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EFFECT OF DIAZEPAM ON THE STRUCTURE OF THE RAT PARS DISTALIS

(With 23 Figures)

By

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تأثير عقار الديازيبام على تركيب الغده النخاميه فى الفئران

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أجرى فى هذا البحث دراسة تأثير عقار الديازيبام على تركيب الغده النخاميه فى ذكور الفئران البيض البالغه باستخدام كل من المجهر الضوئى والمجهر الألكترونى وكذلك الطرق الهستوكيميائيه . ويستخدم هذا العقار فى تهيئة المرضى اعدادا لتخديرهم فى العمليات الجراحيه . وقد وجد أن هذا العقار يؤدى الى زياده فى عدد ونشاط الخلايا الموجوده بالغده النخاميه عند استخدامه لفترات قصيره (١٠ ، ١٥ يوما) حيث أن الخلايا الحافزه لكل من الغده اللبنيه والمناسل والغده فوق الكلويه أظهرت زياده فى حبيبات الافراز وأجسام جولجى والشبكه الاندوبلازميه الحبيبيه . بالاضافه الى ذلك فقد أظهرت الخلايا مدمج خلوى وبالأخص الخلايا الحافزه للمناسل . أما استخدام العقار لفترات أطول (٢١ يوما) فقد أدى الى انخفاض فى نشاط الخلايا التى بدأت فى التحلل حيث ظهرت أنويه هذه الخلايا غير منتظمه ومعتمه أما الشبكه الاندوبلازميه الحبيبيه فقد ظهرت منتفخه . وقد اتضح أيضا أن تاثير العقار المستخدم فى البحث هو تأثير مرتجع حيث أن الخلايا الحافزه للمناسل وتلك الحافزه للغده اللبنيه قد استعادت نشاطها وأظهرت تركيبا مشابها للتركيب الطبيعى بعد شهر من ايقاف استعمال العقار . أما الخلايا الحافزه للغده فوق الكلويه فقد أظهرت زياده فى مظاهر التحلل حيث ظهرت الشبكه الاندوبلازميه منتفخه وبها تجمعات من الافرازات مما يدل على أن تاثرها غير مرتجع ولذا يوصى بالحرص فى استخدام هذا العقار لفترات طويله وخاصة للأطفال والحوامل .

SUMMARY

The effect of diazepam on the morphology of the rat anteriot pituitary gland has been investigated in comparison with the control. Using light and electron microscpic techniques it has been found that diazepam (DZ) causes an increase in the number and activity of mammotrophs, gonadotrophs and corticotrophs when used for short duration (10 and 15 days). The activated cells showed numerous secretory granules, prominant Golgi bodies and well developed RER. However, longer periods of treatment with DZ resulted in a depression of the activity of these cells which exhibited signs of

degeneration. The cell nuclei became irregular with dense chromatin & the cytoplasm contained dilated RER with accumulation of secretory granules. These changes are early stages of degeneration as they were proved to be reversible on withdrawal of DZ. However corticotrophs show more progressive signs of degeneration following DZ withdrawal. The results are discussed in view of the availble literature.

Keywords: Effect, Diazepam, structure, Rat Pars Distalis.

INTRODUCTION

Benzodiazepines (BD) are the most recommended antianxiety drugs *(Mc Evoy & Mc QARRIE1 1984; EADIE & TYERER, 1989).* Diazepam which is a BD derivative is used in a variety of psychosomatic condition. However, there is an increasing evidence that the BD class of drugs may exert neuroendocrine effects, in addition to thier well known profile of central action. Development of gynecomastia during diazepem abuse have been reported *(MOERCK & MAGELUND, 1979).* Besides, treatment with tempazepam results in a marked increase in serum prolactin level in women *(BEARY* et al. *1983).* An increase in plasma LH concentration after DZ treatment have also been reported *(VALLI* et al. *1985).* DZ might exert its effect directly on the anterior pituiatary gland. This view is supported by the demonstraion of specific binding sites for (H3) DZ on rat pituitary cells in culture *(GRANDISON 1982).* An indirect effect of BD has also been suggested *(RACAGNI* et al., *1982).* Here they act centrally via an enhancement of (GABA) ergic transmission which might have a role in the control of hormone release from the anterior pituitary gland. However, the effect of DZ on the morphology of the anterior pituitary gland has not been investigated.

Therefore, the present work was conducted to study the fine structure of mammotrophs, gonadotrophs as well as corticotrophs in rats treated with DZ. This might reveal cytological changes that would be correlated with the reported alteration of the hormonal profiles mentioned above.

MATERIAL and METHODS

One hundred and twenty five male adult albino rats weighing 200-250 gm were used in this study. Animals were divided into five groups. Groups 1,2 & 3 were injected intraperitoneally with pure DZ dissolved in propylene glycol in a daily dose of 0.18 mg/200 mg body wt. for 10,15 and 21 days and sacrificed one day after the last injection. Group 4 received the same dose of DZ for 21 days and sacrificed one month after the last injection. Group 5 served as control. They received a dialy intarperitoneal injection of propylene glycol in a dose of 0.2 ml/200mg body wt.At each period studied 10 of control and 15 of the experimental groups were sacrificed by decapitation. The pituitary was dissected out. For L.M., the specimens were fixed, in Bouin's fixative and processed to

prepare paraffin blocks *(DRURY & WALLINGTON, 1980)*. Paraffin sections of 5-7mm thick were stained with the following staing:

1-Perfomic acid - alcian blue - periodic acid Dchiff - orange G stain PFA/AB/PAS/OG for the differentiation of the various types of pituitary cells mainly mammotrophs, gonadotrophs and thyrotophs *(HEALTH, 1965)*.

