قسم الباثولوجيـــــا
كلية الطب البيطري - جامعة أسيوط
رئيس القسم : أ٠د / حمدى سالم

التغيرات الدقيقة فى الغدد اللبنية (فى حالة عدم الارضاع) تحت تأثير هرمون الاستروجين ومخلوط من الاستروجين والبروجستيرون

علام نفادى ، رقية شامخ ، نعمان نصر *، محمـود صالح *، مديحة محمـود *

فى دراسة على التركيب الدقيق وكذلك تأثير الاستروجين ومخلوط من الاستروجين والبروجسيترون على الغدد اللبنية لاناث الفئران البيضاء البالغة الغير مرضعة والغير حامل باستخدام الميكروسكوب الاليكترونـى النافـذ وجـد الاتـى : -

١ - أن التركيب الدقيق لفصوص الغدة اللبنية للمجموعة الضابطة عبارة عن بعض القنوات اللبنيـة مبطنة بثلاث أنواع من الخلايا وهى خلايا معتمة وخلايا غير معتمة وخلايا طلائية منقبضـــة ٠ وهذه القنوات محاطة بكمية كبيرة من النسيج الدهـنى ٠

٢ - فصوص الغدد اللبنية للحيوانات المعالجة بهرمون الاستروجين وجد بها نمو للحويصلات اللبنيـة مع نشاط افرازي قليل ٠ أما الافراز الموجود فى تجاويف الحويصلات فهو عبارة عن مواد بروتنيـة متجانسة مختلط بها كمية قليلة من الدهـــون ٠

٣ - فصوص الغدد اللبنية للحيوانات المعالجة بمخلوط من الاستروجين والبروجستيرون وجـد بهـا أيضا نمو للحويصلات اللبنية بينما كان النشاط الافرازي أكثر من المجموعة السابقة والمـــواد المفرزة فى تجاويف الحويصلات عبارة عن مواد بروتينية محببة وتحتوي على كمية قليلة مـــن الدهـــون ٠

٤ - النسيج المبـين خلوي فى كلتا المجموعتين كان عبارة عن كمية قليلة من النسيج الضام وخلايـــاه مثل الفيروبلاست والخلايا المتخمه وخلايا البلازما والايزينوفيـــل ٠

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ULTRASTRUCTURAL CHANGES IN THE NONLACTATING MAMMARY GLAND UNDER THE EFFECT OF ESTROGEN AND A COMBINATION OF ESTROGEN AND PROGESTRONE

(With One Table & 5 Figs.)

By
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SUMMARY

Effect of estrogen as well as a combination of estrogen and progesterone on the nonlactating mammary gland of female albine rats was studied ultrastructurally by the transmission electron microscope.

The mammary lobules of control nonlactating rats is formed of solitary ducts surrounded by abundant adipose tissue. The lining epithelium of the duct was found to be formed of dark, light and myoepithelial cells.

In estrogen treated rats, lobulo-alveolar proliferation with slight secretory activity could be found, while in rats treated with a combination of estrogen and progesterone, a proliferative and marked secretory activity of the milk alveoli were pronounced.

INTRODUCTION

Hormonal control of mammary growth and activity is of paramount imprtance. The effect of either estrogen alone or estrogen and progestrone in a mixture on mammary glands was studied by some authors such as NAGASAWA, et al. (1986).

A variety of major and minor side effects have been attributed to the use of these hormones (GILMANS, et al. 1985). Moreover previous studies were done on the effect of estrogen and progesterone on the nonlactating mammary gland using the light microscope and pronounced histological and histochemecal changes were found (NASR, et al. 1986 and SHAMIKH, et al. 1987). However, there are no reports available regarding the effect of estrogen and progestrone whether separately or in a mixture on the ultrastructure of adult nonlactating mammary gland using transmission electron microscopy (TEM). Thus the present study was done to investigate the ultrastructure of the resting mammary gland before and after treatment with estrogen alone and with estrogen and progesterone in a mixture.

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MATERIAL and METHODS

Animals:

40 adult female albino rats of 100-120 gm body weight were used.

Hormones:

1- Estrogen (E) in the form of oestradiol benzoate in a dose of 90 ug/200 gm body weight.

2- Progesterone (P) in a dose of 450 ug/200 gm body weight. The dose of the hormone used was calculated according to surface area in relation to man (GHOSH, 1971).

