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EFFECT OF POST-COITAL ADMINISTRATION OF ANTIHISTAMINIC DRUG (DIPHENHYDRAMINE HYDROCHLORIDE) ON FEMALE MOUSE UTERUS AND ITS DRAINAGE LYMPH NODES AFTER IMPLANTATION OF EMBRYO

(With One Table and 28 Figures)

By

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تأثير عقار مضاد الحساسية (داى فين هيدرامين) على رحم أنثى الفأر الأبيض الصغير وعقده الليمفاوية الصارفة بعد التصاق الجنين

محسن عبد الحافظ مصطفي ، أسامه أحمد علي شرف الدين ، سامي عبد اللطيف أحمد صابر ، عبد الرحمن سيد سلطان

اجـري هـذا البحث لدراسة التغيرات الهستولوجية والهستوكيميائية لرحم أنثي الفأر الأبيض الصـغير، وكذلك التغيرات الهستولوجية لعقده الليمفاوية الصارفة بعد التصاق الجنين وذلك لدراسة تأثير تناول عقار مضاد الحساسية (داي فين هيدرامين) من أول يوم في الحمل حتى يـوم الذبح علي هذه التغيرات. وقد وجد بالفحص المجهري أنة عند أربعة أيام ونصف من بداية الحمل، تم التصاق العلقة بالطلائية السطحية، وعند ستة ايام ونصف ظهر تحطم كامل لطـبقة الطلائية السطحية مع بعض التغيرات الهستولوجية والهستوكيميائية في بطانة الرحم عنها في رحم أنثي الفار البكر، كذلك لوحظ تضخم في العقد الليمفاوية الصارفة وعقيداتها مع ظهـور مراكـز منبـتة. كما أظهرت الدراسة أن تناول عقار (داي فين هيدرامين) المضاد للحساسـية أدي إلي تثبيط التغيرات الهستولوجية المصاحبه لعملية زرع الجنين مما أدي إلى حـدوث إجهـاض للجنين وهذا ما اكدته الدراسات الهستوكيميائية. كما أن نقص حجم العقد اللـيمفاوية الصـارفة وعقيداتها عن مثيلاتهم في إناث الفئران الحوامل الطبيعية أكد ما سبق الإشـارة إلـية من التأثير الذي احدثة العقار. توصي الدراسة باتخاذ الحذر من استخدام هذا العقار أثناء فترة الحمل.

SUMMARY

This study was done to reveal the effect of antihistaminic drug (Diphenhydramine) on mouse uterus and its drainage lymph nodes after implantation of embryo. The non-pregnant mice were sacrificed at

oestrus phase and the pregnant mice were randomly divided into two groups: (1) control group and (2) treated group with antihistaminic drug at a dose 0.52 mg ¼ ml/day. These animals were dosed daily from the day of vaginal plug till the sacrificed days 4.5, 6.5 post-coitus. The animals of each group were sacrificed then the uterus and its drainage lymph nodes were taken and stained with H&E and histochemical stains. At 4.5 day of pregnancy, the blastocyst was attached to the luminal epithelium, the stromal cells (fibroblasts) around the blastocyst were proliferated and differentiated into decidual cells, and a marked edema was observed in the endometrial stroma. Histochemical studies showed a decrease in the carbohydrate content of the mouse uterus, and an increase in the activities of alkaline and acid phosphatases compared to those of the normal mouse uterus at oestrus phase. At 6.5 day of pregnancy, the luminal epithelium around the blastocyst was completely destroyed, the uterine glands were disappeared from the decidual zone, and the decidualization of the stroma becomes more extensive forming the primary and secondary decidual zones. Histochemical investigations revealed an increase in the carbohydrate content and in the activities of both alkaline and acid phosphatases compared to those of the previous stage. Also, at this stage, there was a marked enlargement of the uterine drainage lymph nodes with the formation of secondary lymphatic follicles, containing germinal centers. In treated mice, the histological changes associated with implantation were inhibited. Histochemical studies showed a decrease in the carbohydrate content and in the activities of alkaline and acid phosphatases. Also, at 4.5, 6.5 days of pregnancy, there was a decrease in the size of the uterine drainage lymph nodes and their lymphatic follicles compared to those of the normal pregnant mice.

Key words: *Moues uterus, lymph nodes, implantation, decidualization, miscarriage, antihistominic drug.*

INTRODUCTION

Histamine is an important mediator in embryo-uterine interactions during implantation (Paria *et al.*, 1998; Zhao *et al.*, 2000). Since, the process of implantation means the "two-way" interaction between the blastocyst and uterus (Tranguch *et al.*, 2005).

A number of surveys of using the drug in pregnancy have revealed that antihistamines (histamine H1-receptor antagonists) are taken by approximately 10 to 20% of pregnant women, often for extended periods of time (Chiavegatto *et al.*, 1997). The causes for using

of these agents are: (1) to alleviate the symptoms of various allergic disorders, (2) to treat nausea and vomiting, and (3) to aid night-time sleep. Unfortunately several studies suggested that H-receptor antagonists inhibit implantation induced by histamine (Agrawal & Aravinda, 1995; Diav-Citrin *et al.*, 2003).

Most of the previous workers have concentrated their work on the effect of H-receptor antagonists on the implantation macroscopically. Concerning the effect of antihistaminic drugs on the histological and histochemical structures of the uterus after implantation, the published data are very limited and restricted. Accordingly, it necessitates the carrying out of the present investigation to throw some light on:

1- Histological and histochemical structure of the mouse uterus and histological structure of uterine drainage lymph nodes at oestrus phase of the oestrous cycle and after implantation of embryo.
2- Histological and histochemical changes of the mouse uterus and histological changes of the uterine drainage lymph nodes after implantation of embryo under the effect of antihistaminic drug (diphenhydramine hydrochloride).

MATERIALS and METHODS

Albino mice were used from Faculty of medicine, Assiut university animal house. The animals were maintained in a controlled environment. Daily vaginal smears were taken from virgin mice to determine the phases of oestrous cycle according to Fox and Laird (1970). At the oestrus phase, virgin females were caged in the afternoon for overnight with mature males in a ratio of one female/one male/cage. The morning of the vaginal copulation plug was designated as 0.5 day of pregnancy according to Adamson *et al* (2002). The pregnant females were isolated from mating cages and rehoused in groups.

