CHAPTER - VII

EFFECTS OF CORTICOSTERONE TREATMENT ON CORTICOSTERONE CONTENT OF RAT SERUM, BRAIN AND LIVER TISSUE AND MITOCHONDRIA DURING DEVELOPMENT.

Several methods have been described for the determination of corticosterone content of plasma as well as tissue like adrenals. These includes; estimation by fluorimetric (1-3), isotope dilution (4) , radioimmunoassay (5-7) and by high performence liquid chromatography (HPLC) (8).

Peterson (4) has described isotope dilution technique for estimation of plasma corticosterone. Although this method is highly specific for corticosterone, it has no wide application because the procedure is tedious and requires 30 to 40 ml of plasma for estimation of corticosterone in humans.

The quantitative estimation of plasma corticosterone by radioimmunoassay (RIA) is carried out after ethanol precipitaion of proteins (5 -7). In this method, cross reactivity of the antiserum with cortisol, deoxycorticosterone and 11- deoxycortisol was 8, 8 and 2% respectively, while that for 17 α - hydroxyprogesterone, testosterone and 17 β - estradiol was less than 0.1%. The RIA method for corticosterone is very senstive and can detect 10 pg corticosterone / tube (30 n mol /L) (7).

A sensitive and specific high performance liquid chromatographic assay for quantitation of corticosterone in rat plasma has been described by Wong et al. (8). In this method rat plasma is extracted with methylene chloride, washed with 0.1 mM NaOH and then with water. The washed extract is

then analyzed by HPLC on C 18 column with UV absorbance at 254 nm. The detection limit of the assay is 10 ng/ml. The recovery of costicosterone ranged from 87 to 95% .

Sweat (9, 10) has described a method for determination of unconjugated corticosterone and hydrocortisone in plasma by fluorescence developed in sulfuric acid. The author has made certain generalizations about activity relationship of steroid fluorescence based on fluorescence data of various steroids. Presence of hydroxyl group at position 11 of the steroid nucleus is necessary for the production of a high degree of fluorescence. The α,β unsaturated ketone group in ring A also exerts a strong influence on fluorescence. Saturation of this group in 11 - hydroxy series steroids results in a marked decrease in the degree of fluorescence produced. However in the 12 - hydroxy series, the saturated compounds exibit the greater degree of fluorescence. Hydroxyl group at position 17 exerts a minor influence on fluorescence (9)

The method is sensitive only to a fraction of microgram and employs chromatographic separation of cortical steroids on silica gel microcolumn prior to development of fluorescence. Hence although this method demonstrates usefulness of sulfuric acid induced fluorescence for analysis of plasma corticosterone, is not found to be practical (1,11).

According to the procedures described by Zenker and Bernstein (11) and Silber et al. (1) minimun 0.2 to 0.5 ml of plasma is required for quantitative determination of corticosterone by fluorescence. These methods are highly specific for the corticosterone and most of the other steroids except hydrocortisone show minimum or negligible fluorescence under experimental conditions. Hydrocortisone showed 20 to 40% fluorescence compared to that of the corticosterone (1,11). Since corticosterone is the principal glucocorticoid of rat plasma, these fluorimetric methods could be highly specific for corticosterone determinations.

Guillemin et al. (2) assessed the validity of the method described by Silber et al. (1), under various experimental conditions. The authors have also shown that the plasma samples form rats can be stored frozen for a period of up to 100 days without any appreciable variation in corticosterone concentration (2).

Glick et al. (3) have described a micromethod for determination of corticosterone in 20 to 50 µl of plasma or submiligram samples of adrenal tissue. The method is highly sensitive and can detect corticosterone as low as 0.5 ng; but the method requires special adapters for measurments of fluorescence in 0.05 ml final acid solution (12).

Since the fluorimetric methods for the corticosterone estimation are highly sensitive, specific and more practical, these are widely used for quantitative estimation of corticosterone (2, 13 - 16).