Methods:

The hormone was dissolved in sesame oil and injected intramuscularly. Grouping of the animals, experimental duration of hormonal application and sacrifice are summerized in the following table.

Animal Groups	Material & dose injected daily for each rat	Total number of rats used	No. of animals sacrificed at	
			15 days	21 days
Control group	Sesame oil 0.2 ml	8	4	4
E-group	E dissolved in 0.2 ml sesame oil	16	8	8
E & P group	E & P dissolved in 0.4 ml seasame oil	16	8	8

Immediatly after sacrificing the rats, 10-15 small pieces from mammary tissue 2x2 mm were taken and fixed in 5% glutaraldehyde for 24 hours. The specimens were then washed in cacodylate buffer and posifixed in 1% osmeum tetroxide for 2 hours and then washed in the same buffer. By using ascending grades of ethanol, dehydration of the samples was done and they were embedded in Epon 812 as usual (GUPTA, 1983).

From prepared blocks, semithin sections 0.5-1 u thick were prepared, stainded with toluidine blue, examined by light microscope, photographed and the regions for preparation of ultrathin sections 500-800 A° thick were made, fixed on cupper grids, contrasted in uranyle acetate and lead citrate as usual. The grids were examined in the election microscope (Zeiss EM 10 c) and photographed.

RESULTS

Control nonlactating mammary gland:

Semithin sections of mammary tissue stained with toluidine blue (T.B) revealed that, the lobules contain solitary ducts surrounded by abundant adipose tissue. The epithelial lining of the ducts is formed of two layers of cells together with myoepithelial cells lying on the basement membrane (Fig. 1-1).

EFFECT OF ESTROGEN & PROGESTERONE ON RESTING MAMMARY GLAND

Uttrastructurally, the lining epithelium of the ducts of the control nonlactating mammary gland is formed of three types of cells, dark, light and myoepithelial cells. The dark cells (Fig. 1-2) are characterized by a cytoplasm of high electron density. Mitochondria, RER and freeribosomes were observed in the cytoplasm. The nucleus is indented and contains clumped peripheral heterochromatin. The cell membrnes of contiguous cells demonstrate tight junctions.

The light cells (Fig. 1-3) are characterized by a cytoplasm of less electron density with the presence of a large number of various sized vacuoles. Their nuclei are rounded with sparse peripheral and central chromatin and dilated perinuclear space. Occasionally,dilated smooth endoplasmic reticulum, RER, free ribosomes and mitochondria were seen. The abundance of vacules my be the main cause of the slight electron density of light cells.

The myoepithelial cells lie between the alveolar cells and the basement membrane and have a high electron dense cytoplasm (Fig. 1-2). These cells are oval or stellate in shape with an irregular outline presenting narrow cytoplasmic processes. Their cytoplasm show a few organelles, myofilaments and few vacuoles containing electron dense bodies.

The connective tissue stroma surrounding the ducts consists of bundles of collagenic fibers, fibroblasts and mononulear cells such as, lymphocytes, mast cells and macrophages.

Effect of estrogen nda combined estrogen and progesterone on the ultrastructure of the non lactating mammary gland:

Afer 15 days of estrogen treatment, a large number of milk alveoli in the mammary lobules could be seen (Fig. 2-1). Ultrastructurally, the alveoli were found to be lined by tall epithelial cells (Fig. 2-2), the cytoplasm is distended and contain a large number of lipid droplets. RER is not seen very often. Also the cytoplasm contains Golgi and secretory vesicles containing electron dense granules. These secretory vesicles are present close to the apical part of the cells. The nuclei are compressed. The alveoli have narrow lumen containing a very few lipid droplets. The luminal surface of the cell have a few short microvilli. In some regions, intercellular spaces were seen to contain electron dense material. The myoepithelial cells with its characteristic structure lie between the alveolar cells and the basement membrane.

A large number of milk alveoli developed in the mammary lobules after 15 days of combined estrogen and progestrone treatment (Fig. 3-1). The alveolus has a wide lumen and is lined by somewhat short epithelium (Fig. 3-2). In the cytoplasm, numerous fat droplets could be found. Some of these droplets are seen "pinched off" into the lumen. Also a number of secretory vesicles containing electron dense material are found in the cytoplasm. These vesicles are present in close relation to Golgi region. The RER is well developed and the nuclei vary in shape and size depending on the amount of fat droplets present in the cytoplasm. Most of the nuclei show dispersed euchromatin. The myoepithelial cells are flat and compressed. The lumen of the alveoli is wide and filled with proteinaceous secretory granules and some membrane limited fat droplets.

