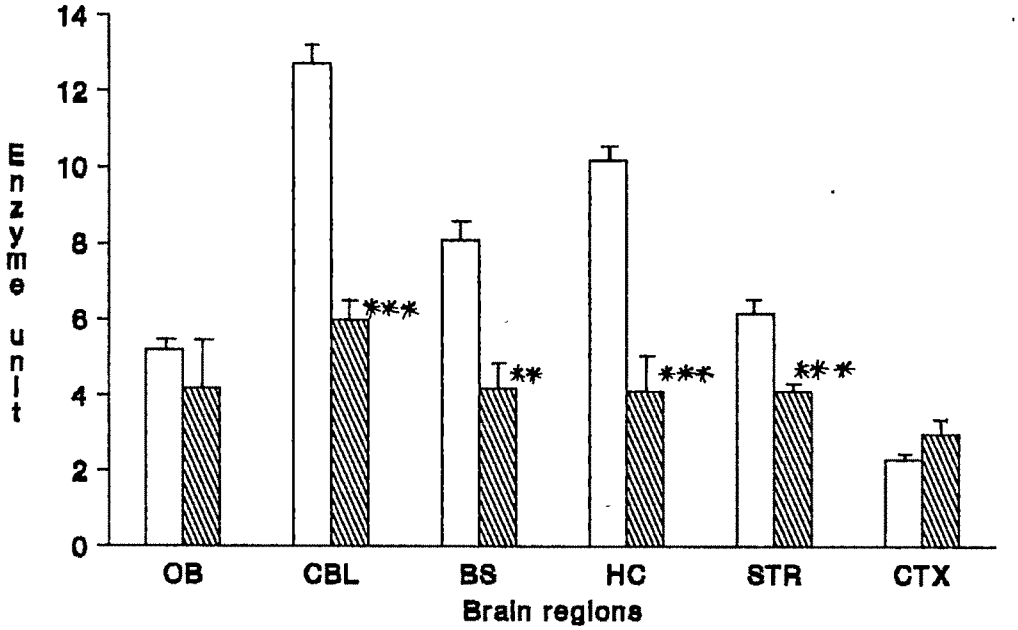
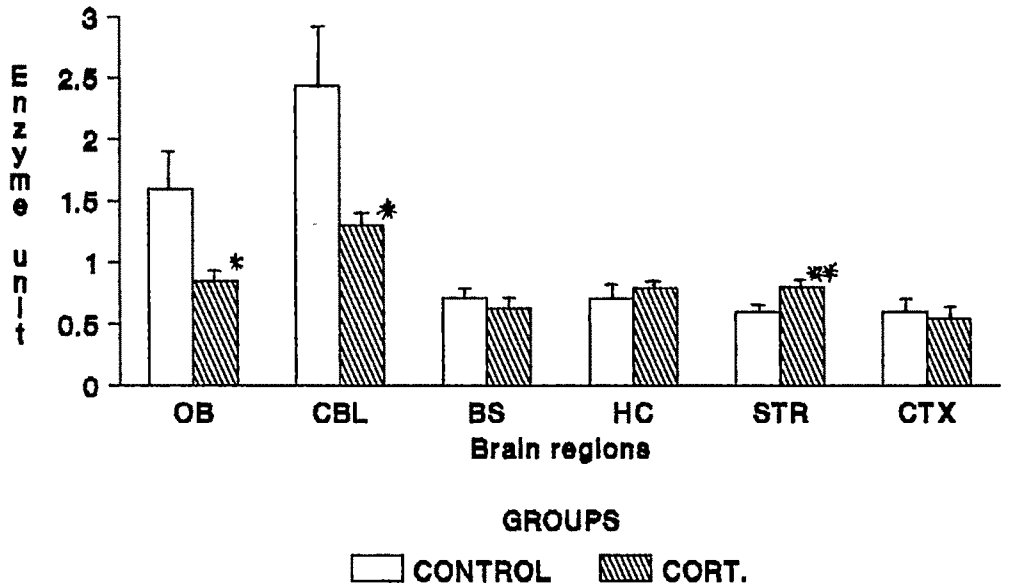


Fig.3.7b 5'Nucleotidase.



Values are expressed in mean ± S.E in enzyme unit. Number of observations are 6. Level of significance by Student's 't' test *P(0.05; **P(0.01; ***P(0.001 from control. Enzyme unit- μmoles of pi liberated/mg protein/ hour. Cort.- Corticosterone. OB- Olfactory bulb, CBL- Cerebellum, BS- Brain stem, HC- Hippocampus, STR- Striatum and CTX- Cortex.

Table: 3.34.

Effect of Corticosterone administration Na+K+ATPase and 5'Nucleotidase in rat brain region subcellular fractions.

Enzymes		Cerebral cortex		Cerebellum	
		Synaptosomes	Myelin	Synaptosomes	Myelin
Na+K+ATPase	Cort.	9.00 ± 0.38**	nd	9.01 ± 0.82**	nd
	Control	12.27 ± 0.92	nd	12.69 ± 0.72	nd
5'Nucleotidase	Cort.	0.47 ± 0.04**	0.67 ± 0.066	1.14 ± 0.10**	0.72 ± 0.14*
	Control	0.61 ± 0.013	0.45 ± 0.089	2.46 ± 0.42	1.14 ± 0.15

Enzyme unit - μ moles of pi liberated /mg protein / hour.

Values are expressed in mean ± S. E. Number of observations are 6.

Levels of significance by Student's 't' test *P<0.05; **P<0.01 from control .

nd- not determined.

table 3.35 and fig. 3.8a,b,c. As evident from the same, the only enzyme which is chiefly affected was Na+K+ATPase. 5'NT was only affected in striatum while AchE activity was not at all influenced in any of the regions studied. Again adrenalectomy uniformly increased the activity of Na+K+ATPase in all the regions except in olfactory bulb and hormone replacement brought it back to normal. The % increase was maximum in cortex followed by brain stem and cerebellum. Hippocampus and striatum were comparatively less affected.

With regard to 5'nucleotidase, adrenalectomy `per se' had no effect but in ADX+H treatment group, the enzyme activity in brain stem was significantly lower compared to control and ADX groups behaved as if the effects were of excess corticosterone. Thus taking all results into consideration, it is obvious that the excess or depletion of corticosterone have variable effects on the membrane bound enzymes. Na^+K^+ATPase is the most sensitive while AchE is the least.

It is well known that during aging the basal levels of corticosterone increases significantly (Sapolsky, 1992). In fact, such elevation has been attributed to be the major cause in further accelerating the aging process when subjected to stress (Sapolsky *et al.*, 1986). It was therefore thought that these enzymes also may be influenced with aging and if so, adrenalectomy should prevent the same. To investigate this, some of the animals which were kept for histological studies were used for enzyme studies.

As can be seen from the table 3.36, aging `per se' has a significant effect on the basal activity of 5' nucleotidase and AchE in certain regions

Table 3.35.

Effect of Adrenalectomy and Cort. replacement on membrane bound enzymes in rat brain region membrane.

Enzyme	Group	OB	CBL	BS	HC	STR	CTX
Na+K+ATPase	ADX+H	8.4 ± 1.00	8.9 ± 0.60@@@	8.9 ± 0.45@@@	10.2 ± 0.50@@	8.4 ± 0.44@@	8.6 ± 0.28@@@
	ADX	8.3 ± 1.13	15.9 ± 1.10***	15.2 ± 0.76***	13.6 ± 0.75**	11.4 ± 0.72**	13.4 ± 0.22***
	SHAM	7.2 ± 0.57	9.8 ± 0.61	7.6 ± 0.58	10.5 ± 0.44	8.0 ± 0.50	5.6 ± 0.30
5'Nucleotidase	ADX+H	1.5 ± 0.40	2.3 ± 0.18	1.1 ± 0.15@	1.3 ± 0.10	1.6 ± 0.28	1.7 ± 0.27
	ADX	1.5 ± 0.18	2.3 ± 0.23	1.9 ± 0.24	1.4 ± 0.07	1.8 ± 0.24	1.2 ± 0.11
	SHAM	1.5 ± 0.27	2.0 ± 0.25	2.1 ± 0.32	1.8 ± 0.27	1.3 ± 0.13	1.2 ± 0.21
Acetylcholine esterase	ADX+H	3.1 ± 0.14	2.3 ± 0.13	8.4 ± 0.52	5.7 ± 0.60	13.7 ± 0.85	5.8 ± 1.00
	ADX	4.4 ± 0.24	3.2 ± 0.33	7.9 ± 0.84	7.0 ± 0.53	14.9 ± 1.80	4.6 ± 0.41
	SHAM	3.7 ± 0.44	3.2 ± 0.36	6.7 ± 0.40	6.9 ± 0.51	11.6 ± 1.24	5.7 ± 0.45

Values are expressed in mean ± S.E in enzyme unit. Number of observations are 6.

Level of significance by Student's't' test @ P<0.05; @@,**P< 0.01; @@@,***P<0.001 from ADX and SHAM respectively.

Enzyme unit- µmoles of pi liberated/mg protein/ hour for both Na+K+ATPase & 5'Nucleotidase.

µmoles of substrate hydrolysed/ mg protein/ hour for acetylcholine esterase.

OB- Olfactory bulb, CBL- Cerebellum, BS- Brain stem, HC- Hippocampus, STR- Striatum and CTX- Cortex.

ADX+H- Adrenalectomy and corticosterone replaced, ADX- Adrenalectomy, SHAM- Operated control.

ADRENALECTOMY & CORT. REPLACEMENT ON ENZYMES IN RAT BRAIN AFTER ADX AT 20.

Fig.3.8a Na+K+ATPase.

