# Chapter 2

# Effect of Dexamethasone Treatment On Biochemical Maturation of Rat Brain

## Introduction

The regulatory role of glucocorticoids for maintaining blood glucose level is well recognized (1,3) To enhance the glucocorticoid as well as anti-inflammatory properties several synthetic glucocorticoid analogues have been prepared by making structural modifications in the original backbone (4) Dexamethasone is one of the potent synthetic glucocorticoids used for several therapeutic purposes It is used not only as an anti-inflammatory agent but also used for the treatment of skin diseases, bronchial asthma, respiratory distress syndrome, rheumatoid arthritis and meningitis in all age groups including children (4-8), toxic effects were seen in 50% of the cases when used in pharmacological doses (9)

The period of fetal brain development (22-24 week) is corresponds to postnatal day 1 of rat (9,10) Thereafter, in the rats, the differentiation of brain cells, connectivity, arborization, continues up to 5 week (11), which can be correlated with the brain development in the children (9,10) Glucocorticoids effects on central nervous system (CNS) are well recognized (12-15) In the light of this attempts were made to check the effects of treatment with the potent glucocorticoid drug dexamethasone on rat brain development at various stages of development These results are summarized in this Chapter of the thesis These includes quantitative estimations of DNA, RNA and protein macromolecules and also the activities of marker enzymes of different cells of the brain i e acetylcholinesterase (AChE) for neuronal cells, glutamine synthetase (GS) for astrocytes and glycerol phosphate dehydrogenase (α G3PDH), for oligodendroglial cells respectively (16-20)

It is anticipated that the results would serve as useful guideline for evaluating dexamethasone effects on macromolecules content as well as metabolic processes in the brains of fetus and children when dexamethasone is used as drug in the early period of the development (21)

## Materials and Methods

### Chemicals

Dexamethasone, sodium salt of adenosine-5'-triphosphate, (Na-ATP), sodium salt of adenosine-5'-diphosphate, (Na-ADP), actylthiocholineiodide, (ACTI), 5,5'-dithio-bis(2-nitrobenzoic acid), DTNB, ethopropazine hydrochloride, ETPZ HCl), L-α-glycerol-3-phosphate, nicotinamide adenine dinucleotide, ($NAD^+$) were purchased from Sigma Chemical Co, St Louis, MO USA Tris-(hydroxymethyl)-aminoethane, (Tris), hydrazine, diphenylamine, orcinol, Folin Ciocalteau reagent and bovine serum albumin (BSA) were from SRL, India

All other chemicals and solvents used were of analytical reagent grade, purchased locally

All the experiments were carried out using double distilled water- distilled in a glass distillation unit

### Animals and treatment with dexamethasone

Albino rats of Charles - Foster strain belonging to different age groups i e 2, 3, 4 and 5 week and adults (8-10 week) were used The animals were injected with dexamethasone phosphate solution subcuteneously (s c), at a dose of 2 mg dexamethasone / Kg body weight for three alternative days prior to the day of killing (5,22) Thus in the 2 week group the animals received dexamethasone treatment on day 8, 10 and 12 and the animals were killed on day 14

Dexamethasone phosphate solutions were prepared fresh daily in saline and the animals were injected between 6.00 AM to 7 00 A M when the plasma corticosterone levels in the rat are the lowest (23,24)

**Extraction of Nucleic acids**

The animals were killed by decapitation and their brains were quickly removed The tissue was repeatedly washed with 0 25M sucrose and 10% (w/v) homogenates were prepared using a Potter-Elvehjem type glass –Teflon homogenizer The extraction of nucleic acids were essentially according to the procedure of Schmidt and Thannhauser (25) as applied to brain tissue by Gourdon et al (26) Thus, briefly, to 1ml of brain homogenate, 2 5 ml of 0 6N cold (0-4 °C) perchloric acid (PCA) was added and the tubes were kept on ice for 10 min The tubes were then centrifuged at 2000 rpm for 10 min The pellet was washed twice more with 2 ml of cold 0 2N PCA The resultant pellet was then incubated with 1 ml of 0 3N KOH solution at 37 °C for 60 min At the end of the incubation period, the proteins and DNA were precipitated by the addition of 1 ml of 1 2N PCA The tubes were kept on ice for 10 min and then centrifuged at 2000 rpm for 10 min to recover the supernatant The pellet was washed twice with 1 ml of 0 2N PCA and the supernatant was pooled The pooled supernatant was used for the determination of RNA content by the orcinol method of Ceriotti (27)

The pellet (after extraction of RNA) was extracted with 1 ml of 0 5N PCA at 37 °C for 20 min by incubating in a water bath, The tubes were then centrifuged at 2000 rpm for 10 mın to collect the supernatant The pellet was washed once with 0 5 ml of 0 5N PCA and the pooled supernatant was used for the determınation of DNA content by the diphenylamine reaction according to method of Burton (28)

**RNA estimation**

1ml of pooled extract was made up to 3 ml with 2ml of distilled water, to which 3 ml orcınol reagent was added The tubes were then boıled in a water bath for 40 min, cooled under running water and extracted with 3ml of isoamylalcohol by vortexing vıgorously The organic phase was removed carefully and the optical density readings were recorded at 675nm (27)

**Orcinol reagent**

200mg of orcinol is dissolved ın concentrated HCl to which 10ml of 0 004M CuCl2 2H2O solution was added The volume was made up to 100ml with concentrated HCl

**DNA estimation**

0 8ml alıquots of pooled supernatant were made up to 1ml by addıng 0 2ml of 0 5N PCA To this 2ml of diphenylamine reagent was added The tubes were left in dark for 24 hours for optımum colour development The optıcal density readıngs were taken at 600 nm (28)

