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ADRENAL ZONA GLOMERULOSA AFTER HYPOPHYSECTOMY IN THE MONGOLIAN GERBIL

ABSTRACT. The ultrastructure of the adrenal zona glomerulosa was examined in hypophysectomized female Mongolian gerbils (*Meriones unguiculatus*). After 3 weeks, the width of the zona glomerulosa increased significantly. As early as 2 weeks, the smooth endoplasmic reticulum became hypertrophic and the Golgi apparatus was well developed. Two populations of coated vesicles were observed with increased frequency in zona glomerulosa cells of hypophysectomized gerbils.

Introduction

FACTORS that regulate the zona glomerulosa have been studied extensively in several species of animals (Gross, 1967; Davis, 1967 and Müller, 1971). Renin originating from the juxtaglomerular apparatus of the kidney and acting through generation of angiotensin exerts a trophic effect on the zona glomerulosa. However, the efficacy of this regulatory system has been questioned in the rat (Cade and Perenich, 1964; Bojesen, 1966). It has been generally agreed that the structure and function of the zona glomerulosa are largely independent of hormones from the anterior pituitary (Deane and Greep, 1946; Muller, 1971). None the less, extremely high doses of ACTH have an effect on biosynthesis of aldosterone in the rat (Muller *et al.*, 1964; Tait *et al.*, 1970).

Deane and Greep (1946) first reported that the width of the zona glomerulosa increases after hypophysectomy despite an atrophy of the zona fasciculata and decreased adrenal weight. In the electron microscope, virtually no change has been reported after hypophysectomy (Sabatini *et al.*, 1962; Idelman, 1966 and Fujita, 1972).

Few studies of the effect of hypophysectomy on zona glomerulosa in other species of animals have appeared. The Mongolian gerbil is native to arid regions (Rich, 1968) and has been introduced as an experimental animal in recent years. Nickerson (1971) has described normal ultrastructure of the zona glomerulosa in the gerbil. The ultrastructure of cells in the zona glomerulosa of the gerbil has been examined at varying intervals after hypophysectomy and correlated with the width of the zone as measured by light microscopy.

Materials and Methods

Thirty-five mature, female Mongolian gerbils (*Meriones unguiculatus*) were obtained from Tumblebrook Farms, Brant Lake, New York. Gerbils were maintained in a Hot Pack Environmental Chamber kept at a constant temperature of 22·5°C and humidity and were provided with lab chow and tap water *ad libitum*. The animals, however, were rarely observed drinking water (Arrington and Ammerman, 1969), even after hypophysectomy.

Gerbils were hypophysectomized by the transaural approach using an H–200 Hoffman-Reiter hypophysectomy instrument† as described previously (Nickerson,

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† Manufactured by the H. Neuman Co., Skokie, Illinois.

1972). Animals were anaesthesized with ether, positioned in the instrument and the pituitary removed. The pituitary was inspected grossly under the 3 × magnification of a dissecting microscope. At sacrifice, the pituitary fossa was inspected grossly, the head fixed in 10% formalin and the area of the sella turcica cut out, embedded and sectioned. Animals that showed remnants of pituitary were excluded from the experiment.

Three hypophysectomized and 2 control animals were sacrificed at 2 and 5 days and at 1, 2, 3 and 4 weeks after hypophysectomy. The left adrenal was trimmed of fat and utilized for electron microscopy, whereas the right adrenal gland was fixed in 10% neutral, phosphate-buffered formalin. For electron microscopy, the adrenal gland was cut into 1 mm thick slices which were fixed in 3% purified glutaraldehyde (Ladd Research Industries, Burlington, Vermont) buffered to pH 7·2 with 0·1 M phosphate. Tissues were fixed at 4°C for 4 hours. While in 0·1 M phosphate buffer (pH 7·3), the zona glomerulosa and zona fasciculata cells were isolated using a microscalpel while working under the 3 × magnification of a dissecting microscope (Nickerson, 1970). Tissues were postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, followed by propylene oxide and embedding in Epon 812 and Araldite (Mollenhauer, 1964). Before thin sections were cut, 1 micron thick sections were cut from plastic embedded blocks to verify the orientation of the tissue. Thin sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome. Sections were stained with methanolic uranyl acetate (Stempak and Ward, 1964) followed by lead citrate (Reynolds, 1963) before examination in a Siemens 101 electron microscope.