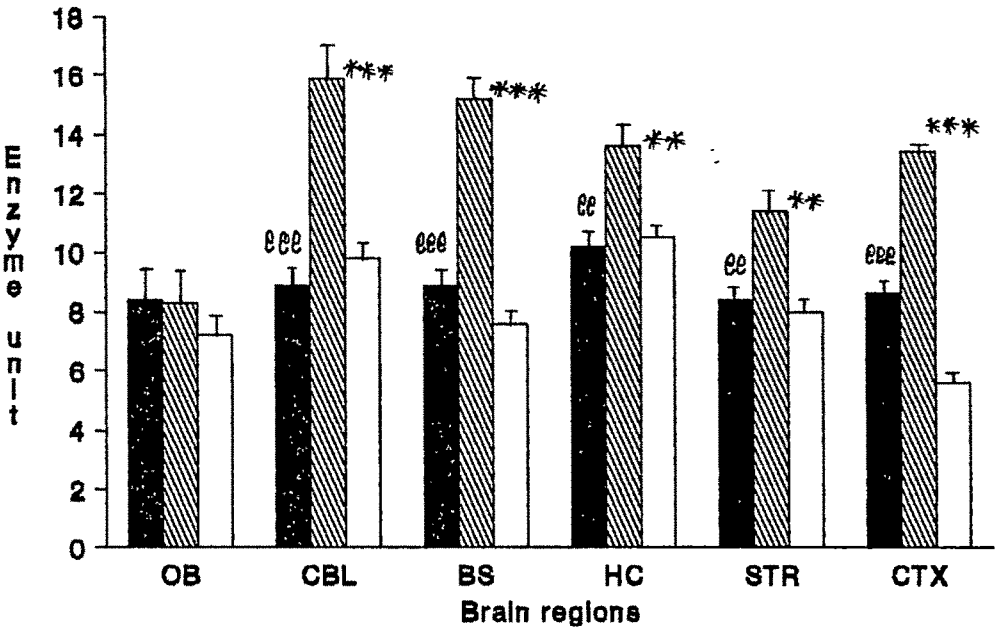


Fig.3.8b 5'Nucleotidase.

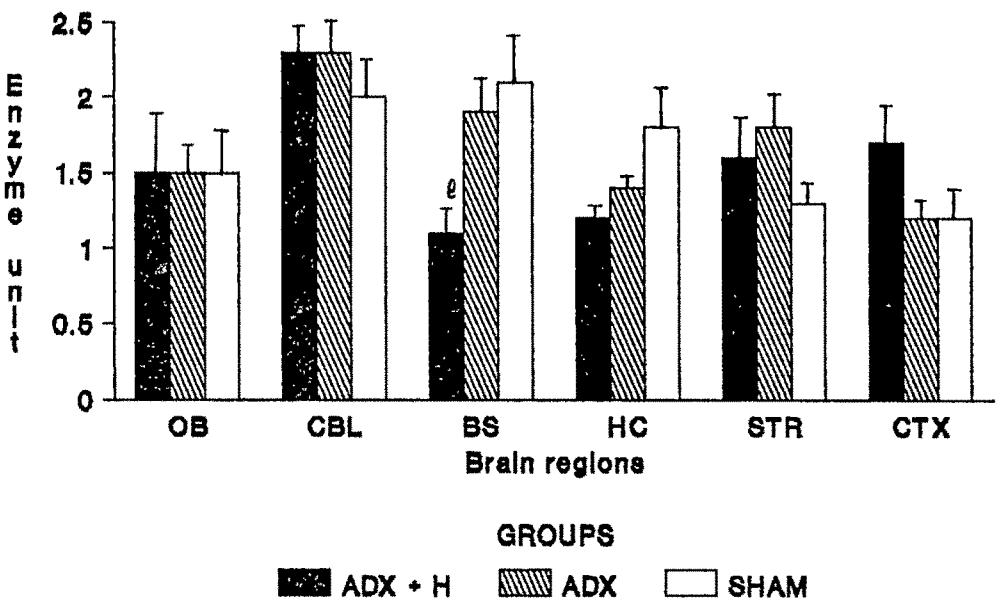
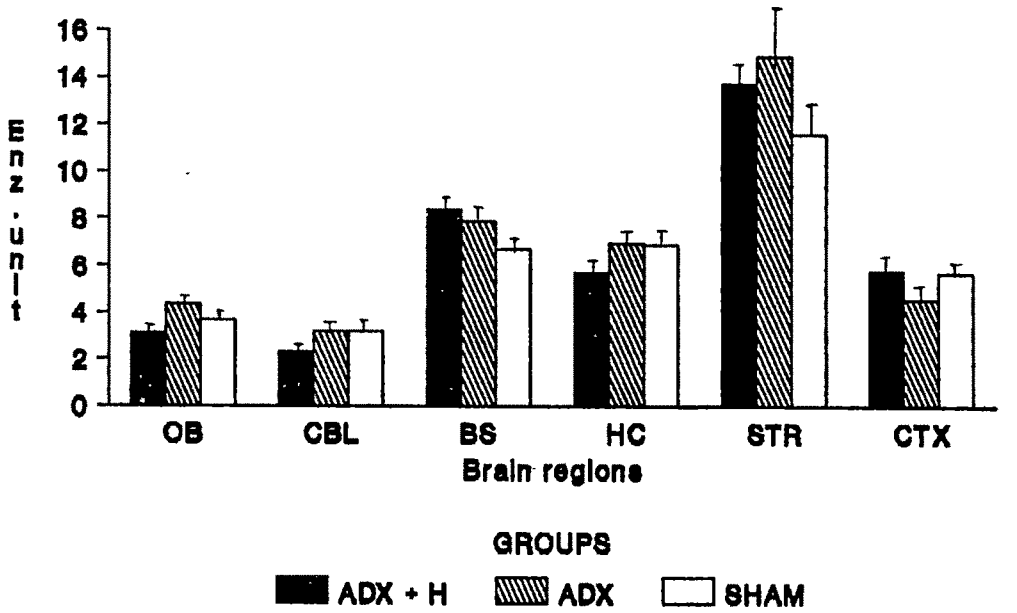


Fig. 3.8c Acetylcholine esterase.



Values are expressed in mean ± S.E in enzyme unit.

Number of observations are 6. Level of significance by Student's't' test @ P<0.05; @@,**P< 0.01; @@@,***P<0.001 from ADX and SHAM respectively.

Enzyme unit- µmoles of pi liberated/mg protein/ hour for both Na+K+ATPase & 5'Nucleotidase.

µmoles of substrate hydrolysed/ mg protein/ hour for AchE.

OB- Olfactory bulb, CBL- Cerebellum, BS- Brain stem, HC- Hippocampus, STR- Striatum and CTX- Cortex.

ADX+H- Adrenalectomy and corticosterone replaced, ADX- Adrenalectomy, SHAM- Operated control.

Table No 3.36.

Activities of membranes bound enzymes in developing rat brain region membranes.

Region	Age (Days)	Na+K+ATPase	5'Nucleotidase	AchE
OB	10	nd	nd	nd
	20	5.9 ± 1.26	1.6 ± 0.32	nd
	40	7.2 ± 0.57	1.5 ± 0.27	3.7 ± 0.40
	22 months	15.5 ± 2.70	1.9 ± 0.26	3.2 ± 0.26
CBL	10	3.8 ± 0.96	1.0 ± 0.08	2.8 ± 0.31
	20	12.7 ± 0.55	2.4 ± 0.47	nd
	40	9.8 ± 0.61	2.0 ± 0.25	3.2 ± 0.40
	22 months	9.5 ± 1.30	1.7 ± 0.40	2.1 ± 0.40
BS	10	3.1 ± 0.42	0.7 ± 0.03	4.9 ± 0.56
	20	8.1 ± 0.65	0.7 ± 0.06	nd
	40	7.6 ± 0.58	2.1 ± 0.32	6.7 ± 0.40
	22 months	12.3 ± 1.20	1.9 ± 0.40	4.1 ± 1.20
HC	10	2.7 ± 0.52	0.3 ± 0.03	2.6 ± 0.20
	20	10.2 ± 0.99	0.7 ± 0.10	nd
	40	10.5 ± 0.44	1.8 ± 0.27	6.9 ± 0.50
	22 months	14.4 ± 1.60	1.5 ± 0.30	3.9 ± 0.90*

Table continued.....

STR	10	2.1 ± 0.33	0.2 ± 0.03	6.6 ± 0.36
	20	6.2 ± 0.29	0.6 ± 0.04	nd
	40	8.0 ± 0.50	1.3 ± 0.13	11.6 ± 1.20
	22 months	13.2 ± 1.70	2.1 ± 0.50	8.2 ± 0.70*
CTX	10	2.7 ± 0.50	0.3 ± 0.04	2.1 ± 0.22
	20	2.3 ± 0.12	0.6 ± 0.08	nd
	40	5.6 ± 0.30	1.2 ± 0.21	5.7 ± 0.50
	22 months	13.6 ± 1.14	1.6 ± 0.30	2.9 ± 0.50**

Values are expressed in mean ± S.E in enzyme unit. Number of observations are 6.
nd- not determined. OB- Olfactory bulb, CBL- Cerebellum, BS- Brain stem,
HC- Hippocampus, STR- Striatum and CTX- Cortex.
Enzyme unit- µmoles of pi liberated/mg protein/ hour for both Na+K+ATPase
& 5'Nucleotidase.
µmoles of substrate hydrolysed/ mg protein/ hour for AchE.
Levels of significance by Student's `t' test * P<0.05; ** P<0.01
from 40 day old rats,AchE- Acetylcholine esterase.

which are consistent with reported studies on aging animals. However, in the present study the effect of aging on Na+K+ATPase were not evident. The animals were 20- 22 months old. As can be seen from the table 3.37, long term adrenalectomy could not prevent age related changes in the activity of any of these enzymes.

The question which arises are- Are these effects due to alteration in the enzyme activity or due to changes in the lipid environment.? Similarly are these effects related to receptor density of this hormone in different regions or is it related to other parameters such as binding of steroid to the membranes?. The other possibility is that of induction or repression at the genetic level.

