Gestational Changes in Guinea Pig Adrenal Cortex: A Morphometric and Histological Study

KOBAY ADRENAL KORTEKSİNDE GESTASYONEL DEĞİŞİKLİKLER: MORFOMETRİK VE HİSTOLOJİK BİR ÇALIŞMA

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Summary

In this study, it was aimed to determine probable structural changes owing to gestation in guinea pig adrenal cortex. For this aim, the adrenal cortices ofpregnant guinea pigs at day 35 of gestation were compared to those of nonpregnants, light- and electron-microscopically. Gestational changes were as follows: I- Light microscopically: an increase in the thickness oftotal cortex and cortical layers, many mitotic figures in the zona glomerulosa (ZG) and external zona fasciculctta (ZF), a decrease in the cell counts per unit area ofinner ZF and outer zona reticularis (ZR), large amounts of lipid vacuoles in the cells of inner ZF and outer ZR, more lipofuscin pigment granules; 2- Electron microscopically, much more developed smooth endoplasmic reticulum (SER) in the cells ofZR and internal ZF, apparent myeline figures in the ZR cells, abundant and polymorphic mitochondria, many lysosomes, more lipofuscin pigment granules and multivesicular bodies in the cells of deeper cortex, many fibroblast-Iike cells around cortical capillaries, many lipid-fi-ee cells with short cytoplasmic processes and a heterochromatic nucleus in the perisinusoidal spaces of deeper cortex were observed.

In conclusion, the reason of the gestational changes occurring in the adrenal cortex may be the stimulation of adrenal cortex by adrenocorticotropic hormone released from hypophysis and/or adrenal gland by the effect of increased estrogen level in pregnancy.

Key **Words:** Adrenal cortex, Pregnancy, Morphometry, Guineapig

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Özet

Bu araştırmada, kobay adrenal korteksinde gebeliğe bağlı olarak meydana gelebilecek olası yapısal değişiklikler saptanmaya çalışıldı. Bu amaçla, 35 günlük gebe hayvanların adrenal korteksleri kontrol grubu ile ışık ve elektron milavskopik düzeyde kıyaslandı. Gebe hayvanların adrenal korteksinde: 1- İşık mikroskobik olarak; total korteksin ve korteks tabakalarının kalınlığında artış, zona glomerulozada (ZG) ve zona fas i kü latan m (ZF) dış bölümünde çok sayıda mitoz, ZF'nın iç ve zona retikülarisin (ZR) dış bölümünde birim alana düşen hücre sayısında azalma ve buradaki hücrelerde bol miktarda lipid vakuolü, derin korteks hücrelerinde daha fazla lipofussin pigmenti; 2- Elektron mikroskobik olarak; ZF'nın iç bölümündeki ve ZR'deki hücrelerde çok daha iyi gelişmiş graniilsiiz endoplazma retikulımu (SER), ZR hücrelerinde yer yer miyelin figürleri, derin korteks hücrelerinde bol miktarda ve bazen değişik şekilli mitokondriyonlaı; çok sayıda lizozom, daha fazla lipofussin pigmenti ve multiveziküler cisim, kortikal kapillerler çevresinde fibroblasta benzeyen çok sayıda hücre, derin kortekste perisinüzoidal alanlarda sitoplazmasında lipid damlaları bulunmayan, kısa sitoplazmik uzantıları ve heterokromatik nukleusu olan hücreler saptandı.

Sonuç olarak, gebe adrenal korteksinde gözlenen bu değişikliklerin, gebelikte yükselen östrojenin etkisiyle hipofizden ve/veya adrenal bezden salgılanması artan adrenokortikotrop hormonun adrenal korteksi uyarmasından kaynaklanıyor olabileceği düşünüldü.

Anahtar Kelimeler: Adrenal korteks, Gebelik, Morfometri, Kobay

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The cortex of adrenal gland is divided into three layers: outer zone, the zona glomerulosa (ZG); midd-le zone, the zona fasciculata (ZF) and inner zone, the zona reticularis (ZR). Adrenal cortex secretes steroid hormones which can be classified in three main categories ie. mineralocorticoids, glucocorticoids and gonadocorticoids (sex steroids). Mineralocorticoids are secreted by the cells of ZG. Glucocorticoids are released from ZF and outer-ZR Gonadocorticoids are secreted by ZR and

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inner-ZF. Cortical secretion is controlled principally by adrenocorticotropic hormone (ACTH) released from hypophysis (1,2). Various endogenous and exogenous factors such as species, age, sex, gonadal functions, physical and chemical factors effect adrenocortical activity (2-7). In this study, it was aimed to observe the microscopic adrenocortical changes due to the consequences of the possible functional changes occurring in adrenal cortex in relation with hormonal alterations in pregnancy.