The right adrenal gland from each animal was divided in half. One half was processed by conventional procedures for light microscopy and sections were stained with hematoxylin and eosin. Six-micron thick frozen sections were cut from the other half of adrenal and stained by the method of Hartroft and Hartroft (1955). All slides were coded for measurement of glomerulosa width. The thickness of the zona glomerulosa was measured at 400 × magnification with an American Optical Filiar micrometer.

Results

By 4 weeks, the weight of the gerbil adrenal gland was 21 ± 2 mg for hypophysectomized animals and 30 ± 3 mg for controls ($p < 0{\cdot}001$). Frozen sections were cut from the adrenal in addition to paraffin sections to ascertain whether or not the embedding procedure altered the width of the glomerulosa. The width of paraffin embedded and frozen sections was largely comparable except at 14 days. A significant increase in width of the zona glomerulosa was observed at 3 weeks and at 4 weeks after hypophysectomy (Table 1). No difference in the number of lipid droplets was observed in glomerulosa cells of control and hypophysectomized animals at 4 weeks.

Table 1. *Effect of hypophysectomy on width of Zona Glomerulosa in the adrenal gland of the Mongolian Gerbil.*

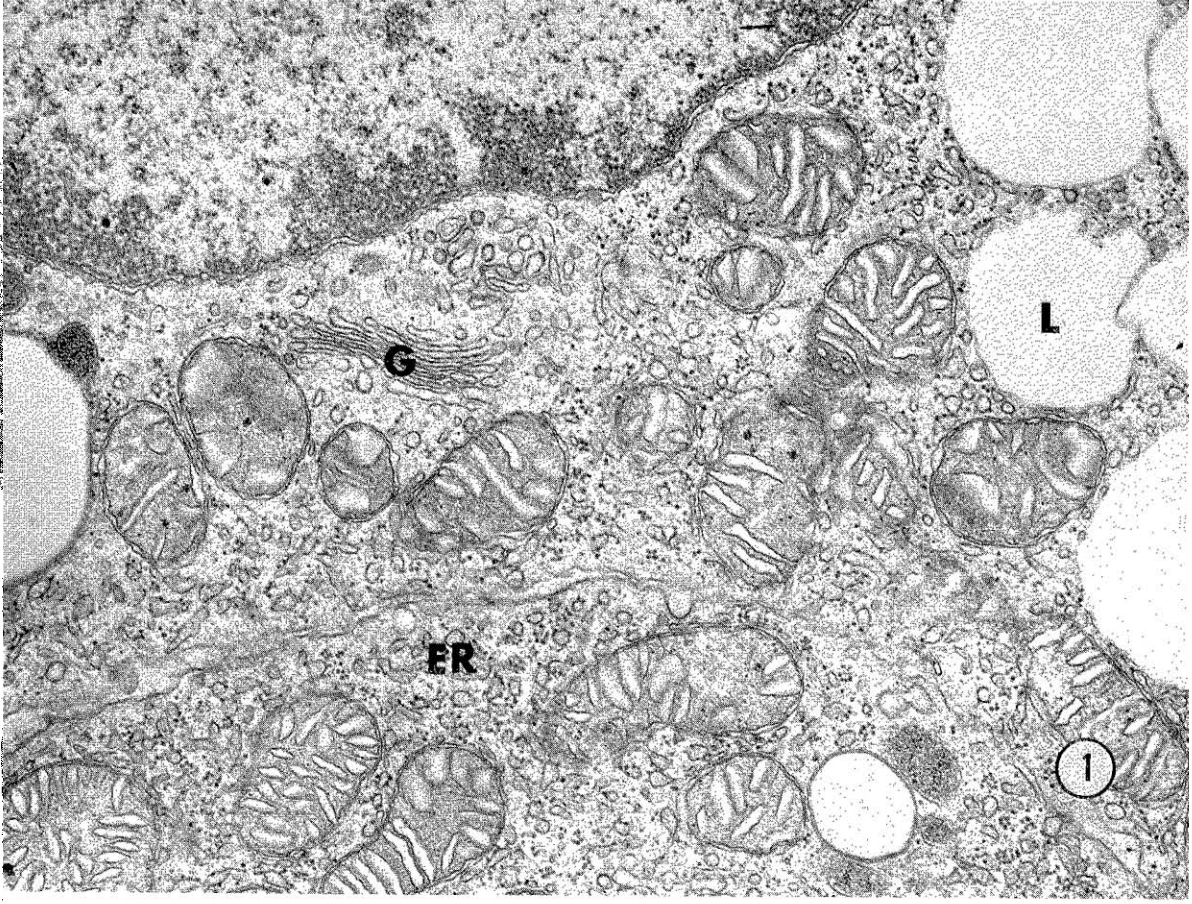
Days	Paraffin sections (μ)	Frozen sections (μ)
Control	82·1 ± 2·2[a]	76·7 ± 2·1
2 days	85·8 ± 1·9	82·9 ± 1·4
5 days	80·2 ± 2·1	82·6 ± 2·8
7 days	79·1 ± 1·6	79·9 ± 1·4
14 days	88·5 ± 2·8	75·5 ± 1·2
21 days	115·1 ± 5·2[b]	119·1 ± 4·3[b]
28 days	109·2 ± 2·5[b]	112·5 ± 1·1[b]

