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Histology and Histopathology

Electron microscopic studies on the pars intermedia of the rat under stress

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Summary. Using electron microscopy, we have studied the pars intermedia (PI) of the pituitary gland of Wistar rat under psychogenic stress, finding a considerable increase in dark cells. The cells of the PI present striking vacuoles in all their cytoplasms, increasing up to a phase of total cellular disintegration. We have also observed a cell type, not found in the animal control group, of regular size and electrodense matrix of medium contrast.

Key words: White rat - Pars intermedia - Pituitary gland - Electron microscopy - Stress

Introduction

The effects and changes induced by psychogenic stress in the pituitary gland, and particulary in its posterior lobe and pars intermedia (PI), have already been studied by us with light microscopy (Blasco et al., 1985). Results were very interesting such as migration of PI cells to the posterior lobe, and even the setting of «interadenoidal transcavernous bridges», or cellular exchanges between the pars distalis and intermedia through the cavern. All these findings showed clearly the great reactive capacity of the PI.

By all these means, the study of this phenomena with electron microscopy seemed convenient, since the PI has been extensively reported in this field under normal conditions (Amat et al., 1976; De Bold et al., 1980; Gosbee et al., 1970; Howe et al., 1973; Kobayashi, 1964; Kurosumi et al., 1961; Steckel, 1981; Terr, 1983), and even under experimental conditions (Kobayashi, 1974a, 1974b, 1974c; Ziegler, 1963), but reports about animals under stress are rare and inconclusive (Moriarty and Moriarty, 1975, Moriarty et al., 1975; Pollard, 1983); therefore, this work was undertaken to obtain some more data about the experimental psychogenic stress.

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Materials and methods

A series of pituitary glands of 16 Wistar white rats previously stressed and coming from our Department's Laboratory, was used for this study. The age of these male and female animals was between 3 and 4 months and their weight was 200-250 grs.

The psychogenic stress was produced by intermittent water-immersion, at 25° C of temperature to avoid influences of cold or hot. Time of bath depend on each animal, as they were kept swimming until the limit of resistance to create anxiety for survival. The animals underwent these conditions 5 days a week, and the other 2 days they were submitted to a restriction of vital space, after Bonfils' technique. All of these conditions were maintained for 3 weeks, while the animals were kept in presence of depredators (cats). Any other condition of life was normal: food, water, temperature, humidity,...

After stress, the animals were sacrificed by decapitation after ether anaesthesia. Pituitary glands were obtained, and fixation was performed by immersion in mixture of 1% paraformaldehyde and 1,5% glutaraldehyde in 0,1M phosphate buffer at ph 7,4. After fixation, the specimens were postfixed in a 1% osmium tetroxide solution, and then washed 3 times in the buffer solution, continuing with dehydration from 50% ethanol, then with uranyl acetate (2% in 60% ethanol), ascending graded ethanols, until 100%, and then washed in propylene oxide. They were embedded in an Epon-Araldite mixture, after which, semithin sections of 1 micra and ultrathin sections of 300-400 Angstroms were prepared on an ultra-microtome Reichert OM U2. Semithin sections were stained with tolouidine blue, and ultrathin sections, with lead citrate, and examined with a PHILIPS EM-301 electron microscope.

Results

First of all, an increase of dark matrix cells must be

Fig. 1. PI from under stress. Notice the assembling of the light cells (showing contents of electron-dense granules) with dark cells (where the recognition of mitochondria is difficult because of the compact cytoplasmic matrix). Note the increase of dark cells, being rare in a normal animal. ×3400

