Histol Histopath (1989) 4: 443-447

Histology and Histopathology

Compensatory adrenal growth in aldosterone-treated male and female hamsters

Aldona Kasprzak and Ludwik K. Malendowicz
Department of Histology and Embryology, Poznań Academy of Medicine, Poznań, Poland

Summary. The aim of the study was to investigate the compensatory adrenal growth in aldosterone-treated male and female hamsters. Hemiadrenalectomised and sham-operated animals were treated for 5 days with a daily d-aldosterone dose of 25 μg/animal.

In both male and female aldosterone-treated hamsters monoadrenalectomy did not change the relative adrenal weight if compared with sham-operated groups. The fasciculata zonae of monoadrenalectomised aldosterone-treated males was larger and contained more parenchymal cells than in appropriate control group. There was no difference in the volume of adrenocortical zones, average cell volume and in cell number between sham-operated and unilaterally adrenalectomised females. *In vitro* ^{3}H-thymidine incorporation per adrenal was markedly higher in monoadrenalectomised than in sham-operated aldosterone-treated males while the opposite was true for female hamsters.

Thus, the action of aldosterone on CAG in the hamster seems to depend on sex, with no effect in males and inhibitory action in females.

Key words: Compensatory adrenal growth, Hamster, Aldosterone, Stereology, ^{3}H-thymidine incorporation

Introduction

The following types of adrenal cortex growth *in vivo* are presently recognized: normal growth of the gland with age, growth connected with sustained elevated ACTH levels, regeneration of the cortex after enucleation and compensatory adrenal growth evoked by unilateral adrenalectomy (Dallman, 1984-85). A growing body of evidence indicates that each of them is controlled by different factors, mainly of hormonal and neural origin. However, involvement of growth factors in these processes must also be considered.

By definition, compensatory adrenal growth (CAG) is the growth seen in the remaining adrenal of unilaterally adrenalectomised animals, relative to an animal with two adrenals (Phillips et al., 1985). The mechanisms controlling this growth are still unknown. It was generally believed that removal of one adrenal resulted in a decrease in plasma corticoid levels followed by increased pituitary ACTH secretion which in turn was responsible for CAG of solitary adrenal (Tepperman et al., 1943).

However, CAG does occur in hypophysectomised-or dexamethasone-treated rats and hamsters (Engeland et al., 1975; Dallman et al., 1980; Grizzle and Dunlap, 1984; Dunlap and Grizzle, 1984). Recent studies indicate that CAG is a neurally mediated reflex, at least in part. As demonstrated by Dallman and coworkers, a neural arc comprised of afferents from one adrenal, of integration in the ventral hypothalamus, and of efferents to the contralateral gland is necessary for CAG (Engeland and Dallman, 1975, 1976; Dallman et al., 1976; Holzwarth and Dallman, 1979).

Moreover, Kleitman and Holzwarth (1985) provided evidence that sympathetic nervous system mediates the adrenocortical cell proliferation that occurs after monoadrenalectomy.

Regarding the known effects of proopiomelanocortin (POMC)-derived peptides on proliferation, growth and differentiated function of adrenocortical cells Lowry et al. (1983) suggest that the N-terminus of POMC (N-POC) (1-74) is enzymically cleaved in the adrenals into two peptides: N-POC (1-48/49) with a mitogenic effects; and N-POC (51-74) or γ_3-MSH with hypertrophic effect. Lowry et al. (1983) hypothetised that unilateral adrenalectomy stimulated, via the neural arc, the activity of an enzyme responsible for a cleavage of N-POC (1-74). Some experimental data support this hypothesis which explains the hormonal and neural interplay in the control of adrenocortical growth and function.

Offprint requests to: Prof. L.K. Malendowicz, Department of Histology and Embryology, Poznań Academy of Medicine, 6 Święcicki Str., Pl-60-781 Poznań, Poland

Some recent data indicate that mineralocorticosteroids, mainly aldosterone, do modify the CAG in the rat and hamster (Dunlap and Grizzle, 1984; Grizzle and Dunlap, 1984). As shown previously, dexamethasone effect on unilateral andrenalectomy-induced CAG depends on the sex of hamster (Kasprazak and Malendowicz, 1988) and the aim of the present study was to investigate the aldosterone influence on CAG in the male and female hamsters with special emphasis on cellular aspects of this process.