Active ingredient (Diphenhydramine hydrochloride) of antihistominic drug was obtained from the Nile-company for pharmaceuticals and chemical industries – Cairo – Egypt. The drug was dissolved in distilled water in preparation (0.52mg/1/4ml/day) dose. Recommended dose for allergic patients was converted from human dose to mice dose according to Paget and Barnes (1964). The animals were administrated the drug orally, using a curved gastric tube and a tuberculin syringe.

Three non-pregnant mice were sacrificed at oestrus phase and 24 pregnant mice were randomly divided into two main groups:

Group 1: control groups (10 mice). These animals subjected to sacrificed. They were further subdivided into 2 subgroups: 5 animals each.

Group II: Treated groups (14 mice). They were further subdivided into 2 subgroups: 7 animals each. These animals were dosed daily by Diphenhydramine hydrochloride from the day of vaginal plug to the sacrificed day. The animals of each group (group I and groupII) were sacrificed at 4.5, 6.5 days of pregnancy

The uteri were isolated by cutting uniformly at the cervical and tubal junctions, and then trimming a way the mesometrium. The two uterine horns were separated, one horn was immediately fixed in cold Rosman's fixative (Cook, 1996) and the other was immediately fixed in cold acetone for enzymes histochemistry. The uterine drainage (pelvic) lymph nodes were isolated, the right lymph node was fixed in Rosman's fixative. After dehydration and clearing, the tissues were embedded in paraffin. Uteri were transversely oriented and serially sectioned. Sections were cut 5-8μ thick for the uterus and 5μ for the lymph node and subjected to the following staining techniques: 1- haematoxylin and eosin (Stevens & Wilson, 1996), 2- PAS for polysaccharides (Cook, 1996) and 3-Gomori method for alkaline and acid phosphatases (Drury & Wallington, 1980).

RESULTS

Histological structure of mouse uterus at oestrus phase and after implantation of embryo:

The uterine wall consists of endometrium, myometrium, and perimetrium. The endometrium consists of a simple luminal columnar epithelium, uterine gland and stroma. The uterine glands are simple and branched tubular in type. Their lumina are wide and lined with cuboidal epithelium. The myometrium consists of an inner circular and an outer longitudinal layer of smooth muscle. A connective tissue sheet containing blood vessels lies between the two muscle layers. The perimetrium composed of connective tissue and mesothelium (Fig.1).

At 4.5 day of pregnancy, the blastocyst appears in close apposition with the luminal epithelium in the antimesometrial end of the uterine lumen. The stromal cells surrounding the implanted blastocycst are proliferated and differentiated into decidual cells. The uterine stroma around the blastocyst shows a marked edema (Figs. 2 & 3).

At 6.5 day of pregnancy, the implantation site is elongated in a mesometrial-antimesometrial direction, the uterine lumen is closed around the blastocyst, and the implanted blastocyst occupies almost the entire uterine chamber. The luminal epithelium around the blastocyst is completely destroyed. The blastocyst at this stage becomes elongated and tubular in shape. The decidualization of stroma becomes more extensive, forming the primary and secondary decidual zones. The uterine glands are disappeared from the decidual zone. The blood vessels are dilated and surrounded by decidual cells (Figs .4, 5&6).

Histochemical observations of the mouse uterus at oestrus phase and after implantation of embryo:

The histochemical features seen in normal mouse uterus at oestrus phase and implantation sites at 4.5 and 6.5 days of pregnancy are given in Table (1).

Table 1: Results of semiquantitative distributions of PAS-positive material and the activities of alkaline and acid phosphatases in normal mouse uterus at oestrus phase and implantation sites at 4.5 and 6.5 days of pregnancy.

Histochemical observation	mouse uterus at oestrus phase					At 4.5 day of pregnancy					At 6.5 day of pregnancy		
	Endo			Myo		Endo			Myo		Endo	Myo	
	L.E	U.G	S.	L.M	C.M	L E	U G	S	L.M	C.M		L.M	C.M
PAS-positive material	-	+++	-	++	++	+	-	+	++	±	++	+	+
Alkaline phosphatese activity	+	+	-	+++	±	-	++	++	+++	-	+++	+	+
Acid phosphjatase activity	+	+	±	+	-	++	++	++	+	+	+++	+	-

Abbreviations: (+++) Strong, (++) moderate, (+) mild, (±) faint, and (-) negative.
LE lumen epithelium UG uterine gland S stroma
LM longitudinal muscle
CM circular muscle Endo Endometrium Myo myometrium

1- In mouse uterus at oestrus phase:

The lumina of the uterine glands in the normal mouse uterus show a strong PAS-positive reaction (plugs in appearance). The basement membrane of the luminal epithelium and the blood capillaries show a mild PAS –positive reaction. The luminal epithelium and the stromal cells show a negative reaction. The myometrium shows a

moderate PAS-positive reaction with the longitudinal layer more than the circular (Fig 7). Alkaline phosphatase reaction at this phase is strongly positive in some blood capillaries of endometrium and longitudinal muscle layer of the myometrium. It is mildly positive in luminal epithelium and the uterine glands, while the endometrial stroma shows a negative reaction (Fig.8). At this phase a mild reaction for acid phosphatase activity was observed in the luminal and glandular epithelium and in the longitudinal muscle layer of myometrium. A faint reaction was observed in some stromal cells (Fig.9).

2- At 4.5 day of pregnancy

At 4.5 day of pregnancy, there is a decrease in PAS-positive material compared to that of the normal mouse uterus at oestrus phase. The antimesometrial luminal epithelium adjacent to the blastocyst and the subepithelial decidual cells around the blastocyst show a mild reaction. The uterine glands show a negative PAS -reaction, while the longitudinal muscle layer of myometrium shows a moderate reaction (Fig.10). There is an increase in alkaline phosphatase activity compared to that of the non-pregnant mouse uterus at oestrus phase. The endometrial stroma and the uterine glands show a moderate reaction of alkaline phosphatase (Fig.11). At 4.5 day of pregnancy, a moderate reaction of acid phosphatase activity was observed in the endometrial stroma, uterine glands, and the anti mesometrial luminal epithelium, while mild reaction was noticed in myometrium (Fig. 12).