2-Lead Hx. for the demonstration of corticotrophs *(SCOLCIA et al., 1969)*. For electron microscopy the specimens were fixed in glutaraldehyde, then processed for electron microscopy and embedded in epon - araldite. Semithin sections were stained with toluidin blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined on Jeol transmission electron microscope.

RESULTS

I.Light Microscopy

In paraffin sections of the control animals, gonadotrophs are arranged in clusters particularly around blood capillaries & near the pars intermedia (Figs. 1,a & b). These cells are polyhedral in shape. Their cyloplasm is packed with deeply stained coarse purple granules. Their nuclei are eccentric in possition. Mammotrophs were stained orange with orange G.They are round to oval in shape, arranged in groups in the central region of the pars distalis and smaller than gonadotrophs (Figs. 1a & b). Corticotrophs were stained with lead Hx. They appear large & irregular. They showed dark blue to black granules peripherally arranged in the cytoplasm (Fig. 4,a & b).

In semithin sections, corticotrophs have voluminous pale cytoplasm which sends processes embracing somatotrophs. They have vesicular nuclei with one nucleolus in each. The nucleo-protoplasmic ratio is approximately 1:2 (Fig. 7a). Whereas goanadotrophs have large nuclei relative to the cytoplasm with one or two nucleoli. The nucleo-protoplasmic ratio is approximately 1:1.5. The secretory ganules are numerous, faintly stained, and concentrated at one pole of the cell (Fig. 7b). Mammotrophs were hardly detected in the section. Treatement with DZ for 10 or 15 days caused an increase in the number & size of goandotrophs & acidophils (Figs. 2a & b). Corticotrophs also appeared numerous and contain more granules compared with the control (Figs. 5,a & b).

In semithin sections most of the forementioned cells lost their characteristic morphological features and became hard to differentiate. They showed an increase in the number of secretory granules with gradual enlargement of the cell size, vacuolation of the sytoplasm and darkness of the nuclei (Figs. 8 & 9). Whereas after 21 days of drug treatment, these cells showed signs of degeneration. They appeared less in number and smaller in size compared with the previous stages (Fig.3a & b). In semithin sections these cells showed dense irregular nuclei with vacuolation of the cytoplasm which contain large dense granules (Fig. 10).

II. Electron Microscopy :

Ultrastructurally, the control animals possess normal pituiitary cells. Mammottrophs appeared oval in shape with a central large nucleus. The nuclei have an evenely dispersed chromatin. The cytoplasm characteristically contained large pleomorphic secretory granules. Other contents include well developed roungh endoplasmic reticulum (RER) at one side of the cell, mitochondria and numerous microvesicles (Fig. 11).

Following administration of DZ for 15 days, mammotrophs contains fewer and smaller granules compared to the normal cells. These granules are found in association with the internal cisternae of the Golgi apparatus. The cells also showed an increase in the area occupied by the RER which appeared in the form of flattened cisternae arranged in a concentric manner (Fig.12). By 21 days, the cell exhibited irregulary shaped nuclei containing condensed chromatin. The cytoplasmic matrix is electron dense with dilated electron-lucent cisternae of RER and numerous large granules (Fig.13). One month after withdrawal of the drug the mammotrophs nuclei appeared less irreguler with less condensed chromatin. The cytoplasm contained both large and small granules within cisternae of Golgi complex and some cisternae of RER returned back to the normal size (Fig. 14).

Gonadotrophs of the control animals appeared normal. They are vriable in shape being oval, round or polyhedral, present either singly or in clusters. They possess euchromatic nuclei and voluminous cytoplasm. The cytoplasm is characterized by having numerous rounded granules of variable sizes and elctron density, distributed all over the cytoplasm but more concentrated at one side. It contains well developed Golgi bodies, few segments of RER and mitochondria (Fig. 15). Administration of DZ for 10 days resulted in an increase in the number of the secretory granules that varied in size and electrondensity. An increase in the amount of Golgi bodies was also observed (Fig. 16). After 15 days of treatment with DZ, the cells formed a syncytium-like structure with dilatation of RER. The nuclei became small, irregular with peripheral condensation of chromatin (Fig. 17). With longer periods of treatment (21 days), the dilated RER were fused, forming larger vascuoles that were electron lucent. The nuclei were electron dense with highly irregular outlines (Fig. 18). After withdrawal of the drug, the nuclei appeared regular in outline and less electron dense. Vacuolation of the RER was reduced and some segments of RER returned back to normal (Fig. 19).

Corticotrophs of control animal were large and stellate in shape with their cell processes extending among the neighboring cells, usually embracing somatrophs. The cytoplasm contained few organelles which include Golgi bodies, segments of RER, mitochondria and few granules, which have a characteristic row-like arrangement along the cell periphery. Some granules were found in the vicinity of the Golgi bodies. The nuclei have evenely

dispersed chromatin. They are large oval and eccentric in position (Fig. 20). Treatment with DZ for 15 days, caused an increase in the number of secretory granules and Golgi bodies of corticotrophs, where is the nuclei appeared normal (Fig. 22). After 21 days, the nuclei showed margination of chromatin and irregularity of their outline with dilatation of the perinuclear cisternae. RER also appeared dilated (Fig. 18). After withdrawal of DZ, the cell cytoplasm showed dilated RER which contained fibrillar material and numerous lysosomes and Golgi bodies (Fig. 23).