It is very evident from the activity of these enzymes in the control group that the activity of Na+K+ATPase increases sharply between 10 and 20 days, and after that either remains constant or diminishes slightly partly because of the increase in other proteins. Only two regions- namely striatum and cortex showed significant increase in activity after 20 days. With regard to 5'NT, there appears to be a gradual increase from 10 to 20 days and beyond. Only cerebellum showed an attainment of adult value by 20 days itself. The ontogenic changes generally indicate increase in the accumulation of proteins as a consequence of increased protein synthesis.

On going through the literature on the effect of corticosteroids on Na+K+ATPase in the non- neuronal tissues such as kidney in which this enzyme is extensively studied, it is found that glucocorticoid increases the activity in adult renal tissue. Adrenalectomy decreases the same although replacement therapy brings back the activity to basal levels (Jorgensen,

Table: 3.37.

Effect of Adrenalectomy (5 months) on membrane bound enzymes in 22 months old rat brain regions membrane.

Enzyme	Group	OB	CBL	BS	HC	STR	CTX
Na+K+ATPase	ADX	27.1 ± 4.60	11.3 ± 0.80	15.4 ± 1.74	18.7 ± 2.30	15.5 ± 1.90	14.3 ± 0.75
	SHAM	15.5 ± 2.70	9.5 ± 1.30	12.3 ± 1.20	14.4 ± 1.60	13.2 ± 1.70	13.6 ± 1.14
5'Nucleotidase	ADX	2.2 ± 0.46	1.7 ± 0.40	1.4 ± 0.15	1.9 ± 0.40	2.0 ± 0.33	1.6 ± 0.30
	SHAM	1.9 ± 0.26	1.7 ± 0.40	1.9 ± 0.40	1.5 ± 0.30	2.1 ± 0.50	1.6 ± 0.30
Acetylcholine esterase	ADX	2.9 ± 0.40	2.0 ± 0.40	3.2 ± 1.00	5.0 ± 1.10	8.4 ± 0.60	4.0 ± 0.50
	SHAM	3.2 ± 0.26	2.1 ± 0.40	4.1 ± 1.20	3.9 ± 0.90	8.2 ± 0.70	2.9 ± 0.50

Values are expressed in mean ± S.E in enzyme unit. Number of observations are 6.

Enzyme unit- µmoles of pi liberated/mg protein/ hour for both Na+K+ATPase & 5'Nucleotidase.

µmoles of substrate hydrolysed/ mg protein/ hour for acetylcholine esterase.

Adrenalectomized age- 17months; Duration - 5months.

1969). Thus one can say that Na+K+ATPase is under the direct influence of glucocorticoid. The mechanism studied by Klein and Lo (1992) indicates an induction of the enzymes as judged by increase in mRNA levels of α- and β-subunit. On the other hand Manitius *et al.* (1968) showed that with methylprednisolone treatment, increase was not in the plasma membranes but perhaps in endoplasmic reticulum due to altered ionic concentration. But the recent report is fairly clear with better method and direct proof. *In vitro* studies by Deliconstantinos (1985) and a recent report by Orchinick *et al.* (1991) indicate a possibility of corticosterone binding with a very high affinity to the plasma membranes and this can increase Na+K+ATPase activity.

With regard to the nervous system ,very few studies have been reported with glucocorticoid administration or adrenalectomy in adult animals. Hydrocortisone injection for a week resulted in increased Na+K+ATPase activity in cerebral cortex, cerebellum and brain stem (Sadasividu *et al.*, 1977). Braughler and Hall (1981) reported that acute injection of methylprednisolone increased activity of Na+K+ATPase in synaptosomes. These authors have attributed this increase to decreased release of arachidonic acid which is normally released by the action of phospholipase A_2, since prednisolone inhibits the phospholipase A_2 activity. In contrast to these observations, Yamamoto (1978) has reported an inhibition of brain Na+K+ATPase by prednisolone, which has been attributed to its binding to either at oubain site or at K+ site. With regard to adrenalectomy, Gallagher and Glaser (1968) indicates no effect on the activity of Na+K+ATPase in rat brain.

There are few reports in younger animals. Stastny *et al.* (1971) have reported a significant increase in Na+K+ATPase of cerebral hemisphere of chick embryo on day 11 and 13. However, on continuation of the treatment further till the 19th day, the activity was brought back to normal but reduced further, indicating a bimodal action. Administration of methylprednisolone for 4 days to 24 day old rats increased the Na+K+ATPase activity in brain homogenates. In another study by the same group, administration of ACTH to young kittens increased the enzyme activity in cerebral cortex. This increase in enzyme activity is interpreted to occur not due to anticonvulsent action but due to alteration of ionic concentration. However the stimulation of Na+K+ATPase activity resulting in an increase in intraneuronal potassium provides a plausible mechanism for the anticonvulsent effect of steroids in infantile seizure (Huttenlocher and Amemiya, 1978). The studies on cell lines of neuronal and glial cells in culture indicates that the activity of Na+K+ATPase in both cell lines increased in presence of hydrocortisone for 4- 6 days. This study was not extended further to identify the cause of activation. This could be either a direct enzyme activation or a modulation of the transport mechanism.

Taking all these points into consideration,the following factors seem to influence Na+K+ATPase activity.

(1) The binding of corticosterone to synaptic plasma membranes (*in vitro*) results in an increase in Na+K+ATPase activity.

(2) Enzyme has absolute requirement for acidic phospholipids namely PS and PI. Alterations in these phospholipid fractions could affect their action.

(3) The activity decreases with the release of arachidonic acid. Higher

unsaturation also decreases the activity.

(4) The enzyme is also influenced by changes in ionic concentration which is altered due to hormone.

(5) During early development, the increase could be due to induction.

Results obtained on day 10 with corticosterone injection are consistent with the reported literature of increase in activity of Na+K+ATPase in younger animals. This increase was found to be uniform in all the regions but it does not explain whether this increase is due to change in receptor number or stages of development of different regions or concentration of phospholipids. As phospholipid concentration was not changed one could provide two possible explanations.

(1) Corticosterone 'per se' remain physiologically bound to membranes which alters membranes fluidity and thereby increases the Na+K+ATPase.

(2) It induces the enzymes in all regions, possibly by having a transcriptional factor in all the regions with minimum number of corticosterone receptor activation.

Since the studies have been carried out in the total membrane preparation rather than the synaptosomal fraction, it is likely that the effects observed are the additive effects of neuronal as well as glial cell membranes.

In contrast to 10 days of age, at 20 days the glucocorticoid resulted in significantly low activity in all the regions. At this age plasma corticosterone levels reaches an adult pattern as reported in the literature (Sapolsky and Meaney, 1986). Again, the pattern of change is identical in all the regions irrespective of the receptor density. The percentage change

in activity ranged from 30% to 50%. At 20 days of age only phospholipids which are known to be associated with Na+K+ATPase namely PS and PI were increased in hippocampus and striatum respectively whereas regions like brain stem had a decreased PE content. Cerebellum as well as cortex phospholipids were not altered, but cerebellar Na+K+ATPase activity was decreased whereas that of cortex remain unaltered. The cerebral cortex membranes showed extremely low activity which is generally not so when activity of homogenate is estimated. It was suspected that perhaps some factor in membrane preparation specifically affected the activity in the cortex. This is evident from the fact that the activity measurement done on synaptosomes of cortex and cerebellum are comparable with reported values of the controls (Sun and Sun, 1974) and even cortex had a significantly low activity of Na+K+ATPase. Thus corticosterone does affect the cerebral cortex as well.

The question is- why there is a significantly low activity at day 20 whereas at day 10 the activity is elevated?. This is consistent with other reports on whole brain or gross brain region (Sadasividu *et al.*, 1977; Huttenlocher and Amemiya, 1978). The possible reason for such an effect at 20 days can be discussed along with studies on adrenalectomized and hormone replaced animals, where it was observed that adrenalectomy significantly increased the activity of Na+K+ATPase in all the regions except olfactory bulb, and hormone replacement brought back the activity to normal but did not further decrease.

The results were quite intriguing and rather unexpected. Obviously, it is very clear that the permissive affect does not specifically

affect the hippocampus alone as cerebellum and brain stem are not known to have type I receptors (Ahima & Harlan, 1990). Similarly irrespective of the number of receptors present, all regions including cerebellum showed altered Na+K+ATPase activity with either corticosterone treatment or with ADX. Thus factor(s) other than receptor number is/are possibly associated with the altered activity in response to change in the corticosterone levels. It cannot be explained on the basis of changes in the concentration of phospholipid fractions, as adrenalectomy has no effect on the same. In 20 day old rats the phospholipids which are known to influence Na+K+ATPase activity such as PS and PI were altered only in hippocampus and striatum with corticosterone treatment but the enzyme is affected in other regions as well.

The preliminary work on fatty acid composition of various fractions of phospholipids of the synaptosomes from cerebral cortex indicate increased presence of unsaturated fatty acids especially 20:4 which is known to be an inhibitory factor for Na+K+ATPase (Chan *et al.*, 1983). Sun and Sun (1974) had shown that fatty acid deficiency caused an increase in the synaptosomal membrane Na+K+ATPase activity. They showed that a decrease in 20:4 in PI as well as PE fractions and 22:6 in PS suggesting that the increase in the Na+K+ATPase in fatty acid deficient rats is due to the decrease of these polyunsaturated fatty acid. Since an increase in polyunsaturated fatty acids in PI, PS and PE was observed in the present study, it indicates that the fatty acid profile of these phospholipids are responsible for the inhibition of Na+K+ATPase in hormone treated rats. Such an experiment was not done in the ADX rats one can expect an opposite action. Adrenalectomy of

aged rats also didn't show any alteration in Na+K+ATPase indicating that the change in Na+K+ATPase is age dependent but not hormone dependent at old age. However more systematic studies at the kinetics and molecular levels are needed to explain this phenomenon. One possible reason of altered Na+K+ATPase could be that adrenalectomy increased susceptibility to seizures and an increase in Na+K+ATPase could be an adaptive response to perhaps prevent deleterious effects.