Materials and methods

Adult hamsters *(Mesocricetus auratus*, Waterhouse) were employed in the study. They were maintained under standardized conditions of light (14L: 10D) and temperature (22 ± 2° C) and laboratory pellets with the addition of fresh vegetables and tap water were available *ad libitum*.

A left adrenalectomy was performed by dorsal approach under a light ether anaesthesia. Sham operation was performed in similar manner but the adrenal gland was not touched. All animals were treated for 5 days with daily s.c. injection of 25μg aldosterone/hamster (d-Aldosterone, Sigma), the first injection was made within 1 h after surgery.

24 h after the last injection animals were decapitated, adrenals promptly removed, freed of adherent adipose tissue and weighed.

Stereologic methods

Adrenals were fixed for 24 h in Bouin's solution, embedded in paraffin and serially sectioned at 5-6 μm. Hematoxylin and eosin stained sections were evaluated stereologically as described by Weibel (1979). Detailed procedure of measurements was published earlier (Kasprzak et al., 1988). By stereologic methods the following parameters were evaluated: volume of particular adrenocortical zones, average volume of parenchymal cell in particular adrenocortical zones and the number of adrenocortical cells in the zones and in the entire cortex.

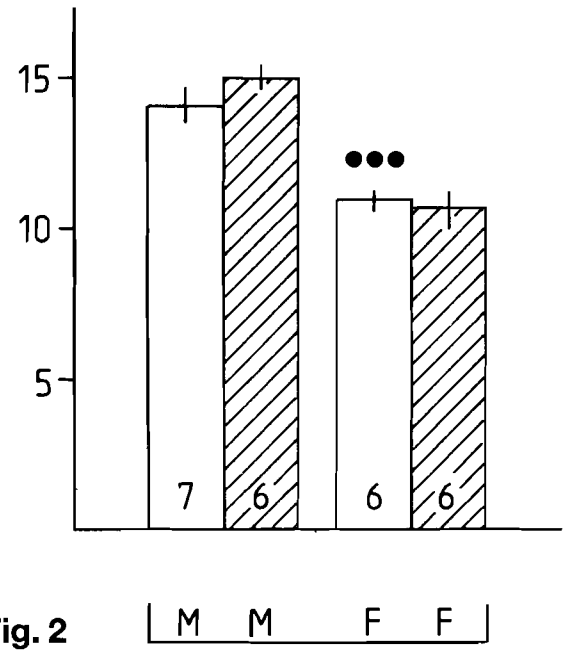

Fig. 2

Fig. 2. Relative left adrenal gland weight (mg/100 g body weight) of male and female hamsters. Adrenals were removed at the time of monoadrenalectomy (stripped bars) and from sham-operated animals treated for 5 days with aldosterone (open bars). Vertical lines represent ± SE. M-male hamsters; F - female hamsters; number of animals in the group is shown in each bar. The only statistically significant differences was found between adrenal of male and female hamsters (Student's t-test ●●● - $p < 0.001$).

In vitro ^{3}H-thymidine incorporation

The method applied was similar to that described by Dallman et al. (1980). The details are given elsewhere (Kasprzak et al., 1988).