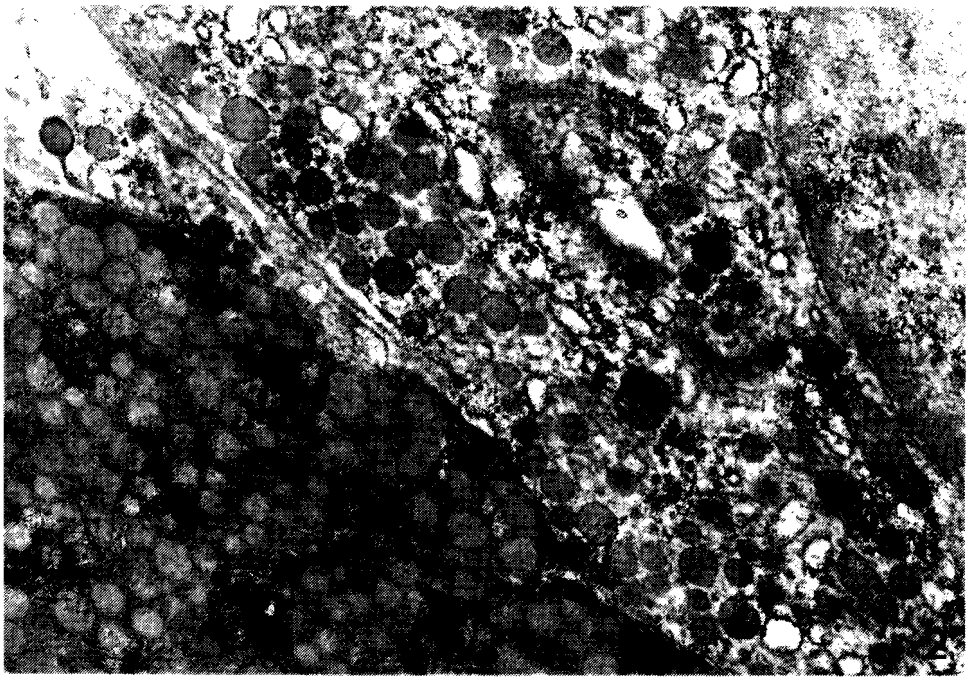

Fig. 2. Partial view of PI cells from stressed rat. Notice the variations of density in the protoplasmic matrix and, instead, the neat delimitation of secretory granules. ×11000

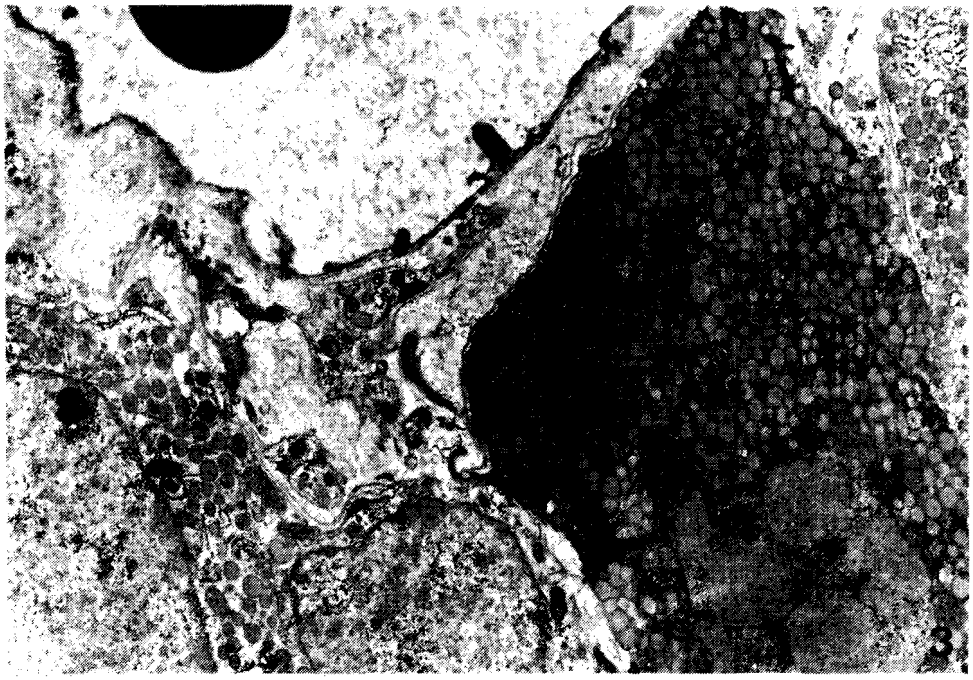

Fig. 3. PI from animal under experimentation. Variations of cytoplasmic density in PI cells surrounding a capillary. Granule size and contents are similar. ×4500