With regard to 5' nucleotidase, results of the enzyme activity after chronic corticosterone treatment, at 10 days, an increase in activity was observed in cerebellum, hippocampus, striatum and cerebral cortex as compared to control. At 20 days the enzyme activity was decreased in olfactory bulb and cerebellum and increase in striatum without any change in the rest of the region membranes. Adrenalectomy showed no change in enzyme activity either in ADX or corticosterone replaced rats in any of the region, except in the brain stem membrane where a decrease in activity was observed. The results obtained after subcellular fractionation of cerebrum and cerebellum showed that 5'NT activity was found to be decreased in synaptosomes of both these regions. Decrease in activity was also found in myelin isolated from cerebellum in corticosterone treated rats as compared to control at 20 day but not in cortex.

There are practically no reports of the effects of glucocorticoid on 5'nucleotidase except a recent report on the effects of corticosterone in neuron enriched cultures. The study indicated that corticosterone stimulated the enzyme activity in culture cells incubated with various concentration of corticosterone for various durations. The increase was concentration

dependent. However the mechanism of increase is not discussed (Pieringer *et al.*, 1991).

Smith *et al* (1980) have studied the effect of hypo and hyper thyroidism on 5'nucleotidase from forebrain and cerebellar membranes during development. The results indicate that thyroxine deficiency decreases the activity of 5' nucleotidase in both fore brain and cerebellum. Hyperthyroidism increases its activity in fore brain but not in cerebellum. The effects were pronounced at day 20 of postnatal age. Again the reason for such a change has not been discussed.

The other hormone which also seems to be influencing 5' nucleotidase in liver plasma membrane is the growth hormone as judged by hypophysectomy and subsequent replacement of the growth hormone. The study indicates that hypophysectomy decreases 5' nucleotidase activity to 75% as compared to control. In this study even Na+K+ATPase was found to be altered (Rubin *et al.*, 1973).

In the present study at 10 days of postnatal age, 5' nucleotidase activity was significantly elevated with corticosterone administration in almost all regions. This increase in activity is consistent with the report on neuron enriched culture studied *in vitro* (Pieringer *et al.*, 1991).

At day 20 however, there was regional variations- the striatum had significantly higher whereas cerebellum and olfactory bulb showed lower activity. The cerebellar synaptosomal and myelin membranes both showed a decrease whereas cortical synaptsomes were not affected. Adrenalectomy had no effect except in brain stem membranes of ADX rats. The effect cannot be explained on the basis of lipids or receptor or age. Perhaps some factor in

the membranes is responsible, since hormone replacement group seem to have no effect. It can be assumed that hormone treatment in adult does not influence 5' nucleotidase as can be seen from adrenalectomized aged rats.

With regard to AchE it is very obvious from the results that it is a peripheral protein and hence is not influenced by hormone status as lipids do not play an important role in maintaining activity. However, discontinuity in Arrhenius plot of this enzyme from brain membranes indicate that the kinetics of this enzyme can perhaps be influenced by lipids (Gorgani and Meisami, 1979). AchE doesn't showed any effect and are consistent with the reported study of Bau and Vernadakis, (1982). Even aged rats showed no effect in AchE activity with adrenalectomy, although, a decrease in activity was evident with age.

In conclusion, this study on membrane bound enzymes clearly indicates that the activity of the two enzymes namely Na+K+ATPase and 5' nucleotidase are influenced by glucocorticoid status. The Na+K+ATPase is responsive to both elevated as well as basal levels of hormone. 5' nucleotidase is influenced only with elevated levels and that too at younger ages only. In addition, the sensitivity to corticosterone status vary with age as enzymes of aged animals didn't show any change.

There are practically no reports on the mechanism by which alteration in these enzymes takes place under the influence of hormone status. In the kidney increase in Na+K+ATPase has been implicated in the increased protein synthesis as judged by increased mRNA levels of α- and β- subunit of Na+K+ATPase (Klein and Lo, 1992). Such reports on brain enzymes are yet to come. In the present study the effects were possibly because of in-

creased protein at 10 days of age as the phospholipids were not altered in any of the regions. It is known that Corticosterone `per se' has some effect because *in vitro* addition of corticosterone in the membrane preparation alters the membrane fluidity. On the other hand, at 20 days of age effects are not only reversal of 10 day but it was unexpected. This perhaps could be due to altered membrane phospholipid composition or changes at the genomic level. The study can further be extended by checking the Arrhenius plot as well as kinetic properties to get some more insight in to the mechanism. But the present study has been limited only to identify whether or not the membrane bound enzymes are also influenced by glucocorticoids.

Experiment IVb:

Effects on synaptosomal membrane fluidity in control and corticosterone treated animals:

It has been known that fluidity of the cell membranes changes with temperature and composition. Lipids may change from solid to a fluid state as they are exposed to a higher temperature and vice versa. Phospholipids in the membranes change from crystalline state to liquid crystal state. These changes are mainly influenced by components like cholesterol, cholesterol/phospholipids ratio, degree of unsaturation and chain length of fatty acid of the phospholipids (Shinitzky & Barenholz, 1978; Nelson 1980). Cholesterol molecule may fit between the fatty acid chain and prevent the formation of either true liquid crystal or crystalline -gel configuration (Kandutsch and Chen, 1977).

It has been seen that with corticosterone treatment there was an

alteration in composition of phospholipids or the ^{32}P incorporation various in it, alteration in the fatty acid composition in cortical synaptosomes, and changes in the membrane bound enzyme of synaptosomes. It appears that these changes would result into a significant alteration in membrane function especially of the synaptosomal plasma membranes which can be assessed by the membrane fluidity. Steady state fluorescence polarization technique provides a variable information concerning the dynamic properties of cellular membranes. Relative difference in the polarization value can accurately reflect changes in the physical properties of these membranes.

In the present study SPM from cerebral cortex were prepared and subjected for steady state fluorescence polarization (P) of the apolar probe 1,6- diphenyl -1,3,5-hexatriene (DPH) used for membrane fluidity. Details of the same are discussed in materials and methods.

As can be seen from the table 3.39 the anisotropy of the SPM at different temperature seems to be altered in the control as evident from 37^{o}C and 25 ^{o}C but at 0^{o}C the values were significantly higher. The anisotropy of the SPM at 21 day seem to be very similar to the reported values (Table 3.38). The SPM from corticosterone treated rats indicates that microviscocity of the SPM was more or less similar to the control. If one looks at the values of (P) at 37^{o}C or at 25^{o}C, rather there appears to be a significant increase in the microviscosity at 0^{o}C. This difference between experimental and control groups at 0^{o}C indicated that corticosterone increases microviscosity and hence membranes are less fluid. However, the same membranes at 25^{o}C and 37^{o}C do not show any difference from that of the control. Thus the membrane fluidity remains unaltered due to 3 days of

Table: 3.38.

Comparison of anisotropy and microviscosity of the biomembranes.

Anisotropy (r_s)	0°C	25°C	37°C
Present study (SPM)	0.208	0.187	0.188
Synaptosomes*	---	0.220	0.190
Microviscosity (P)			
Present study (SPM)	2.92	2.58	2.59
Dog SPM**	---	---	1.156
Leukemia cells @@	---	---	0.188
Liposomes !@	---	5.40	2.900
HeLa cells !@*	---	---	0.245

* Viani *et al.* (1991); ** Delicontsantinos (1985); @@ Kiss *et al.* 1990
!@ Shinitzky & Inbar (1975); !@* Johnston & Melnykovych (1980).

Table: 3.39.

Effect of Corticosterone in anisotropy and microviscocity of synaptomal plasma membranes from cerebral cortex at day 20 rat.

Anisotropy (r_s)	0°C	25°C	37°C
Cort.	0.218 ± 0.005	0.190 ± 0.008	0.182 ± 0.002
Control	0.208 ± 0.008	0.187 ± 0.004	0.188 ± 0.003
Microviscosity (P)			
Cort.	3.430 ± 0.080**	2.670 ± 0.255	2.410 ± 0.068
Control	2.920 ± 0.036	2.580 ± 0.130	2.590 ± 0.086

Levels of significance by Student's 't' test ** P <0.01 from control.

Number of observations are 3.Values are expressed in mean ± S.E.

corticosterone treatment.

As mentioned earlier membrane fluidity is a complex term which combines statical and dynamic factors of lipid composition. Except for one *in vitro* study on dog synaptosomal membranes with cortisol treatment, there are no reports regarding effects of *in vivo* hormonal effect on the brain membranes fluidity. On the other hand several lines of studies in humans, who are exposed to elevated level of glucocorticoid for a therapeutic purpose have been reported. Few studies on cell cultures incubated with different levels and time periods of synthetic or natural glucocorticoid have also been reported.

The effect of glucocorticoid can be divided in two classes The studies on membranes incubated with glucocorticoid for a short period (15-60 mins.). Such studies would imply the direct influence of the glucocorticoid binding to membranes and hence influencing membrane fluidity. On the other hand studies on cells/ isolated cell membranes from patients /animals treated with glucocorticoid for various periods of time indicate a possible alteration in the membrane either only due to genomic effect (indirect) or combined with the binding of the steroid to the membrane as well. The latter possibility is remote as most studies involved extensive washing to remove physical association.

The reports available so far indicates that the *in vitro* or short term incubation with glucocorticoid effects vary with the types of cell. For example, in BRM as well as CEM leukemia cells, a T lymphoid leukemia cells lines, show a dose dependent significant increase in fluorocence polarization (P) by incubating them at 37^{O} C with dexamethasone (Kiss *et al.*,

1990). On the other hand *in vitro* incubation of erythrocyte ghost cells with cortisol indicated an increase in membrane fluidity (Massa *et al.*, 1975). Similarly the *in vitro* incubation of cortisol in the dog synaptosomal membranes has the same effect (Deliconstantinos, 1985).