The interalveolar connective tissue stroma are formed of mature collagen fibers and fibroblasts. Aslo eosinophil, plasma cells and mast cells could be seen.

After 21 days of estrogen treatment, the mammary lobules appeared to be containing large numbers of milk alveoli (Fig. 4-1). The alveolar epithelium is tall and its cytoplasm contains numerous fat droplets (Fig. 4-2). Two types of secretory vesicles can be distinguished. The first shows partially or completely condensed contents, while the other appear to contain a fine fibrillar material together with lipid globules (Fig. 4-3). Mitochondria are present in the vicinity of rough endoplasmic reticulum. Also numerous and well developed Golgi vesicles are present at the apical

pole of the cells. These vesicles contain electron dense granules which represent the proteinaceous part of the secretory material. The nuclei are variable in shape and size and most of them show indentations of the nuclear envelope. In some places the intercellular space is slightly distended and contains electron dense materials (Fig. 4-2). The lumen of the alveoli is narrow and filled with dense secretory granules admixed with very few membrane-bound fat droplets. The connective tissue stroma contains eosinophils, macrophages, plasma cells, fibroblasts, fat cells and a large number of blood capillaries.

After 21 days of combined E & P. treatment, the milk alveoli became distended with secretionand lined with flat cells (Fig. 5-1). A marked increase in the amount of lipid droplets and secretory vesicles were found in the alveolar cells (Fig. 5-2). The secretory vesicles contain granules varying in size and electron density. The electron dense granules vary in their electron density from one vesicle to another even in the same cell. They seem to be formed of aggregations of fine linear particles or micelles within the sacs of a dilated Golgi body. The luminal surface of the alveolar cells show short microvilli. The alveolar lumen is dilated and filled with mostly proteinaceous milk secretion containing a few lipid droplets. The myoepithelial cells appeared discontinuous inbetween the alveolar epithelium and the basement membrane. Mature collgen fibers and connective tissue cells such as fibroblasts, mast cells, plasma cells and eosinophils could be seen in the connective tissue stroma. Also numerosu blood capillaries were observed adjacent to the basement membrane of the milk alveoli.

DISCUSSION

Solitary milk ducts surrounded by abundant adipose tissue are the main constituents of the nonlactating mammary lobules. The lining epithelium of the ducts is formed of light, dark and myoepithelial cells. Similar description of the resting mammary gland was forwarded by MURAD (1970); TOBON, et al. (1972) and SALAZAR, et al. (1975). The cytoplasm of light cells contains a large number of vacuoles. These vacuoles indicate that either this type of cells are envolved in active fat formation and or these is a delayed release of this fat; and since the lumen of the ducts contains a very few fat droplets delayed release is the main cause of the accumulation of fat in the light cells.

Lobulpalveolar proliferation was noticed to be prominent after 15 daily injections of estrogen. Nearly all the alveoli have narrow lumen containing a small amount of homogenous electron lucent proteinaceous secretion. Beside the well developed cellular organelles, a large number of fat droplets fill the cytoplasm which may be considered as the main cause of the nuclear compression observed in these results. Similar results were observed in rat and mouse mammary glands on the 12th day of gestation (MILLS and TOPER, 1970 and MURAD, 1970) and on mid pregnancy in human (SALAZAR, et al. 1975). Also TOBON, et al. (1972) found the same changes in rabbit mammary gland after human chorionic gonadotrophin injections for 7 days.

In case of injection of estrogen for 21 days, the most peculiar ultrastructural change was the appearance of proteinaceous granules in the luminal secretion and in the cytoplasm of the secretory cells. The latter cells contain two types of secretory vesicles; the first of them are fat globules while the second showed partially condensed granules. The alveolar lumen is still narrow and well developed cell organelles together with fat droplets could be found. The present results are in agreement with those described by TOBON, et al. (1972) after intraductal injection of ovine prolactin for 5 days into pseudopregnant rabbit mammary gland.

EFFECT OF ESTROGEN & PROGESTERONE ON RESTING MAMMARY GLAND

The effect of estrogen on the mammary gland has been attributed to the direct action of estrogen on estrogen receptors found in the mammary epithelial cells or through indirect effects of estrogen on mammary glands through stimulation of pituitary prolactin secretion (NAGASAWA, et al. 1986).