**Diphenylamine reagent**

1 5 g diphenylamine was dissolved in 100ml of glacial acetic acıd and 1 5 ml of concentrated sulfuric acid was then added Just before use, 0 1ml of 1 6% aqueous acetaldehyde was added for every 20ml of the reagent

## Enzyme assay

### Acetylcholinesterase (AChE) activity

The assay procedure was essentially as described previously by Ellman et al (29) and Swegert et al (30) Briefly, the assay medium (total volume 1 ml ) contained 100mM phosphate buffer, pH 8 0, 0 32 M DTNB, pH 7 0 and 1μM of ETPZ HCl The post nuclear fraction (75-150 μg protein) was used as the source of the enzyme The reaction was started by adding 5mM ACTI and the increase in absorbance at 412 nm was measured at 37 °C at 5 sec intervals The enzyme activity is expressed as μmoles of ACTI hydrolyzed/ min/ g tissue. The molar extinction coefficient for chromophore 13 6 $lit^{-1}cm^{-1}$ is used for calculation

### Glutamine Synthetase (GS) activity

Assay of glutamine synthetase activity was according to the method described by Bruce Rowe et al (31) The assay system (total volume 1ml) contained, 50mM imidazole-HCl buffer, pH 7 2), 20mM $MgCl_2$, 50 mM $Na^+$-L-glutamate, 2 5mM β-mercaptoethanol, 10mM hydroxylamine, 0 5mM $Na^+$-ATP The reaction was started by addition of enzyme (150 to 300μg protein) After incubation for 30min, the reaction was terminated by the addition of 1 5ml of $FeCl_3$ reagent containing 0 37M $FeCl_3$, 0 67N HCl and 0 2M TCA Precipitated protein is removed by centrifugation at 2000 rpm for 5 min and the optical density of the supernatant measured at 535nm against reagent blank Under these conditions, 1μmole of γ-glutamylhydroxamic acid gives an absorbency of 0 340 One unit of glutamine synthetase activity is defined as the amount of enzyme which catalyzes the synthesis of 1 μmole of γ-glutamylhydroxamic acid under the given condition described above (31)

**Glycerol-3-phosphate dehydrogenase (α -G3PDH) assay**

The assay procedure was as described by Lee and Lardy (32) To total volume of 1ml, 0 5ml of glycine buffer (0 2M, pH 10.2), 0 1ml hydrazine (100mM), 0 1ml glycerol-3-phosphate(60mM), 10µl of 10% (v/v) Triton-X-100 and 100-200 µg protein as the source of enzyme The reaction was started with addition of 0 1ml of $NAD^+$(5mM) and the increase in absorbance was measured at 340nm The total activity is calculated by using millimolar extinction coefficient of $NADH^-$ at 340nm is 6.23$lit^{-1}cm^{-1}$the enzyme activity is expressed as nmoles/mg protein

The protein estimation was carried out according to Lowry et al (33) using BSA as the standard

Statistical evaluation of the data was by Students' t-test

## Results

The effects of dexamethasone treatment on body weights of rats belonging to different age groups are shown in Fig 1 The initial body weights in the control and dexamethasone treated groups were matched With increase in age a standard pattern of weight gain is obvious from the data presented At the end of 4 day experimental period, the controls belonging to all age groups showed significant weight gain The extent of weight gain decreased after dexamethasone treatment Thus in the dexamethasone treated groups the final body weight decreased from 8-28%

The data of brain weight and relative brain weight are given in Table 1 from which it is apparent that the brain weight was not affected in general except in the 3 week group where a small but reproducible 10% decrease was seen However due to lowering in

**Fig. 1** Effects of dexamethasone treatment on body weights of rats belonging to different age groups The three bars in a set represents initial body weight, final body weights in control rats and dexamethasone treated rats respectively The error bar gives the S E M of 20 independent observations