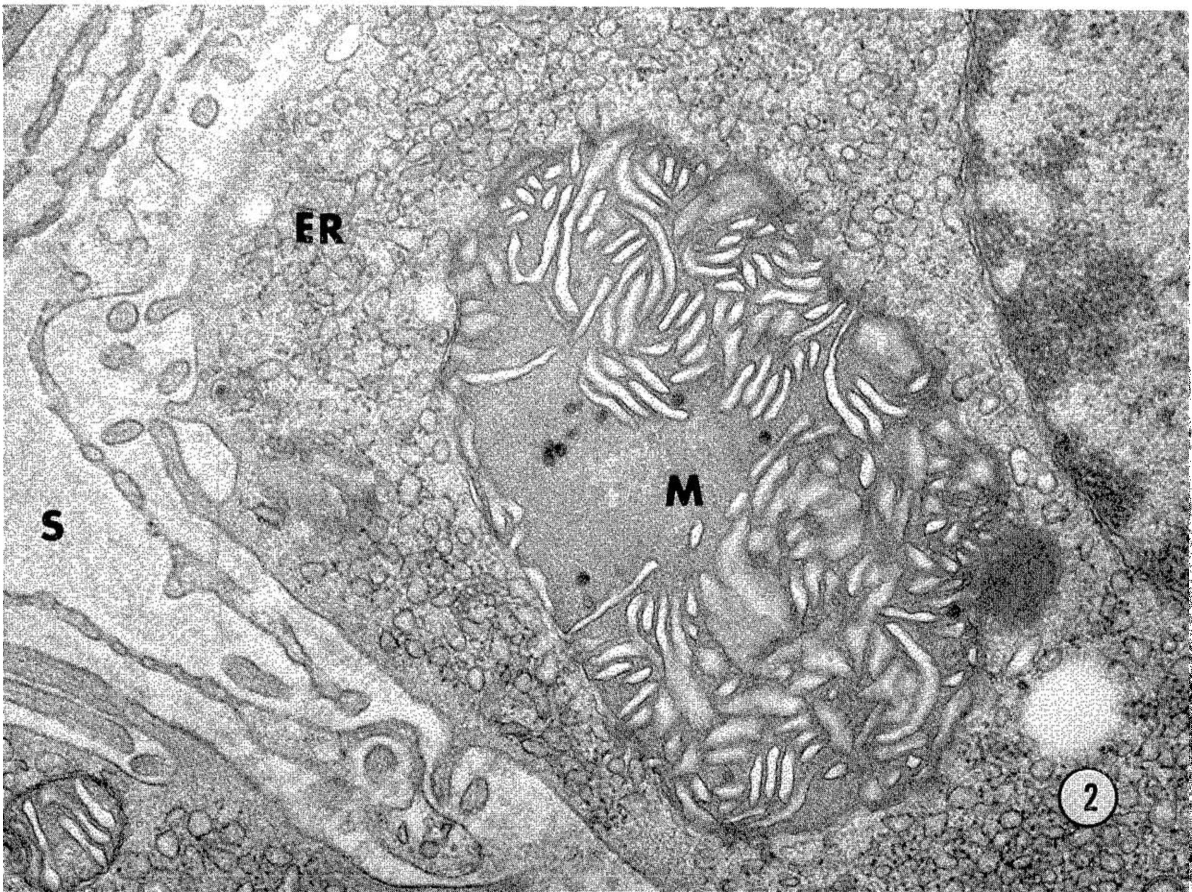
[a] Mean ± standard error of the mean.
[b] $p < 0{\cdot}001$.

