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MORPHOLOGICAL AND FUNCTIONAL OBSERVATIONS ON THE CYCLIC CORPUS LUTEUM IN EGYPTIAN BUFFALOES (BUS BUBALIS)

(With 2 Tables and 10 Figures)

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مشاهدات شكلية ووظيفية على الجسم الأصفر في الجاموس المصري

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أجريت هذه الدراسة على خمسين من إناث الجاموس المصري، يتراوح عمرها من ثلاث إلى ست سنوات. تم تجميع عينات دم قبل ذبح هذه الحيوانات أما الأجسام الصفراء فقد أخذت بعد الذبح في مجزر موشا بأسيوط. أستخدمت عينات الدم في التحليل الهرمونى لكل من البروجيسترون والإستروجين ، أما الأجسام الصفراء فقد أستخدمت في دراسة الصورة الشكلية لها بإستخدام الميكروسكوب الضوئي والإلكتروني. يتكون الجسم الأصفر في الجاموس من خلايا صفراء وأنسجة غير صفراء، حيث مثلت الأخيرة نسبة متزايدة من الجسم الأصفر أثناء عملية اضمحلاله. وقد كانت هناك صعوبة فى التفرقة بين الخلايا الصفراء المحببه والأخرى المحفظيه نظرا للتشابه الكبير بينهما فى التركيب. هذا وقد أظهرت الدراسة وجود ارتباط موجب بين وزن الجسم الأصفر ونشاط الخلايا الصفراء ومستوى هرمون البروجيستيرون في مرحلة الجسم الأصفر الناضج. على أنه صاحب ذلك ارتباط سالب مع هرمون الإستروجين.كما كان نشاط الخلايا الصفراء ممثلا بوجود كميات كبيرة من الشبكة الإندوبلازمية الملساء وأعداد كبيرة من الميتوكوندريا مع وجود عدد من الأجسام الداكنة الصغيرة. أما اضمحلال الجسم الأصفر فقد ظهر بنقص وزنه وانخفاض نشاط الخلايا الصفراء، كما قل مستوى هرمون البروجيستيرون وإرتفع مستوى هرمون الإستروجين في الدم. ومن النتائج الملفته للنظر في هذه الدراسة وجود خلايا داكنة في مراحل اضمحلال الجسم الأصفر الأخيرة. كانت هذه الخلايا تحتوى على كميات كبيرة من الشبكة الإندوبلازمية الخشنة والتي قد تساهم في إنتاج الكولاجين الذي يحل محل الخلايا المضمحلة أو إنتاج إنزيمات هاضمة لهذه الخلايا.

SUMMARY

This study was conducted on 50 Egyptian buffalo-cows aging 3-6 years. The material employed for this study was obtained from Mosha slaughter house near Assiut. Corpora lutea were collected and used for studying the histomorphological picture of corpus luteum during different phases of luteal regression. Blood samples were obtained before slaughtering and used for hormonal assay. The corpus luteum was made up of luteal and non-luteal constituents. The latter represented an increasing volume percentage of the total CL mass during luteal regression (about 13% in CL_3 to about 20% in CL_1). There was difficulty to differentiate between granulosa and theca luteal cells because of their structural similarity. The present investigation ascertained a positive correlation between the histomorphologically active luteal cells in CL_3 and the level of progesterone in the blood as well as the weight of corpus luteum. Well developed SER, abundant mitochondria and many dense granules accompanied high progesterone level and low estradiol-17β in the blood during the stage of CL_3. Luteal regression was revealed by reduced activity of luteal cells, decrease weight of corpus luteum, decrease progesterone hormone with an increase in estradiol-17β levels in the blood. In CL_1, dark cells were demonstrated displaying the features of protein secretion (well developed RER). These cells were junctioned with normal luteal cells and may play a role in formation of collagen and production of proteolytic enzymes to help in disintegration of luteal cells.