From the studies described in Chapters II to VI, it is clear that corticosterone treatments - acute and chronic - had differential effects on lipid/phospholipid content and composition and functional attributes of liver and brain mitochondria. Therefore it was of interest to examine the levels of corticosterone in serum, tissues and subcellular organelles. Results of these studies are summarized in this Chapter.

MATERIALS AND METHODS

Chemicals

The details are as described in Chapter II.

Extraction of corticosterone from serum, liver and brain tissues and mitochondria

The extraction of corticosterone from the samples was carried out according to the procedure described by Silber et al. (1) with some modifications. The volumes were proportionately reduced to increase the sensitivity of detection.

In the initial experiments, the samples were first treated with petroleum ether and then extracted with methylene chloride (1). It was found that vigorous vortexing led to formation of gel at the interface and recovery was poor and highly variable. The petroleum ether step in the procedure was hence omitted. Instead, the proteins were first denatured using TCA and then the samples were directly extracted with methylene chloride. Other researchers have also used the direct extraction procedures employing methylene chloride or chloroform but without denaturing proteins (4,8,11,17). The exact extraction procedureused for corticosterone form the samples was as follows:

Firstly, 50 µl of serum sample or the sample of tissue homogenate or mitochondria corresponding to about 500 ug protein was taken to which 0.2 ml of 5% TCA (chilled, 0-4°C) was added and the final aqueous volume was made up to 1.5 ml with distilled water.

After keeping for 20 minutes (0-4°C), 6.0 ml of methylene chloride was added followed by vigorous vortexing for 2 to 3 minutes. The two liquid phases were allowed to separate and the bottom layer was removed carefully with the help of a syringe with a long needle attached to it and transferred into a fresh tube.

To this solvent extract, 1.0 ml of 0.1 N NaOH was added and the tubes were vortexed vigorously for 15 seconds for mixing. The top aqueous layer of alkali was immediately removed completely and discarded.

Finally 3.0 ml of 30 N H_2SO_4 was added and tubes were vortexed vigorously for 1 to 3 minutes. The upper solvent layer was removed and discarded. Fluorescence of corticosterone in sulfuric acid was measured after 45 minutes incubation at room temperature (1,3). The fluorescence was found to be stable at least for 3 hours. The blanks contained only distilled water whereas the standard tubes contained 50 to 1000 ng of corticosterone. All the tubes were processed in a similar manner as descrided above for samples and the fluorescence was read against the blanks.

This extraction procedure gives recovery of corticosterone in the range of 80 to 100 % for serum and tissue homogenates as well as mitochondria form liver and brain. The recovery of corticosterone after methylene chloride extraction was found to be independent of concentration. A similar finding has also been reported by Wong et al. (8).

Fluorimetric estimation of corticosterone

Corticosterone fluorescence in sulfaric acid was determined in a Shimadzu RF-5000 spectrophotofluorimeter. The

excitation and emisssion wavelengths were determined form the excitaion and emission spectra of authentic samples and were found to be 472 nm and 523 nm respectively., Excitation and emission bandwidths were set at 5 and 10 nm respectively. standard graph of corticosterone fluorescence was plotted using the concentrations in the range of 50 to 1000 ng. It was found that 1.0 ng of corticosterone gives the 0.45 units of fluorescence.

RESULTS

Table 1 summarizes the results of corticosterone levels in serum from animals of different age groups in control and corticosterone treated rats. The results indicate that in the control animals the corticosterone level was highest in the 7-day group and decreased gradually till the adult stage. The value decreased by a factor of 16 compared to the 7-day-old rats.

Acute treatment with corticosterone resulted in significant increase in the serum corticosterone levels in amimals of all the age groups. The extent of increase was higher (2 to 3 fold) in young than in the adults (only 35 % increase). On the other hand, chronic treatement resulted in decreased serum levels from 20 to 80% in the different age groups except adults where no change was seen.

Table 1

Effect of corticosterone treatment on levels of corticosterone in rat serum during development.