The picture of the mammary gland after estrogen treatment as well as combined estrogen and progesterone in the present study is comparable to that present prior to parturition as described in the report of MURAD (1970). This is logical because just before parturition there is a high level of estrogen and lower levels of progesterone. There may be an indirect action of the hormene through the thyroid and adrenal glands but the role of the pituitary prolactin is not probable.

Concerning the effect of compound estrogen and progesterone on the resting mammary gland, our results indicate that both proliferative and secretory processes were prominent. An obvious lobuloalveolar development with the secretion of large amounts of milk were observed after 15 and 21 daily injection of estrogen and progesterone compound. Ultrastructurally, the alveolar cells showed well developed cellular organelles as well as fat droplets and secretory vesicles containing polymorphic electron dense granules similar to those found in the lumen.

Shortening or flattening of the alveolar cells observed in there results seen to be a reault of pressure from accumulated secretion in the lumen. Also the variation in shape and size of the epithelial nuclei can be attributed to the large amount of fat droplets and secretory vesicles in the cells. A similar description was founded by MURAD (1970); SALAZAR, et al. (1975) and SINOWATZ, et al. (1980) in rat, human and Beagle respectively during late pregnancy. Also MILLS and TOPPER (1970) found that the addition of insulin, hydrocortisone and prolactin to the media of mid pregnant mouse mammary gland culture induced the same changes.

CERIANI, et al. (1970) found that progesterone with prolactin and aldosterone induce a secretory state to fetal rat mammary gland in tissue culture. Also RICHERDS, et al. (1983) found that combined estrogen and progesterone added to mammary tissue culture give similar secretory changes.

Concerning the large amount of fat droplets observed in our results inthe secretory cells under the effect of estrogen or estrogen and progesterone simulate what has been reported by MURAD (1970) concerning the accumulation of lipid droplets in the cytoplasm early in pregnancy and their released into the lumen at or after delivery.

CONCLUSION

It may be concluded that estrogen alone affects the resting mammary gland by inducing lobuloalveolar proliferation with a slight secretory activity in which the secretion is homogenous, electron lucent proteinaceous and admixed with a few lipid droplets. However, when combined with progesterone these is also proliferation and the secretory activity is more prominant and the secretion is in the form of dense proteinaceous granules together with lipid droplets.

This indicates that progesterone has a potentiating effect with estrogen on the secretory mateerials as revealed by its extent and nature of its protein contents. The proliferative effect of estrogen alone on the alveoli in particular is mainly due to its direct action through cellular receptors is much less probable.

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DISCRIPTION OF FIGURES

Fig. (1): Ductal wall of nonlactating mammary gland.

1-1 Semithin section showing milk duct and periductal connective tissue T.B. stain Mag. (40 X 12.5).

1-2 Electron micrograph showing, Dark cell (DC), myoepithelia cell (Mc), and collagenic fiber (Co) Orig. Mag. (8800).

1-3 Electron micrograph showing, light cell (Lc).

Fig. (2): Mammary gland of nonlactating rat after 15 day of Estrogen treatment.

2-1 Semithin section of mammary lobules showing numerous milk alveoli T.B. stain Mag. (40 X 12.5).

2-2 Electron micrograph of milk alveolus showing narrow lumen (L), the secretory cells having fat droplets (F) and short microvilli (M.V). Orig. Mag. (8800).

Fig. (3): Mammary gland of nonlactating rat after 15 day of combined estrogen and progestrone treatment.

3-1 Semithin section showing a part from lobule and formed of numerous compact milk alveoli T.B. stain Mag. (40 X 12.5).

3-2 Electorn micrograph of milkalveolus showing the alveolar cell containing fat droplets (F), well developed R.E.R. and the lumen contain secretory granules (G) and fat globules (F) Orig. Mag. (8800).

Fig. (4): Mammary gland of nonlactating rat after 21 day of estrogen treatment.

4-1 Semithin section showing the milk alveoli and fat cells T.B. stain (40 X 12.5).

4-2 Electron micrograph of milk alveolus showing narrow lumen (L) containing milk. the secretory cells having partially condensed granules in secretory vesicle (SV), fat droplets (F), electron dense material in intercellular space (E.D). Orig. Mag. (8800).