DISCUSSION

Treatment with DZ for 10& 15 days resulted in stimulation of mammotrophs secretion. This is indicated by the increase in their number and size by L.M.By E.M. the activity is indicated by the presence of small granules which are considered as immature granules *(PEREZ & LAWZIWITACH, 1984)*, and an increase in organelles involved in the process of secretion which include Golgi bodies and RER. However, the treatment for prolonged periods caused depression of the cellular activities as indicated by the decrease in the number & size of the cells by L.M.and accumulation of secretory granules and dilatation of RER by E.M. In addition, the nuclei became highly irregular and dense. These findings indicate that the cells are stimulated to hypersecretion and further to functional exhausion and cell degeneration. These changes are proved to be reversible. Following winthdrawl of DZ, the cell nuclei became more or less regular in outline and of normal electron density. The secretory granules in the cytoplasm, were of the small immature type and few in number. These findings indicate a restoration of the process of synthesis and secretion. Similar findindings have been reported in rat prolactin cells following pimozide (a dopamin recepor blocking agent) treatment (*SANTOLAYA et al, 1979*). However, BD are considered to exert their action via an enhancement of GABA ergic transmission *(MOERCH & MAGELUND, 1979)*. There is a considerable evidence that GABA may have a role in the control of hormone releae from the anterior pituitary *(RACAGNI et al, 1982)*. Regarding the effect of DZ on gonadotrophs, it was quite similar to its effect on mammotrophs regarding the stimulation of secretion that was less evident with longer periods of treatment. However, the dilatation of RER found after 21 dayes of treatment was more pronounced in gonadotrophs than in mammotrophs. The dilated RER fused to form a large vacuole. These findings are quite similar to what was reported in the castration cells seen after a surgical or chemical damage of the gonads *(ALLANSON & DEANESLY, 1962; MIETKIEWSKI et al., 1969, PEREZ & VON LAWZIWITSCH, 1984)*. Therefore,it can be suggested that DZ might induce a sort of chemical castration by affecting the metabolism of testosterone. Peripheral BD binding sites in the interstitial cells of Leydig has been reported *(ANHOLT, 1985)*. However an increase in the plasma testosterone levels have been reported 2 weeks following oral

adminstration of DZ *(ARGUELLES & ROSNER, 1975)*. These findings could be attributed to liver enzymes induction and alteration in the clearance of blood testosterone. In support of the present work, is the finding of *COOK et al., 1979*. They reported a significant reduction of both the weight of the ventral prostate and serum testosterone level in rats treated with large dose of DZ (50 mg/Kgm). Gonadotrophs also formed a syncytium after 15 and 21 days of treatment. It is Known that Dz stimulates the mitotic activity of the anterior pituitary gland *(PAWLIKOWSKI, et al. 1987)*. However, the exact mechanism of the effect is still unknown. *PAWLIKOWSKI et al., 1986* found that the rat hypothalamic extracts contain an antimitogenic factor, the activity of which is blocked by DZ. Involvement of peripheral and central BD receptors in the control of pituitary cell proliferation has been suggested *(PAWLIKOWSKI et al. 1987)*. DZ affected corticotrophs in a way similar to that of mammotrophs and gonadotrophs. That is an initial stimulation followed by an inactivation. The mechanism by which DZ can increase ACTH secretion could be related to its action on the central noradrenergic system. The latter is inhibitory to ACTH secretion. Therefore, lowering the inhibitory effect by DZ could lead to a greater release of corticotropin releasing factor and consequently ACTH *(CHABOT et al., 1982)*.DZ effect on corticotrphs was proved to be irreversible. After withdrawal of the drug, corticotrophs exhibited dilated dilated RER & numerous lysosomes. The dilated RER appeared in the form of microvesicles containing a rarified material which is considered as an accumulated secretion. Presence of numerous lysosomes indicates their involvement as regulators of the secretory process (crinophagy). Therefore, it is evident from the present work that DZ causes alterations of the morphology of the pars distalis. This has to be taken into consideration particularly, when such a drug is to be recommended for long periods for young individuals and pregnant women.

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LEGENDS

Fig. (1a): Photomicrograph of a paraffin section of pituitary of control adult male rat showing the large deep purple stained gonadotrophs (G) and the small mammotroph (M) which stain orange.
PFA,AB,PAS,OGx320

Fig. (1b): Higher magnification of the previous section showing gonadotrophs (G) with large deeply stained purple granules and mammotrophtrophs (M) with orange granules
PFA/AB/PAS/OG x 1250

Fig. (2a): Photomicrograph of a paraffin section of pituitary of adult male rat treated with DZ for 10 days. Note the increased number of gonadotrophs (G) and mammotrophs (M).
PFA/AB/PAS/OG x 320.

Fig. (2b): Higer magnification phohotomicrograph showing gonadotrophs (G) & mammotrophs (M).
PFA/AB/PAS/OG x 1000

Fig. (3a): A paraffin section of pars distalis after treatment with diazepam for 21 days. Note the marked decrease in the cellular numer and size which are indicative of the degenerative process.
PFA/AB/PAS/OG x 320.

Fig. (3b): High magnification photomicrograph showing gonadotrophs (G) & mammotrophs (M).
x 1250.

Fig. (4a): A paraffin section of an adult male cotrol rat showing the positively stained corticotrophs distributed close to pars intermedia (C).
Lead Hx x320.