a, p< 0 01, and b, p<0 001 as compared to initial body weight of controls

*, p< 0 01, and **, p<0 001 as compared to final body weight of controls

Fig.1

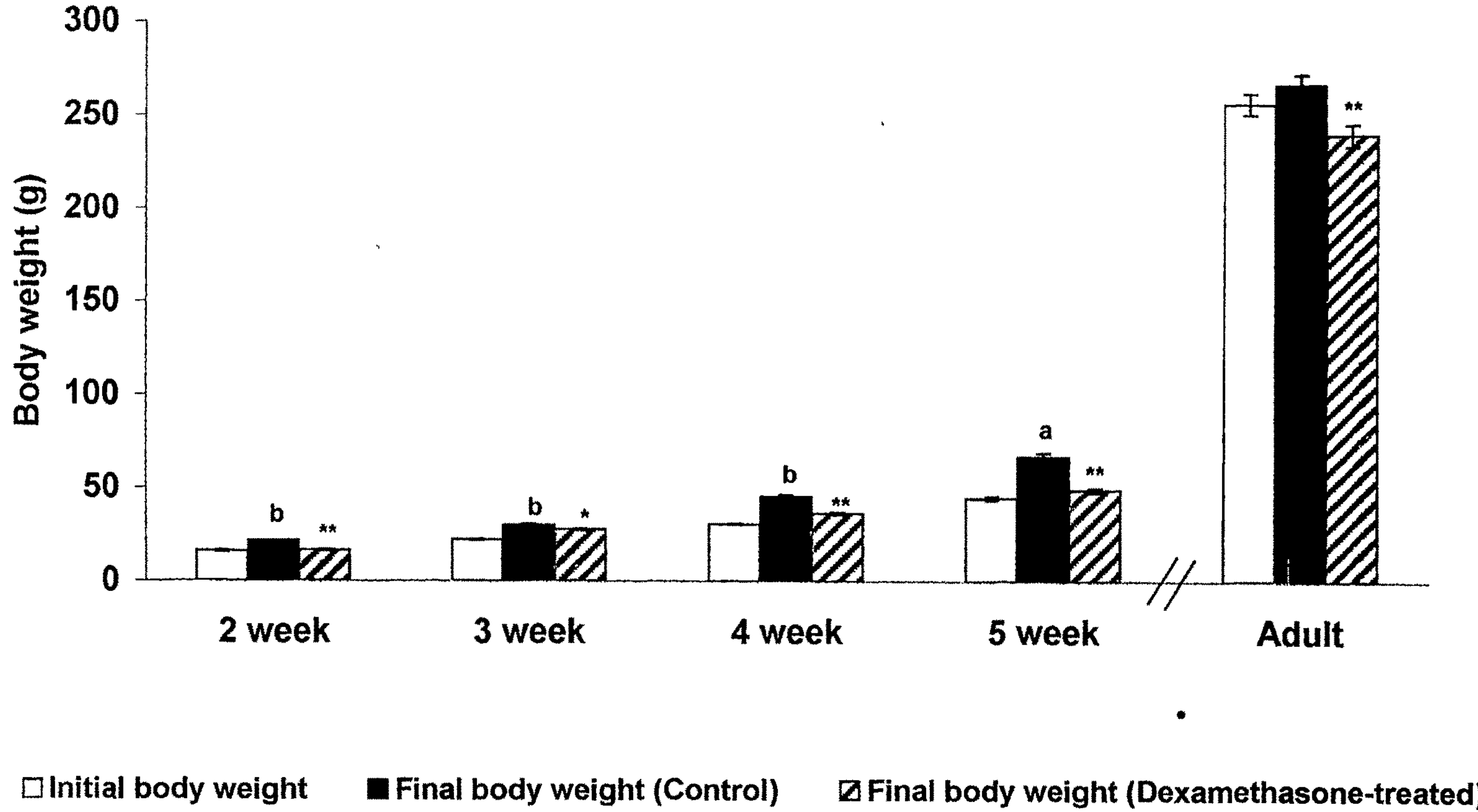

Body weight (g)
300
250
200
150
100
50
0
b
**
b
*
b
**
a
**
**
2 week
3 week
4 week
5 week
Adult
Initial body weight
Final body weight (Control)
Final body weight (Dexamethasone-treated)

Table 1 The effect of dexamethasone treatment on brain weight and relative brain weight

| Group | Treatment | Brain weight (g) | Relative Brain weight, (%) |
|---|---|---|---|
| 2 week | Control (22) | 0 95 ± 0 014 | 4 49 ± 0 075 |
| | Dex (16) | 0 92 ± 0 017 | 5 17 ± 0 131 [a] |
| 3 week | Control (18) | 1 28 ± 0 012 | 4 41 ± 0 105 |
| | Dex (22) | 1 15 ± 0 020 [a] | 4 28 ± 0 105 |
| 4 week | Control (16) | 1 33 ± 0 031 | 2 96 ± 0 058 |
| | Dex (22) | 1 28 ± 0 020 | 3 59 ± 0 083 [a] |
| 5 week | Control (22) | 1 40 ± 0 029 | 2 18 ± 0 148 |
| | Dex (28) | 1 43 ± 0 036 | 2 97 ± 0 071 [a] |
| Adult | Control (24) | 1.79 ± 0 046 | 0 67 ± 0 012 |
| | Dex (16) | 1 70 ± 0 029 | 0 71 ± 0 014 |

The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis
[a]$p < 0\ 001$ compared with the corresponding control

weight gain following dexamethasone treatment (e.g see Fig 1) the relative brain weight was higher by 21 to 36% in 2, 4 and 5 week animals It may also be noted that the controls displayed a expected pattern i e the relative brain weight decreased with development Thus the 2 week value was 4 5%, the adult value was 0 7%

The tissue protein, RNA and DNA contents are shown in Fig 2-4 As is evident, in the controls the protein content was high initially up to 3rd week but decreased thereafter to the extent of 35-40% The dexamethasone treatment resulted in decreased protein content in 2 and 3 week groups Thus in effect the protein content in all the age groups was fairly constant at around 105 mg /g tissue in the dexamethasone treated animals

The brain RNA content (expressed in mg / g tissue) decreased up to 4th week in the control group The value increased again in 5 week group and was somewhat higher in the adults Dexamethasone treatment had no effect on the RNA content till 4th week However, in the 5 week group and the adults 20-35% increase in RNA content was evident

In the control group the DNA content decreased with development up to the 5 week but showed a significant rise in the adults Dexamethasone treatment significantly lowered the tissue DNA content in the initial stages i e up to 4th week with the decrease amounting to 40-60% DNA content in 5 week old animals and in adults were unaffected

The total macromolecular contents of the brain are shown in Fig 5-7 These values were derived by multiplying the brain weight with the specific content of each macromolecule i e protein, RNA and DNA Thus the protein content in the control group was higher in 3

**Fig. 2** Effects of dexamethasone treatment on tissue protein content in brain of rats belonging to different age groups The straight line represents the control group whereas the dashed line represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observations

**Fig. 3** Effects of dexamethasone treatment on tissue RNA content in brain of rats belonging to different age groups The straight line represents the control group whereas the dashed line represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