3- At 6.5 day of pregnancy

At 6.5 day of pregnancy, there is an increase in PAS-positive reaction in the endometrial stroma including the decidua compared to that of the previous stages. A moderate PAS-positive reaction was observed in the cells mesometrial and lateral to the embryo forming the glycogen wings. A mild reaction was observed in the myometrium. The antimesometrial decidua shows a negative PAS-reaction. (Fig. 13). At this stage, there is a marked increase in the reaction for alkaline phosphatase activity in the endometrial stroma including the decidua compared to that of the previous stages. A strong reaction was observed throughout most of the endometrial stroma including the deciduas, while a decrease in the reaction was observed in the myometrium which shows a mild reaction (Fig. 14). The activity of acid phosphatase reaches its maximum at this stage. A strong reaction was observed in the antimesometrial decidual zone around the embryo, while the mesometrial decidual zone and the longitudinal muscle layer of myometrium show a mild reaction (Fig. 15).

Histological changes of the mouse uterus under the effect of post – coital diphenhydramine treatment:

The oral administration of the antihistaminic drug diphenhydramine hydrochloride, causes both pre and post implantation miscarriages. The pre -implantation miscarriage before 4.5 day is associated with intact luminal epithelium, there is no blastocyst in the uterine lumen, the stroma exhibits small number of the decidual cells, and the stromal edema, which is characteristic of the attachment phase of pregnancy has been inhibited (Fig. 16).

The post- implantation miscarriage after 6.5 day is characterized by an open the uterine lumen, the blastocyst is absent, the antimesometrial luminal epithelium is degenerated, cellular infilteration can be seen inside the uterine lumen, and the uterine glands are still present throughout the stroma (Fig. 17).

Histochemical changes of the mouse uterus under the effect of post – coital diphenhydramine treatment:

1- Pre- implantaion miscarriage (at 4.5 day):

The basement membrane of the luminal epithelium, the lumina of some uterine glands, and the myometrium show a mild PAS-reaction, while the endometrial stroma shows a negative reaction (Fig.18).

A decrease in alkaline phosphatase activity was observed in the endometrium of the treated mice compared to that of the normal pregnant uteri. Also, no change in myometrial alkaline phosphatase activity was observed. The uterine glands and some regions of the stroma show a mild reaction for alkaline phosphatase, while the luminal epithelium shows a negative reaction (Fig. 19).

Administration of diphenhydramine hydrochloride causes a decrease in the endometrial acid phosphatase activity compared to that of the pregnant mice uteri.

A mild staining of acid phosphatase was observed in the luminal epithelium, the uterine glands, and the sub-epithelial stromal cells (Fig. 20).

2- Post- implantaion miscarriage (at 6.5 day):

A strong PAS-positive material was observed in the degenerated luminal epithelium and in the subepithelial stroma. Also, there are small quantities of PAS-positive material in the lumina of some uterine glands. The myometrium shows a mild reaction (Fig. 21).

A strong reaction of alkaline phosphatase activity was observed in the uterine glands and in the degenerated luminal epithelium, and a moderate staining was observed in some regions of the stroma. (Fig. 22).

A decrease in the endometrial acid phosphatase activity compared to that of the pregnant mice uterus was also observed in this stage. The degenerated luminal epithelium and the sub-epithelial stromal cells show a moderate reaction for acid phosphatase activity, while the uterine glands show a mild reaction (Fig. 23).

Histological structure of the uterine drainage (pelvic) lymph node of the mouse at oestrus phase of the oestrous cycle:

The uterine drainage lymph node at oestrus phase consists of a relatively thin connective tissue capsule, cortex, and medulla.

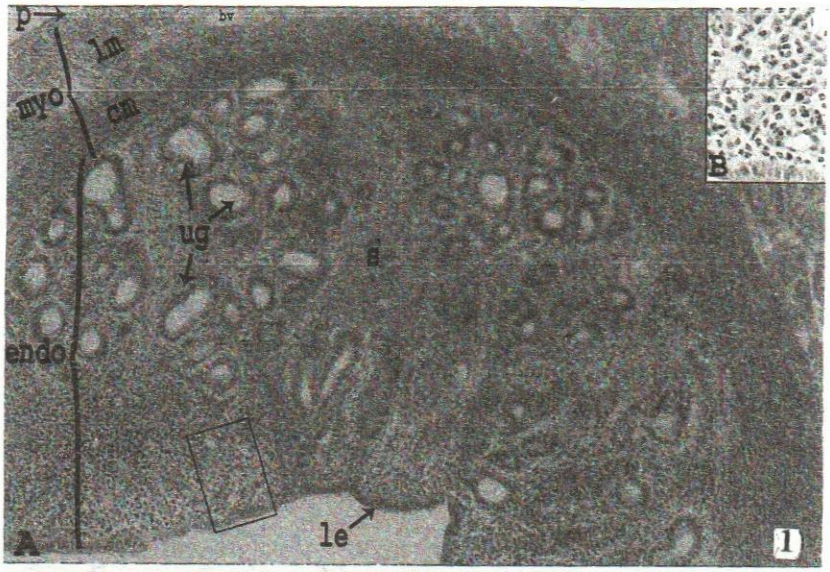
The cortex is composed of the lymphoid tissue and the sub-capsular sinus which communicates with the medullary sinuses through intermediate sinuses. Within the lymphoid tissue there are the primary lymphatic follicles which are formed of aggregation of small lymphocytes without a germinal centre. The medulla is composed of the medullary cords which are irregular collections of lymphocytes. The medullary cords are separated from each other by medullary sinuses (Fig. 24).