Fig. (4b): A magnified part of the previous section, showing corticotrophs with an irrigular outline and peripherally located secretory granules.
x 1250.

Fig. (5a): A paraffin section of pars distalis following DZ treatment for 10 days. Note the increased number of corticotrophs (C).
Lead Hx x320.

Fig. (5b): Corticotrphs of rats treated with DZ for 10 days at higher magnification.
x 1250.

Fig. (6a): A paraffin setion of pars distalis following treatment with DZ for 21 days. It shows the decreased number of corticotrophs (C).
Lead Hx x320.

Fig. (6b): A magnified part of the previous section showing corticotrophs which are reduced in size and contain deeply stained granules.
x 1250.

Fig. (7a): Semithin section of pars distalis of control rat stained with toluidin blue. It shows a corticotroph (c) embracing a somatotroph (s) .(7b).A group of gonadotrophs (G) around a blood capillary.
x 1250.

Fig. (8): Semithin section of pars distalis after treatment with DZ for 10 days stained with toluidin blue. Note the increase in the size & number of secretory grnules of chromophils.
x 1250.

Fig. (9): Semithin section of pars distalis after DZ treatment for 15 days, stained with toluidin blue. Note the marked increase in the number of secretory granules & the vacuolation of the cytoplasm of chromophils.
x 1250.

Fig. (10): Semithin section of pars distalis after treatment with DZ for 21 days. Note the reduction in size of chromophils which show dark irrigular nuclei.
x 1250.

Fig. (11): Electron micrograph of a control mammotroph characterized by having large sized pleomorphic secretory graules. RER (rough endoplasmic reticulum), mt (mitochondoria).
x 4,000.

Fig. (12): Electron micograph of a mammotroph after treatment with DZ for 15 days. The mammotroph cytoplasm contains few small granules within the Golgi citerane. It also shows an increase in RER which are arranged in a concentric manner. RER (rough endoplasmic reticulum), G (Golgi bodies).
x 6,600.

Fig. (13): Electron micrograph of a mammotroph after treatment with DZ for 21 days. It shows an irregular dense nucleus (N0, large plesmorphic granules, dilated RER and dense cytoplasm.
x 6,700.

Fig. (14): An electron micrograph of a rat mammotroph after withdrawal of diazepam The nuclear outline is more or less regular with less densitiy. The cytoplasm shows small pleomorphic granules within the Golgi cisternae, and dilated RER cisternae(*).
x 10,000.

Fig. (15): An electron micrograph of a gonadotroph of a control rat. The cytoplasm contains secretory granules of variable sizes and densities. G (Golgi bodies), mt (mitochondria).
x 6,000.

Fig. (16): An electron micrograph of a gonadotroph of a rat treated with diazepam for 10 days. Note the marked increase in the number of secretory granules and prominant Golgi bodies (G). mt (mitochondria).
x 6,000.

Fig. (17): An electron micrograph of a rat treated with DZ for 15 days. Syncytial formation of gonadotrophs with dilatation of RER (*) can be noted.
x 4,000

Fig. (18): An electron micrograph of a rat treated with DZ for 21 days. Note the syncitial formation of gonadotrophs. The nuclei are highly irregular in outline with dense chromatin. Marked dilatation of RER (*) can also be noted.
x 4,000.

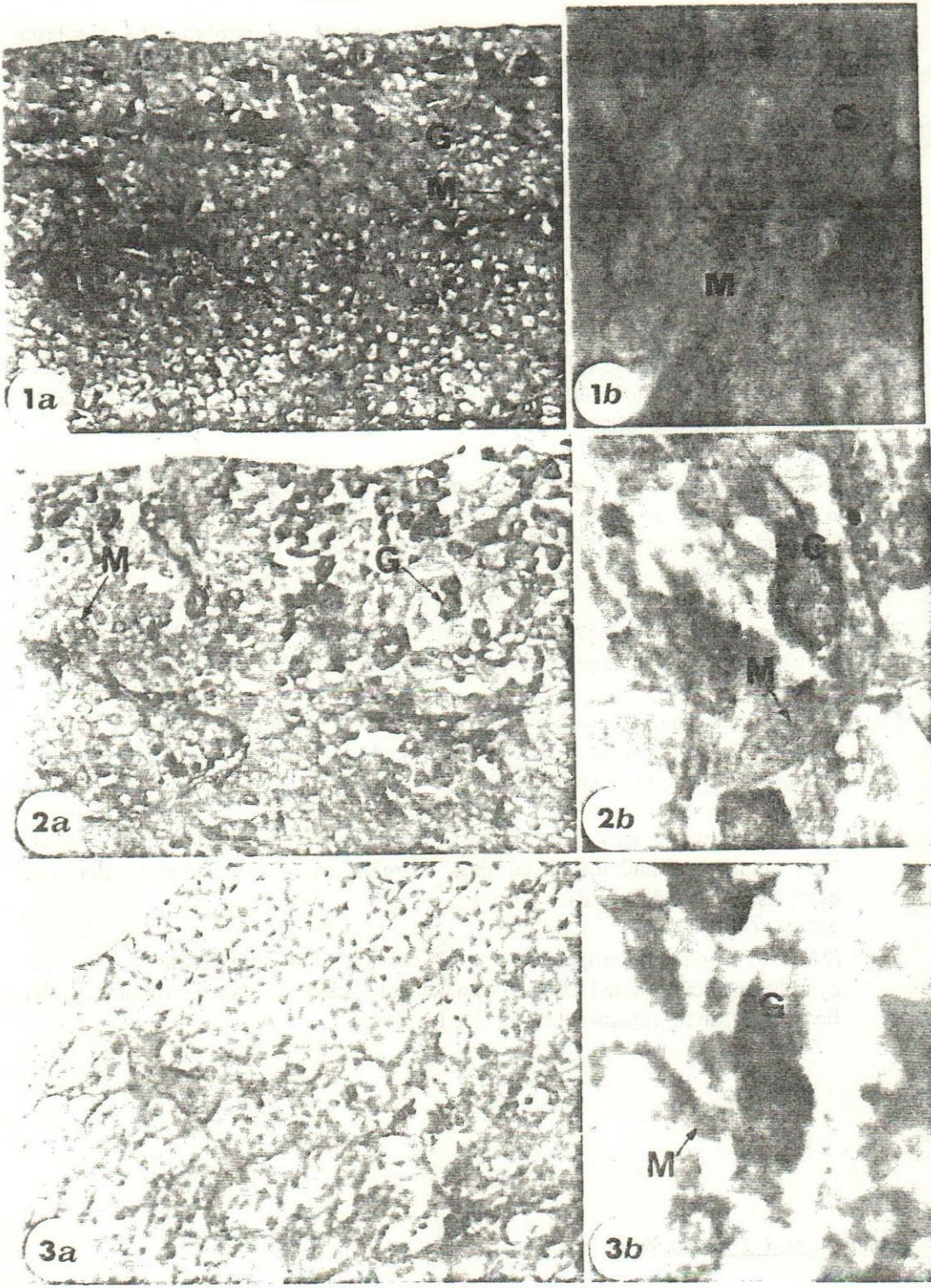
Fig. (19): An electron micrograph of rat gonadotroph after DZ withdrawal. The nuclear outline is more or less regular with an evenely dispersed chromatin and few clumped ones. Some citernae of RER appear normal (arrow head), while others are still dilated (*).
x 4,000.