Age	Corticosterone content (µg/100 ml)		
	Control	Acute	Chronic
7 Days (10)	558 ± 42	N.D	N.D
14 Days (10)	384 ± 55	844 ± 50[b]	178 ± 13[b]
21 Days (10)	147 ± 07	358 ± 20[b]	118 ± 11[a]
35 Days (12)	63 ± 06	172 ± 17[b]	27 ± 03[b]
Adults (12)	35 ± 04	48 ± 04[a]	36 ± 04

Results are given as mean ± SEM of number of independent observations for each group as indicated in the parentheses.

[a]$P < 0.05$ and [b]$P < 0.001$ compared to the corresponding controls.

N.D = not done.

Data on corticosterone content of the brain tissue as influenced by corticosterone treatment are given in Table 2. It is clear that in the controls, the content was the highest on day 21 but decreased by about 40% in the adults. Acute treatment did not have any significant effect except in the adults where the values increased by 28%. By contrast, chronic treatment caused about 20 to 30 % decrease in corticosterone content in 14- and 21-day groups, whereas in 35-day-old and adult animals there was about 25 to 50% increase.

When the corticosterone content of brain tissue was expressed as ug /mg protein (Table 3), a more or less similar picture was observed for the acute treatement group (e.g. compare Table 2). In case of 21-day-old animals, chronic treatment caused 70% decrease in corticosterone content. This reduction could be attributed to significant (82%) increase in protein content of brain in this group (Table 4).

Data on mitochondrial corticosterone content in the brain as affected by the two corticosterone treatments are shown in Table 5. Upon acute treatment the hormone content in the brain mitochondria form 14- and 21-day-old animals increased by about 40% while the adults showed about 60% decrease; the 35-day-old rats were not affected. Chronic treatment caused significant (50 to 65%) reduction in mitochondrial corticosterone content in brain from 14- and 21-day-old rats, but had no effect on 35-day-old and adult animals.

Table 2

Effect of corticosterone treatment on corticosterone content of rat brain during development.

Age	Corticosterone content (μg/g tissue)		
	Control	Acute	Chronic
14 Days	350 ± 11	360 ± 05	283 ± 12[b]
21 Days	470 ± 10	475 ± 12	340 ± 15[b]
35 Days	391 ± 28	401 ± 18	495 ± 26[a]
Adults	294 ± 21	377 ± 22[a]	443 ± 21[b]

Results are given as mean ± SEM of 8 independent observations in each group.

[a]$P < 0.05$ and [b]$P < 0.001$ compared to the corresponding controls.

Table 3

Effect of corticosterone treatment on corticosterone content of rat brain during development.

Age	Corticosterone content (µg/mg protein)		
	Control	Acute	Chronic
14 Days	2.43 ± 0.08	2.26 ± 0.11	1.16 ± 0.06[b]
21 Days	4.16 ± 0.20	4.01 ± 0.14	1.24 ± 0.15[b]
35 Days	4.44 ± 0.44	4.52 ± 0.27	6.34 ± 0.61[a]
Adults	2.60 ± 0.03	3.85 ± 0.22[b]	4.11 ± 0.24[b]

Results are given as mean ± SEM of 8 independent observations in each group.

[a] $P < 0.05$ and [b] $P < 0.001$ compared to the corresponding controls.

Table 4

Effect of corticosterone treatment on protein content of rat brain during development

Age	Protein content (mg/g tissue)		
	Control	Acute	Chronic
14 Days	145 ± 2.2	160 ± 10.4	131 ± 9.0
21 Days	114 ± 5.3	117 ± 8.4	207 ± 10.4[b]
35 Days	90 ± 3.4	90 ± 6.2	98 ± 5.6
Adults	113 ± 4.6	98 ± 2.4[a]	102 ± 5.4

Results are given as mean ± SEM of 8 independent observations in each group.

[a] $P < 0.01$ and [b] $P < 0.001$ compared to the corresponding controls.

Table 5

Effect of corticosterone treatment on corticosterone content of rat brain mitochondria during development.