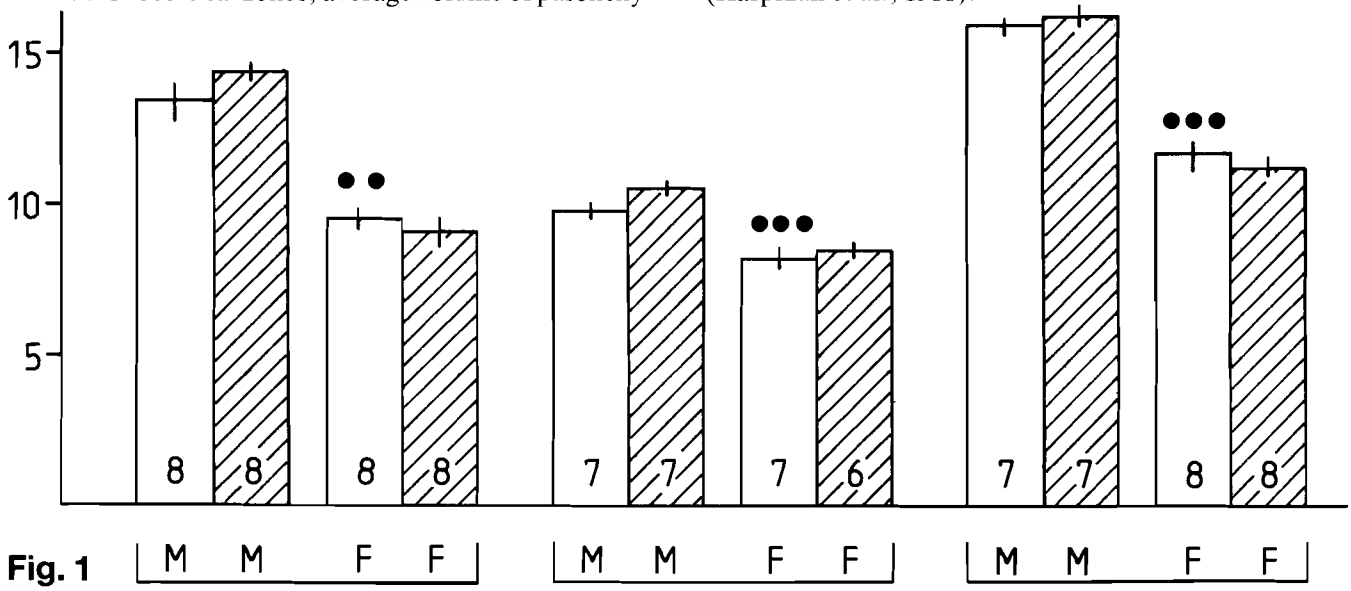

Fig. 1

Fig. 1. Relative right adrenal gland weight (mg/100 g body weight) of hemiadrenalectomised (stripped bars) and sham-operated (open bars) male and female aldosterone-treated hamsters. Results of 3 independent experiments are shown. Vertical lines represent ± SE. M-male hamsters; F - female hamsters; number of animals in the group is shown in each bar. Statistical evaluation of differences by Student's t-test. Comparisons were made between hemiadrenalectomised and sham-operated animals of the same sex (no statistically significant differences) and between sham-operated male and female hamsters (marked above female group).

Table 1. The effects of monolateral adrenalectomy and aldosterone treatment on the weight of the right adrenal gland, volume of adrenocortical zones, average volume of adrenocortical cells, number of adrenocortical cell and on in vitro ^{3}H-thymidine incorporation by the gland of the male and female hamster. Results expressed as mean ± SE.

	Males		Females	
	S	HA	S	HA
n	8	7	9	8
Body weight (g)	92 ± 5	96 ± 2	111 ± 7	107 ± 4
Adrenal weight (mg)	12.5 ± 0.9	14.1 ± 0.5	10.4 ± 0.8	10.3 ± 0.9
" " (mg/100 g)	13.7 ± 0.9	14.7 ± 0.5	9.3 ± 0.5[d]	9.6 ± 0.8
Volume of the zones (mm^3)				
zona glomerulosa	1.394 ± 0.100	1.408 ± 0.333	1.220 ± 0.095	1.075 ± 0.096
zona fasciculata	7.129 ± 0.512	8.475 ± 0.305[a]	5.139 ± 0.389[c]	5.242 ± 0.479
zona reticularis	2.047 ± 0.147	2.096 ± 0.075	1.750 ± 0.132	1.931 ± 0.173
Volume of cell (μm^3)				
zona glomerulosa	1072 ± 62	1038 ± 48	1150 ± 56	1051 ± 38
zona fasciculata	1988 ± 91	1885 ± 78	1655 ± 96[a]	1852 ± 86
zona reticularis	1191 ± 53	1169 ± 37	852 ± 27[d]	890 ± 21
Number of parenchymal cells (1×10^3)				
zona glomerulosa	1272.5 ± 91.5	1474.3 ± 53.0	1034.8 ± 81.0	990.4 ± 88.6
zona fasciculata	3571.2 ± 256.7	4473.0 ± 160.8[b]	3066.3 ± 231.9	2765.4 ± 252.8
zona reticularis	1676.8 ± 120.4	1691.8 ± 60.7	1946.0 ± 147.1	2112.3 ± 188.8
total	6520.5 ± 468.7	7639.0 ± 274.5	6047.2 ± 458.2	5868.1 ± 530.2
^{3}H-thymidine incorporation (^{3}H-CPM)				
per mg	9.8 ± 0.7	23.3 ± 3.4[d]	24.0 ± 1.8[d]	18.4 ± 2.2
per adrenal	122.4 ± 8.8	329.5 ± 11.8[d]	248.5 ± 19.4[d]	188.6 ± 16.9[a]

n = number of animals; in case of cell volume and ^{3}H-thymidine studies in each group n = 7; S = sham-operated; HA = hemiadrenalectomised. Statistical analysis of differences by Student's t test: a = $p<0.05$; b = $p < 0.02$; c = 0.01; d = $p< 0.001$ (HA versus S group and S females versus S males).