Fig. (20): An electron micrograph of a corticotroph of a control rat. The cytoplasm shows the row like arrangement of secretory granules. G (Golgi bodies).
x 5,000.

Fig. (21): An electron micrograph of a corticotroph after treatment with DZ for 10 days. It shows an increase in the number of secretory granules which are peripherally arranged and proniment Golgi bodies (G).
x 4,000.

Fig. (22): An electron micrograph of a corticotroph after treament with DZ for 21 days. It is binucleated and the nuclei show margination of clumped chromatin. The cytoplasm shows dilated RER (/).
x 4,000.(↑↑).

Fig. (23): An electron micrograph of a corticotroph after withdrawal of DZ. The cytoplasm shows dilated RER (*) which contains dark substance, Prominant Golgi bodies (G), and lysosomes (ly) can also be noted.
x 6,000.


G
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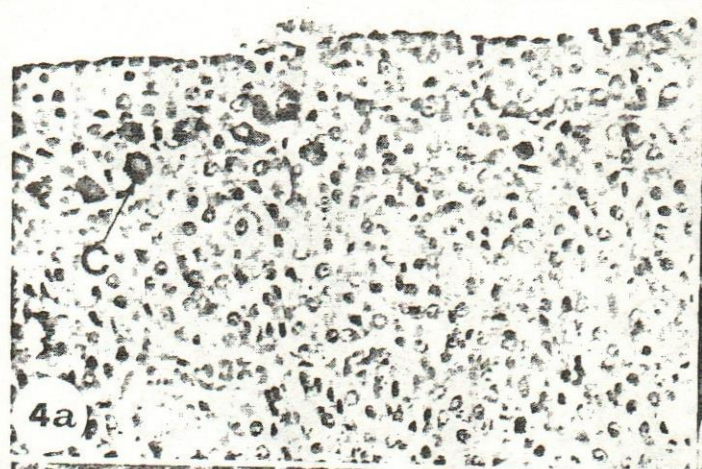
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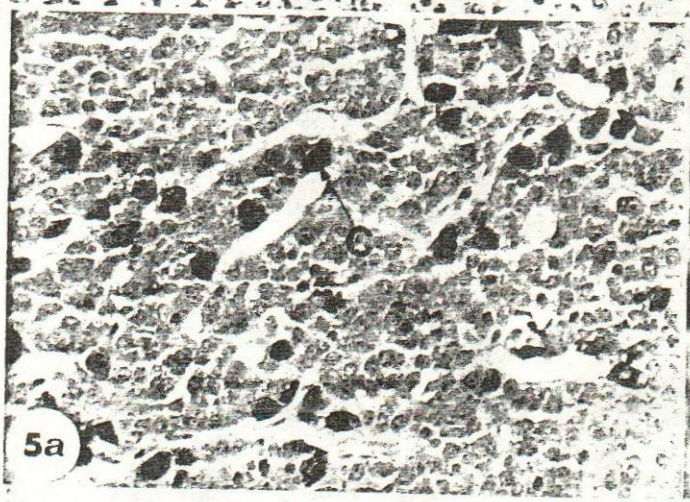
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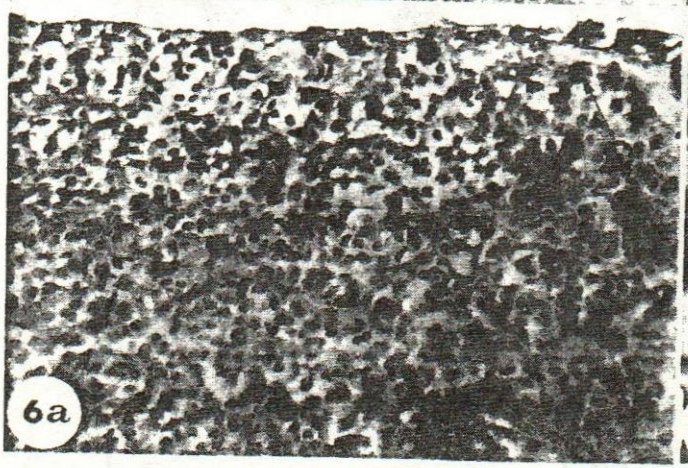
5b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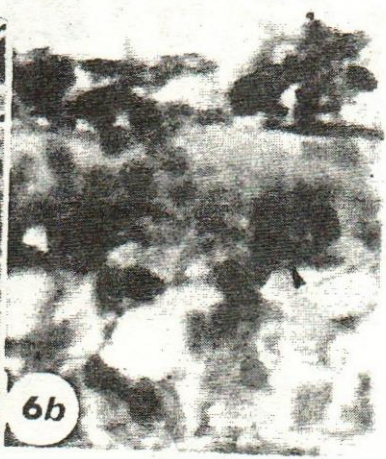
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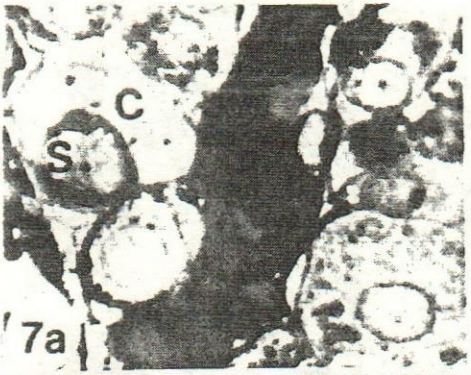