Age	Corticosterone content (µg/mg protein)		
	Control	Acute	Chronic
14 Days	0.58 ± 0.02	0.83 ± 0.03[c]	0.20 ± 0.04[c]
21 Days	3.10 ± 0.40	4.28 ± 0.21[a]	1.50 ± 0.13[b]
35 Days	1.60 ± 0.09	1.62 ± 0.07	1.85 ± 0.08
Adults	2.60 ± 0.07	1.10 ± 0.08[c]	2.50 ± 0.09

Results are given as mean ± SEM of 8 independent observations in each group.

[a]$P < 0.02$ and [b]$P < 0.002$ and [c]$P < 0.001$ compared to the corresponding controls.

The results on corticosterone content of liver are summarized in Table 6. Acute treatment caused from 20 to 50 % increase in 14- and 21-day-old animals but in case of the 35 day group about 15% decrease was observed; the adults did not show any significant change. chronic treatment caused 35% increase in the 14-day-old animals, but rest all the age groups showed significant (30 to 45%) reducation.

When the data were expressed as corticosterone content, ug/mg protein in liver, (Table 7), acute treatment caused 25 to 80 % increase in 14-day-old and adult rats but in case of 35-day group the corticosterone content had decreased by about 50%. Chronic treatment had no effect on 14- and 21-day-old animals but the 35-day-old and adult rats showed about 30 to 40 % reduction. The observed differences in the corticosterone contents by two different ways of expressing the results was due to alterations in the protein content of liver in corticosterone treated rats (Table 8).

From the data in Table 9 it can be seen that the acute treatment had no effect on mitochondrial corticosterone content in all the age groups except about 50% increase in 14-day-old animals. On the other hand, chronic treatment caused 100 and 250% increase in corticosterone content of liver mitochondria form adults and 14-day-old animals; in 21- and 35-day groups it remained unaffected.

Table 6

Effect of corticosterone treatment on corticosterone content of rat liver during development.

Age	Corticosterone content (μg/g tissue)		
	Control	Acute	Chronic
14 Days	154 ± 6.0	237 ± 13.0[d]	209 ± 13.0[c]
21 Days	195 ± 10.0	232 ± 10.0[a]	136 ± 17.0[b]
35 Days	105 ± 3.4	87 ± 4.8[b]	58 ± 4.0[d]
Adults	94 ± 4.0	98 ± 7.0	50 ± 3.0[d]

Results are given as mean ± SEM of 8 independent observations in each group.

[a]$P < 0.02$ [b]$P < 0.01$; [c]$P < 0.002$ and [d]$P < 0.001$ compared to the corresponding controls.

Table 7

Effect of corticosterone treatment on corticosterone content of rat liver during development.

Age	Corticosterone content (µg/mg protein)		
	Control	Acute	Chronic
14 Days	1.21 ± 0.07	2.18 ± 0.12[b]	1.16 ± 0.06
21 Days	1.62 ± 0.07	1.71 ± 0.12	0.58 ± 0.05
35 Days	1.06 ± 0.05	0.56 ± 0.03[b]	0.62 ± 0.04[b]
Adults	0.66 ± 0.03	0.82 ± 0.05[a]	0.44 ± 0.04[b]

Results are given as mean ± SEM of 8 independent observations in each group.

[a] $P < 0.02$ and [b] $P < 0.001$ compared to the corresponding controls.

Table 8

Effect of corticosterone treatment on protein content of rat liver during development.

Age	Protein content (mg/g tissue)		
	Control	Acute	Chronic
14 Days	141 ± 5	115 ± 8[b]	169 ± 11[a]
21 Days	121 ± 3	147 ± 12	212 ± 5[c]
35 Days	103 ± 3	157 ± 8[c]	94 ± 5
Adults	132 ± 11	123 ± 17	114 ± 7

Results are given as mean ± SEM of 8 independent observations in each group.