Materials and Methods

In the present study, 8 nonpregnant adult guinea pigs and 8 pregnant ones which were at day 35 of pregnancy were employed. The time of the beginning of gestation was defined as midpoint of a 24-hour mating period. Gestational period in the guinea pig is 59-72 days (8). The anhnals were maintained under standardized conditions of light (14 hour on/10 hour off) and temperature (22±2 °C), with free access to laboratory pellets and tap water. Nonpregnant (control) and pregnant animals were killed by decapitation. The abdominal cavities of anhnals from both groups were opened with a ventral midline incision and adrenal glands were promtly removed. The left adrenal glands were fixed in Boiiin's solution, dehydrated in ethanol and routinely embedded in paraffin. The paraffin sections (5-6 mm in thick) were stahied with haematoxylin-eosin and examined in an Olympus BH-2 light microscope. On these sections, the thicknesses of adrenocortical layers were measured by using an oculometer, and the parenchymal cell counts per unit area (0.01 mm^2) were calculated in the area where the cells were tightly together and stroma was not apparent (9). Cortical pieces taken from the right adrenal glands were fixed in 3% glutaraldehyde in 0.2 M phosphate buffer, postfixed in 2% phosphate-buffered osmium tetroxide, dehydrated in acetone and propylene oxide, and embedded in Araldite CY 212. Sections were cut with LKB III ultramicrotome. Semi-thin sections were stained with toluidine blue and observed under the light microscope to select the three adrenocortical zones. Ultra-thin sections were stained with uranyl acetate and lead citrate, examined in a Jeol 100 C electron microscope (10,11).

Statistics: Nonparametric Wilcoxon test was used for statistical evaluation.

Results

For morphometric comparison of adrenal cortices of pregnant and nonpregnant guinea pigs, the thicknesses of total cortex and ZG and combined thickness of ZF and ZR (the thickness of ZF+ZR) were measured by using an oculometer; parenchymal cell counts per unit area in the ZG and in external and internal compartments of ZF and ZR were calculated (Table 1).

The thicknesses of total cortex, ZG and ZF+ZR were found to be increased in the pregnant animals compared to those of the nonpregnants ($p<0.05$) (Table 1). Although no statistically significant differences were demonstrated between two groups in terms of cell counts per unit area of ZG, outer ZF and inner ZR, cell counts in inner ZF and outer ZR were significantly fewer in the pregnants than those of the nonpregnants ($p< 0.05$) (Table 1).

Table **1.** Morphometric values of adrenal cortex of pregnant and nonpregnant guinea pigs

	In Nonpregnants	In Pregnants	p value
The thickness of total cortex (jim)	1614.25± 146.25	2412.5 ±208.35	0.0010*
The thickness of ZG (Jim)	68. 975 ± 7.4	110 ± 10.525	0.0007*
The thickness of ZF+ZR (jim)	1545.275 ±77.32	2302.5 ± 96.45	0.0051*
Number of cells / 0.01 mm2 / ZG	41.30 ±4.35	43.20 ±5.53	0.3743
Number of cells / 0.01 mm^2 / outer ZF	29.00 ±6.41	31.50 ±4.28	0.3139
Number of cells / 0.01 mm^2 / inner ZF	25.30 ±2.16	19.00 ±3,13	0.0051*
Number of cells / 0.01 mm^2 / outer ZR	39.00 ±4.92	33.60 ± 5.34	0.0284*
Number of cells / 0.01 mm^2 / inner ZR	48.50 ±6.64	46.90 ±4.18	0.4990