4-3 Secretory cells containing secretory vesicle having fat globules (SVF) and partially condensed granules (SVG) and fat fat droplet (F) Orig. Mag (8800).

Fig. (5): Mammary gland of nonlactating rat after 21 day of combined estrogen and progestrone treatment.

5-1 Semithin section showing the milk alveolus become distended with milk and lined with flat secretory cells. T.B. stain (40 X 12.5).

5-2 Electron micrograph of milk alveolus showing flat epithelium containing numerous fat droplets (F), secretory vesicles with polymorph condensed granules (SVG), compact nucleus (N), short microvilli (MV), wide lumen (L) Orig. Mag. (8800).


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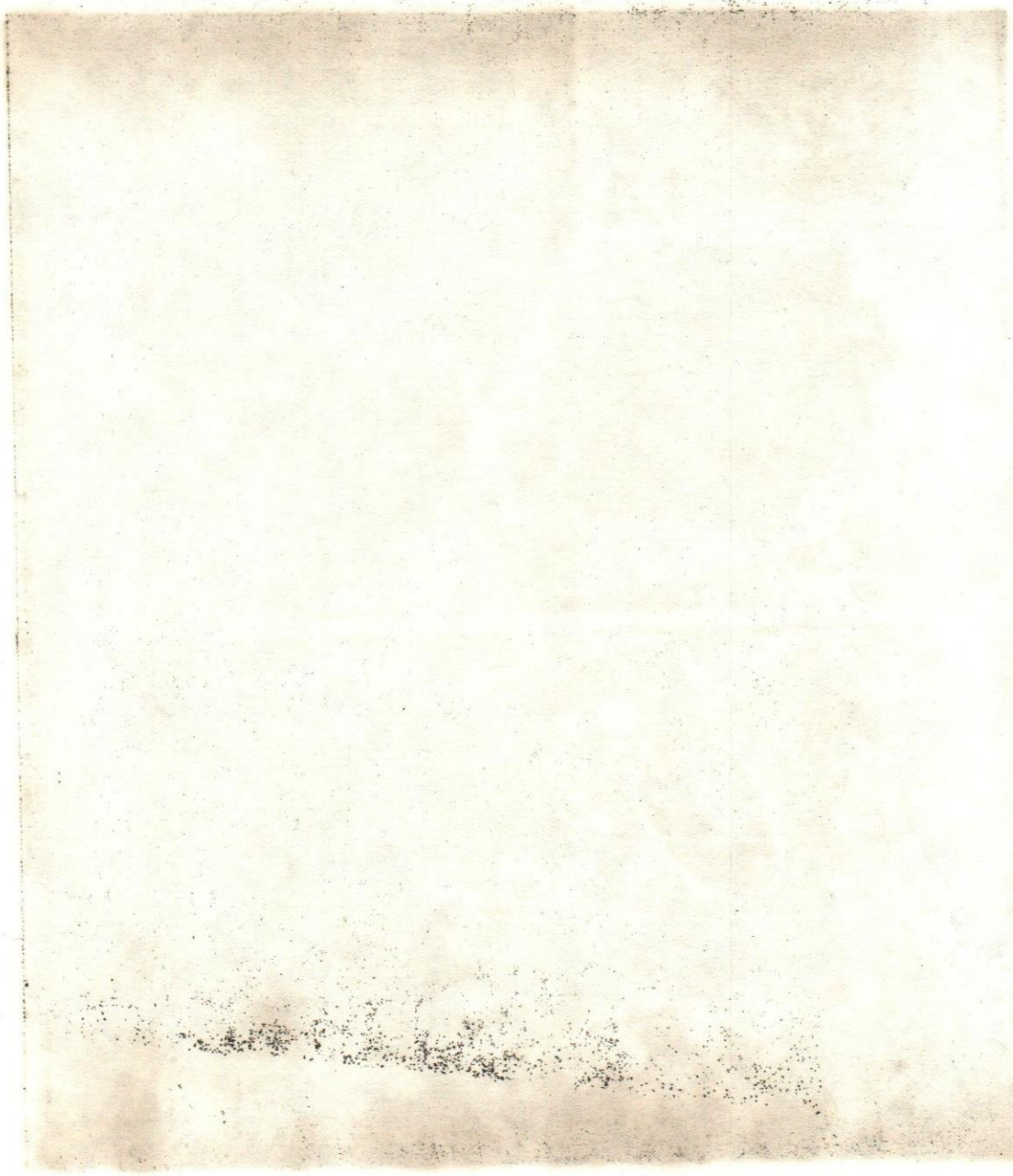
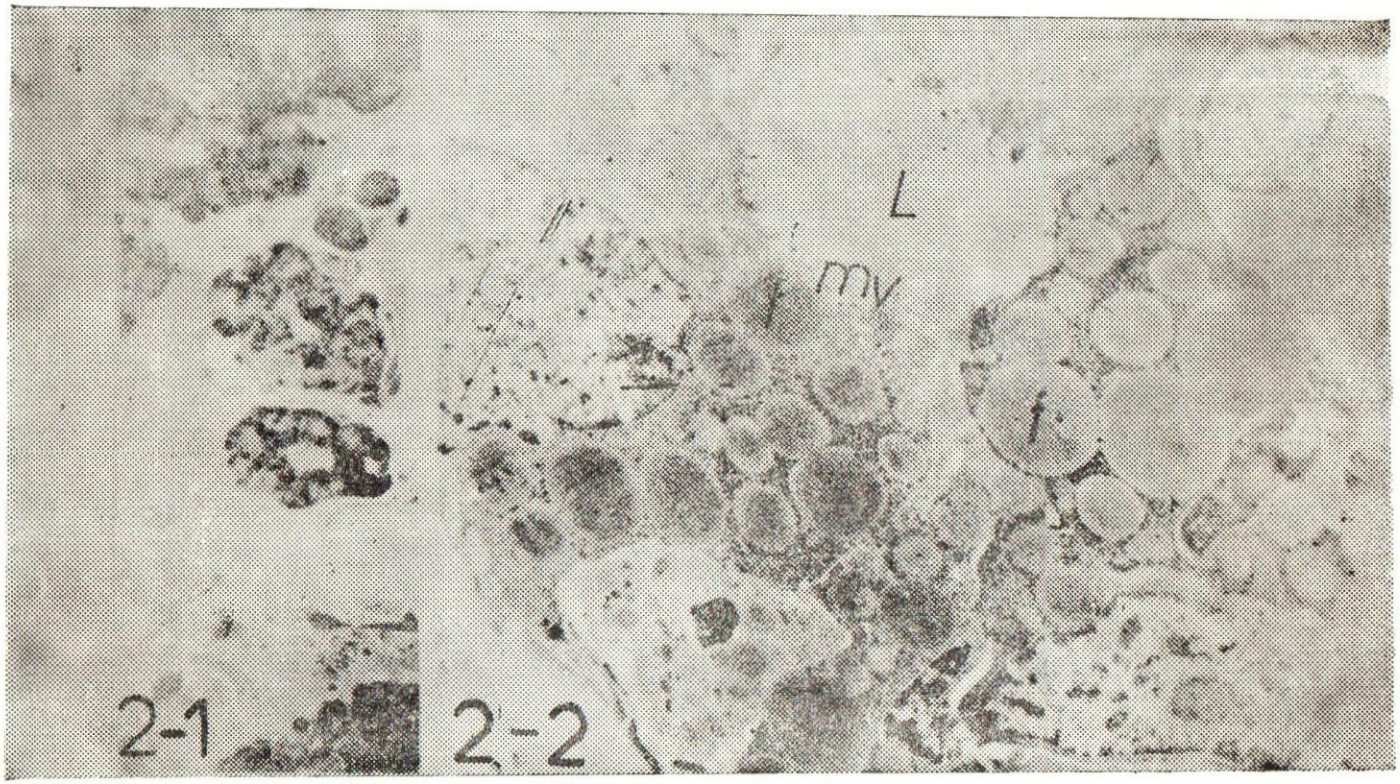
Lc