**Fig. 4** Effects of dexamethasone treatment on tissue DNA content in brain of rats belonging to different age groups The straight line represents the control group whereas the dashed line represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

[a] $p < 0\ 05$. [b] $p < 0\ 02$, [c] $p < 0\ 01$ and [d] $p < 0\ 001$ as compared to controls (Fig 2-4 and Fig 8,9)

Fig. 2

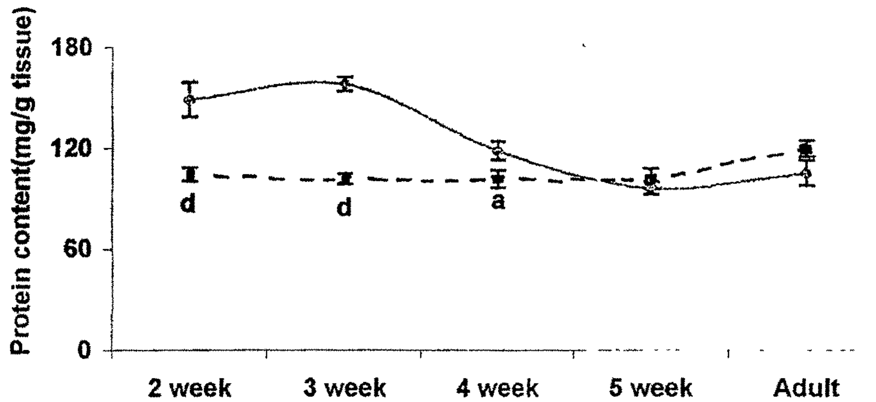



Fig. 3

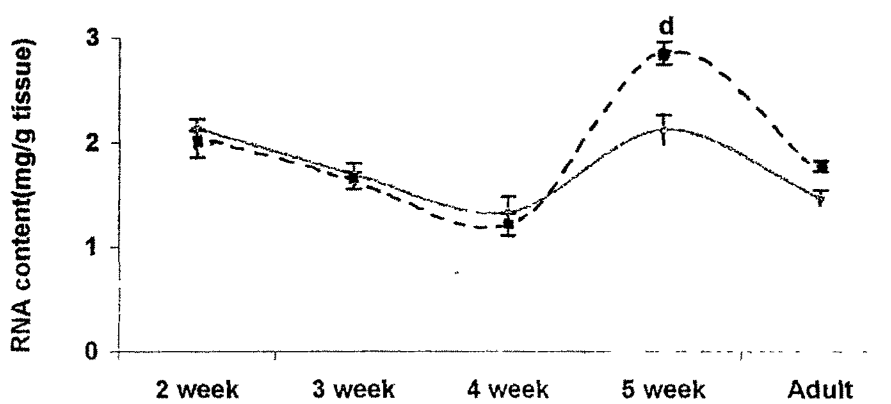



Fig. 4

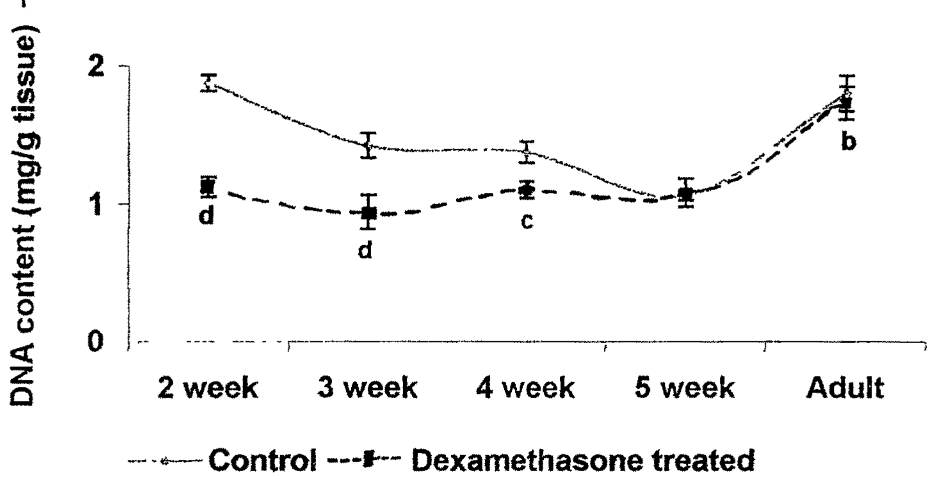


**Fig. 5** Effects of dexamethasone treatment on total DNA content in brain of rats belonging to different age groups The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

**Fig. 6** Effects of dexamethasone treatment on protein/ DNA ratio in brain of rats belonging to dıfferent age groups The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 ındependent observation

**Fig. 7** Effects of dexamethasone treatment on total RNA/DNA ratio ın brain of rats belonging to dıfferent age groups The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observatıon

$^{a}$ p < 0 05, $^{b}$ p < 0 01, $^{c}$ p < 0 002 and $^{d}$ p < 0 001 as compared to controls (Fig 5-7)