[a]P < 0.05; [b]P < 0.02 and [c]P < 0.001 compared to the corresponding controls.

Table 9

Effect of corticosterone treatment on corticosterone content of rat liver mitochondria during development.

Age	Corticosterone content (ng/mg protein)		
	Control	Acute	Chronic
14 Days	241 ± 28	354 ± 19[a]	846 ± 30[b]
21 Days	709 ± 73	858 ± 44	575 ± 14
35 Days	578 ± 66	419 ± 19	369 ± 27
Adults	402 ± 26	517 ± 33	823 ± 71[b]

Results are given as mean ± SEM of 8 independent observations in each group.

[a]$P < 0.01$ and [b]$P < 0.001$ compared to the corresponding controls.

DISCUSSION

The fluorimetric method for corticosterone estimation described here is rapid, specific, highly sensitive and economical. It could detect corticosterone contents in subnanogram quantities. 50 µl of serum or 500 µg of tissue/mitochondrial protein provided sufficient materials for the assay, and the method was linear upto 2000 ng of corticosterone. In separate experiments, it was found that cholesterol up to 10 µg and phospholipids (phosphatidylcholine and phosphatidyl- ethanolamine) up to 100 µg concentration did not show any fluorescence. Interference by other steroidal compounds has already been reported to be negligible (1,11).

Results of the present studies have shown the effect of the two corticosterone treatments (acute and chronic) on the serum levels and contents of corticosterone in brain and liver tissues and mitochondria during postnatal development of rats.

Young animals showed very high levels of serum corticosterone, which decreased with advancement of age. In contrast to this, Sapolsky and Meaney (18) have reported that in early postnatal period, the adrenocortical response to stress is greatly reduced (14, 19-27). Begining on about postnatal day 2 and continuing into the second week of life, rat pups failed to respond or responded weakly to a variety of stressors that reliably elicit a dramatic increase in

corticosterone secretion in animals at other stages (18). This period of adrenocortical quiescence has been termed the "stress non-responsive period" (SNRP) and it is defined by the relative inability of the adrenocortical axis to respond upon exposure to stress (20). The SNRP can be considered as a protective mechanism to avoid deleterious effects of glucocorticoids on growth and development of the central nervous system.

From the data presented in this chapter on the serum corticosterone levels in rats during postnatal development, it would seem that the young animals fail to reaspond to any stressors during SNRP because the serum corticosterone levels are already high. Interestingly, Schapiro (28) has reported that the half-life of corticosterone in the rat during first week of postnatal life is about 3 times higher than that in the adults. This could explain the higher levels of serum corticosterone in young pups reported here. The adult values reported are in general agreement with the values reported by others (1,3,11). However, the values of plasma corticosterone reported by Sapolsky and Meaney (18) are low even in adult animals (15 µg/100 ml) than what are reported here (35 µg/100 ml).

The acute corticosterone treatment caused significant increase in the serum corticosterone levels. However, the extent of increase was variable depending on the age of the

animals. Similarly, Dickinson et al. (15) have also reported that when rats were given acute treatment with corticosterone (50 mg/Kg body weight), after 2 to 3 hours the plasma corticosterone levels increased by about 3 fold.

In contrast to acute treatment, the chronic treatment caused significant reduction in serum corticosterone levels in animals of all the age groups except adults. The observed decrease in serum corticosterone levels could be due to increased distribution of corticosterone in the tissues or because of increased metabolism i.e. conjugation and excretion.

It was interesting to note here that the corticosterone concentration in brain tissue as well as mitochondria was 2 to 3 times higher compared to their liver counterparts in control animals. This could be due to the fact that it is the liver and not the brain, which is the major site for conjugation and inactivation of corticosterone (29).

Acute treatment did not have much effect on corticosterone content of brain but chronic treatment caused significant alterations in age-specific manner. The effects of corticosterone treatments on corticosterone content of brain mitochondria were treatment specific. Acute treatment caused significant increase whereas the chronic treatment decreased significantly the corticosterone content of brain mitochondria

from young animals. These observations may explain the age-dependent and treatment-specific effects of corticosterone on mitochondrial oxidative energy metabolism and lipid content/ composition of brain mitochondria (Chapter II and IV).