The ultrastructure of zona glomerulosa cells in the gerbil has been described in a

Fig. 1. Zona glomerulosa cell from control gerbil adrenal gland. The Golgi apparatus (G) is composed of several cisternae and is located in a juxtanuclear position. Smooth endoplasmic reticulum (ER) is predominant. Mitochondrial cristae are plate-like. A few lipid droplets (L) are observed. × 26,000.

Fig. 2. Portions of zona fasciculata cells at 7 days after hypophysectomy. A giant mitochondrion (M) is observed with tubular and plate-like cristae and several dense granules. Numerous tubules of smooth endoplasmic reticulum (ER) are dispersed throughout the cytoplasm. Sinusoid (S). × 34,000.


G
L
ER
1


ER
S
M
2

previous study from our laboratory (State University of New York) (Nickerson, 1971). Mitochondria are elongate and the cristae plate-like. Although smooth endoplasmic reticulum predominated, only a few tubules were dispersed throughout the cytoplasm. The Golgi apparatus was usually inconspicuous and composed of several cisternae (Fig. 1) with one or two coated vesicles. Several lipid droplets and an occasional lysosome were observed in glomerulosa cells (Fig. 1).

Zona glomerulosa cells first showed changes at 14 days after hypophysectomy; similar changes at 21 and 28 days after operation were observed. The Golgi complex became enlarged and was composed of several dictyosomes, although the number of cisternae per dictyosome was unchanged (Fig. 3). More small coated vesicles than in controls were dispersed throughout the Golgi region. Frequent fusions of a larger sized coated vesicle with the cell membrane were observed (Fig. 3). The contents of the large coated vesicles were apparently continuous with the extracellular space (Fig. 3). Membrane of coated vesicles fused with cell membrane and spines of the coated vesicles were still connected with the cell membrane (Fig. 3). Smooth endoplasmic reticulum became hypertrophic in many zona glomerulosa cells (Fig. 4).

Atrophic changes in zona fasciculata cells were apparent at 7 days. Several giant mitochondria were observed in zona fasciculata cells (Fig. 2), apparently arising from fusion of smaller mitochondria as observed by Volk and Scarpelli (1966) in the rat.