Keywords: *Corpus luteum, morphology, Luteal cells, Progesterone, Estradiol.*

INTRODUCTION

The corpus luteum is a suitable organ for correlating structure with endocrine function, since it undergoes a series of defined structural and secretory phases within a short life-span (Parry et al., 1980). A high correlation has been reported between the plasma level of progesterone and the corpus luteum mass volume (Marciel et al., 1992) as well as the histomorphological picture of the gland (Gasse et al. 1984).

It is well established that the corpus luteum is formed by lutinization and vascularization of both granulosa and theca cells of the ovarian follicle after ovulation. This steroid secreting gland has been the

subject of many morphological studies (Lei et al., 1991; Field et al., 1992; Yamada et al., 1994; Singh and Roy, 1995). The steroidogenic interaction, which occurs between the theca interna and granulosa cells during the development and growth of ovarian follicles has been well documented (Fortune, 1986). Origin and types of luteal cells, their ultrastructure and their luteolysis have been studied recently by several investigators (O'shea et al., 1990; Garcia-Iglesias et al., 1992; Field et al., 1992; Shah et al., 1992; Yamada et al., 1994). The concentration of the secretory granules in the cytoplasm of luteal cells has been correlated with the progesterone secretion throughout the cycle (Parry et al., 1980). Moreover, the intercellular communication among large and small luteal cells may play a significant role in the regulation of corpus luteum function (Del Vecchio et al., 1995).

The histomorphological picture of the cyclic corpus luteum of buffalo received little attention among the researchers (Shah et al., 1991; Singh and Roy, 1995). The aim of the present study is to correlate between morphological features of the corpus luteum and the hormonal levels in the blood plasma (progesterone and estrogen) during the process of luteal regression in Egyptian buffaloes.

MATERIALS and METHODS

This study was conducted on 50 Egyptian buffalo-cows aging 3-6 years as determined by their dentition (Kartha, 1975). The material employed for this study was obtained from Mosha slaughter house near Assiut. Blood samples were collected from all animals through jugular veinpuncture before slaughtering in clean sterile centrifuge tubes without anticoagulant. Sera were separated after centrifugation at 3000 rpm for 20 minutes, then stored at -20°C for hormonal assay. Immediately after slaughtering, the genitalia were removed and the stage of the estrous cycle was determined according to Eissa (1996). Corpora lutea were collected from the same animals, kept in cool box and transferred immediately to the laboratory. The collected corpora lutea represented the different phases viz., the fully developed corpus luteum (CL_3) and regressing corpora lutea (CL_2 and CL_1) as well as corpus albicans (CA). The corpora lutea were weighed and small pieces were taken from them and fixed in paraformaldehyde-gluteraldehyde mixture (Karnovesky, 1965). Washing by 3 successive changes of 0.1M phosphate buffer (pH

7.3) was performed before the materials were osmicated (1% OsO_4). The samples were then dehydrated and embedded in a mixture composed of Epon and Araldite as described by Mollenhauer (1964). Semithin sections were cut and stained with toluidine blue and examined with light microscope. Ultrathin sections were mounted on copper grids, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with Joel EM at the electron microscopy unit of Assiut University.

Morphometric studies including nuclear diameter, nucleus/cell ratio of luteal cells were recorded. The volume percentage of the non-luteal constituents in the whole gland was also determined in all stages of corpus luteum using image analysis system (Leica Q500). Moreover, serum progesterone (P4) and estradiol-17β (E2) were determined by using DELFIA kits and folurometer LBK.

Data were expressed as means ± S. D., then analyzed statistically using ANOVA test and means ± S. D. were tested at least significant difference (LSD). All tests were done by using PS-Stat computer program. Results considered significant at $P< 0.05$ or less.