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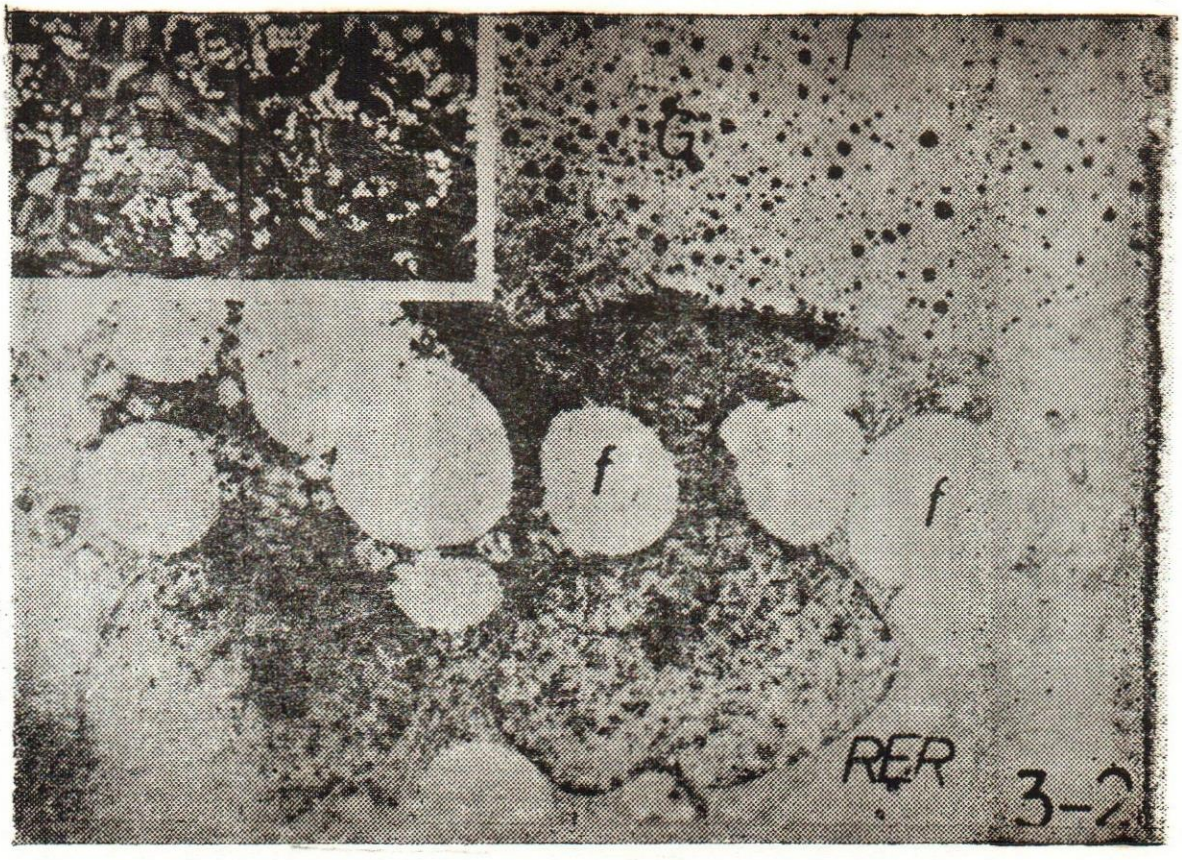
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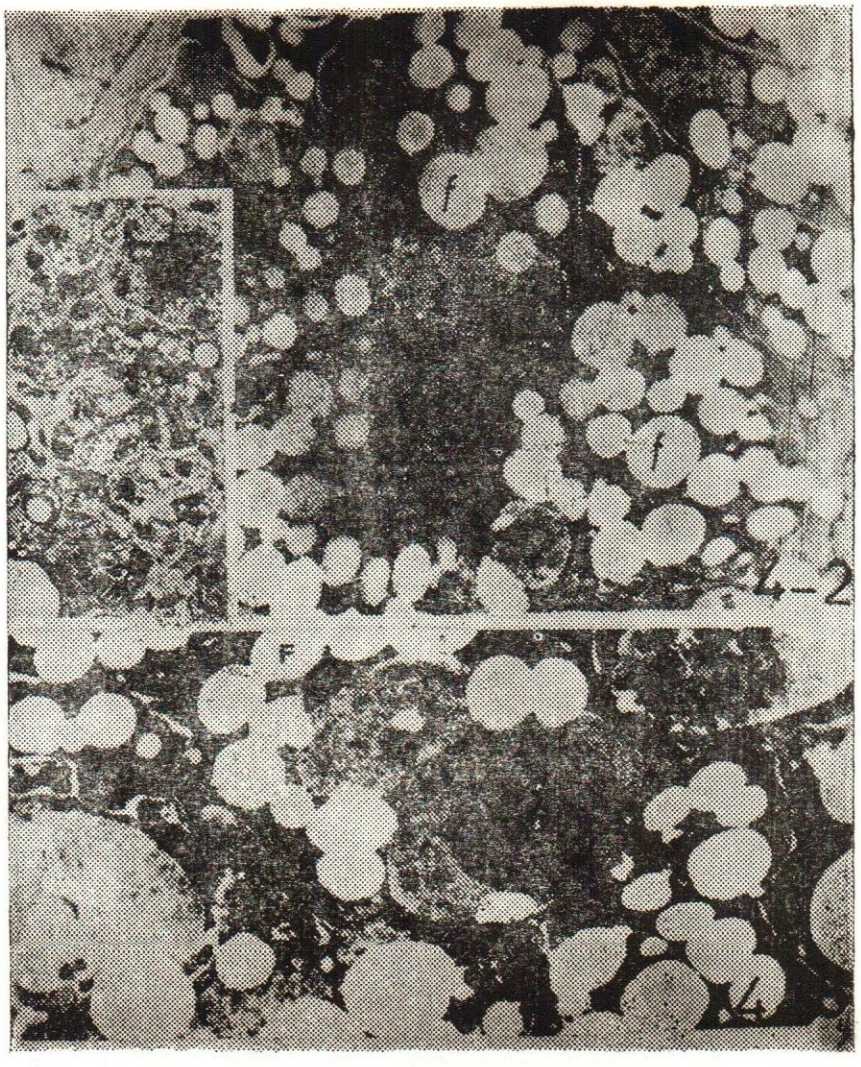