Histological changes of the uterine drainage (pelvic) lymph nodes after implantation of embryo:

At 4.5 day of pregnancy the uterine drainage lymph nodes become large in size compared to those of the normal mouse at oestrus phase. Also, at this stage, the small lymphocytes are developed into activated medium-sized lymphocytes forming the secondary lymphatic follicles with germinal centres (Fig. 25). At 6.5 day of pregnancy the uterine drainage lymph nodes reach their maximum size. Also, the secondary lymphatic follicles are increased in size compared to those of the previous stage (Fig. 26).

Histological changes of the uterine drainage (pelvic) lymph nodes under the effect of postcoital diphenhydramine treatment:

At 4.5 and 6.5 days post-coitus, there are marked decreases in the size of the uterine drainage lymph nodes and their lymphatic follicles of treated animals comparing to those at 4.5 and 6.5 days of pregnancy (Figs. 27, 28).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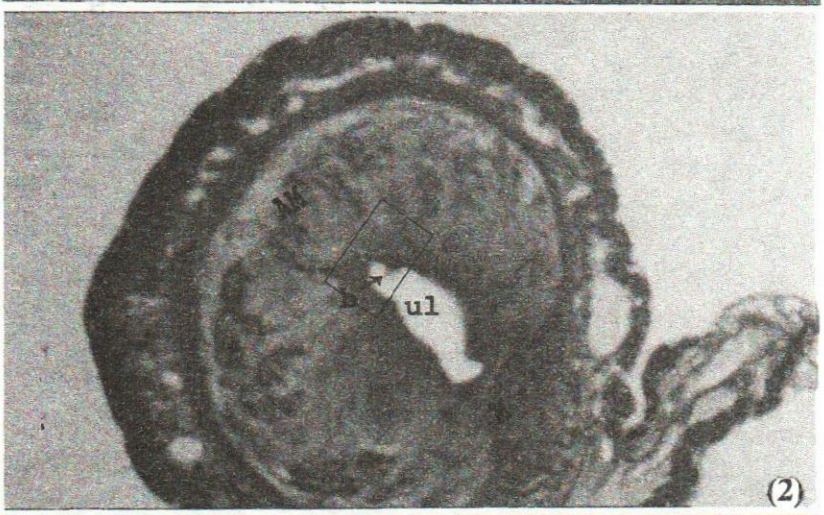
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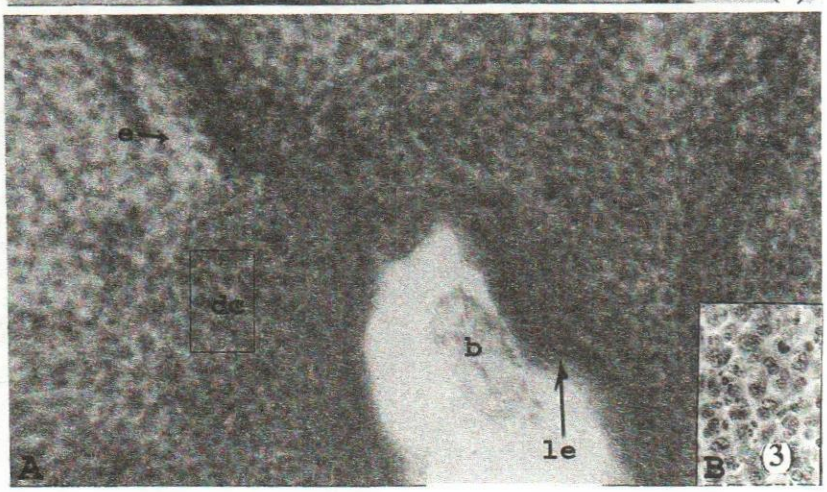
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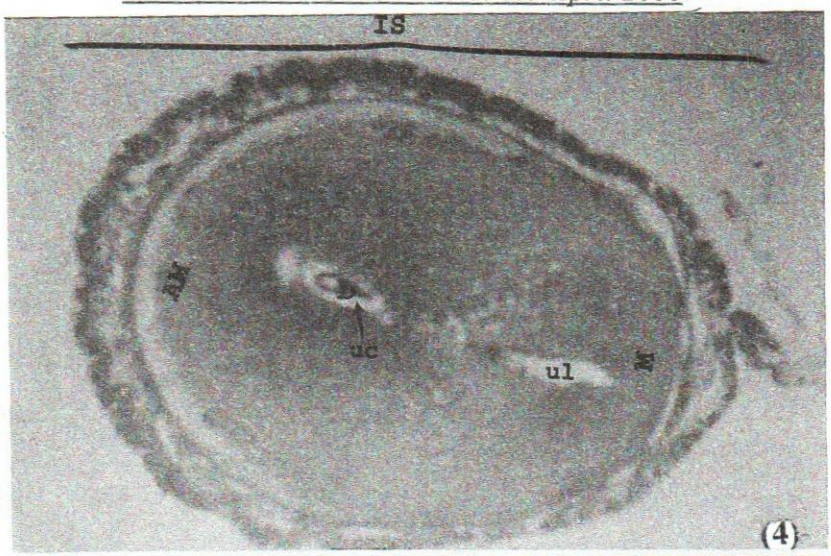
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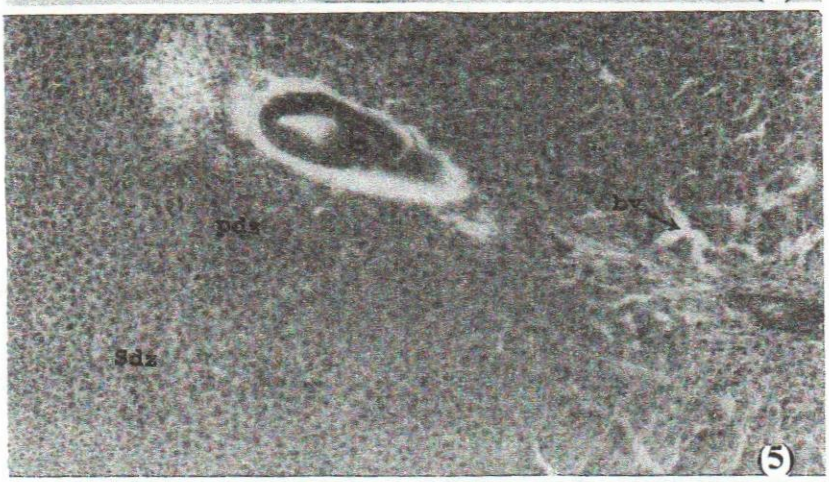
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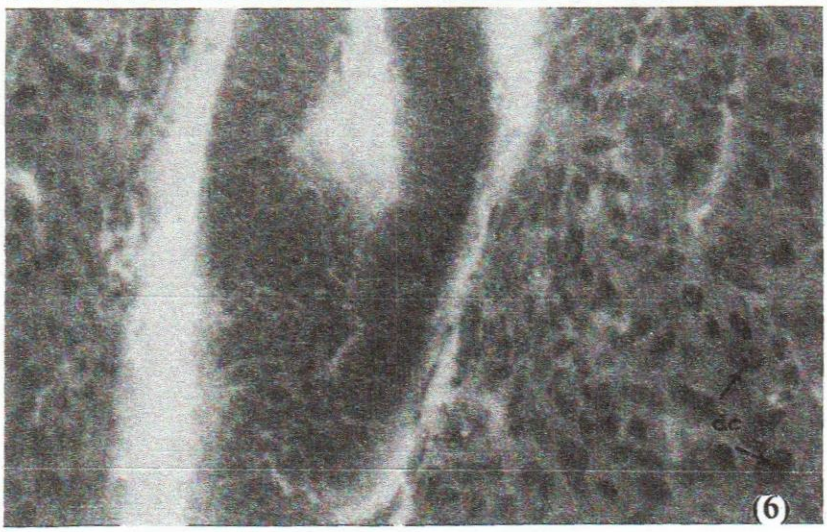
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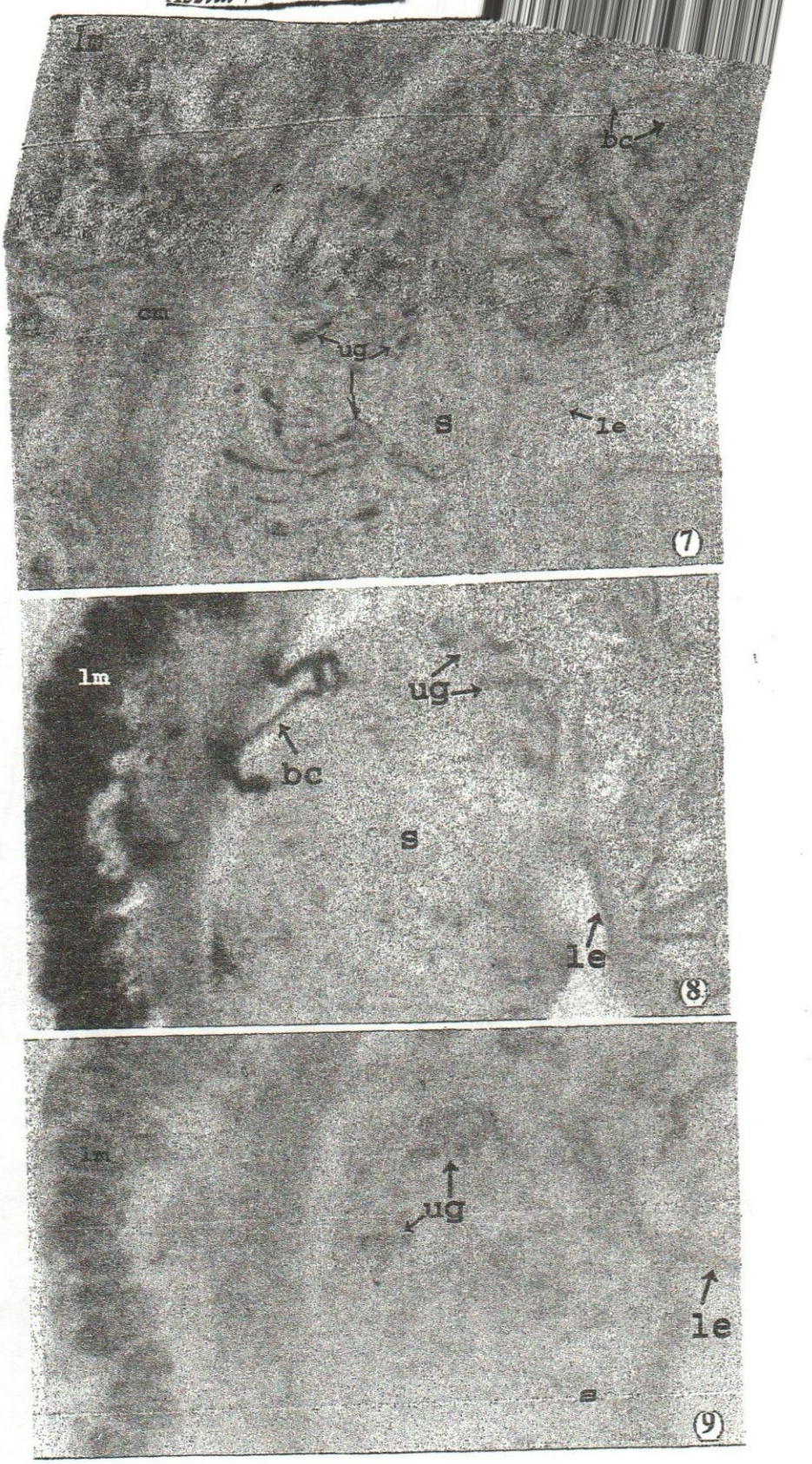
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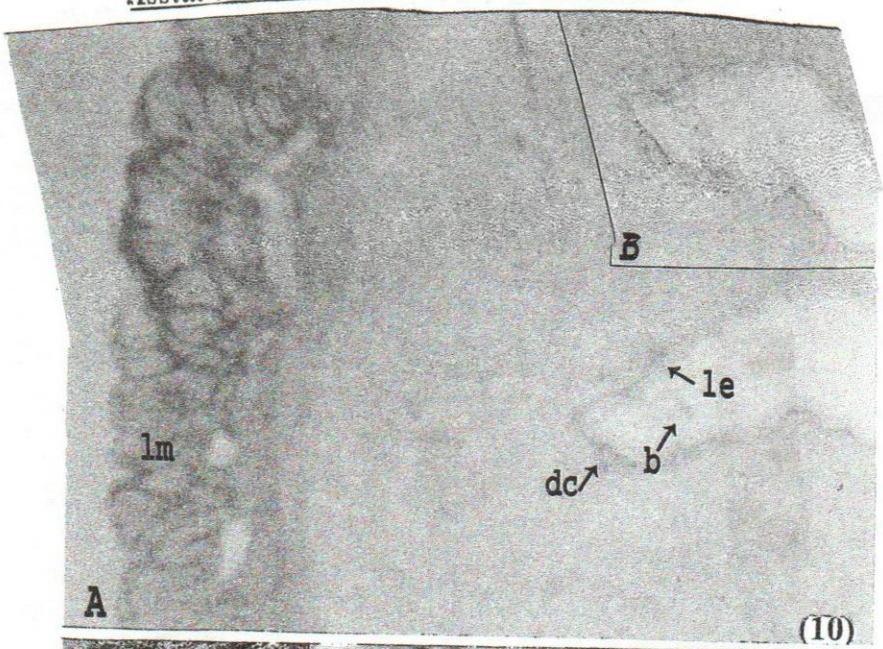