* $p<0.05$

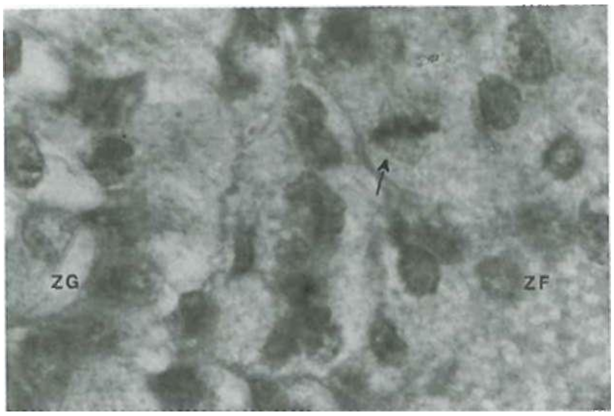


Figure 1. The photomicrograph of adrenocortical cells of a pregnant guinea pig. ZG: zona glomerulosa; ZF: zona fasciculata externa; the arrow indicates a mitotic figure. Haematoxylin-eosin (H-E), Original Magnification (O.M.) x 100.

In comparison with nonpregnant controls, more mitotic figures were observed in the ZG and outer ZF of pregnants (Figure 1). In spite of the fact that big lipid vacuoles were scarcely seen in the inner ZF- and outer ZR-cells of nonpregnants, the same cells of pregnants were crowded with large lipid vacuoles (Figure 2,3). Ultrastructural characteristics of the ZG-cells and outer ZF-cells of pregnants were similar to those of nonpregnant controls, hi the cytoplasms of inner ZF-cells in pregnants, there were abundant small mitochondria, many free and bound ribosomes, more developed SER situated in close proximity to mitochondria, many lysosomes and large lipid droplets (Figure 4). In the ZR-cclls of pregnants, polymorphic and dividing mitochondria, many lipid droplets and lysosomes, numerous free and bound ribosomes were observed (Figure 5,6). In these cells, SER tubuli were more abundant (Figure 6-8); in some spots, myeline figu-

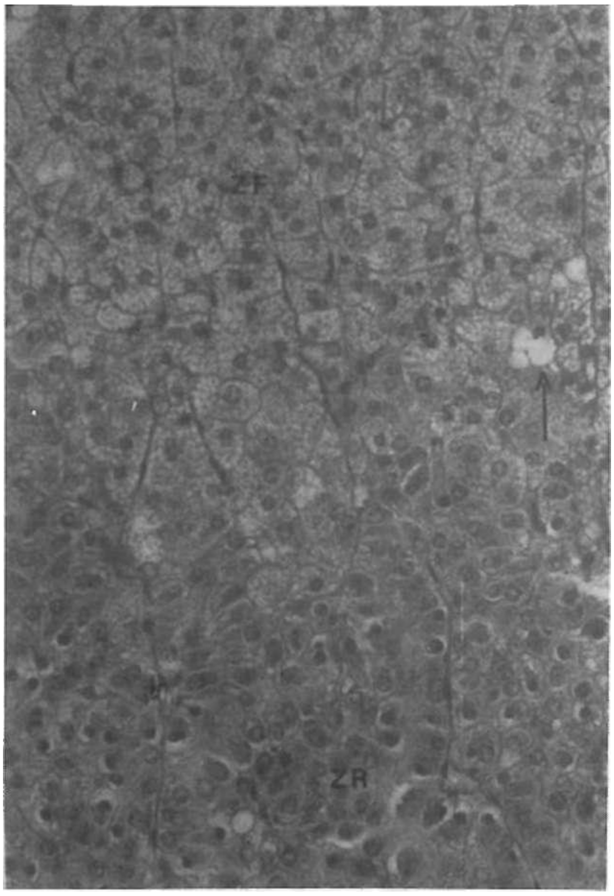


Figure 2. The photomicrograph of adrenal cortex of a nonpregnant (control) guinea pig. ZF: zona fasciculata; ZR: zona reticularis; arrows indicate large lipid vacuoles. H-E, O.M. x 10.