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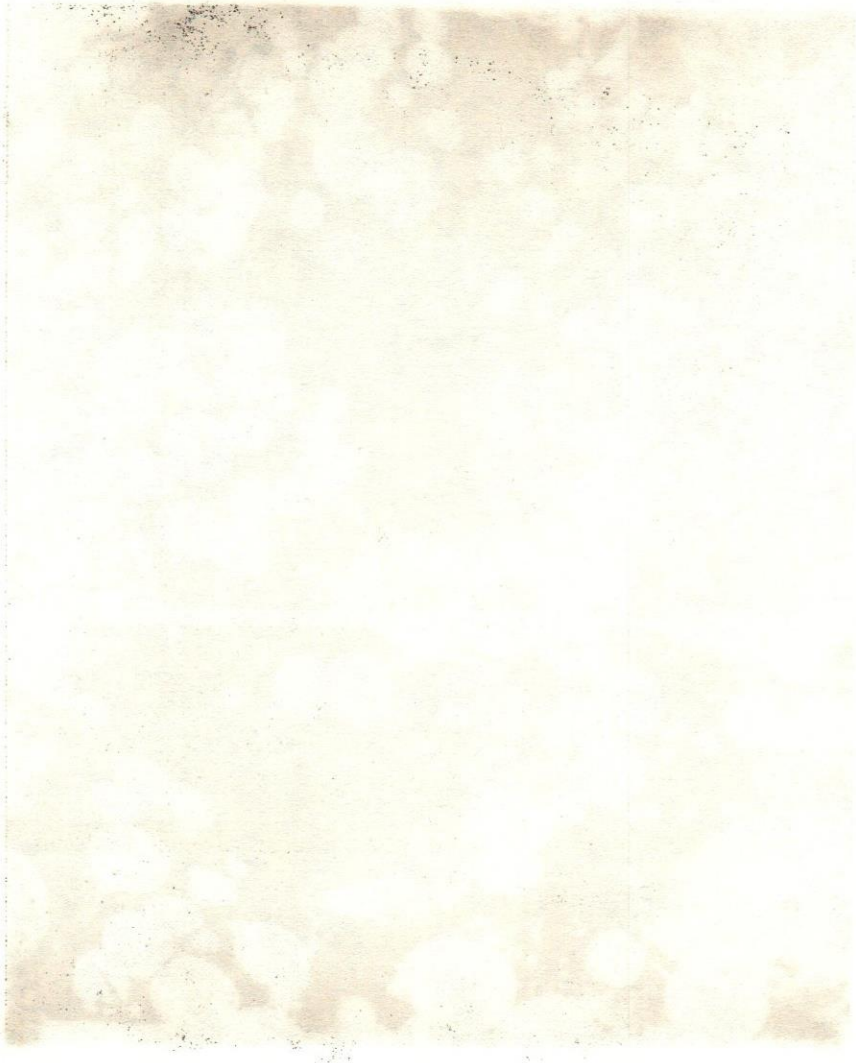


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