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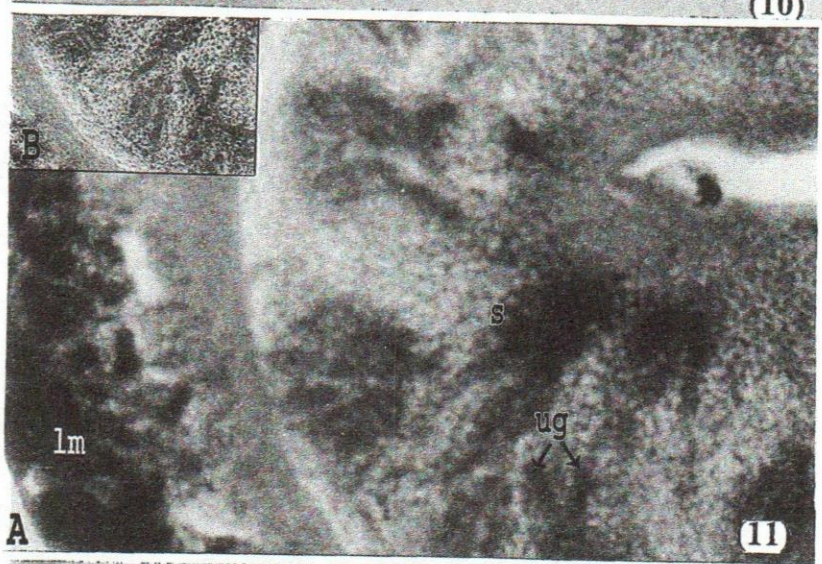

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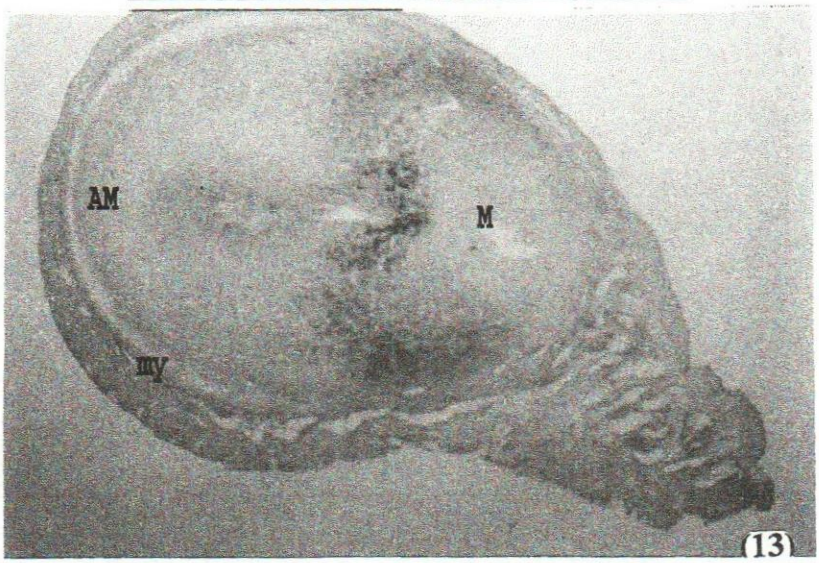

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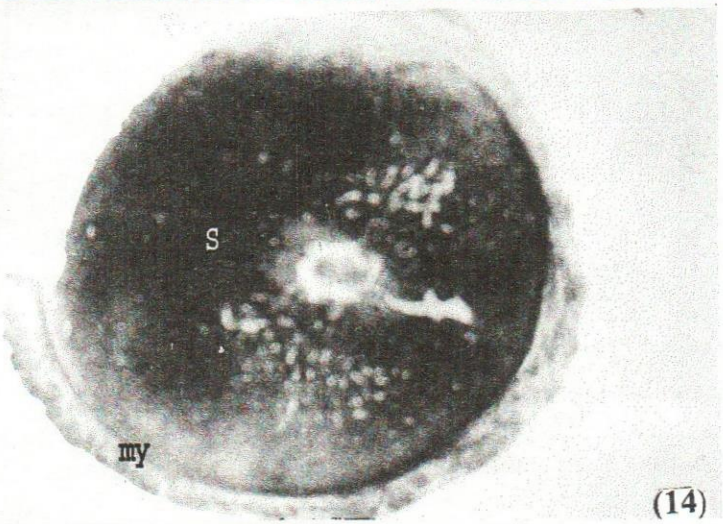