Discussion

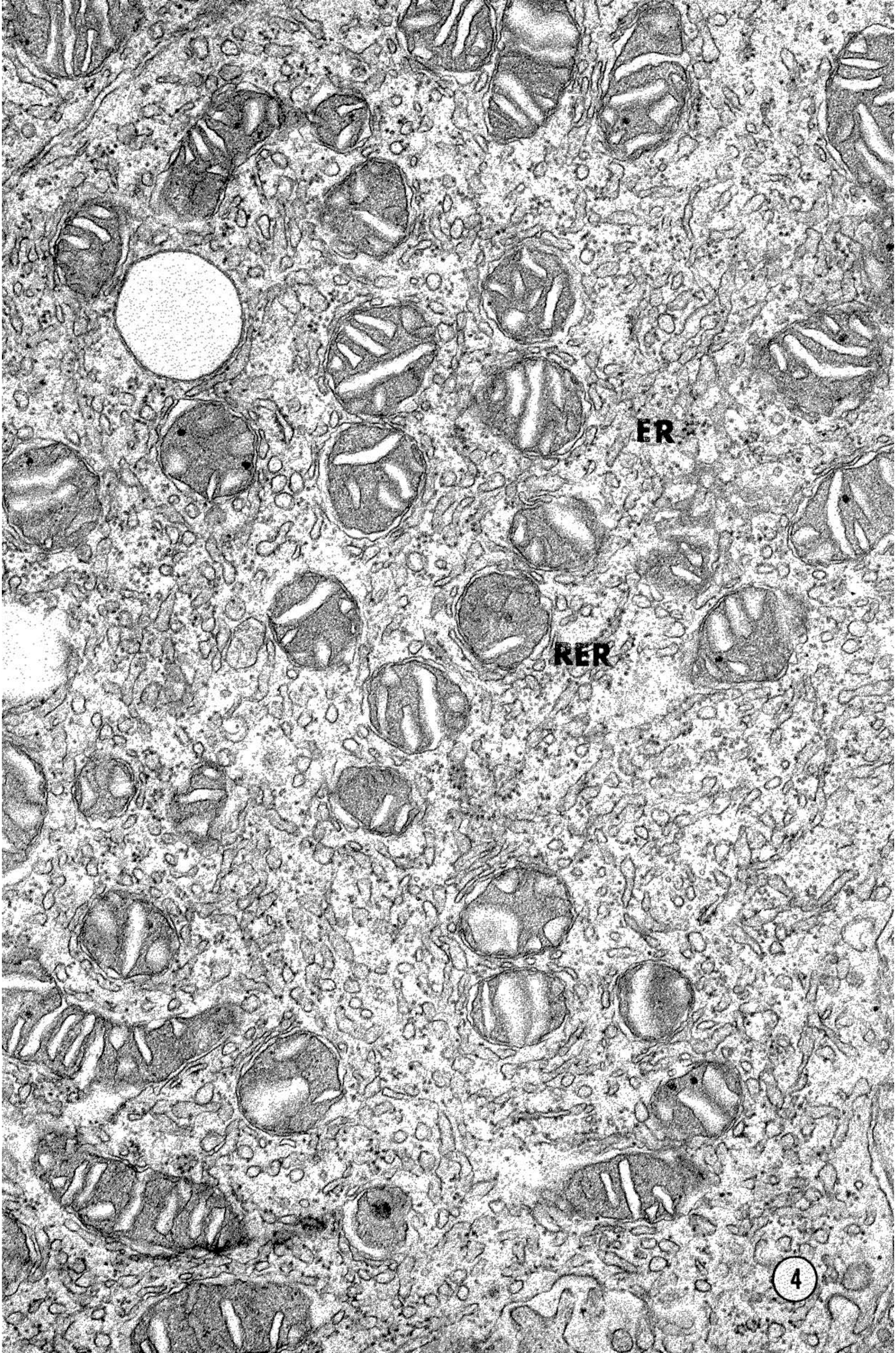
The ultrastructure of zona glomerulosa cells did not become atrophic as did adjacent zona fasciculata cells. In fact, the width of the zona glomerulosa increased at 3 weeks. The width of the zona glomerulosa has been utilized as an approximate index of zona glomerulosa activity. Stimulation causes an increase (Hartroft and Eisenstein, 1957) and suppression, a decreased (Hartroft and Hartroft, 1955; Nickerson and Molteni, 1972) width of the zone. Originally, the apparent increase or lack of effect of hypophysectomy on glomerulosa cells and decreased width of inner cortical zones provided evidence for independence of the glomerulosa from control of the pituitary (Deane and Greep, 1946). However, Müller (1971) has indicated that width is not a reliable indication of zona glomerulosa activity.

Hypophysectomy stimulated hypertrophy of smooth endoplasmic reticulum in zona glomerulosa cells. A similar hypertrophy of the smooth reticulum was reported in the opossum after sodium depletion by Long and Jones (1967). Concomitant increases in synthesis of aldosterone occur *in vivo* and *in vitro* after sodium depletion (reviewed by Davis, 1967). Aldosterone, the principal secretory product from zona glomerulosa cells in numerous species of animals (Müller, 1971) including the gerbil (Oliver and Peron, 1964), is active in controlling blood electrolytes as well as body water balance. There is no indication that hypophysectomy exerted any effect on body water balance in the gerbil since no increased consumption of water or increased urine production was discerned after hypophysectomy. This is of interest inasmuch as the gerbil is native to arid regions. Furthermore, the gerbil has an efficient mechanism for water conservation and can obtain virtually all of its bodily needs for water from the diet without provision of a source of drinking water in the

Fig. 3. Zona glomerulosa cells at 28 days after hypophysectomy. The Golgi apparatus (G) is well developed and numerous vesicles are associated with and observed near the Golgi apparatus. Several coated vesicles are observed; one population of coated vesicles is small (arrow) whereas the other is large and observed near the cell membrane (double arrow). Numerous tubules of smooth endoplasmic reticulum (ER) are observed. Centriole (C). A lipid droplet (L) appears completely surrounded by a lysosomal-like structure. In the insert, coated vesicles are fused with the cell membrane and the spiny coat is clearly visible. ×29,000 (insert ×37,000).