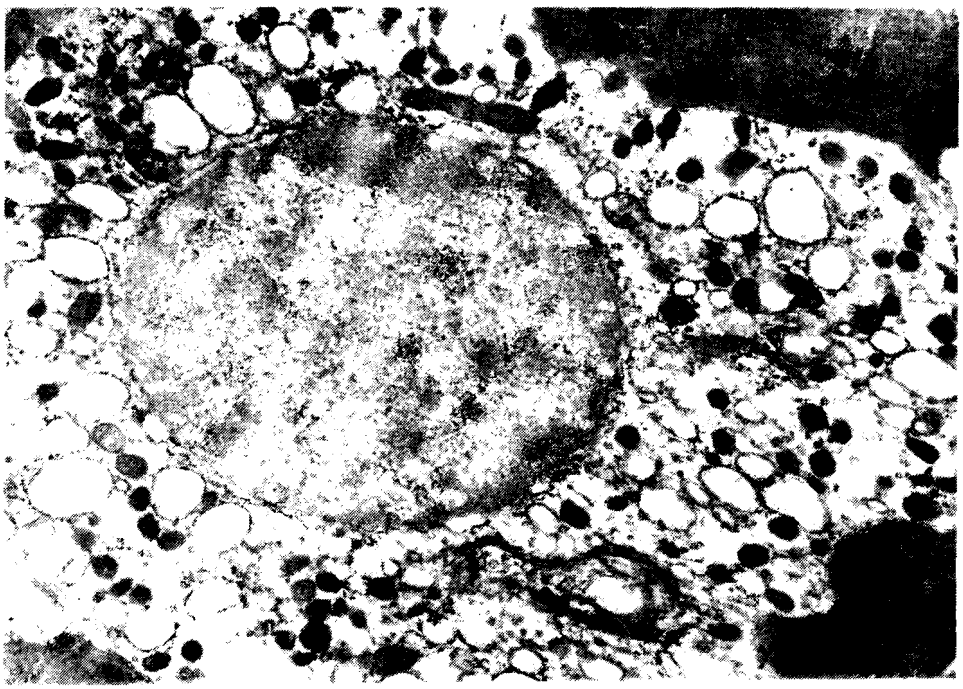

Fig. 4. PI from animal under experimentation. Advanced cell-degeneration, with the nucleus floating in a licuated cytoplasm among some dispersed organelles (mitochondria, Golgi complex, secretory granules, vacuoles). ×9100

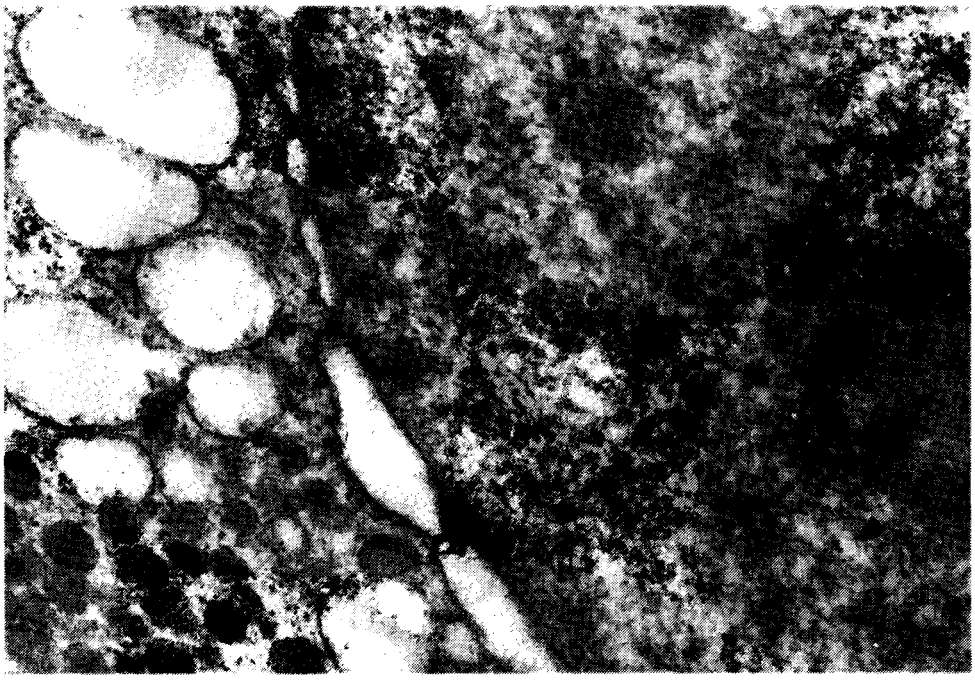

Fig. 5. Aspect of a PI cells in an advanced stage of degeneration, from stressed rat. Notice the large cytoplasmic vacuoles and the expansion of the perinuclear space. ×19000