In contrast to *in vitro* results, the *in vivo* effects or incubation of cells with glucocorticoid for a longer duration may have different effects. HeLa cells on incubation for 24 hours with dexamethasone indicated a significant increase in membrane fluidity. This was attributed to decrease in cholesterol synthesis as dexamethasone inhibits this synthesis, which is very similar to 25- hydroxy cholesterol, a known inhibitor for cholesterol synthesis (Johnston and Melnykovych, 1980). On the other hand studies on leukemia patients, who were on chronic cortisol treatment, have circulating lekeumia cells with decreased fluidity (Ben-Bassat *et al.*, 1977). Similarly Kiss and colleagues have shown that even longer period of exposure to dexamethasone of the cultures also have decreased fluidity. The membrane composition seems to be an important factor for the *in vitro* effects of the glucocorticoids. These responses can have variable effects depending on several factors including the regulation of transcriptional factors and metabolic state of the cell.

The present study seems to be first of its kind with *in vivo* effects studied in the SPM membranes of corticosterone treated rats. The results indicate that the SPM of treated animals has undergone changes in composition and these changes, as mentioned earlier, would have altered the membrane composition in such a way that SPM at physiological temperature maintains its fluidity. Thus the membrane is able to carry out the function

in as normal fashion as possible. This is perhaps an adaptive mechanism in response to stress.

Experiment V

Histology of the Hippocampus - Comparative study of young, young ADX, Aged and aged ADX animals:

During 1978- 1981, Landfield group and McEwen-Sapolsky groups independently have attempted to correlate brain functional deficit such as in synaptic transmission and alteration of neuroendocrine control mechanism as an important pacemaker of the mammalian aging process. Since then several reports have appeared relating this aspect. The hippocampus is the target of investigation because of the following reasons, (1) functional deficit in aging correlates with hippocampal damage, (2) there is selective loss of hippocampal cell with aging (Landfield *et al.*, 1978; Sapolsky *et al.*, 1985), (3) increased gliosis in response to neuronal death (Landfield *et al.*, 1978; 1981), (4) aging is associated with increased circulating levels of glucocorticoids, (5) this region is also the target of glucocorticoid as it has the highest number of glucocorticoid receptor which diminishes with aging (Sapolsky *et al.*, 1983a), (6) hippocampus is known to have inhibitory influence on hypothalamic nuclei which release CRF (Moberg *et al.*, 1970) and thus regulate the circulating levels through feed-back mechanism, (7) aging is also associated with persistent high levels of circulating glucocorticoid even after removal of stress in rats (Sapolsky *et al.*, 1983b). All these observations led Sapolsky and McEwen to put forward the hypothesis implying feed-back cascade to explain the hippocampal cell death (Sapolsky *et al.*, 1986). Several reports have been appeared for testing the

hypothesis.

It is observed that type I and type II receptors of glucocorticoid are highest in hippocampus (McEwen, 1982) and altered morphology of hippocampal cell has been noticed with aging (Landfield *et al.*, 1981). It has been proposed that decrease in glucocorticoid receptors in these cells make them ineffective in functioning i.e, their inhibitory influence on CRF release is diminished hence persistent release of CRF leading to increase in circulating glucocorticoid. This deficit in negative feed-back control seems to be the major cause in aged male rats for having a delay in terminating corticosterone secretion at the end of the stress (Sapolsky *et al.*, 1984a). However, it is not yet clear the cause-effect relationship between loss of glucocorticoid receptors and hippocampal cell death with that of persistent elevation of circulating levels of GC and functional damage with aging.

Normally, a mechanism of down regulation operates for all hormones and neurotransmitters. Corticosterone receptors are known to be down regulated in the event of exogeneously administrated GC or stress-induced GC elevation. This phenomenon of down regulation seems to be anatomically specific only in the hippocampus and the amygdala and not in the hypothalamus or pituitary. This effect is reversible. Thus protective mechanism operates in the hippocampus at least in young adult animals of 3-5 months of age (Sapolsky *et al.*, 1984). Since elevated levels of GC accelerates aging, does this mean that there is a failure in the adaptive mechanism?. No attempt has been made to answer this question.

Corticosteroids have potent long lasting anti-inflammatory actions

as it has been used clinically for therapeutic purpose. Since excess glucocorticoid can have deleterious effects and could result in lasting effects if used in the early stages of brain development, scientists have been interested in identifying the early exposure to excess of corticosterone on hippocampal cells. Foetal Rhesus monkeys exposed to high levels of glucocorticoid - dexamethasone through maternal circulation and brain tissue of such foetus when subjected on light and electron microscopic observations, revealed decreased number of pyramidal neurons in the hippocampal CA region and granular neurons of the dentate gyrus. These effects were evident even after 30 days of post insults recovery indicating that both granule cells and pyramidal cells are sensitive to excessive circulation of glucocorticoids (Uno *et al.*, 1990). On the other hand, Bohn (1980) observed granule cells genesis of hippocampus at various postnatal age after hydrocortisone injection. This study revealed that the granule cell loss was transient and there was full recovery of granule cells by postnatal day 60. Such differential effects in recovery may be due to difference in maturation or time required for recovery. Monkeys are more mature than rats with regard to brain development after birth. It is likely that these two different result indicate rather development stage. In this connection interesting observation made by Sapolsky and his colleagues (Meaney *et al.*, 1988) indicates that neonatal handling retards age related impairments. The effects of neonatal handling resulted in the appearance of greater number of glucocorticoid receptors on hippocampal cells. Subsequently when these animals aged, they possessed relatively higher number of total receptors than those which were not handled and hence had a less age related effects.

This is also indicate that hippocampal cells have varying adaptability with age and the effects of elevated corticosterone would depend on previous exposure.

In the present study a very similar phenomenon of differential sensitivity of the biochemical parameters was observed in young and aged animals as for example Na+K+ATPase activity. It was observed in young adult animals that adrenalectomy resulted an increase in Na+K+ATPase activity but same was not the case with aged animals. Therefore it was thought to examine whether the adrenalectomy at different ages will have differential effects on different cell types of hippocampus.

General experimental design used by various groups to test this hypothesis has two ways. Either administration of excessive glucocorticoid to identify whether excess glucocorticoids accelerates the age related changes in the hippocampus or aging animals were adrenalectomized and followed up to compare with the age matched non-ADX animals to see whether the ADX prevented the aging process. In the present study the latter approach was selected. Animals were adrenalectomized at 20 days of postnatal age, at 2 months (60 days) and at 17 months of age and kept for five months. The animals were killed at the end of the experiment and examined histologically. The details of which are given in the materials and methods. The type and the connectivity of the hippocampal region and names of different areas are given in fig. 3.9.

Due to technical problems the samples of 20 day old adrenalectomized animals could not be processed and hence not examined. In the present study only the comparison of young adult (2+5= 7 months) and aged (17+5= 22

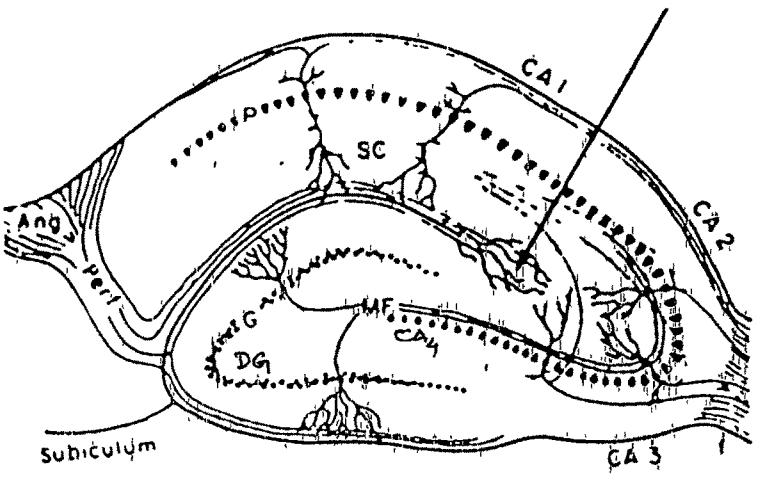


Fig. 3.9. Schematic representation of a coronal slice from hippocampus of a rodent. DG- Dentate gyrus. (Taken from Landfield *et al.* 1978).

months) animals has been carried out. Among the young and aged group the only conspicuous difference was in the distance between DG cell layer and CA_1 layer, which was found to be greater in aged animals (Fig. 3. 10). This can be correlated to the fact that as the age advances, the brain size increases with an increase in myelin.

Dentate gyri: (Fig. 3.11)

This region is known for the presence of granule cells and have high number of type I receptors. It has been shown first by Sloviter *et al.* (1989) that adrenalectomy resulted in a selective loss of granule cells in the dentate gyrus in adult rats after 4 months. The comparison between DG of young and aged sham showed a reduction in cell number, increase in cell size, diffused nucleus and poor cytoplasmic staining. This may be due to the loss of Nissl granules, as cresyl violet is specific stain for Nissl substance. In both young and aged ADX animals the granule cells showed a decreased staining with respect to controls. The number of glial cells were increased in both ADX groups. The increased glial cell is an index of neuronal cell loss. The glial reactivity was found to be higher in young ADX animals than aged ADX animals. There was no change in size of the cells after ADX in young animals.