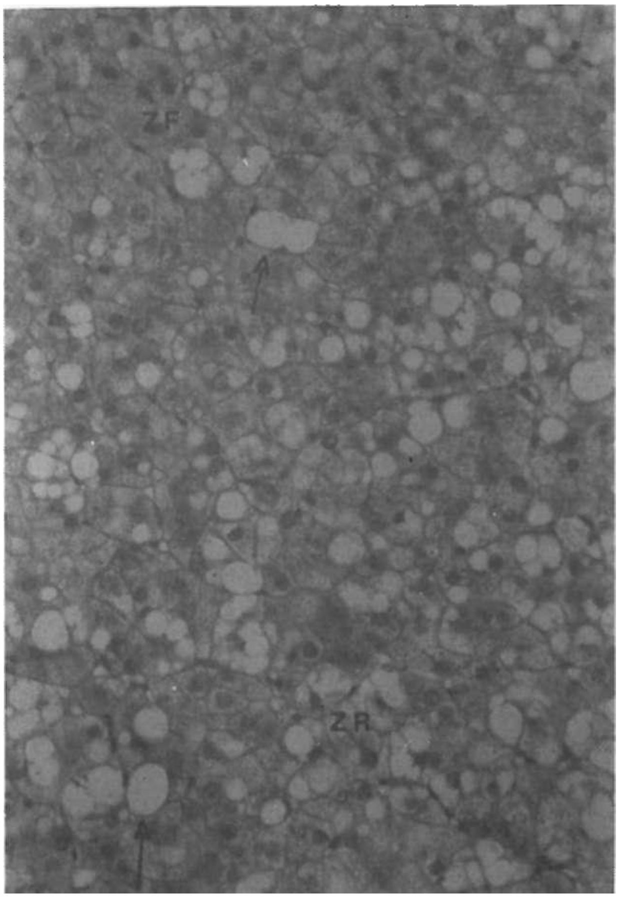
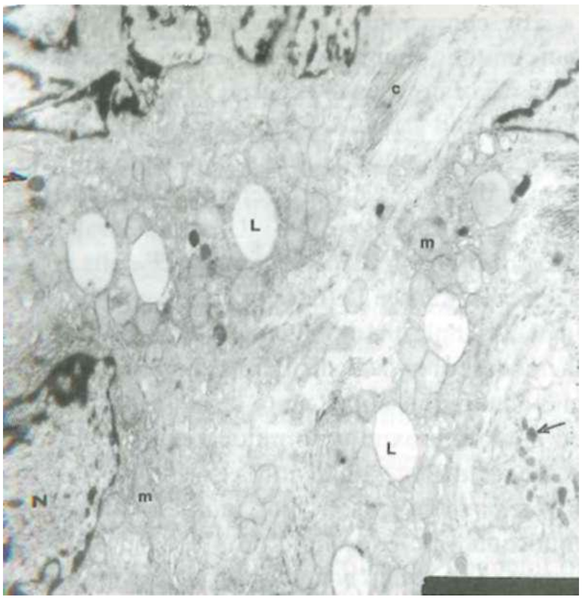
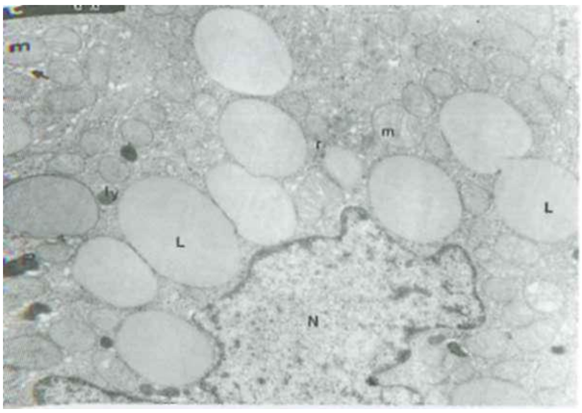


Figure 3. The photomicrograph of adrenal cortex of a pregnant guinea pig. ZF: zona fasciculata; ZR: zona reticularis; arrows indicate large lipid vacuoles. H-E, O.M. x 10.



-e 4. The election micrograph of a cell from ZF interna of drenal cortex of a pregnant guinea pig. N: nucleus; L: droplets; arrows indicate SER tubuli; m: mitochondria, r: ^irid bound ribosomes; LP: lipofucsin pigment granules; sosomes, x 6600.



j-tz 5. The election micrograph of outer ZR-cells from the al cortex of a pregnant guinea pig. N: nucleus; L: lipid j ˛ ts; m: polymorphic mitochondria; ly: lysosomes; r: free >tind ribosomes; c: collagen fibres, x 6600.

ere demonstrated among SER tubuli (Figure ! comparison with nonpregnants, more abun- lipofuscin pigment granules were observed in 2^R-cells of pregnants (Figure 9). Even more secondary lysosomes and multivesicular bodies increasing from outer ZR to inner ZR were seen in pregnants (Figure 6,8). In addition, many fibroblast-like cells containing an oval or discoid nucleus were demonstrated around cortical capillaries of pregnants. Collagen fibres were visible adjacent to these cells (Figure 10). Apart from these, in pregnant animals, many lipid-free cells with short cytoplasmic processes and a hcterochromatic nucleus were seen in the perisinusoidal spaces of deeper