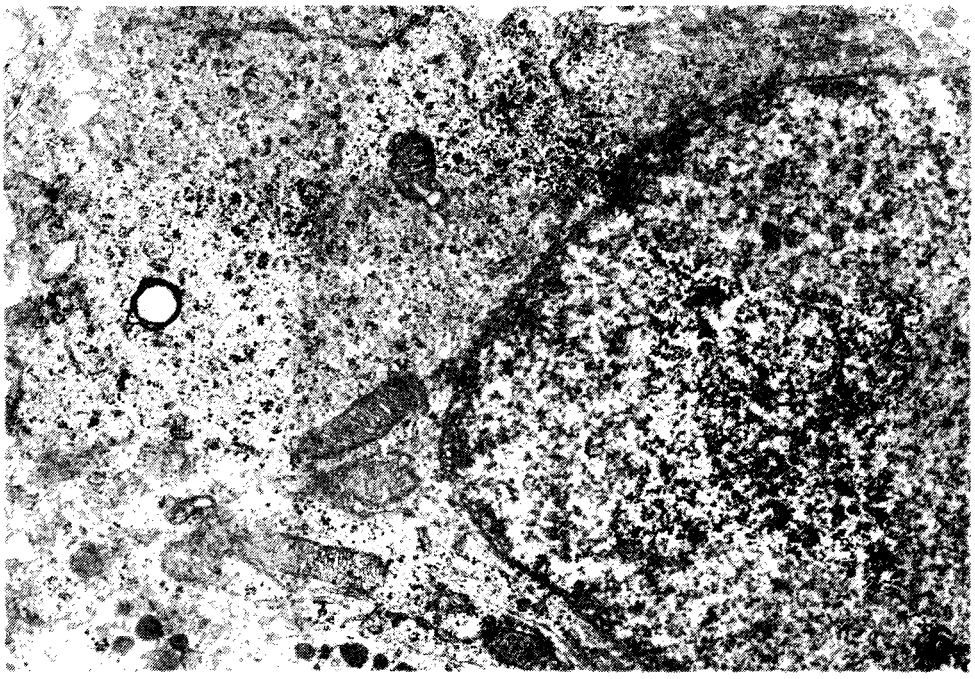

Fig. 6. PI cell from stressed rat, showing a dense, uniform cytoplasmic matrix, with frequent mitochondria and polyribosomes, scattered around. This is a special cell we have not found in animals that were not under experimentation. ×11000