Fig.5

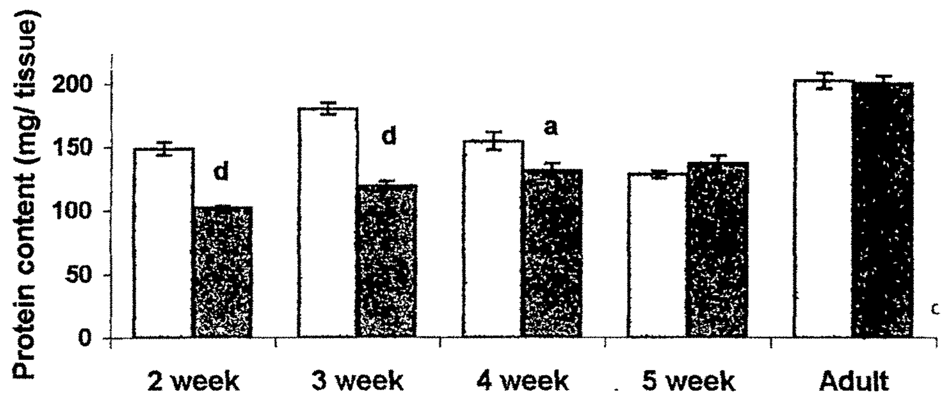



Fig. 6

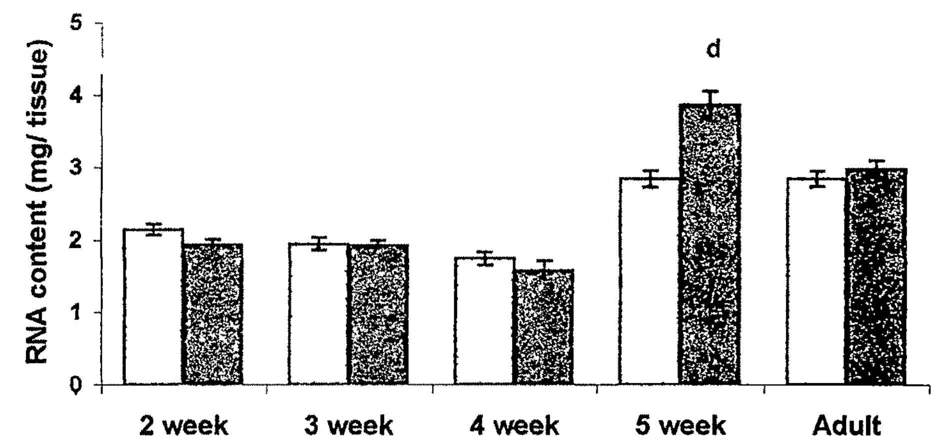
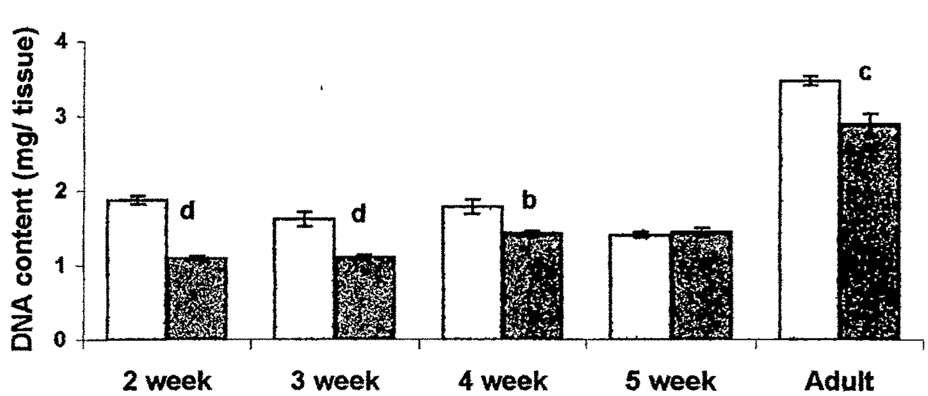



Fig. 7



week animals but declined by 5th week in the adults there was a significant increase Dexamethasone treatment in general caused lowering of total protein content up to the age of 4 week

The total RNA content in the brain decreased up to 4th week but reached a steady state value at latter stages Dexamethasone treatment had no effect in general on the total RNA content except for 5 week animals where the content was higher by 36%

The total DNA content in controls remained fairly constant up to 4th week, declined somewhat in 5 week animals and then almost doubled in adults Dexamethasone treatment resulted in lowering of the DNA content from 17-41% in all the groups except for the 5 week animals

In the view of the differential changes in the content of the macromolecules ( Fig 2-4 and 5-7) attempts were made to estimate the cell size in terms of protein/DNA ratio and the metabolic activity in terms of RNA/DNA ratio (34) (Fig 8-9)

The protein/DNA ratio was highest in 3 week animals but decreased by about 35% in the adults Dexamethasone treatment resulted in increased protein/DNA ratio in 2 week and adult animals

The RNA/DNA ratio in the controls was highest in the 5 week animals For the remaining groups the ratio was almost comparable Dexamethasone treatment brought about increase in RNA/DNA ratio from 24 to 55% in all the groups signifying increase metabolic activity, no changes noted for 4 week animals

**Fig. 8** Effects of dexamethasone treatment on total protein content in brain of rats belonging to different age groups The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

**Fig. 9** Effects of dexamethasone treatment on total RNA content in brain of rats belonging to different age groups The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated The error bar gives the S E M of 20 independent observation

$^{a}$ $p < 0.05$, $^{b}$ $p < 0.02$, $^{c}$ $p < 0.01$ and $^{d}$ $p < 0.001$ as compared to controls (Fig 2-4 and Fig 8,9)