RESULTS

Light microscopy:

The buffalo corpus luteum consisted of luteal and non-luteal components. The latter (vascular and connective tissue elements) constituted increasing values during the process of the luteolysis (Table1). The luteal cells (Figs 1-3) were variable in size and were almost polygonal in shape with spherical to oval centrally located nuclei. The nucleus was usually vesicular with an average diameter of 7.36μm in CL_1 to 8.16 μm in CL_3 and having one or two nucleoli. The cytoplasmic mass was lightly stained and decreased gradually during luteal regression where the nuclear/cytoplasmic ratio ranged from 8.91 in CL_3 to 11.76 in CL_1 (Table 1). Lipid inclusions displayed an increasing existence during the luteolysis, being more prevalent in CL_1 (Fig.3). Dark cells taking usually an elongated form surrounding islets of light luteal cells were demonstrated in CL_1 (Fig.3). In the CA (Fig.4), the luteal cells lost their original picture seen in CL_3. They became small in size leaving large intercellular spaces filled with collagenous fibers. Many cells were seen packed with lipid inclusions.

Electron microscopy:

The electron microscope observation clarified the picture of luteal cells in different stages of luteal regression starting from the fully developed corpus luteum (CL_3) to the regressing corpora lutea (CL_2 and CL_1) and the CA. In the CL_3 (Fig.5), luteal cells were large with vesicular nuclei having peripherally situated nucleolus. These cells demonstrated the features of steroid secreting cells. A well developed smooth endoplasmic reticulum (SER), appearing in cross sections, and large number of round mitochondria were seen filling most of the cytoplasmic area. Few short tubules of rough endoplasmic reticulum (RER) were seen in a juxtanuclear position. Small dense bodies were also demonstrated throughout the cytoplasm. In CL_2 (Fig. 6), the luteal cells were comparatively smaller than in CL_3 showing the early signs of regression. The nucleus had somewhat irregular outlines with peripheral chromatin aggregations. The amount of the SER tubules and the number of mitochondria were decreased. Additionally, the mitochondria showed swelling and partial destruction. Few scattered free ribosomes were also demonstrated. In CL_1 (Figs.7&8), many luteal cells attained reduced cytoplasmic area in comparison to those in CL_3, some lipid droplets were frequently seen in their cytoplasm. Partial destruction of some cell organelles leaving small spaces were also experienced. An interesting and striking feature was the increase in the contents of the luteal cells from the RER and free ribosomes. The darkly stained cells seen by light microscope appeared containing abundant RER tubules with many small dense vesicles in between. At the final step of luteal regression (CA), the luteal cells lost their integrity, and showed very large number of variably-sized lysosomal bodies and lipid inclusions. The cell organelles were nearly absent in the greatly reduced cytoplasmic mass. The nuclei showed more shrinkage and nuclear condensation, but many nuclei looked quite healthy. Collagen material and collagen fibrils filled most of the intercellular spaces between the shrunken luteal cells (Figs. 9&10).

Table 1: Some histomorphometrical data of the cyclic corpus luteum of buffaloes.

Corpus luteum	Non-luteal components (volume percentage)	Nuclear diameter of luteal cells (μm)	Nucleus/cell ratio In luteal cells
CL3	13.18 ± 3.94^{b}	8.16 ± 1.22^{a}	8.91 ± 1.47^{a}
CL2	14.37 ± 4.08^{b}	8.00 ± 1.57^{a}	9.63 ± 1.26^{a}
CL1	20.29 ± 3.64^{a}	7.36 ± 2.37^{a}	11.76 ± 2.78^{a}

Values with different superscripts in the same column are significantly different ($p<0.01$)

Weight of CL and Hormonal analysis:

The weight of the corpus luteum varied clearly in different stages (Table 2). It ranged from 2.13 gm in CL_3 to 0.27 gm in CA. The concentration of hormones is shown in table (2). The level of progesterone (P_4) was significantly high ($P<0.01$) in the stage of CL_3 when compared with other stages of corpus luteum. However, estradiol-17β was significantly low ($P<0.01$) during the same stage. Correlation of the levels of both hormones with the CL weight showed significantly positive correlation ($P<0.05$) between the weight of the CL and the progesterone level and a significantly negative correlation ($P<0.01$) between the weight of the corpus luteum and the level of estradiol-17β.