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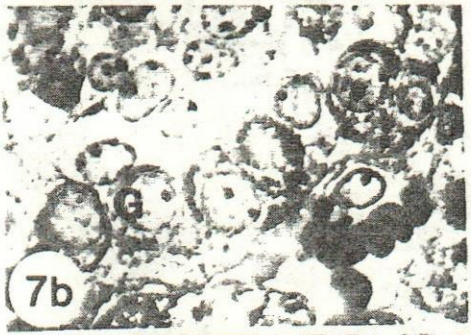

7b

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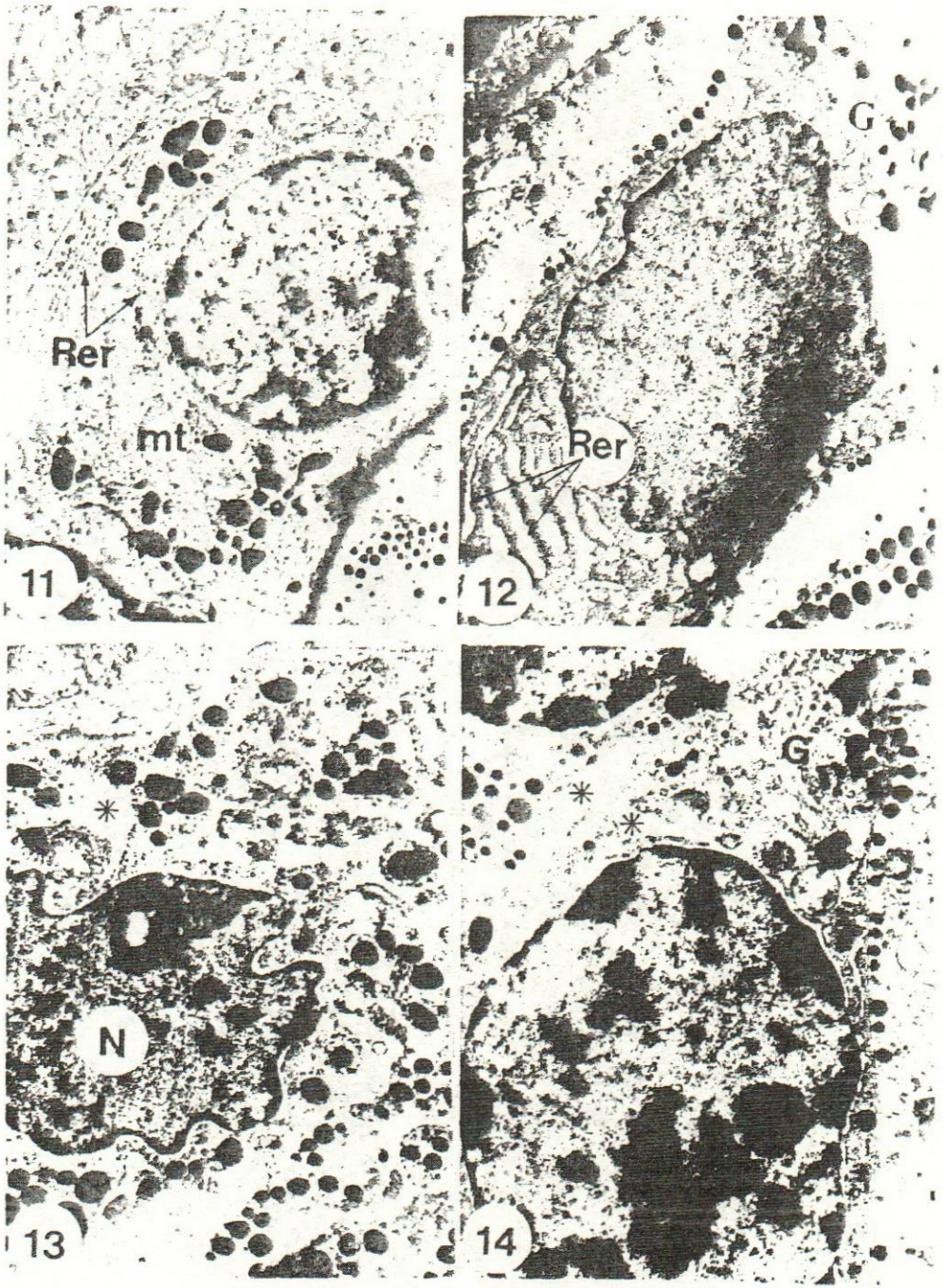
Rer
mt
11
G
Rer
12
N
13