Comparison of young and aged ADX reveals that in young ADX cell degeneration was higher than in aged animals. The granule cells of dentate gyrus which are not only having highest number of corticosterone type I receptors but also have common have type II receptors. Earlier reports have shown granule cell death in dentate gyrus with short- term and long- term adrenalectomy (Sloviter *et al.*, 1989; Gould *et al.*, 1990). These studies

Explanation for Figures

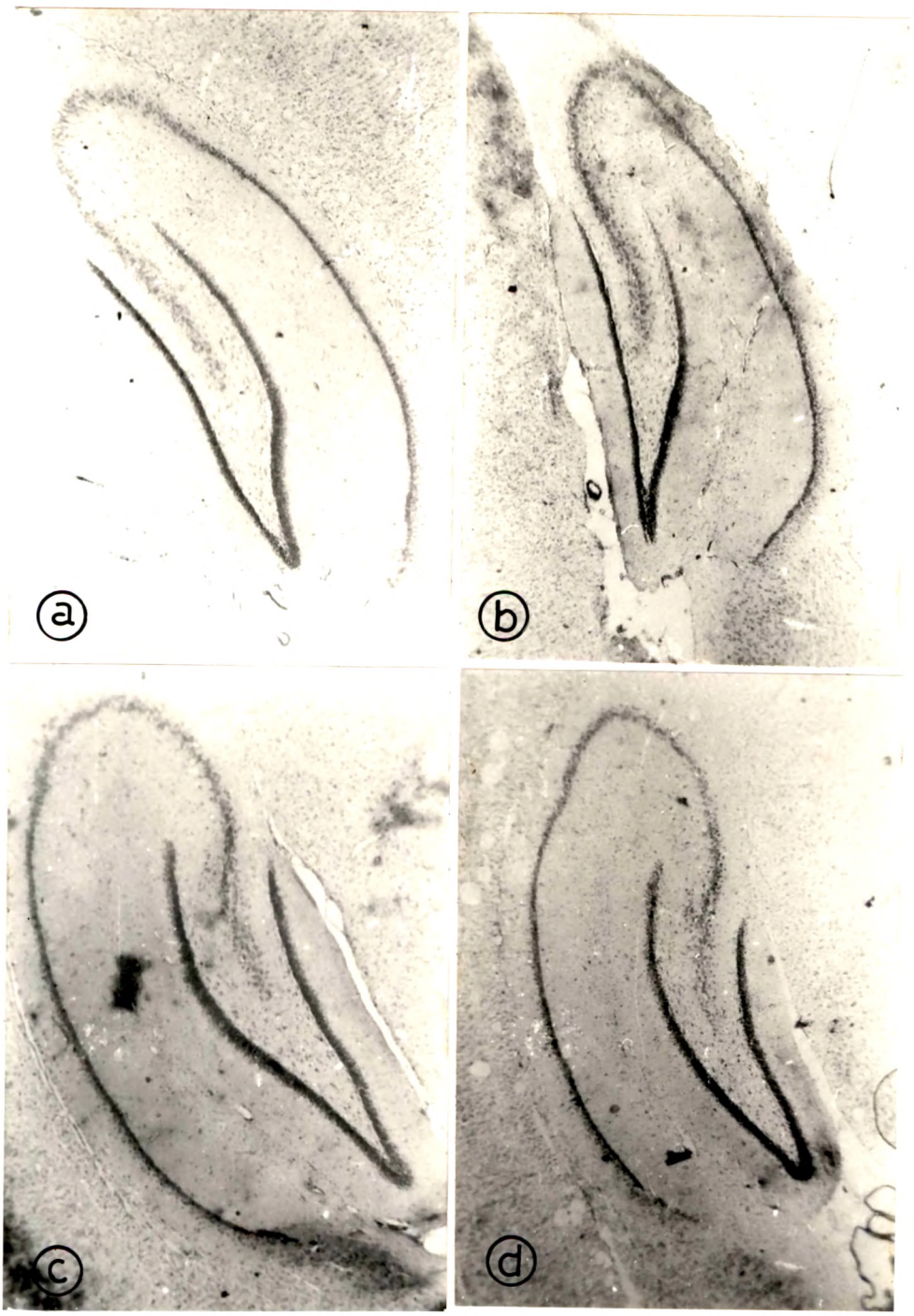
Fig 3.10. Low power photomicrographs of hippocampus in rats of different treatment groups. All X 28.

a. Young sham

b. Young ADX. The dentate gyrus shows cell degeneration.

c. Aging animals

d. Aging ADX animals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Explanation for Figures

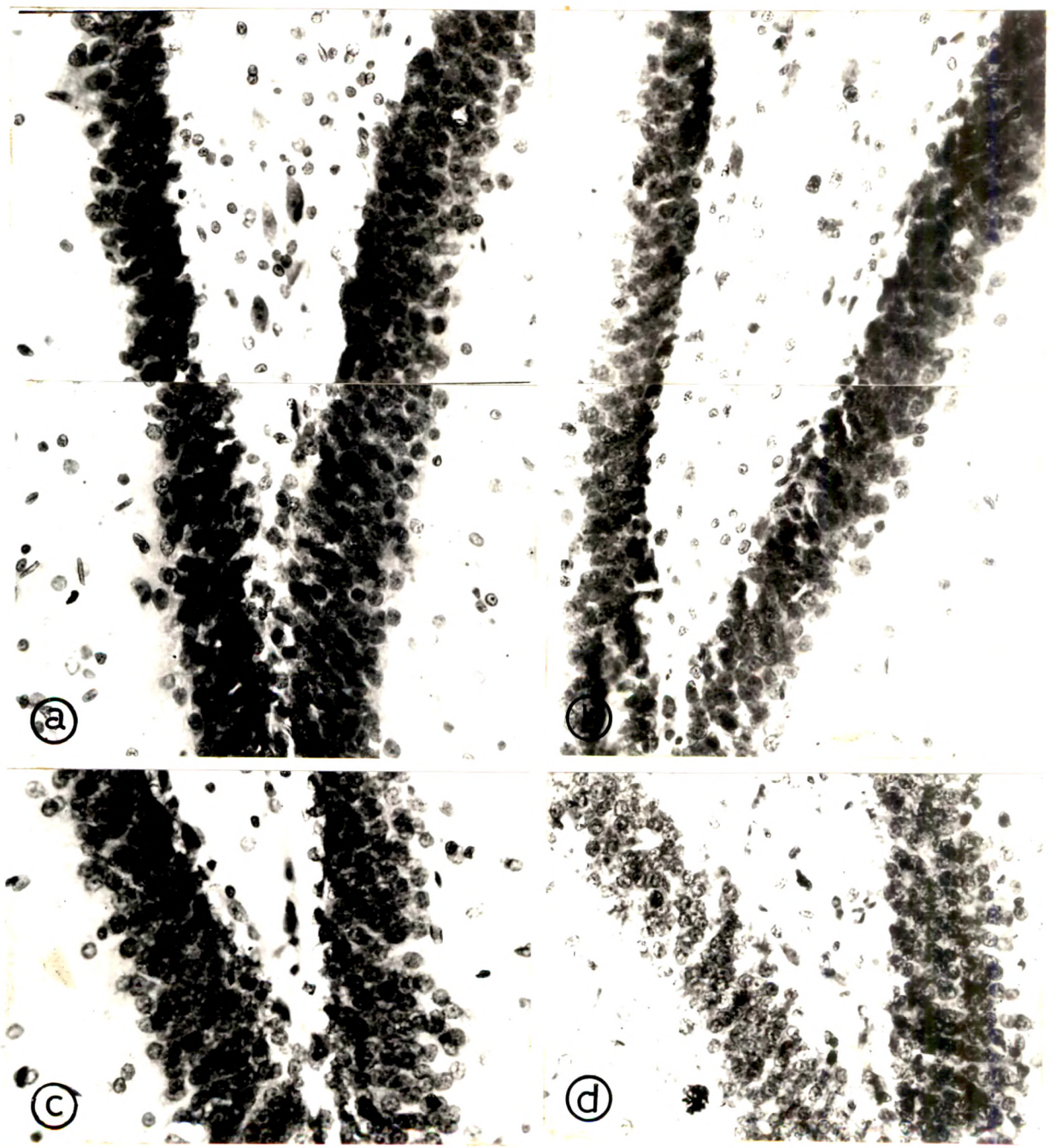
Fig 3.11. Dentate Gyrus in different treatment groups. All X 270.

a. Young sham,the granule cells are highly packed and intensely stained.

b. Young ADX. The cells show degeneration, the number glial cells are increased.

c. Aging animals. The basophilia of the cells are reduced, cell size is reduced and nucleus is diffused.

d. Aging ADX. The basophilia is reduced (compare with c).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indicated that basal levels of corticosterone is essential for granule cells survival. However, the survival of these cells can be observed by administration of aldosterone (Woolley *et al.*, 1990). This suggests that stimulation of type I receptor is essential for the survival of these cells. Thus it appears that granule cells are the sensors of the levels of circulating corticosterone. In this connection Sloviter *et al.*(1989) observed long-term adrenalectomy induced granule cell loss in young ADX rats. In the present study also the cell loss was observed but not as high as reported by the above group. One possible reason of this difference could be that the animals of the reported literature were purchased just before study while in the present case animals were obtained from our stock colony and reared by the investigator and had been frequently handled. Handling seem to have beneficial effect on hippocampal cell as suggested by Meaney *et al* (1988). The second possibility is that the strain used in the present study was Charles Foster where as in Sloviter's study it was Long-Evans.

CA_4 area: (fig. 3.12)

The CA_4 area has predominent number of pyramidal cells. Comparison between young and aged sham animal indicates that the cells in aged animal were larger but the morphology of the cells were altered. This might indicate less RNA content and reduction in Nissl granules. The number of microglial cells was found to be relatively increased after ADX in both young and aged animals. In young animals ADX does not seem to affect the cell morphology. There appears to be greater reduction in the number of pyramidal cells, decrease in cell size and reduction in staining indicates the sign of neuronal damage with aged ADX.