Table 2: Weight of the corpus luteum, levels of progesterone (P4) and estradiol-17β (E2) during stages of luteolysis. (means ± SD)

Corpus luteum	Weight (gm)	P4 (ng)	E2 (pg)
CL3	2.13 ± 0.32 a	1.87 ± 0.21 b	8.24 ±1.16 c
CL2	1.27 ± 0.19 a	1.25 ± 0.29 b	19.28 ± 0.72 c
CL1	0.99 ± 0.15 a	0.51 ± 0.14 b	26.96 ±0.97 c
CA	0.27 ± 0.01 a	0.17 ± 0.02 b	35.62 ± 3.55 c

Insignificantly different means are followed by the same letter in the same column.

DISCUSSION

Light and electron microscopical observations revealed that the two luteal cell types (granulosa and theea cells) were difficult to differentiate in the buffalo corpus luteum. Both cell types were almost similar structurally. In the same concern, Parry et al. (1980); Delmann

and Brown (1981) and Banks (1993) reported that the two luteal cell types are difficult to distinguish when they become mixed in the corpus luteum of cow. Additionally, Delmann and Brown (1981) added that the small luteal cells make up a minor part of the corpus luteum and occupy mainly trabecular and peripheral areas. However, these findings disagree with the observations of Singh and Roy (1995) in buffalo who described three main types; small, large and regressing luteal cells.

The present investigation ascertained a high correlation between the histomorphological picture of corpus luteum and the level of progesterone as well as the weight of CL during its different luteolytic phases. This finding supports the previous observations of O'Shea et al. (1989); Del-Vecchio et al. (1995); Moutos et al. (1995) and Mohammed and Megahed (1998).

In the present study, a very high progesterone level concured with the well developed luteal cells in CL_3. In this stage voluminous luteal cells were characterized, on the electrone microscope level, by the features of steroid secretion i.e. abundant SER and numerous mitochondria with few short tubules of RER. Unlike other steroid secreting cells the SER, in the buffalo luteal cells, were never seen in whorls but they appeared in the form of crossly cut tubules. This observation simulates that of Parry et al. (1980) in bovine, who mentioned that although the bovine luteal cells contained abundant agranular endoplasmic reticulum, it was only rarely seen in whorls, and even then the whorls were not as extensive as those observed in the luteal cells of other animals.

The role of mitochondria in steroid synthesis has been explained by Parry et al. (1980) who reported that the mitochondria are known to contain enzymes involved in oxidative metabolism associated with steroid biosynthesis. In the same connection, Green and Maques (1965) mentioned that mitochondria contain enzymes for generating NADPH from NADP and Kreb's cycle metabolites. Moreover, NADPH has been shown to accelerate the conversion of cholesterole to progesterone in CL_3.

The presence of few short tubules of RER and number of small sized dense granules (supposed to be secretory granules) in the well developed luteal cells of CL_3 suggests that these cells perform some sort of protein synthesis. The latter process is an important feature of steroid secreting cells as it plays a role in the steroid synthesis (Hansel et al., 1987 and Field et al., 1992). Additionally, Gemmell et al. (1974) mentioned that the bovine corpus luteum contains binding proteins for

progesterone which may be involved in its intracellular synthesis. The presence of a progesterone-binding protein in the secretory granules of the CL_3 may be the cause of the electron density of these granules (Willcox, 1979 and Mohammed and Megahed, 1998). Progesterone has been located in a unique particular luteal fraction (Quirk et al., 1979) and further biochemical studies have led to the suggestion that progesterone may be sequestered in a granular membrane (Rice et al., 1986 and Gemmell, 1995).