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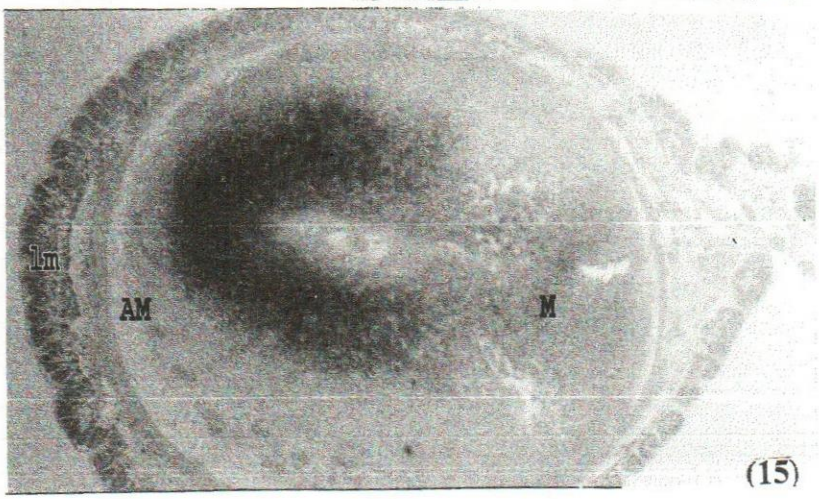

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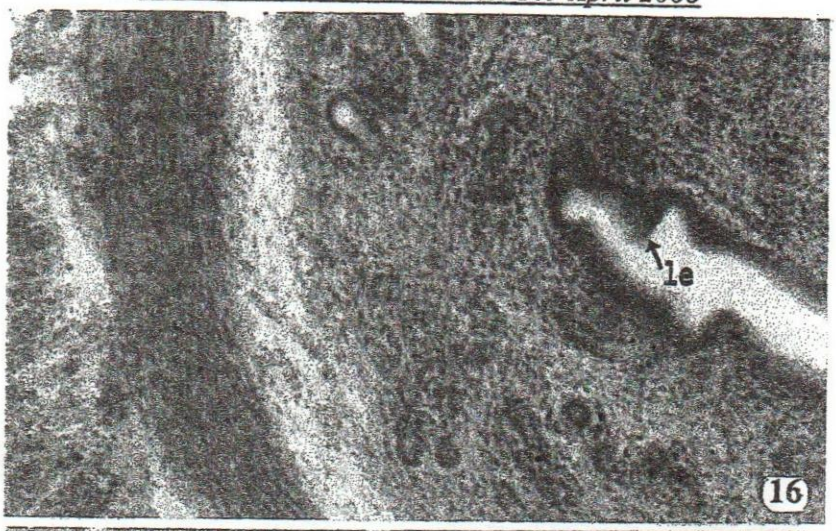

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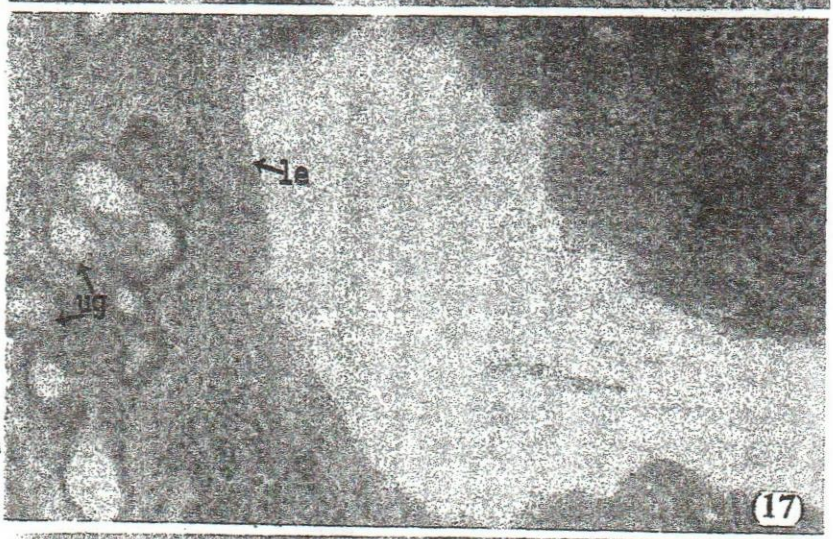