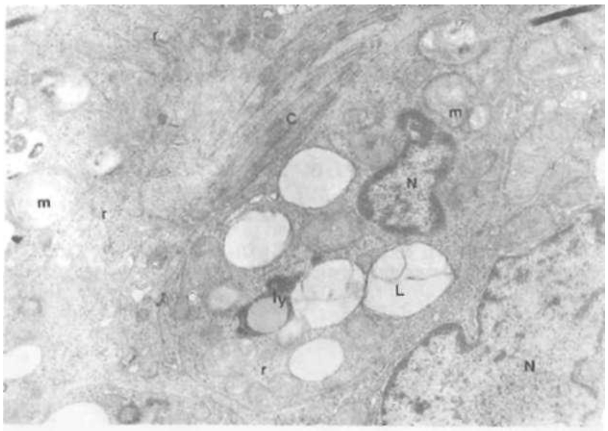


Figure 6. The electron micrograph of two cells from outer ZR of the adrenal cortex of a pregnant guinea pig. N: the nucleus of the parenchymal cell; n: the nucleus of the fibroblast-like cell; c: capillary; L: lipid droplets; Mv: a multivesicular body; s: SER; m: a dividing mitochondrium; ly: lysosomes; LP: lipofucsin pigment granules; cf: collagen fibres, x 4900.

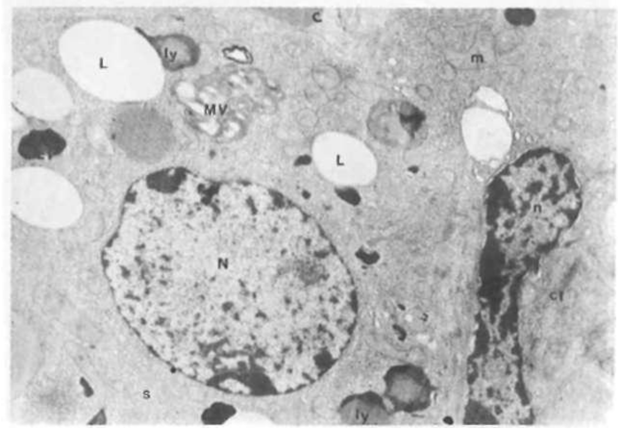


Figure 7. The electron micrograph of a ZR-cell from the adrenal cortex of a pregnant guinea pig. M: myelin figures; s: SER tubuli; ly: lysosomes; m: mitochondrium; LP: lipofucsin pigment granules, x 6600.

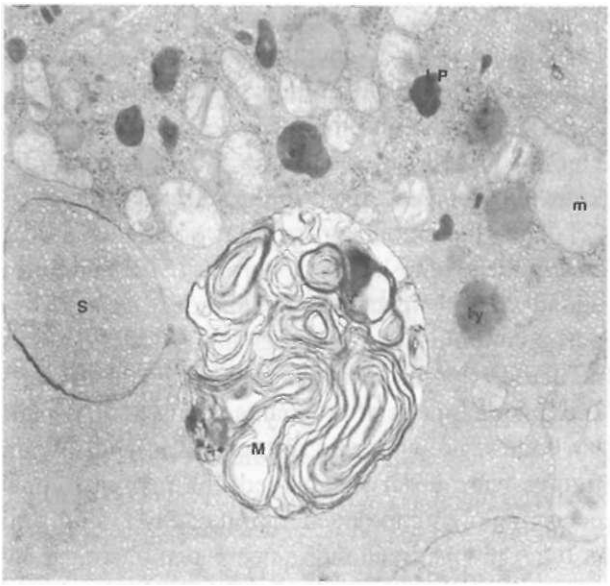


Figure 8. The electron micrograph of an area from the inner ZR of the adrenal cortex of a pregnant guinea pig. N: parenchymal cell nucleus; Mv: multivesicular bodies; Ly: lysosome; LP: lipofucsin pigment granules; s: SER. x 4900.