High vascularization of the CL_3 may be another reason for increasing progesterone level during diestrus. Increased blood flow in this stage may allow utilization of serum-derived lipoprotein as a source of cholesterol for steroidogenesis as stated by Grummer and Carroll (1988).

During luteal regression, (CL_2 & CL_1), the present observation demonstrated that the luteal cells decreased gradually in size due to reduction of the cytoplasmic mass and the concomitant decrease in the weight of the corpus luteum. Ultrastructurally, the amount of SER and number of mitochondria showed obvious inclination. Contrarily, the amount of RER and lipid droplets were increased. This was accompanied by reduction in the level of progesterone in the blood. These results are in agreement with those reported by Singh et al. (1997); Young et al. (1997) and Mohammed and Megahed (1998).

The cessation of progesterone secretion in late stages of luteolysis, in the present study, may be interpreted by the explanation of Baird (1992) who mentioned that, in the mid-luteal phase, the frequency of endogenous LH pulses is markedly reduced owing to the effect of the high levels of progesterone. He added that the uterus starts to secrete increasing quantities of $PGF_{2\alpha}$, which reach the corpus luteum and interfere with the coupling of LH to the adenyl cyclase system and luteal regression occurs.

A striking feature in the regressing corpus luteum (CL_1) of buffalo, in the present work, was the appearance of dark cells that displayed abundant RER at the level of electrone microscope. These cells were adjacent to the typical luteal cells and were junctioned by desmosomes, indicating that both cell types are sister cells. This feature has not been mentioned, in the available literature, in the corpus luteum of human being or domestic animals. It is well known that cells with abundant RER are protein secreting cells. It can be postulated that these cells may be, on one hand, responsible for synthesis of collagen needed to substitute the degenerating luteal cells and on the other hand produce

proteolytic enzymes to facilitate disintegration of luteal cells. The last postulation may be supported by the picture of the regressed luteal cells in CA in the present study. These cells demonstrated a peripheral disintegration of the luteal cells cytoplasm and loss of their organelles despite the integrity of their nuclei.

In conclusion, the luteal cells were demonstrated at different stages of development and regression. Large luteal cells of the buffalo corpus luteum during the developed stage (CL_3) which decrease in size during the regression stage (CL_2 & CL_1) have been shown to parallel progesterone production and secretion. The high progesterone level, recorded at CL_3 stage, decreased gradually at CL_2 then sharply at CL_1. At CA stage, the luteal cells showed degenerative changes (starting peripherally) and accumulation of lysosomes and lipid inclusions.

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LEGENDS

Fig. 1-4: Semithin sections of corpus luteum stained with toluidine blue. X 1000.

Fig. 1: Developed CL_3 showing many large luteal cells with large vesicular nuclei (N) and clear cell boundaries. Notice a binucleated luteal cell (B).

Fig. 2: Regressing CL_2 showing the early signs of luteal regression. The luteal cells have nuclei with irregular outlines (N) and some lipid droplets (L).

Fig. 3: Regressing CL_1 . Most luteal cells have many small lipid droplets (L). Notice the presence of many dark cells (D) in the form of cords.

Fig. 4: Corpus albicans showing many fibroblastic elements (F). The regressed luteal cells are packed with lipid inclusions (L).

Fig. 5-10: Electron micrographs of luteal cells during different stages of luteolysis.

Fig. 5: A luteal cell in a CL_3 stage. The nucleus (N) is vesicular with peripherally located nucleolus. The cytoplasm is filled with crossly cut SER, numerous mitochondria (M) and few RER tubules. Some dense bodies (D) are scattered throughout the cytoplasm. X 5000.

Fig. 6: A luteal cell showing early signs of luteolysis in CL_2. The nucleus (N) shows irregular outlines and emargination of chromatin (C). Swollen mitochondria (M) with partial breakdown are also demonstrated. X 5000

Fig. 7 & 8: Two adjacent light and dark luteal cells in a regressing CL_1. The dark cell demonstrated abundant RER, lipid droplets (L). The light cell contains relatively large amount of RER tubules and lipid inclusions (L). The two cells show desmosome junctions (J). x 5000 (Fig. 7) and x 10000 (Fig. 8).