Fig. 8

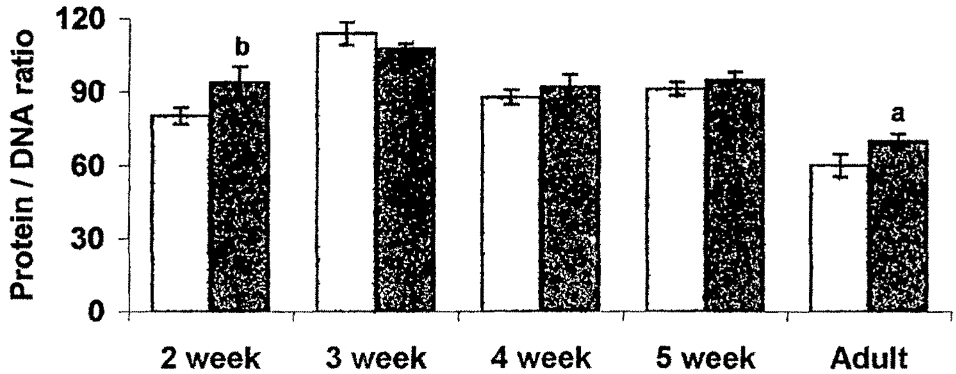
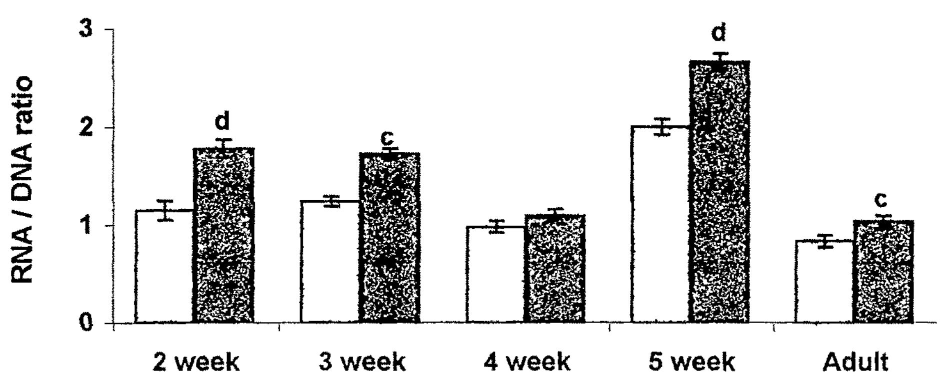



Fig. 9




Control    Dexamethasone treated

In next set of experiments the effects of dexamethasone treatment on brain development were by assessed by checking the marker enzymes Thus in the control the AChE activity in the control was highest in the 5 week stimulation but declined somewhat in the adults Dexamethasone treatment caused early stimulation of AChE activity up to 4 week but had no effect on adults ( Fig 10)

The αG3PDH activity in the controls could be detected only at the age of 4 week, and animals showed a peak at 5 week, in the adults the level became comparable to the 4 week group Dexamethasone treatment caused significant stimulation of αG3PDH activity in 3 week animals (Fig 11)

The GS activity in the controls increased up to 5$^{th}$ week but declined considerably in the adults Dexamethasone treatment caused significant stimulation of activity in 2 and 3 week animals Following dexamethasone treatment the activity in 5 week and adults were almost comparable (Fig 12)

## Discussion

The overall adverse effect of dexamethasone on body weights of rats belonging to different age groups are apparent from data in Fig 1 Dexamethasone treatment also differentially affected the protein, RNA and DNA content of the brain tissue Interestingly however, the brain weight was practically unchanged except for the 3 week group (10 % decrease) Nevertheless, the total protein content of the tissue in the growing animals decreased up to 4$^{th}$ week (Fig 5), the RNA content was high in 5 week group (Fig 6), the total DNA content showed an overall decrease practically in all age groups

**Fig. 10** Effects of dexamethasone treatment on tissue activity of acetylcholinesterase in brain of rats belonging to different age groups The activity is given as μmoles/ min/ gm tissue The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

**Fig. 11** Effects of dexamethasone treatment on tissue activity of glutamine synthase in brain of rats belonging to different age groups The activity is given as μmoles/gm tissue The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

**Fig. 12** Effects of dexamethasone treatment on tissue activity of α GPDH in brain of rats belonging to different age groups The activity is given as μmoles/ gm tissue The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

a, $p< 0\ 002$, b, p, 0 001 as compared to initial body weight of controls (Fig 10-12)