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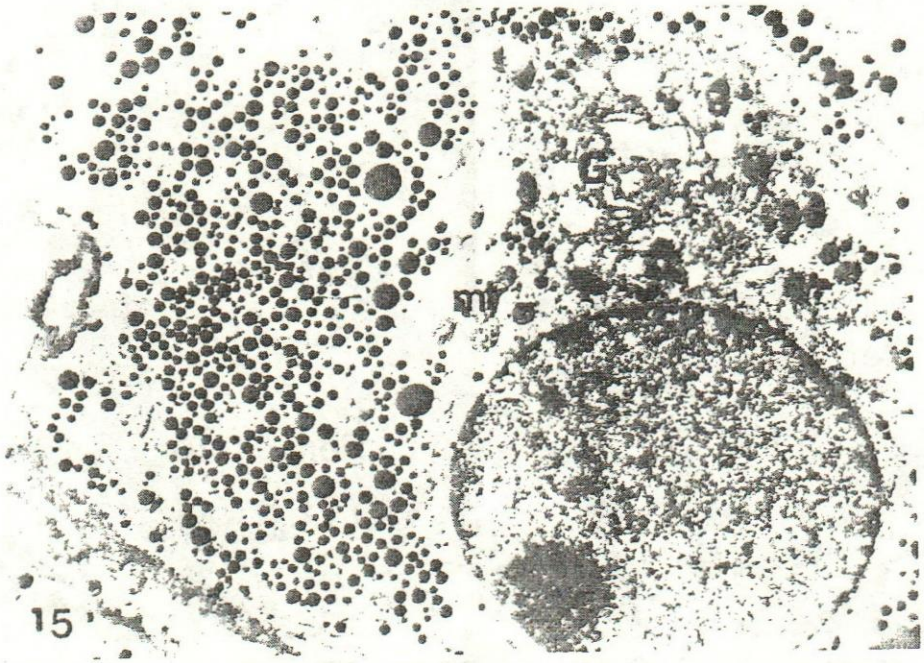
14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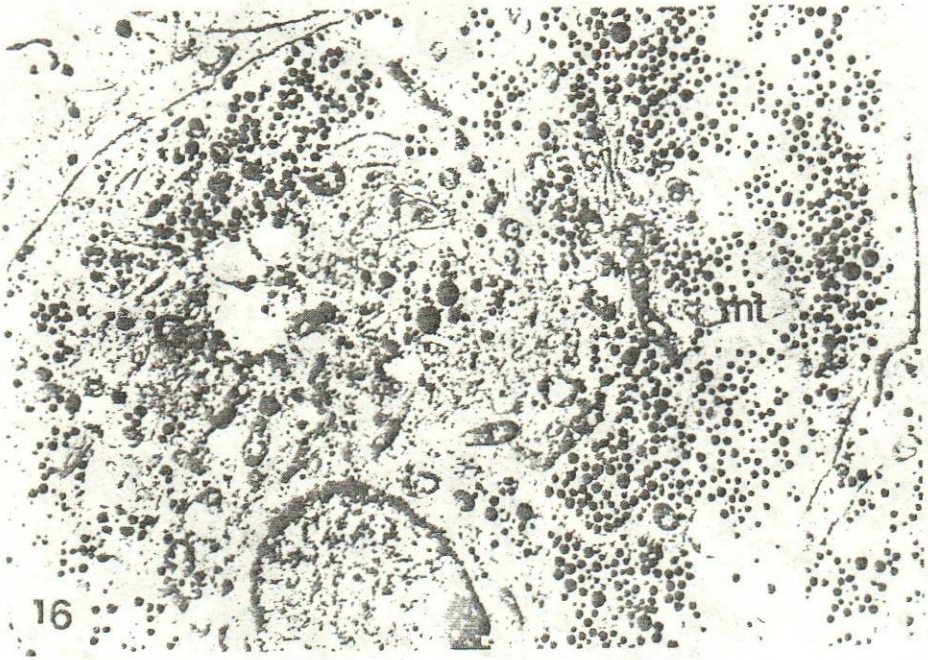
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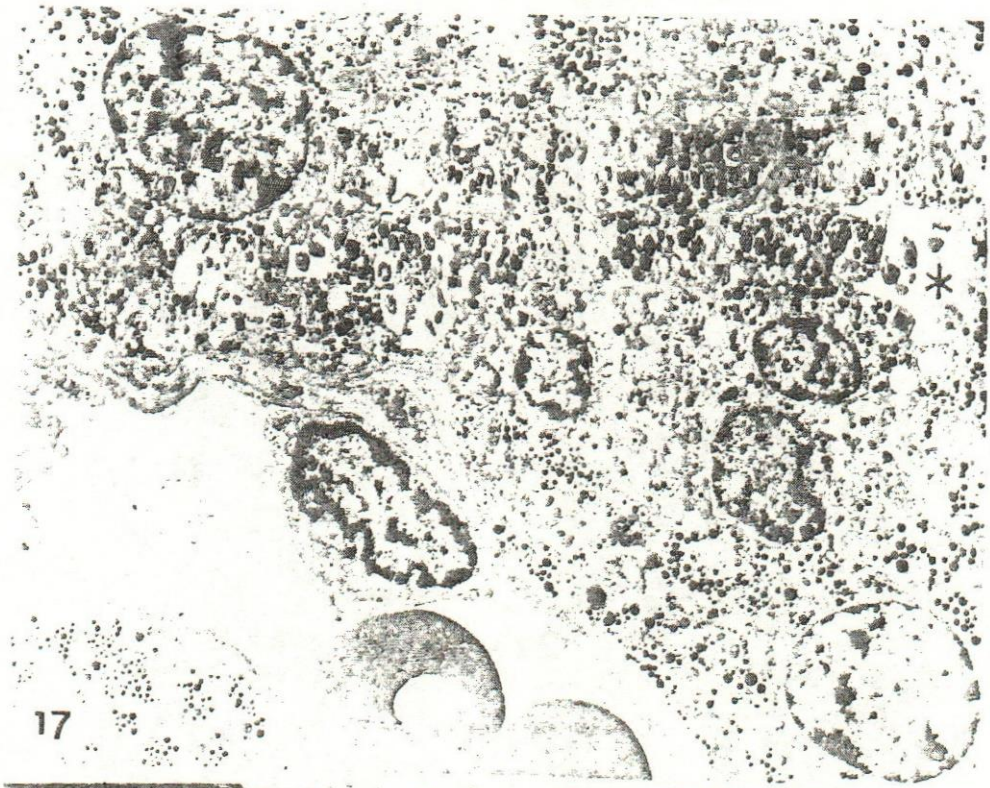