Figs. 9 & 10: Regressed luteal cells in the corpus albicans. Note the accumulation of lysosomal bodies (L) and fat globules (G). The cytoplasmic area is completely disintegrated while the nuclei (N) are still intact. Collagen fibrils (F) appear filling the intercellular spaces. x 5000 (Fig. 9) and x 6700 (Fig. 10).

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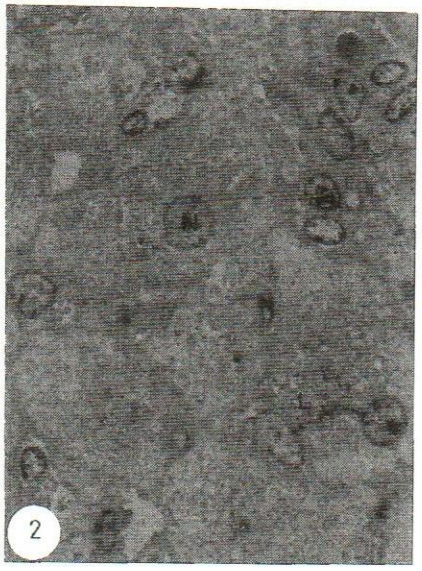

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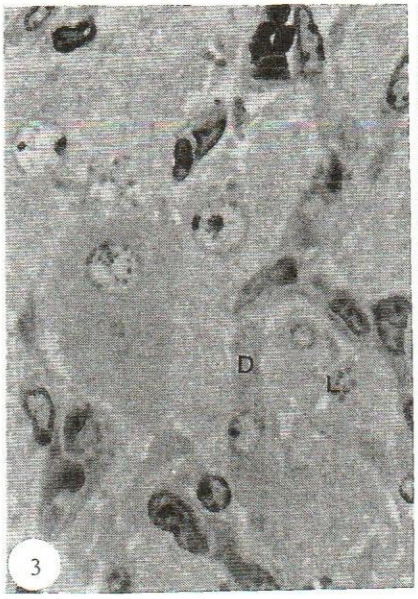

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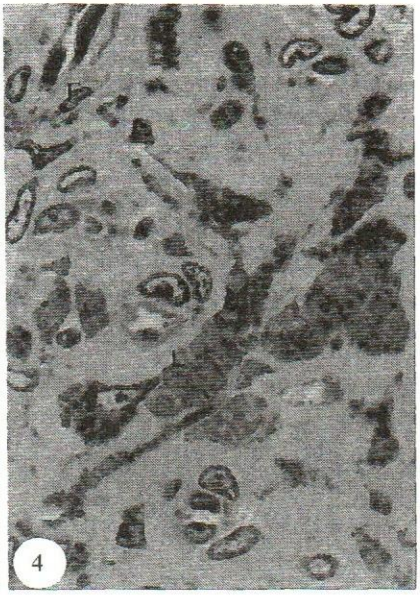

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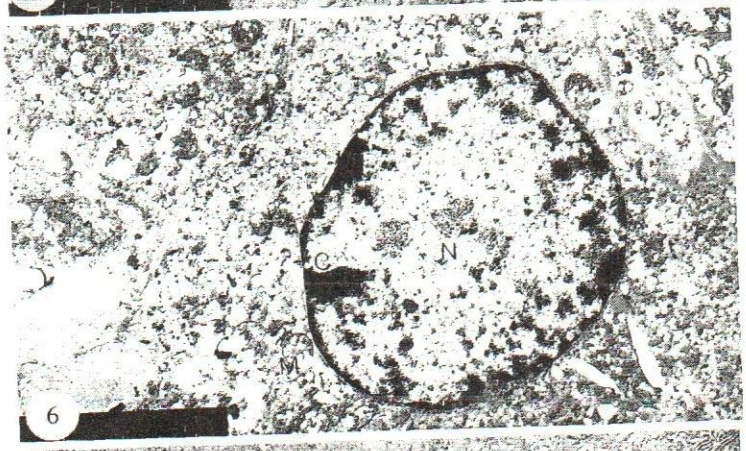