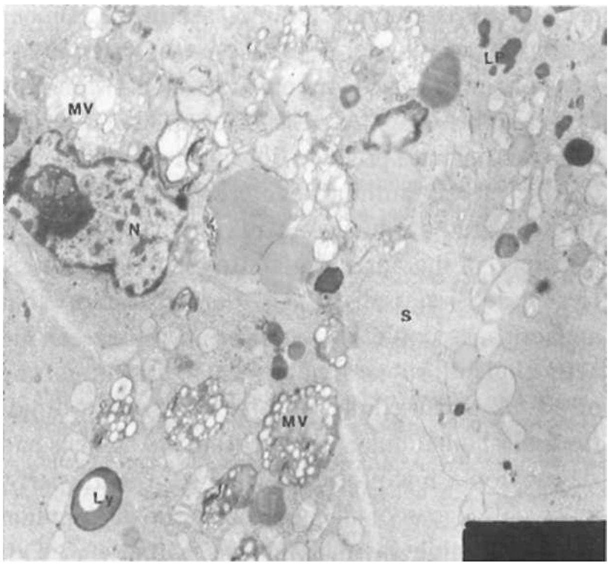


Figure 9. The photomicrograph of inner ZR of the adrenal cortex of a pregnant guinea pig. s: sinusoid; arrows indicate lipofucsin pigment granules. H-E, O.M. x 20.

cortex, but particularly in the transition zone of cortex and medulla.(Figure 11).

Discussion

Plasma concentrations of maternal steroid hormones as estrone (E1), estradiol (E2), androstcnedione (D4) and testosterone (T) increase during the course of pregnancy (12,13). Accordingly, direct or indirect functional and related morphological changes might be expected to occur in the adrenal cortex that secretes corticosteroids. In this study, an increase in the thickness of cortical layers of pregnants was found. Kovzun (14) demonstrated that estradiol causes an anabolic effect on metabolism

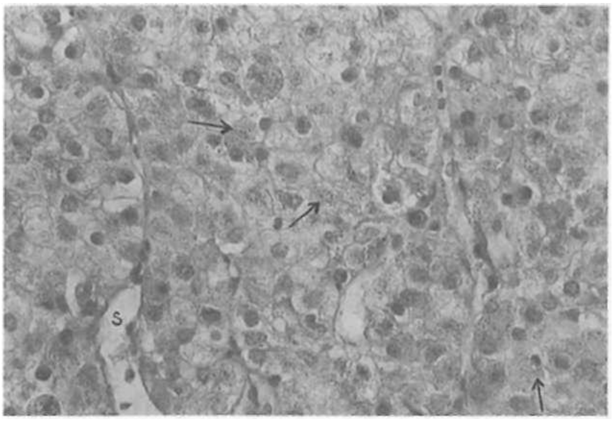


Figure 10. The electron micrograph of an area from the inner ZF of the adrenal cortex of a pregnant guinea pig. Arrows indicate fibroblast-like cells; c: capillary; E: endotelium; cf: collagen fibres; L: lipid droplets of a ZF-cell. x 4900.

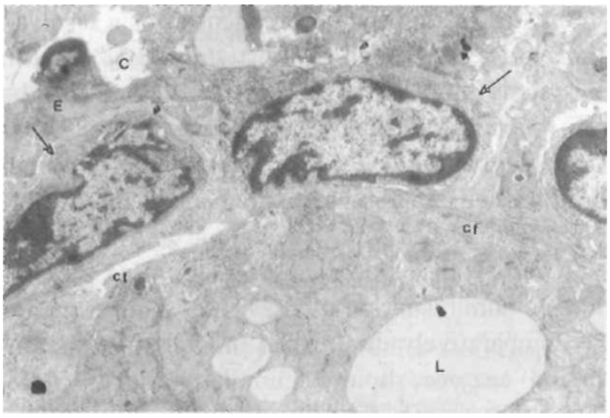


Figure 11. An electron micrograph from the transition zone of adrenal cortex and medulla of a pregnant guinea pig. M: medullar cells; c: capillary; the arrow indicates the lipid-free cell with short cytoplasmic processes and a heterochromatic nucleus, x 5500.