Results from individual hamsters were averaged per group and standard error of the mean calculated. Differences in means of the monoadrenalectomised and sham groups and between sham males and sham females were analysed using the Student's one-tailed t-test.

Results

In all three experiments with male and female aldosterone-administered hamsters unilateral adrenalectomy did not change the relative weight of the solitary adrenal gland. In all cases the relative weight of the right adrenal gland of sham-operated aldosterone-treated females was lower than in males (Fig. 1). Comparison of the relative adrenal weight of the left gland removed at the time of monoadrenalectomy with the weight of the left gland of sham-operated aldosterone-treated hamsters revealed no difference and suggested that within 5 days aldosterone doses applied did not change the weight of the gland *per se* (Fig. 2).

The fasciculata zonae of monoadrenalectomised aldosterone-treated males was larger and contained more parenchymal cells than in appropriate control group (Table 1). All the remaining stereologic parameters studied did not show differences between both groups of male hamster. ^{3}H-thymidine incorporation was markedly higher in monoadrenalectomised than in sham-operated aldosterone-treated males.

In aldosterone-administered female hamsters there was no difference in the volume of adrenocortical zones, average cell volume or in cell number between sham-operated and unilaterally adrenalectomised groups. However in this case ^{3}H-thymidine incorporation per gland was lower in hemiadrenalectomised than in sham-operated animals.

If compared in sham-operated aldosterone-treated hamsters, the relative adrenal gland weight, volume of the zona fasciculata, average volume of fasciculata and reticularis cells all were lower in females than males, while the opposite was true for ^{3}H-thymidine incorporation.

Discussion

Structure and function of hamster adrenal cortex differs in several aspects from that of other rodents. The gland is almost completly devoid of lipid droplets and stored cholesterol esters, and it secretes cortisol as a principal glucocorticosteroid. In this species biosynthesis of steroid hormones depends mainly on intraadrenal synthesis of cholesterol from small molecules of substrate and corticoid secretion rate is lower than in other mammals (Frenkel et al., 1965; Lehoux and Lefebvre, 1980; Jansen and Birkenhagen, 1981; Malendowicz and Nussdorfer, 1984; Spady and Dietschy, 1985; Albers et al., 1985; Iwaki et al., 1985).

Unlike in rats, adrenals of the male hamster are larger and secrete more cortisol than in females, the differences depending on stimulatory action of testosterone on hypothalamo-pituitary-adrenal cortex-liver axis of the hamster (Gaskin and Kitay, 1970, 1971; Malendowicz et al., 1982a,b; Malendowicz and Nussdorfer, 1984; Nikicicz et al., 1984a,b). As shown in the present study, some of these sex-related differences in the structure and function of the gland are also seen in aldosterone-treated sham-operated hamsters.

The occurrence of unilateral adrenalectomy-induced CAG in the hamster is a subject of considerable dispute. The most frequently used parameter depicting CAG in this species is the weight of the gland; however, due to great variations in adrenal weight of hamsters of comparable size and age this parameter is of minor importance.

The increase in adrenal gland weight due to hemiadrenalectomy was observed only in some reports (Yonetsu, 1966; Dunlap and Grizzle, 1984; Kasprzak et al., 1988) while others did not find such changes (Reiter and Hoffman, 1967; Kasprzak and Malendowicz, 1985, 1988; Nowak et al., 1989). Therefore, the others parameters depicting CAG in the hamster are required. As early as in 1967 Reiter and Hoffman found a marked increase in the number of cells in the S-phase of the cell cycle in the solitary adrenal of hemiadrenalectomised male hamsters. On the other hand, Yonetsu (1966) observed ultrastructural features of hypertrophy of the zona fasciculata cells of monoadrenalectomised male hamsters. Our experiences show that estimation of the average cell volume by means of sterology gave very useful data characterizing CAG in the hamster. The response of the solitary adrenal in the hamster to monoadrenalectomy depends on sex. In the male, cells of the zona fasciculata undergo hypertrophy while in the female reticularis cells enlarge and these cellular alterations are independent of changes in the weight of the solitary adrenal gland (Kasprzak and Malendowicz, 1985; Kasprzak et al., 1988; Nowak et al., 1989). Moreover, the modulatory effect of dexamethasone on CAG in the hamster is a sex depending event (Kasprzak and Malendowicz, 1988).