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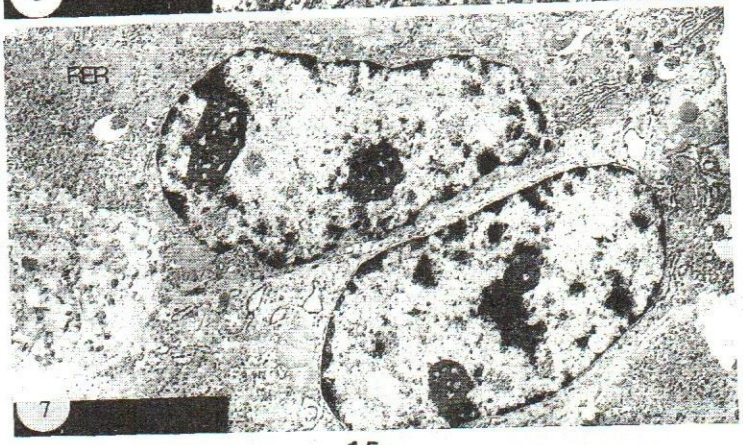

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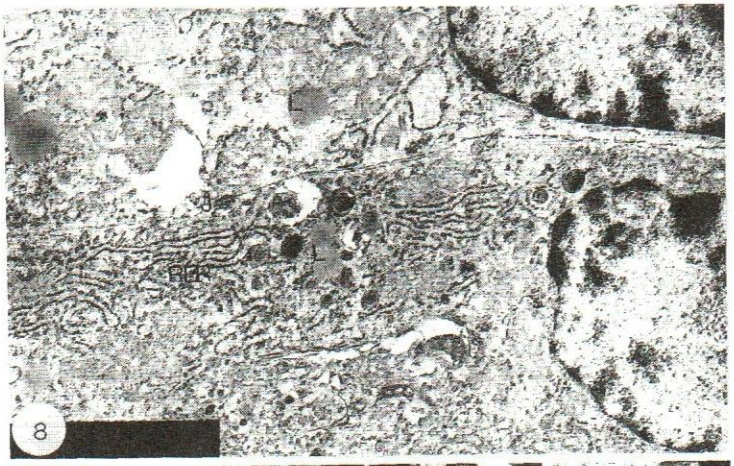
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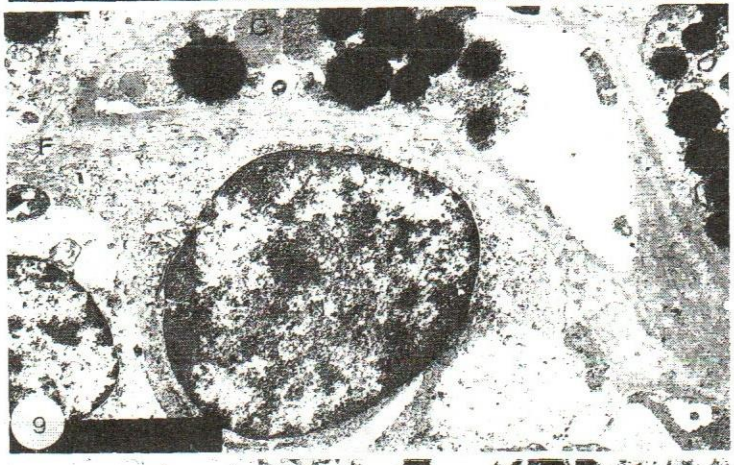
G
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N
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