of DNA, RNA and protein in the adrenal cortex and an increase in the adrenal weight. Some researchers showed that estradiol stimulates pituitary-adrcnocortical axis resulting in increment in plasma concentration of ACTH (6,7). This hormonal effect naturally gives rise to enlargement of adrenal cortex controlled by especially ACTH. Some authors reported that ACTH stimulates proliferation of ZG- and outer ZF-cells and centripetal migration of these proliferating cells (15). Although no significant difference was demonstrated between two study groups in terms of cell counts per unit area of ZG, ZG in pregnants was thicker than that of the nonpregnants and had many mitotic figures. So it was concluded that the increase of the thickness of ZG in the pregnants might be related to the hyperplasia stimulated by ACTH. In pregnants, it was found that the thickness of ZF+ZR was more, but cell counts of inner ZF and outer ZR were significantly fewer than those of the nonpregnants; therefore, it was concluded that the cells of inner ZF and outer ZR were hypertrophic in pregnants. It was reported that ACTH resulted in adrenocortical hypertrophy and hyperplasia by means of increasing RNA and protein syntheses (16,17). Thus the observation of numerous free polyribosomes and well developed RER in the adrenocortical cells of pregnants is not surprising. Cholesterol, a steroid hormone precursor, is converted to cholesteryl ester (CE) in adrenocortical cells and accumulates in lipid droplets. Some authors reported the volume of lipid-droplcl compartment decreased because of CE hydrolysation for steroidogenesis as a result of the short-term ACTH administration (2,18). But in this study lipid accumulation was observed in the ZF- and outher ZR-cells of pregnants. Indeed it was declared that the volume of lipid droplets in deeper cortical cells increases in pregnancy and ACTH stimulates the enzymes mediating both hydrolisation and synthesis of CE (19). But during the prolonged administration of ACTH, as a consequence of comparatively diminished production of degradating enzyme, lipid accumulation takes place. Thus in pregnancy lipid accumulation related to the prolonged ACTH stimulation is a naturally occurring process. Comparatively more abundant mitochondria and SER in the cells of ZF interna and ZR were demonstrated in pregnants. Dehidroepiandrosteron (DHEA), a precursor of E1, E2, D4 and T hormones known to increase during pregnancy is synthesized in the ZR of the adrenal cortex of pregnants due to the absence of necessary enzymes in placenta (12,13). In addition to ZR, sex steroids are known to be synthesized in the internal ZF as well (2). Thus the amount of cellular organelles taking part in the synthesis of sex steroids (SER and mitochondria) are expected to be abundant in these hyperfunctioning cells in pregnants (2,16). Besides numerous lipofuscin pigment granules in the ZR- and inner ZF-cells of pregnants, many multivesicular bodies, polymorphic mitochondria and myelin figures were observed. Indeed, the myelin figures occurring owing to degeneration of SER tubuli and polymorphic mitochondria were demonstrated by some researchers as a result of ACTH stimulation (16). So, it was decided that the occurrence of degeneration in the hyperactive organelles and accumulation of metabolic residue in the hyperfunctioning cells are natural processes. Many fibroblast-like cells were observed around adrenocortical capillaries of pregnants. In the enlarging cortices of pregnants, there is an expected increase in the number of fibroblasts in order to synthesize connective tissue fibres that have a supportive function for cells and vascular structures. Miller et al. (20) reported that macrophages produce fibroblast growth factors (FGF). Some authors declared that there are macrophages, producing a wide range of biological products, in the adrenal gland (3,4,21). Gonzalez-Hernandez et al. (22) demonstrated that macrophages could be found in all regions of the adrenal gland, but particularly in the transition zone of cortex and medulla. In pregnants, many lipid-free cells containing a heterochromatic nucleus and short cytoplasmic processes were seen in the perisinusoidal spaces of deeper cortex. In my opinion these cells might be macrophages. Miller et al. (20) reported that during pregnancy the number of human macrophages increase with rising concentrations of estrogen. Many investigators reported that there was a local production of corticotropin-releasing hormone (CRH) and ACTH in the adrenal gland (3,4,23-25). Some researchers reported that infra-adrenal ACTH was produced by resident adrenal macrophages (26) and estrogens stimulated "adrenal macrophage system" (27).

In view of the above references, as a result of an increase in estrogen level in pregnancy, ACTH secretion is expected to be increased. It was reported that ACTH is secreted by adrenal gland apart from hypophysis, also and was shown to stimulate hyperplasia and hypertrophy in adrenocortical cells.

In conclusion, increased intraadrenal-ACTH level might be the reason of the morphological changes occur in the adrenal cortex in pregnancy.

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