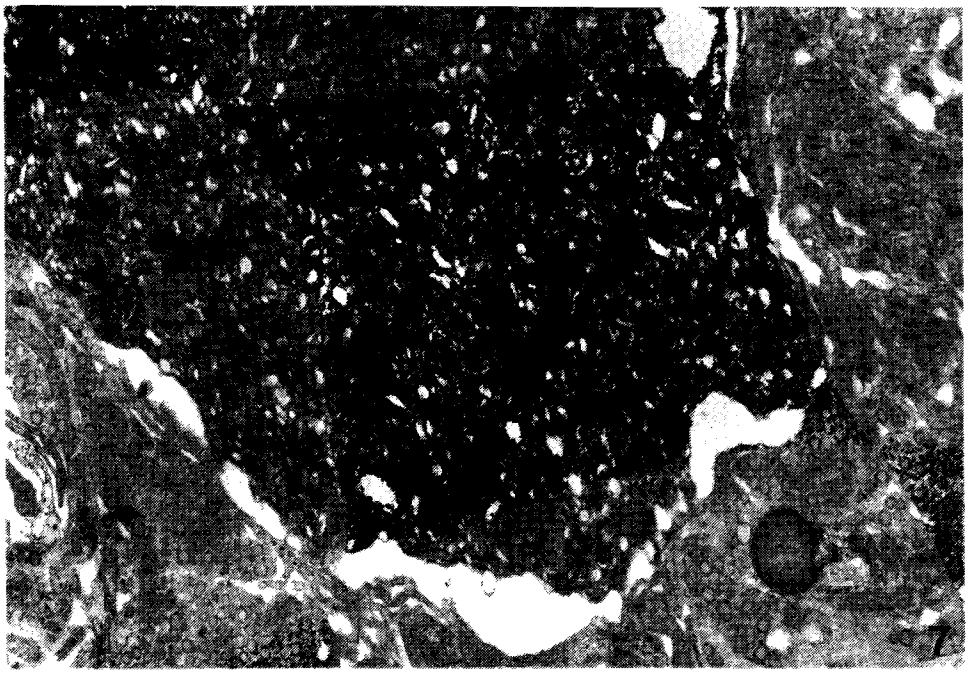

Fig. 7. PI cell from stressed animal, in the latest degenerative stages. Its cytoplasm is an electron-dense mass where recognition of organelles is really difficult. ×7100

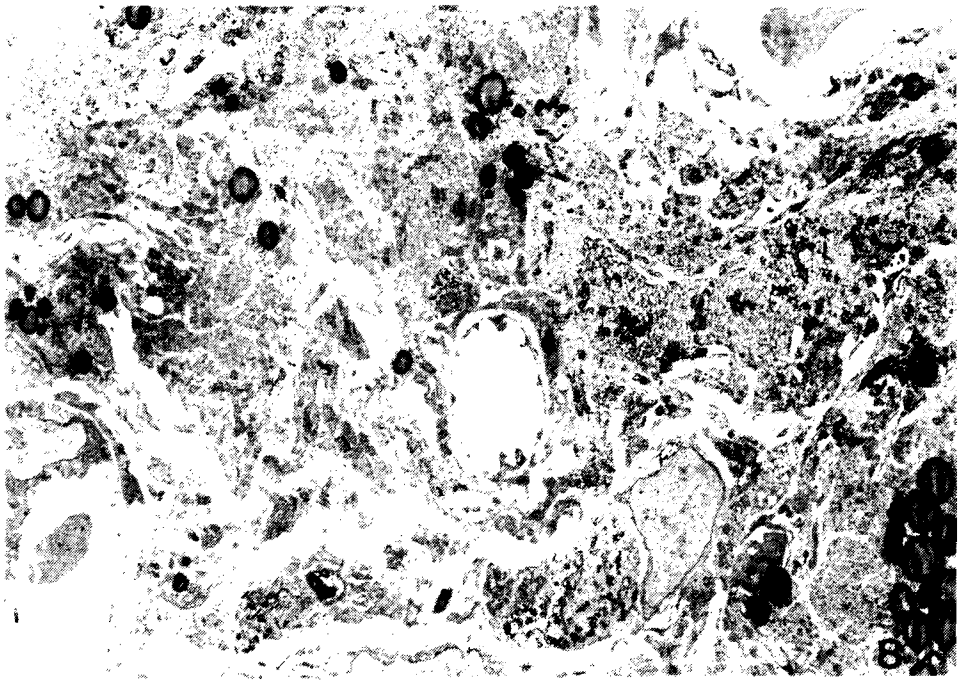

Fig. 8. Vascular connective tissue septum separating the neural lobe from the PI in stressed rat. Intense vasodilatation and lipidic accumulation in connective cells. ×2500