Estimating the changes in the weight of decapsulated adrenal gland of the male hamsters, Dunlap and Grizzle (1984) found that administration of either aldosterone alone or in combination with dexamethasone and ACTH completely blocked CAG after monoadrenalectomy.

Similar results have been reported for intact or hypophysectomised male rats (Grizzle and Dunlap, 1984).

On the other hand Phillips et al. (1985) reported that in both male and female rats aldosterone did not change CAG evaluated by alterations in adrenal gland weight. Results of present study did not totally support the findings of Dunlap and Grizzle (1984) on inhibitory effect of aldosterone on CAG in the male hamster. Although in male hamsters treated with aldosterone the adrenal gland weight of monoadrenalectomised animals did not differ from sham-operated ones, this parameter does not adequately characterize CAG in the species studied. On the other hand, a marked increase in ^{3}H-thymidine incorporation was found in adrenals of hemiadrenalectomised aldosterone-treated male hamsters, an effect paralelled by an increase in the number of fasciculata cells and with tendency to an increase in the number of parenchymal cells in the entire gland. Under these conditions increased proliferative activity of adrenal cortex can be responsible for a lack of enlargement of fasciculata cells like that observed in non-treated monoadrenalectomised male hamsters (Kasprzak and Malendowicz, 1985; Kasprzak et al., 1988; Nowak et al., 1989).

The opposite conclusion may be drawn from experiments with aldosterone-treated female hamsters. A significant drop in ^{3}H-thymidine incorporation by the gland and a lack of enlargement of reticularis cells in monoadrenalectomised-females suggest the inhibitory action of aldosterone on CAG in the female hamsters. Thus the action of aldosterone on CAG in the hamster seems to depend on sex, with inhibitory action in females.

The mechanism of aldosterone action on CAG is not clear and Grizzle and Dunlap (1984) suggest that this mineralocorticoid acts either directly on adrenals or via intracranial type I receptors that bind aldosterone; however, studies of Phillips et al. (1985) do not support these hypotheses.

Acknowledgements. This paper has been supported in part by a grant No X-15 from Poznań Academy of Medicine, Poznań, Poland.

Part of the paper was presented in partial fulfilment of Ph.D. thesis requirements (A.K.) of Poznań Academy of Medicine, Poznań, Poland.

References

Albers H.E., Yogev L., Todd R.B. and Goldman B.D. (1985). Adrenal corticoids in hamsters: Role in circadian timing. Am. J. Physiol. 248, R434-R438.

Dallman M.F. (1984-85). Control of adrenocortical growth in vivo. Endocr. Res. 10, 213-242.

Dallman M.F., Engeland W.C. and Shinsako J. (1976). Compensatory adrenal growth: A neurally mediated reflex. Am. J. Physiol. 231, 408-411.

Dallman M.F., Engeland W.C., Holzwarth M.A. and Scholz M.

(1980). Adrenocorticotropin inhibits compensatory adrenal growth after unilateral adrenalectomy. Endocrinology 107, 1397-1404.

Dunlap N.E. and Grizzle W.E. (1984). Golden Syrian hamster: A new experimental model for adrenal compensatory hypertrophy. Endocrinology 114, 1490-1495.

Engeland W.C. and Dallman M.F. (1975). Compensatory adrenal growth is neurally mediated. Neuroendocrinology 19, 352-362.

Engeland W.C. and Dallman M.F. (1976). Neural mediation of compensatory adrenal growth. Endocrinology 99, 1659-1662.

Engeland W.C., Shinsako J. and Dallman M.F. (1975). Corticosteroids and ACTH are not required for compensatory adrenal growth. Am. J. Physiol. 229, 1461-1464.

Frenkel J.K., Cook K., Grady H.J. and Pendleton S.K. (1965). Effects of hormones on adrenocortical secretion of golden hamsters. Lab. Invest. 14, 142-156.

Gaskin J.H. and Kitay J.I. (1970). Adrenocortical function in the hamster: Sex differences and effects of gonadal homones. Endocrinology 87, 779-786.

Gaskin J.H. and Kitay J.I. (1971). Hypothalamic and pituitary regulation of adrenocortical function in the hamster. Effects of gonadectomy and gonadal hormone replacement. Endocrinology 89, 1047-1059.

Grizzle W.E. and Dunlap N.E. (1984). Aldosterone blocks adrenal compensatory hypertrophy in the rat. Am. J. Physiol. 246, E306-E310.