In the case of liver, the effects of corticosterone treatments were quite different from those mentioned above for the brain. Increased corticosterone content of livers from corticosterone treated 14- and 21-day-old animals seems to be due to the reduction in the protein content. In case of 35-day-old and adult rats, the observed significant decrease in corticosterone content could possibly be due to activation of the system for conjugation of corticosterone in liver upon treatment with corticosterone. Corticosterone content of liver mitochondria in general remained unaltered upon acute treatment; chronic treatment caused significant increase in age-dependent manner. This could possibly explain the treatment-specific effects of glucocorticoid overload on mitochondrial function and lipid/phospholipid content and composition of mitochondrial membrane in liver (Chapter V and VI).

REFERENCES

1. Silber,R.H., Busch,R.D. and Oslapas,R. (1958) Practical procedure for estimation of corticosterone or hydrocortisone. Clin.Chem. 4, 278-285.

2. Guillemin,R., Clayton,G.W., Smith,J.D. and Lipscomb,H.S. (1958) Measurement of free corticoids in rat plasma : physiological validation of a method. Endocrinology 63, 349-357.

3. Glick,D., Redlich,D.V. and Levine,S. (1964) Fluorimetric determination of corticosterone and cortisol in 0.02-0.05 milliliters of plasma or submilligram samples of adrenal tissue. Endocrinology 74, 653-655.

4. Peterson,R.E.(1957) The identification of corticosterone in human plasma and its assay by isotope dilution. J.Biol.chem. 225,25-37.

5. Abrahm,G.E., Manlimos,F.S. and Garza,R.(1977)Radioimmuno-assay of steroids. In: Handbook of radioimmunoassay. (Abrahm,G.E. ed.), Marcel Dekker, New York. pp.591-656.

6. Przegalinski,E., Budziszewaska,B. and Grochmal,A.(1991) Oxaprotiline enantiomers stimulate ACTH and corticosterone secretion in the rat. J.Neural.Transm. 85, 211-222.

7. Przegalinski,E., Budziszewaska,B. and Grochmal,A. (1992) Effect of adenosine analogues on plasma corticosterone concentration in rats. Acta Endocrinologica 127, 471-475.

8. Wong,Y.N., Chien,B.M. and Dinello,A.P.(1994) Analysis of corticosterone in rat plasma by high- performance liquid chromatography. J. Chromatography, B : Biomed. Appl. 661, 211-218.

9. Sweat,M.L. (1954) Sulfuric acid - induced fluorescence of corticosteroids. Anal. chem. 26, 773-777.

10. Sweat,M.L. (1954) Silica gel microcolumn for chromatagraphic resolution of cortical steroids. Anal. Chem. 26, 1964-1967.

11. Zenker,N. and Bernstein,D.E. (1958) The estimation of small amounts of corticosterone in rat plasma. J. Biol. chem. 231, 695-701.

12. Glick,D. and Redlich,D.V.(1963) Fluorometer cuvette adapters for measurements on 0.05 or 0.01 milliliter volumes. Anal. chem. 6, 471-474.

13. Haltmeyer,G.C., Denenberg,V.H., Thatcher,J. and Zarrow,M. X. (1966) Response of the adrenal cortex of neonatal rat after subjection to stress. Nature 212, 1371-1373.

14. Gray,P. (1971) Pituitary - adrenocortical response to stress in the neonatal rat. Endocrinology 89, 1126-1128.

15. Dickinson,S.L., Kennett,G.A and Curzon,G.(1985) Reduced 5 - hydroxytryptamine - dependent behaviour in rats following chronic corticosterone treatment. Brain Res. 345, 10-18.