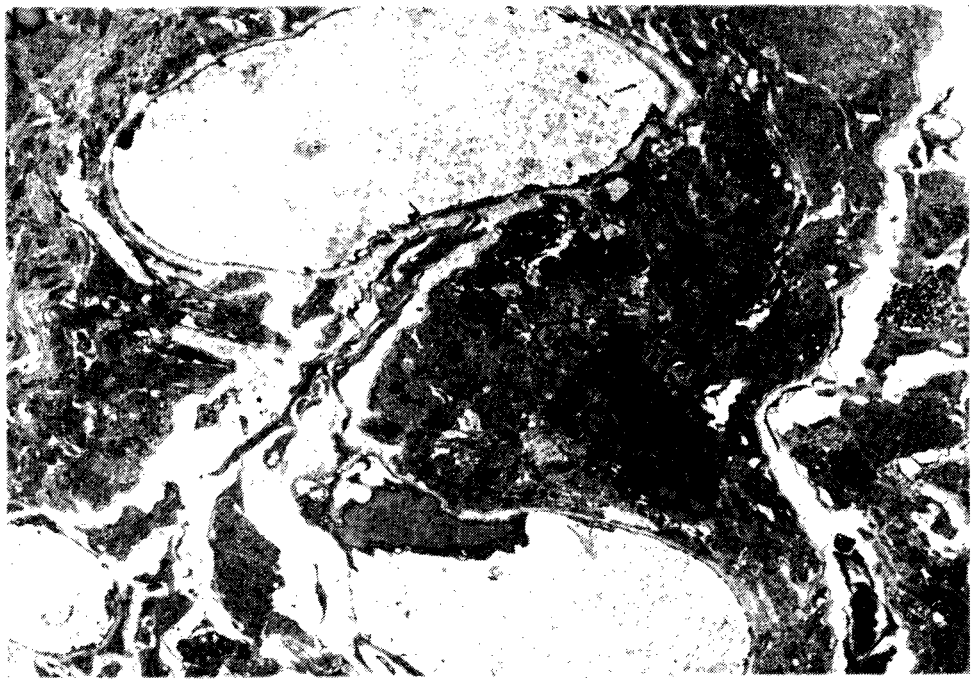

Fig. 9. A greater magnification of Fig. 8. ×3500

emphasized (Fig. 1), considering, under this notation, cells with cytoplasmic elements of variable electron-density degrees, with plenty of granulation in some cases (the cytoplasmic granules being of similar density and size in both types, that is, dark cells and light cells, as shows fig. 2). In some other cases, the recognition of any organelles becomes impossible. There is, as well, a striking increase in distended capillaries, in close contact PI cells (Fig. 3).

Vacuoles are scattered through the cytoplasm, occasionally being so vacuolated that well-conserved nuclei keep floating in the centre of the cell (Fig. 4). In previous stages, expansion of perinuclear space is the indicator of evolution towards the limit situation shown above (Fig. 5).

We have also observed on these animals under stress, a strange cellular type that we have not seen in the animals of the control group. We cannot risk an interpretation yet, but it is a kind of cell of regular size, with an ellipsoidal nucleus of smooth outline, and occupied by slender granulated chromatin, uniformly distributed, with a slight reinforcement of membrane. The cytoplasm is organised over an electrodense matrix of medium contrast, where we find clusters of densely stained material of polyribosomic aspect standing out, and a scarcely fasciculated material in the surroundings of the nucleus. We have been able to visualize neither endoplasmic reticulum nor Golgi complex, but only well-conserved mitochondria (Fig. 6).

In the latest stages of the experiment, degenerated cells on the way to disintegration are frequent, and they include mitochondria, cellular detritus, phagosomes, pigments,... and a big pericellular halo, separating them from more or less normal tissue. We suppose that these areas, seen with light microscopy as «empty of cells», are occupied by a colloid (Fig. 7).

Another characteristic of these PI cells, belonging to pituitary glands of stressed animals, is that their protoplasm contains several lipidic droplets, also found in the animals of the control group, but not as frequently and never in such a quantity as in stressed animals (Figs. 8,9).

Discussion

Talking about experimental stress, we would like to discuss several points still in conflict. The general answer of hypophysis, and particulary, that of the hypothalamus-hypophyseal system, or even that of PI, have been known since long ago. So, there are conclusions about the answer of the hypothalamus-hypohyseal system, but contradictory ones about the PI's.

We think we can assure that, in the rat, the first 4-5 days are days of hyperactivity of the PI, both in animals under water deprivation and under psychogenic stress. From that point, the PI is forced either to migrate or to die. The structure of PI undergoes a degeneration with cell death, and a colloid takes place in the so-called «empty-of-cells areas», while, at the same time and perhaps because of the prior hypertrophy stage, PI cells can be observed on their way to the neural lobe. These facts occur almost independently of the cause that triggers off the process, emphasizing the low degree of specificity.