Fig. 4. Zona glomerulosa cell at 21 days after hypophysectomy. The smooth endoplasmic reticulum (ER) is hypertrophic. Only a few cisternae of rough endoplasmic reticulum (RER) are observable. ×32,800.

G
C
ER
L
3


ER
RER
4

laboratory (Arrington and Ammerman, 1969). The mechanism for water conservation in the gerbil is obscure however (Oliver and Peron, 1964).

Smooth endoplasmic reticulum is prominent in many steroid secreting cells (Christensen and Fawcett, 1966; Blanchette, 1966; Nickerson, 1971). The smooth reticulum possesses several enzymes for synthesis of steroids. Enzymes for cholesterol synthesis are found in smooth endoplasmic reticulum (Chesterton, 1968) as well as enzymes for pregnenolone to progesterone (Beyer and Samuels, 1956). Progesterone is converted either to deoxycorticosterone (Ryan and Engel, 1957) or 17-hydroxyprogesterone which in turn is transformed to 11-deoxycortisol (Plager and Samuels, 1954) by enzymes localized to the microsomal fraction of the cell. It is unknown, however, whether or not aldosterone secretion was increased in the gerbil, although reduced aldosterone has been reported in the rat after hypophysectomy (Singer and Stack-Dunne, 1955).

The number of dictyosomes increased in zona glomerulosa cells. The function of the Golgi apparatus in steroid secreting cells is largely unknown. Long and Jones (1967) have suggested that steroids could be conjugated in the Golgi apparatus. Presumably such a mechanism might occur preparatory to release and would undoubtedly increase solubility of steroid molecules which are particularly water insoluble. However, the mechanism for secretion of steroids has not been determined; the enlargement of the Golgi apparatus in zona glomerulosa cells of the gerbil is unclear. In zona fasciculata cells, ACTH induces hypertrophy of the Golgi apparatus (Reese and Moon, 1938). Rouiller and Schimmer (1969) observed a dramatic hypertrophy of the Golgi apparatus and associated vesicles in cultured mouse adrenocortical cells treated with ACTH.

Numerous coated vesicles were observed in the region of the Golgi apparatus. Two populations of coated vesicles have been reported in protein-absorbing cells of the vas deferens (Friend and Farquhar, 1967). The larger coated vesicle is formed at the cell surface by pinocytotic invagination and functions in transporting absorbed protein to multivesicular bodies. The smaller coated vesicle originates in the Golgi apparatus and moves peripherally, presumably transporting acid hydrolysases to the multivesicular body. Coated vesicles may have multiple transport functions. Maul and Brumbaugh (1971) demonstrated tyrosinase, a non-acid hydrolyase, in coated vesicles. Two types of coated vesicles were observed in zona glomerulosa cells of the gerbil; the first was located near the Golgi apparatus and the second was near or fused with the cell membrane. The identity of the contents of the coated vesicles is unknown however.

Identification of the system involved in stimulation of the zona glomerulosa in the gerbil is unresolved; species differences in the regulation of the zona glomerulosa have been reported (Davis, 1967; Muller, 1971). Hypophysectomy, however, exerts little, if any, effect on the renin-angiotensin system in the rat (Palkovits *et al.*, 1970). The effect of hypophysectomy on the renin-angiotensin system in the Mongolian gerbil must be examined in future studies.

Summary

The ultrastructure of cells in the zona glomerulosa has been investigated in hypophysectomized Mongolian gerbils. The width of zona glomerulosa was measured in frozen and paraffin embedded sections. By 3 weeks after hypophysectomy, the width of the zona glomerulosa increased significantly. By electron microscopy, glomerulosa cells first exhibited an increase in smooth endoplasmic reticulum and in the number of Golgi dictyosomes at 2 weeks. Numerous vesicles and a large- and small-sized population of coated vesicles were observed near the Golgi apparatus. Some coated vesicles fused with the cell membrane. Adjacent zona fasciculata cells became atrophic showing a few giant mitochondria and small cell size after hypophysectomy.

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