Explanation for Figures

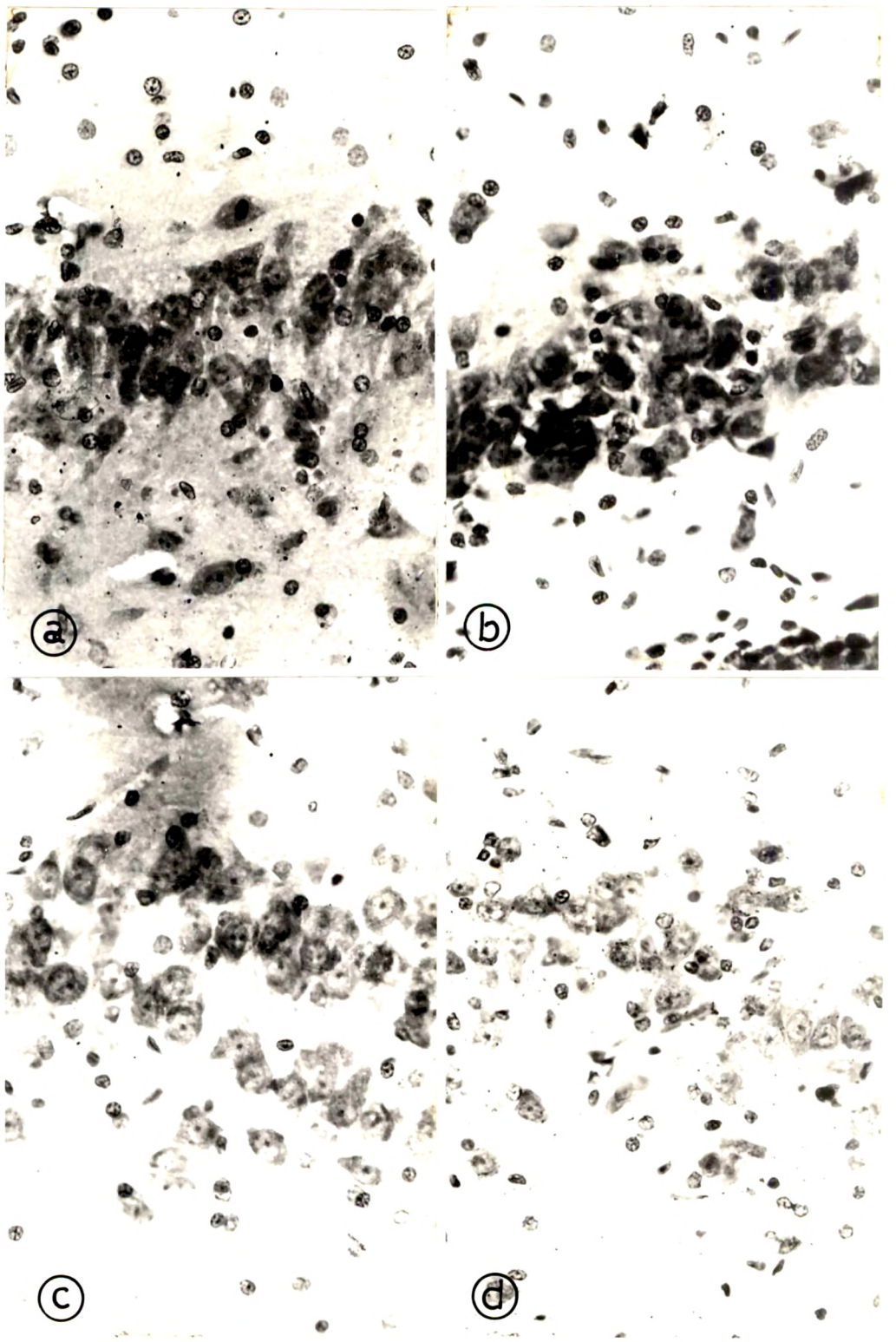
Fig 3.12. CA_4 area of the hippocampus. All X 270.

a. Young sham. The pyramidal cells are highly basophilic

b. Young ADX.

c. Aging animals. The cell size is increased but the basophilia is reduced compared to young animals.

d. Aging ADX. Adrenalectomy in aged animals decreased the number of pyramidal cells in this area. The cells are shrunken and lost basophilia, an index of neuronal damage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CA_3 area: (fig. 3.13)

This area is having typical pyramidal cells. Previous reports indicate that the cells in this region are extremely sensitive to elevated levels of corticosterone. The granule cells from DG make contact with the apical dendrite of the CA_3 area and elevated corticosterone diminishes the dendritic length and size of the apical region that eventually result in death of CA_3 cells due to persistent excitatory impulse (Woolley *et al.*, 1991). The mechanism of pyramidal cell death due to excitotoxicity mechanism has been demonstrated using *in vitro* model system by Sapolsky (1988). It appears to be mediated through glutamate receptors namely NMDA and Kainate type (Aramanini *et al.*, 1990; Behrens *et al.*, 1992). The effects are due to the depletion of energy source since corticosterone is known to reduce glucose transport in the hippocampal neurons as well as astrocytes (Virgin *et al.*, 1991). Thus elevated levels of corticosterone would make cells more susceptible to hypoglycemia or hypoxic condition as well as other metabolic stress (Tombaugh *et al.*, 1992).

The present study revealed that in young animals adrenalectomy does not induce any noticeable change in pyramidal cells. In aging animals, ADX decreased cell size while basophilia was found to be increased with a change in cell shape. Comparing young and aged animals, in aged animals, the cells were round and lost basophilia.

Current hypothesis suggest that CA_3 pyramidal cells are target cells of age related increase in glucocorticoid, resulting in hyperexcitability and eventually cell death. Therefore, ADX should prevent age related cell death. Unfortunately, no studies have been undertaken so far

Explanation for Figures

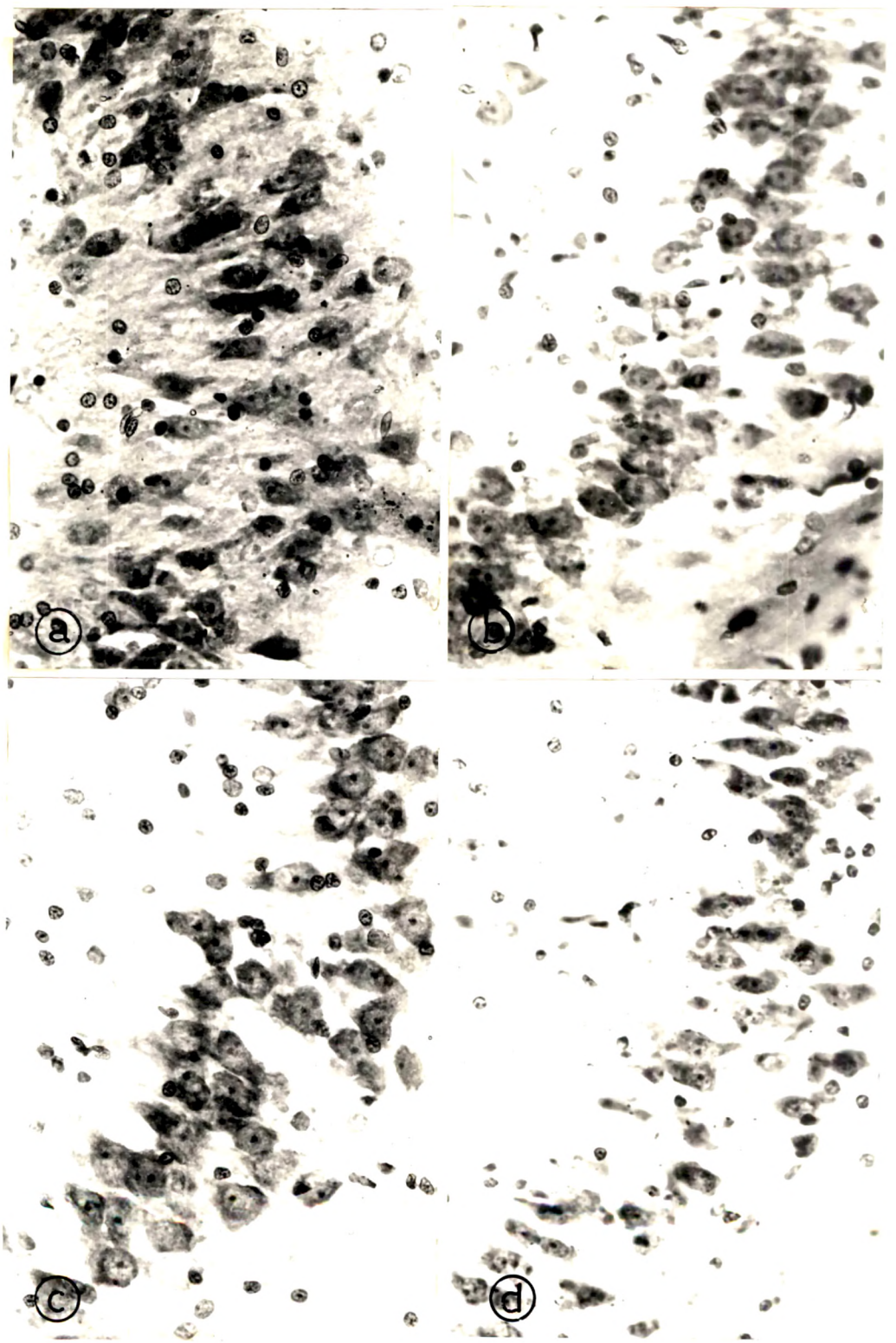
Fig 3.13. CA_3 area of the hippocampus. All X 270.

a. Young sham

b. Young ADX. Adrenalectomy do not alter the morphology of the cells.

c. Aging animals. The cell size is increase during aging but the basophilia is decreased.

d. Aging ADX. Adrenalectomy in aging animals induces cell degeneration.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comparing the effects of ADX in CA_3 hippocampal cells of young and aging animals. The present study indicates that no appreciable cell loss occurs in pyramidal cells of CA_3 region with aging. Young ADX had no effect while ADX aged showed change in cell size and increased basophilia. Perhaps higher basophilia indicates better cell status compared to age matched controls.

CA_2 area: (fig. 3.14)

The cell size was not found to be changed with aging. ADX in young animals slightly increased the cell size and their number. In aging animals, ADX caused a reduction in cell size and loss of basophilia.

CA_1 area: (fig. 3.15)

During aging, the size of CA_1 cells was found to be increased while the cells lost their basophilia. In young ADX animals an increase in cell size and basophilia was observed. In aged ADX animals there was no noticeable change in cell size or basophilia.

The table 3.40 depicts the size of the cells in different area of the hippocampai recorded in various treatment groups. In young and aged controls, the size of the granule cells in the DG has increased with age while there was no change in CA_1 pyramidal cells. The size in young ADX was not significantly affected except in CA_1 area whereas in aged animals, ADX resulted in a decrease in cell size practically in all areas except in CA_1. Thus there are some apparent difference in response to ADX in young and aged animals. This indicates that although corticosterone has been implied in aging process, the sensitivity to depleted levels of corticosterone seem to be significantly higher in aged animals compared to young ADX.