Fig.10

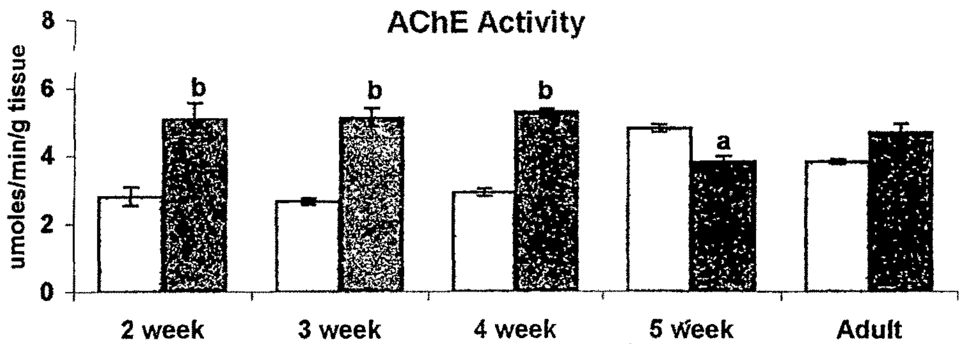



Fig. 11

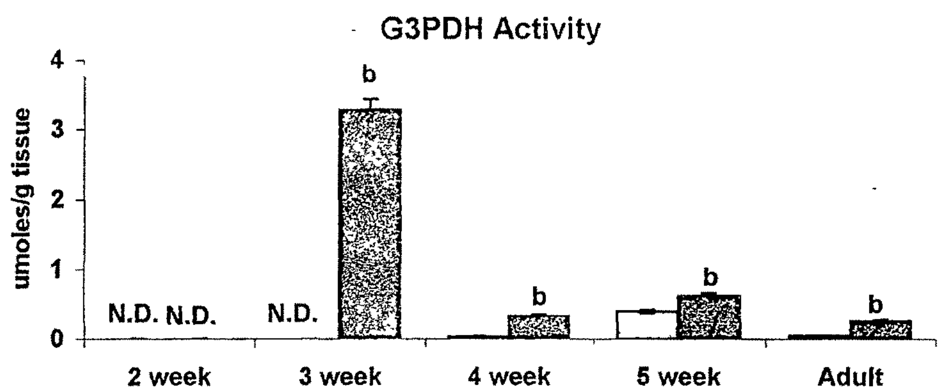
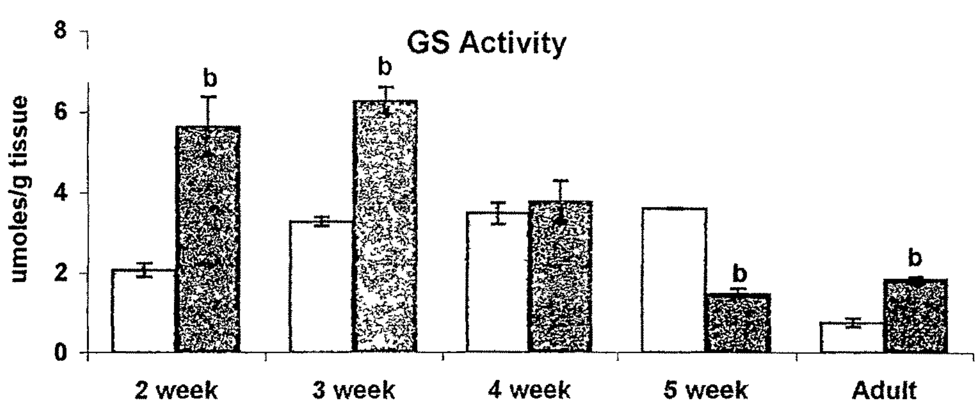



Fig. 12



The DNA content of the individual cell is constant i e 6 pg/cell (35) Hence, the data in Fig 7 suggest that the total vὸlume of cells in the brain decrease ın general following dexamethasone treatment

The ratio of protein/DNA is taken as an index of cell size (34) The data represented ın Fig 8 would suggest that the cell size was marginally affected by dexamethasone treatment which is consistent with the data in Fig 2 and Fig 4

The ratio of RNA/DNA is indicatıve of the metabolic activity The data in Fig 9 showed that dexamethasone treatment resulted in increasıng this ratıo, the effect was maximum in the early age groups i e in 2 week and 3 week The results thus suggest that the dexamethasone treatment was effective in enhancing the metabolic activity in the early age, perhaps suggesting an early maturational effect

Such an assumption is also born out by the data in the Fig 10-12 where early sıgnificant stimulation was noted for AChE, αG3PDH and GS activıties The response in the 5 week and adult was of lesser magnitude or negatıve

In conclusion our results suggest that dexamethasone treatment used for therapeutic purposes in growing organisms can adversely affect the brain maturational process In the light of this caution in indiscriminate use of dexamethasone for therapeutic purposes is warranted

## Summary

Effects of dexamethasone treatment on brain development were examined Animals belonging to different age groups were treated chemically (3 doses alternate day) with dexamethasone and the DNA, RNA and protein contents were quantified and the activities of marker enzymes i e. AChE (neuronal cells), glutamine synthetase (astrocytes) and glycerol 3 phosphate dehaydrogenase (oligodendroglia) were determined Dexamethasone treatment caused 8-28% decrease in body weight, only in the 3 week group, the brain weight decreased by 10% Dexamethasone treatment in general resulted in decreased protein content in the younger animals (up to 4 week) while the DNA content decreased from 20-40% up to 3rd week of age signifying the decrease in cell number RNA content increased only in 5 week old and adults The protein/DNA ratios were high in very young (2 week) and adult animals Dexamethasone also resulted in early stimulation of AChE, αG3PDH and GS activities The results thus suggest that treatment with dexamethasone at different ages interferes with the process of brain development

## References


1 Weber, G , Srivastava, S K and Singhal, L (1965) Role of enzymes in homeostasis J Biol Chem 240, 750-756

2 Edwin, M , (1990) Steroid hormones In Hormones form molecules to disease Baulieu, E E and Kelly, P A eds Hermann publishers in arts and science, Champmann and Hall, New York and London, pp 385-442

3 Muller, M , Renkawitz, R , 1991 The glucocorticoid receptor Biochim Biophys Acta 1088, 171-182

4 Gilbertson, E O , Spellmann, M C , Piacquidio, D J , Mulford, M I , 1998 Super potent topical corticosteroid use associated with adrenal suppression Clinical considerations J Am Acad Dermatol 38, 318-321

5 Tsuneishi, S , Takada, S , Motoike, T , Ohashi, T , Sano, K , Nakamura, H , 1991 Effects of dexamethasone on the expression of myelin basic protein, proteolipid protein,and glial fibrillary acidic protein genes in developing rat brain Experimental Brain Res 61, 117-123

6 Rastogi, A , Akintorin, S M , Bez, M L , Morales, P , Pildes, R S , 1996 A controlled trial of dexamethasone to prevent bronchopulmonary dysplasia in surfactant-treated infants Pediatrics 98, 204-210

7 Romagnoli, C , Zecca, E , Vento, G , Maggio, L , Papacci, P , Tortorolo, G , 1999 Effect on growth of two different dexamethasone courses for preterm infants at risk of chronic lung diseases a randomized trial Pharmacology 59, 266-274

8 Schimmer B P and Parker, K L (2001) adrenocorticotropic Hormone, Adrenocortical steroids and their synthetic analogs, Inhibitors of the synthesis and

actions of adrenocortical hormones Ch. 60 In The pharmacological basis of therapeutics Harman, J G and Limbird ( Eds ) 10th edition pp 1649-1678

9 Flagel, S.B , Vazquez, D.M , Watson, Jr S J , Neal, Jr C R (2002) Effects of tapering neonatal dexamethasone on rat growth, neurodevelopment, and stress response Am J. Physiol Integrtative Comp Physiol 282, R55-R63.