Holzwarth M.A. and Dallman M.F. (1979). The effect of hypothalamic hemi-islands on the compensatory adrenal growth. Brain Res. 162, 33-43.

Iwaki T., Noguchi A. and Sekimoto T. (1985). Sources of extramitochondrial corticoidogenic cholesterol in the adrenal cortex. Jpn. J. Pharmacol. 38, 207-214.

Jansen and Birkenhager J.C. (1981). Liver lipase-like activity in human and hamster adrenocortical tissues. Metabolism 30, 428-430.

Kasprzak A. and Malendowicz L.K. (1985). Sex differences in adrenocortical structure and function. XIX. Differences in the compensatory hypertrophy of the adrenal cortex in the male and female hamster after unilateral adrenalectomy. J. Anat. 143, 161-165.

Kasprzak A. and Malendowicz L.K. (1988). Compensatory adrenal growth in dexamethasone treated male and female hamsters. In vivo 2, 409-414.

Kasprzak A., Nussdorfer G.G. and Malendowicz L.K. (1988). Sex differences in adrenocortical structure and function. XXVI. Further studies on the differences in the compensatory growth of the adrenal cortex of the male and female hamster after unilateral adrenalectomy. In Vivo. 2, 119-122.

Kleitman N. and Holzwarth M.A. (1985). Compensatory adrenal cortical growth is inhibited by sympathectomy. Am. J. Physiol. 248, E261-E263.

Lehoux J.G. and Lefevre A. (1980). De novo synthesis of corticosteroids in hamster adrenal glands. J. Steroid Biochem. 12, 479-485.

Lowry P.J., Silas L., McLefan C., Linton E.A. and Estivariz F.E. (1983). Pro-melanocyte-stimulating hormone cleavage in adrenal gland undergoing compensatory growth. Nature. (London), 306, 70-73.

Malendowicz L.K. and Nussdorfer G.G. (1984). Sex differences in adrenocortical structure and function. XV. Cellular composition and quantitative ultrastructural study on adrenal cortex of adult male and female hamster. J. Submicrosc. Cytol. 16, 715-720.

Malendowicz L.K., Kasprzak A. and Nikicicz H. (1982a). Sex differences in adrenocortical structure and function. IX. Stereologic studies on the effects of gonadectomy and testosterone or estradiol replacement on adrenal cortex of adult male and female hamsters. Z. mikrosk.-anat. Forsch. 96, 91-102.

Malendowicz L.K., Nikicicz H. and Kasprzak A. (1982b). Sex differences in adrenocortical structure and function. VIII. Karyometric and stereologic studies on adrenals in intact adult male and female hamsters. Z. mikrosk.-anat. Forsch. 96, 81-90.

Nikicicz H., Kasprzak A. and Malendowicz L.K. (1984a). Sex differences in adrenocortical structure and function. XIII. Stereologic studies on adrenal cortex of maturing male and female hamsters. Cell Tissue Res. 235, 459-462.

Nikicicz H., Kasprzak A., Majchrzak M. and Malendowicz L.K. (1984b). Sex differences in adrenocortical structure and function. XVII. Analysis of adrenal growth rate in maturing male and female rats and hamsters. Gegenbaurs morph. Jahrb. (Leipzig) 130, 572-582.

Nowak M., Kasprzak A., Leśniewska B. and Malendowicz L.K. (1989). Studies on the compensatory growth of the adrenal cortex of the male hamster after unilateral adrenalectomy. Exp. Clin. Endocrinol. (in press).

Phillips R., Crock C. and Funder J. (1985). Effects of mineralocorticoids and glucocorticoids on compensatory adrenal growth in rats. Am. J. Physiol. 248, E450-E456.

Reiter R.J. and Hoffman R.A. (1967). Adrenocortical cytogenesis in the adult male golden hamster. A radiographic study using tritiated-thymidine. J. Anat. 101, 723-729.

Spady D.K. and Dietschy J.M. (1985). Rates of cholesterol synthesis and low-density lipoprotein uptake in the adrenal glands of the rat, hamster and rabbit in vivo. Biochim. Biophys. Acta 836, 167-175.

Tepperman J., Engel F.L. and Long C.N.H. (1943). A review of adrenocortical hypertrophy. Endocrinology 32, 373-402.

Weibel E.R. (1979). Stereological methods. I. Practical methods for biological morphometry. Academic Press. New York. 1-415.

Accepted April 22, 1989