About the hyperactivity signs, the findings in our animals are opposite to those of Moriarty and Moriarty, (1975); Moriarty et al. (1975), and Pollard, 1983, perhaps because their images belong to the first stage of the PI reactivity. Our animals had large lipidic deposits, an abnormally dense cytoplasm, considerable width of the

perinuclear space, and a excess of vacoules; becoming, in the latest stages, to necrotic cells, composed of electron-dense clusters not allowing the observation of any organelles.

Another discussion point is the increase of dark cells found in these animals, in contrast, as well, to the abovementioned authors. These authors find, in their material under stress, the change of every cell to a single type, without any distinction between light and dark cells being possible, intead of our increase in dark cells. Now, we do not have enough data to see the reasons for this phenomenon.

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Ultrastructure of the tubular nephron of *Testudo graeca* (Chelonia). A comparison between hibernating and non-hibernating animals

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Summary. The tubular nephron of hibernating and non-hibernating specimens of *Testudo graeca* (Chelonia) was studied by means of conventional light and electron microscopy and histochemistry. The tubular nephron was composed of proximal, intermediate, distal and collecting tubules in both hibernating and non-hibernating animals. The cells of the proximal tubule showed long microvilli, cytoplasmic vacuoles, a developed endoplasmic reticulum and abundant mitochondria. Fat droplets were also observed. The intermediate segment was lined by ciliated and non-ciliated cells. The lining cells of the distal tubule presented few microvilli, abundant dense mitochondria and clear vesicles of mucous appearance in the terminal portion. Collecting ducts are composed of mucous and non-mucous cells. Mucous cells presented strong reaction to the histochemical techniques detecting sialo- and sulpho-mucins.

During hibernation, a progressive vacuolar degeneration of the endoplasmic reticulum was observed in all the segments of tubular nephron, which may be caused by a massive intake of extracellular water into the cell.

Key words: Ultrastructure - Tubular nephron - Hibernation - *Testudo graeca*

Introduction

In recent years the reptilian kidney has been increasingly studied due to the pivotal role in the phylogenetical scale of this group of vertebrates (Anderson, 1960; Roberts and Schmidt-Nielsen, 1966; Davis and Schmidt-Nielsen, 1967; Schmidt-Nielsen and Davis, 1968; Davis et al., 1976; Fernández et al., 1978, a, b; Peek and McMillan, 1979, a, b; Gabri, 1983, a, b,; Gabri and Butler, 1984; Soares and Fava de Moraes, 1984; Solomon, 1985) Previous reports described the reptilian nephron as composed of: renal corpuscle, proximal, intermediate and distal tubules and collecting duct (Bishop, 1959; Kahlil et al., 1974, a, b; Gabri, 1983 a); however, marked variability has been found in the ultrastructure of the tubular cells which has been attributed to the enormous variety of species and habitats of the animals included in this group (Gabri, 1983 a). Most studies, however, have been concerned with the ultrastructure of the Lacertidae. Little attention has been given to the Chelonia (Solomon, 1985) and no studies have been conducted to elucidate the alterations in the nephron produced by the hibernation period.

This study was undertaken in an attempt to establish the ultrastructural characteristic of the tubular component of the nephron of *Testudo graeca* and the morphological changes which take place during the hibernation periods. A similar study was previously carried out in the renal corpuscle of the same specie (Zuasti et al., 1986).

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Materials and methods

Adult specimens of *Testudo graeca* (n=10) of both sexes were caught in their own habitat in August (non hibernating period) and February (hibernating period). The animals were anaesthetized by intraperitoneal injection of 3 mg of pentobarbital for 100g of body weight. The specimens were sacrified and the kidneys were exposed.

Samples for light microscopic study were fixed in 10% buffered formalin or Bouin's solution for 24h and processed routinely. 5μm thick sections were stained by the following methods, H.&E., PAS (Martoja and Martoja, 1970), alcian Blue (AB) at pH 2.5 and pH 1 (Pearse, 1985), aldehyde fuchsin (AF) (Gabe, 1968), alcian Blue (ph 2.5)-PAS (AB-PAS) (Ganter and Jolles, 1969), alcian Blue at pH 2.5 after methylation and saponification (Met + Sap + AB 2.5) (Pearse, 1985), high iron diamine (HID) (Spicer, 1965), high iron