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

17. Henkin,R.I., Casper,A.G.T., Brown,R., Harlan,A.B. and Bartter,F.C.C. (1968) Presence of corticosterone and cortisol in the central and peripheral nervous system of the cat. Endocrinology 82, 1058-1061.

18. Sapolsky,R.M. and Meaney M.J. (1986) Maturation of the adernocortical response : Neuroendocrine control mechanisms and the stress hypohresponsive period. Brain. Res. Rev. 11, 65-76.

19. Schapiro,S., Geller,E. and Eidason,S. (1962) corticoid response to stress in the steroid - inhibited rat. Proc. Exp.Biol.Med. 109, 935-937.

20. Schapiro,S. (1968) Maturation of the neuroendocrine response to stress in the rat. In : Early experience and behavior (Newton,G. and Levine,S.eds.),Thomas Springfield, IL.

21. Hiroshige,T. and Sato,T. (1970) changes in hypothalamic content of corticotropin - releasing activity following stress during neonatal maturation in the rat. Neuroendocrinology 7, 257-270.

22. Milkovic,S. and Milkovic,K. (1972) Responsiveness of the pituitary - adrenocortical system during embryonic and early postnatal periods of life. In : Physiology and pathology of adaptive mechanisms (Bajusz,S.ed.) Pergamon Press, Oxford, pp. 28-47.

23. Butte,C., Kakihana,R., Farnham,M.L. and Nobe,E.P. (1973). The relationship between brain and plasma corticosterone stress in developing rats. Endocrinology 92, 1775-1779.

24. Cote,T.E. and Yasumura,S. (1975) Effect of ACTH and histamine stress on serum corticosterone and adrenal cyclic AMP levels in immature rats. Endocrinology 96, 1044 - 1047.

25. Tang,F. and Philips.J.G. (1977) pituitary-adrenal response to ether stress in the neonatal rat J.Endrocrinol. 75, 188-184.

26. Guillet,R. and Michaelson,S.M. (1978) corticotropin responsiveness in the neonatal rat. Neuroendocrinology 27, 119-125.

27. Guillet,R., Saffran,M.and Michaelson,S.M.(1980) pituitary -adernal response in neonatal rats. Endocrinology 106, 991-994.

28. Shapiro,S. (1971) Hormonal and environmental influences on rat brain and behaviour. In : Brain development and behavior (Sterman and McGinty eds.) Academic Press, New York, pp. 307-334.

29. Bottoms,G. and Goetsch,D.D (1967) Subcellular distribution of the (^{3}H) coticosterone fraction in brain, thymus, heart and liver of the rat. Proc.Soc.Expt.Biol. Med. 124, 662 - 665.

SUMMARY

1. Micromethod for fluorimetric estimation of corticosterone in rat serum, tissues and mitochondria was standardized. The method is sensitive, rapid, economical and specific for corticosterone.

2. Serum corticosterone levels in control animals were found to be highest in the 7-day-old rats and decreased gradually with advancement of age. Also,the corticosterone content of brain tissue as well as mitochondria was 2 to 3 times higher than that of the liver.

3. Acute treatment caused significant increase in serum corticosterone levels in animals of all the age groups; the extent of increase was higher in young animals compared to adults. Upon chronic treatment serum corticosterone levels decreased significantly in young animals; adult did not show any significant change. Corticosterone content of the brain tissue remained more or less unaltered by acute treatment. Chronic treatment caused significant alterations in age-dependent manner. In young animals acute treatment inrease and chronic treatment decreased significantly the corticosterone content of brain mitochondria.

4. Upon acute treatment young animals showed significant increase in corticosterone content of liver tissue but adults did not show any change. On the other hand, chronic treatment decreased significantly the liver corticosterone content in animals of all the age groups except the 14-day-old rats. Some of these changes were due to alterations in protein content of liver from corticosterone treated rats. Acute treatment had no effect on mitochondrial corticosterone content of liver except in 14-day-group. Chronic treatment caused significant increase in mitochondrial corticosterone content in 14-day-old and adults; animals of 21- and 35-day groups remained unaffected.