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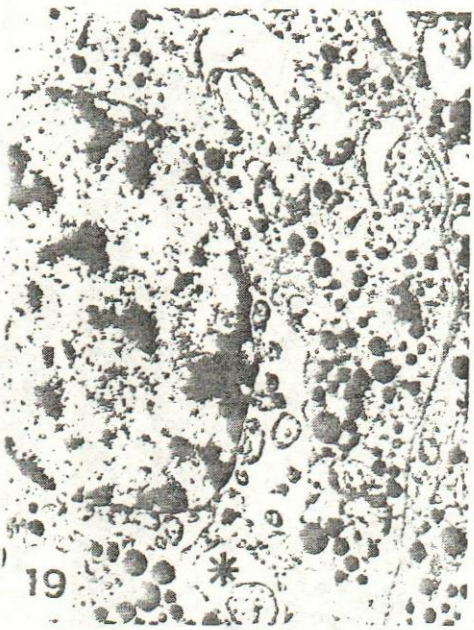

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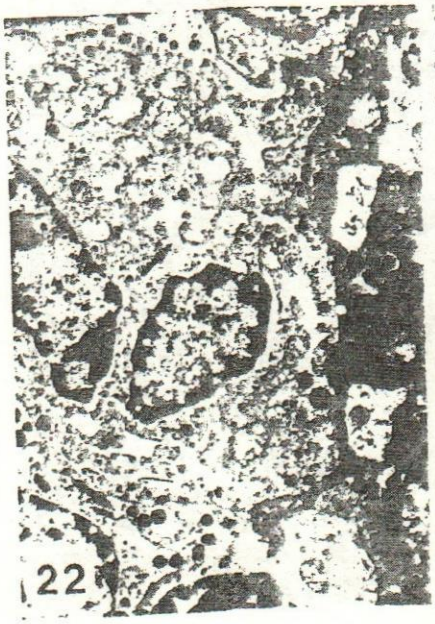

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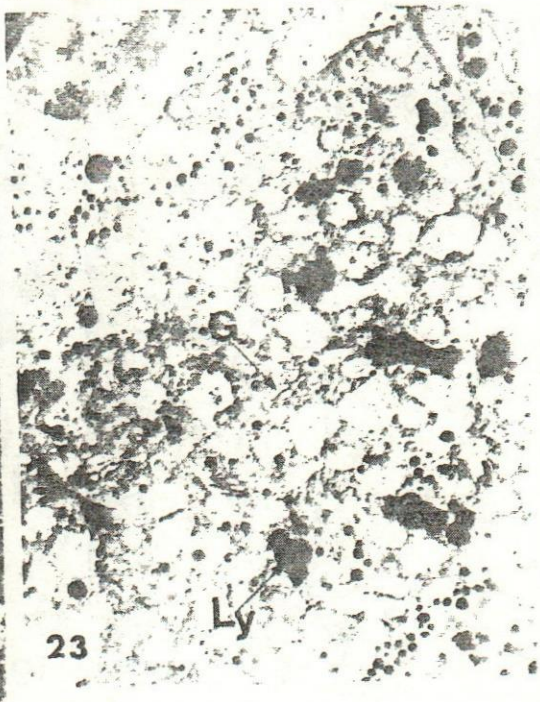

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