10 Whitelaw, A , Thorensen, M (2000) Antenatal steroids and the developing brain Arch Dis Child Fetal Neonatal Ed 83, F154 – F157

11 Balazs, R., Lewis, P D and Patel, A J (1975) Effects of metabolic factors on brain development Growth and Development of the Brain (Brazier, M A B ed ) Raven Press, New York pp 83-115

12 Field, E J (1954) Effect of cortisone on the neonatal rat Nature 174, 182

13 McEwan,B S , De Kloet, E R and Rostene, W (1986) Adrenal steroid receptors and actions in the nervous system Physiol Rev 66, 1122-1188

14 Meijer, O C and DeKloet, E R (1998) Corticosterone and serotonergic neurotransmission in the hippocampus Functional implications of central corticosteroid receptor diversity Critic Rev Neurobiol 12, (1&2) 1-20

15 Reul, J M H M et al (2000) The brain mineralocorticoid receptor greedy for ligand, mysterious in function Eur J Pharmacol 405,235-249

16 Vellis, J D and Inglish, D (1968) Hormonal control of glycerolphosphate dehydrogenase in rat brain J Neurochem. 15,1061-1070

17 Tardy, M , Rolland, B , Fages, C and Caldani, M (1984) Astroglial cells Glucocorticoid target cells in the brain Clin Neuropharmacol 7, 296-302

18 Zajc-kreft, K , Brank, M , Weber, U and Grubic, Z (1998) Glucocorticoid control of acetylcholinesterase and butyrylcholinesterase expression in the mammalian

organisms, In Structure and Function of cholinesterase and related proteins, (Doctors et al ed ), Plenum Press, New York, pp 79-85

19 Patel, A J , Hunt, A. and Faraji-Shadan, F , (1986) Effect of removal of glutamine and addition of dexamethasone on the activities of glutamine synthetase, ornithine decarboxylase and lactate dehydrogenase in primary cultures of forebrain and cellular astrocytes Dev Brain Res 26, 229-238

20 Kumar, S , Cole, R., Chiapelli, F and Vellis, J D (1989) Differential regulation of oligodendrocyte markers by glucocorticoids Post-transcriptional regulation of both proteolipid protein and myelin basic protein and transcriptional regulation of glycerol phosphate dehydrogenase Proc Natl Acad Sci 86, 6807-6811

21 Howard, E (1965) Effects of corticosterone and food restriction on growth and on DNA, RNA and cholesterol contents of the brain and liver in infant mice J Neurochem 12, 181-191

22 Ferguson, S A and Holson, R (1999) Neonatal dexamethasone on day 7 causes mild hyperactivity and cerebellar stunting Neurotoxicol Teratol 21, 71-76

23 Jani,M S , Telang, S D and Katyare S S (1991) Effect of corticosterone treatment on energy metabolism in rat liver mitochondria J Steroid Biochem Molec Biol 38, 587-591

24 Ahlersova, E , Ahlers, I . and Smajda, B (1992) Influence of light regimen and time of year on circadian oscillations of insulin and corticosterone in rats Physiol Res 41, 307-314

25 Schmidt G , and Thannhauser S J (1945) J Biol Chem 161, 83-89

26 Gourdon, J , Clos, J , Coste, C , Dainat J and Legrand, J (1973) Comparative effects of hypothyroidism, hyperthyroidism and undernutrition on the protein and

nucleic acid contents of the cerebellum in the young rat J Neurochem 21, 861-871

27 Ceriotti G (1955) J.Biol Chem 214, 59-70

28 Burton, K (1956) Biochem J 62, 315-323

29 Ellman, G L, Courtney, K V., Andres, V and Featherstone, R M (1961), A new and rapid colorimetric determination of acetylcholinesterase activity Biochim Pharmacol 7, 88-95.

30 Swegert, C V, Dave, K R, Katyare, S S, 1999 Effect of aluminium-induced Alzheimer like condition on oxidative energy metabolism in rat liver, brain and heart mitochondria Mech Age Dev 112, 27-42

31 Bruce Rowe, W, Ronzio, R A, Wellner, V P, Mester, A, [129], Glutamine synthetase ( Sheep Brain), 1970 In Tabor, H, Tabor, C W (eds ), Methods in Enzymology, 17a, Academic Press, New York, pp 900-904

32 Lee, Y P and Lardy, H A, (1965) Influence of thyroid hormones on L-α-glycerophosphate dehyderogenases and other dehydrogenases in various organs of the rat J biol Chem 240, 1427-1436

33 Lowry, O H, Rosebrough, N J, Farr, A L, Randall, R J, 1951 Protein measurement with the Folin phenol reagent J Biol Chem 193, 265-275

34 Balazs R and Cotterell, M (1972) Effect of hormonal state on cell number and functional maturation of the brain Nature, 236, 348-350.

35 Zomenhof, S and Marthems, E V, (1974) Study of factors influencing prenatal brain development 4, 157-168