Explanation for Figures

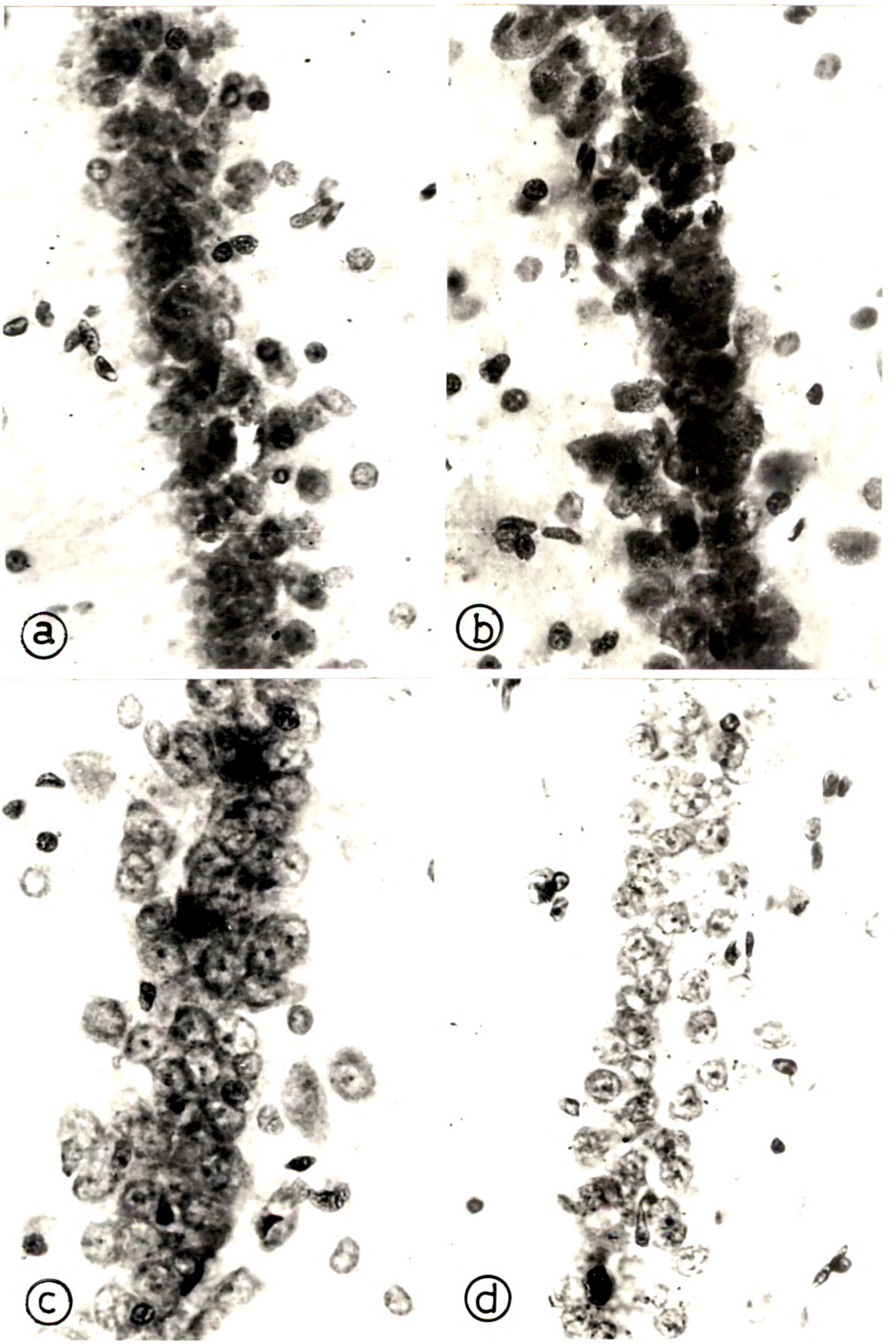
Fig 3.14. CA_2 of hippocampus. All X 470.

a. Young sham.

b. Young ADX. Adrenalectomy had a positive influence on CA_2 cells. The cell size and number are increased.

c. Aging animals. During aging the cells loose their basophilia.

d. Aging ADX. Adrenalectomy does not have any positive influence on CA_2 cell.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Explanation for Figures

Fig 3.15. CA_1 area of hippocampus. All X 470.

a. Young sham

b. Young ADX. Adrenalectomy increases the cell size and basophilia.

c. Aging animals

d. Aging ADX. Adrenalectomy does not have any effect on CA_1 cells.

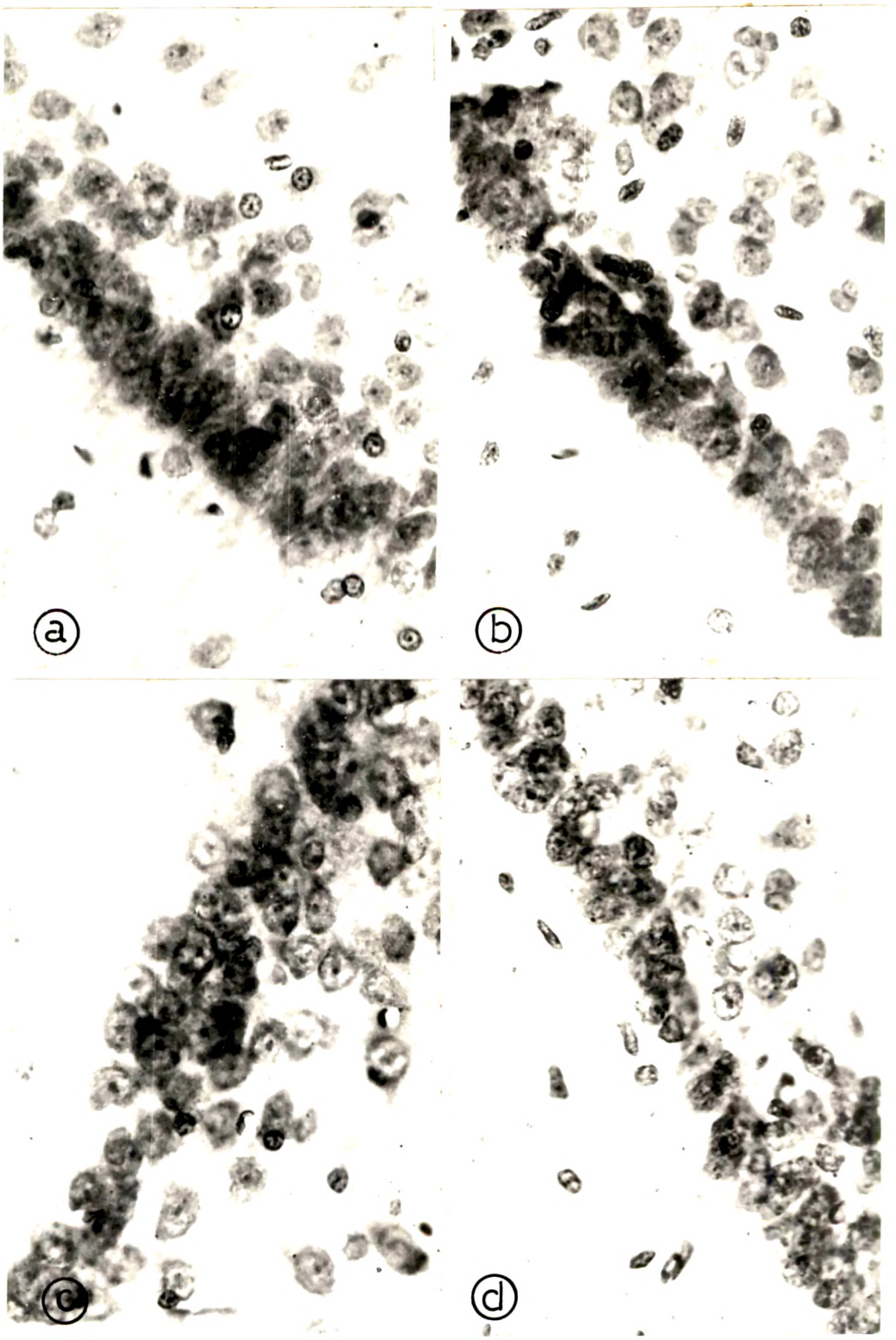
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Table: 3.40.

Cross sectional diameter of cells in hippocampal areas (μm).

	Young	Young ADX	Aging	Aging ADX
DG	6.74 ± 0.19	6.68 ± 0.25	7.21 ± 0.09	5.50 ± 0.18***
CA_4	10.43 ± 0.30	10.30 ± 0.36	12.56 ± 0.35	9.20 ± 0.49**
CA_3	9.96 ± 0.36	9.20 ± 0.28	11.86 ± 0.46	7.40 ± 0.25***
CA_2	7.30 ± 0.19	8.90 ± 0.28	8.20 ± 0.51	5.80 ± 0.29**
CA_1	6.50 ± 0.33	9.30 ± 0.35**	7.90 ± 0.22	7.60 ± 0.41

Diameter measured of 15-20 cells,n- 15-20,Levels of significance by Student's 't' test ** P<0.01;*** P<0.001 from respective control.

DG - Dentate gyrus.

One reason for such difference could be the loss of adaptability to change in the corticosterone levels. It is also likely that aged animals are in continuous exposure to elevated corticosterone and therefore cell biochemistry is adapted to such high levels of corticosterone and hence maintain homeostasis of the cell. When the animals were adrenalectomized, titers of corticosterone decreased; since there is impairment in the synthesizing mechanisms the cells were not able to adapt to the levels of corticosterone hence the depletion showed greater reactivity. In our earlier experiments, it was seen that the regions respond differentially with age and to the duration of ADX as judged by the ^{32}P incorporation in phospholipid fractions and with regard to Na+K+ATPase.

This study thus emphasizes that the effects of corticosterone are more age dependent rather than on receptor number. The present study has taken only genomic action into consideration. Since ADX depletes the levels of corticosterone, the membrane associated or direct effect of corticosterone does not play any role.