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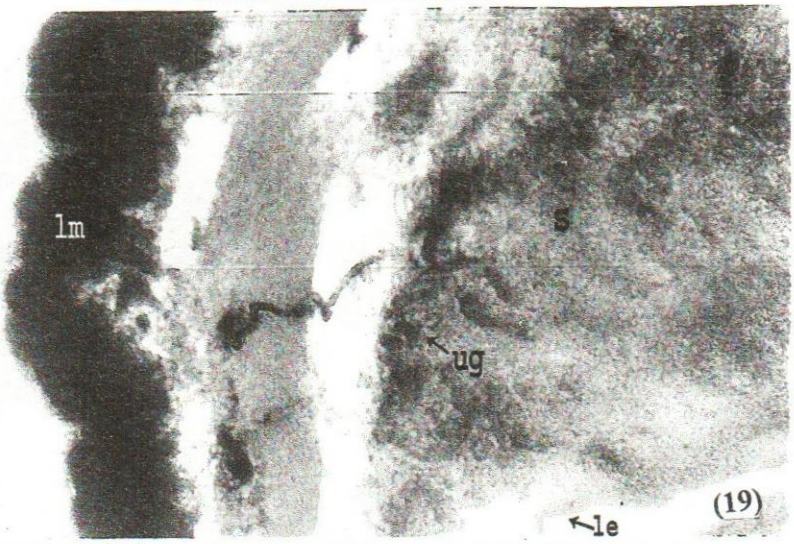
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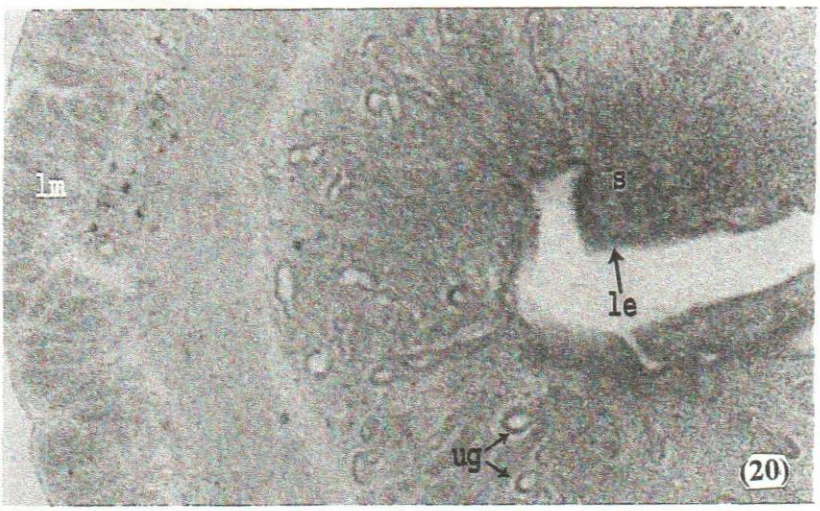
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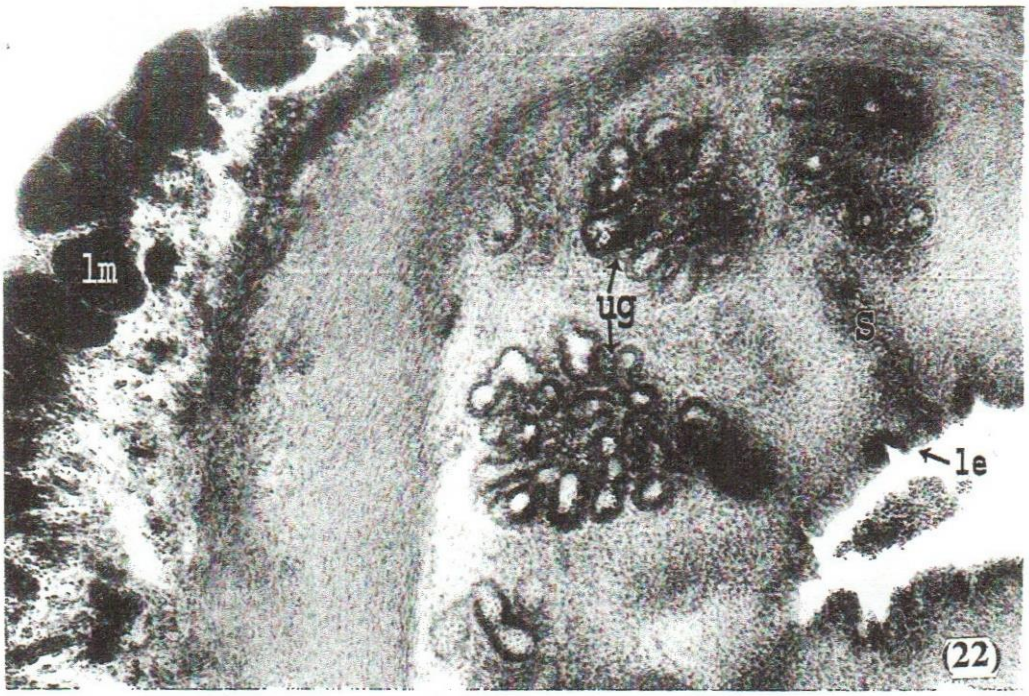
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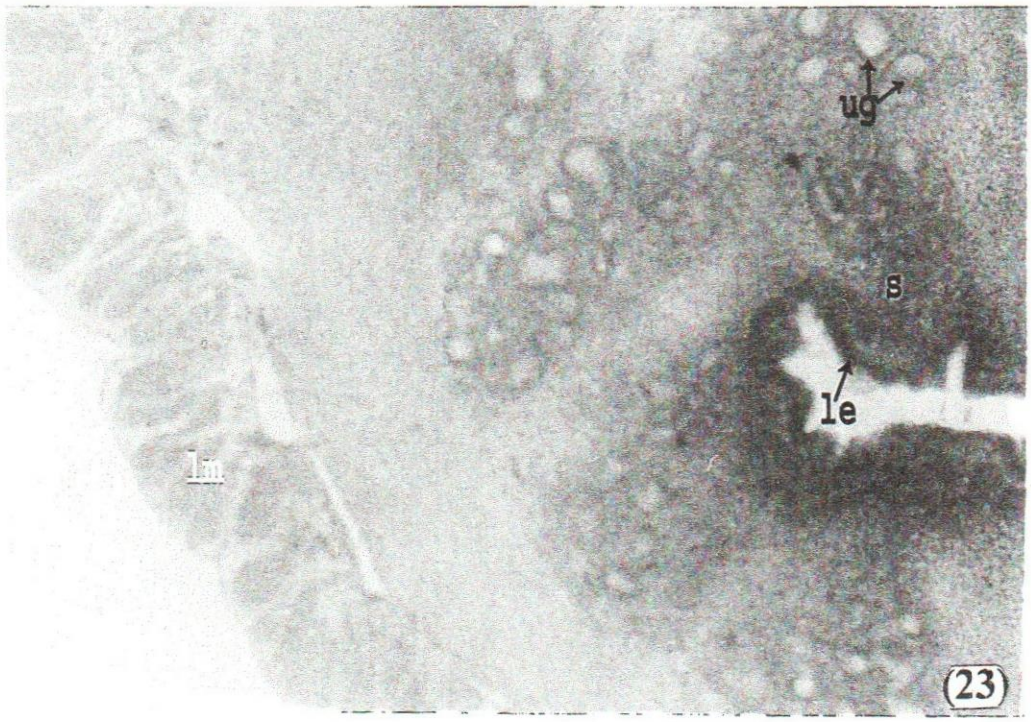
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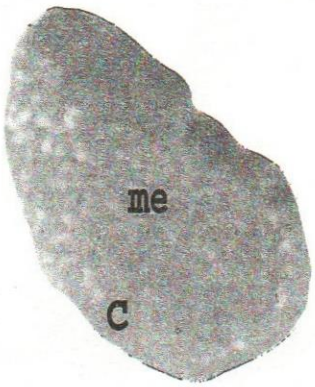

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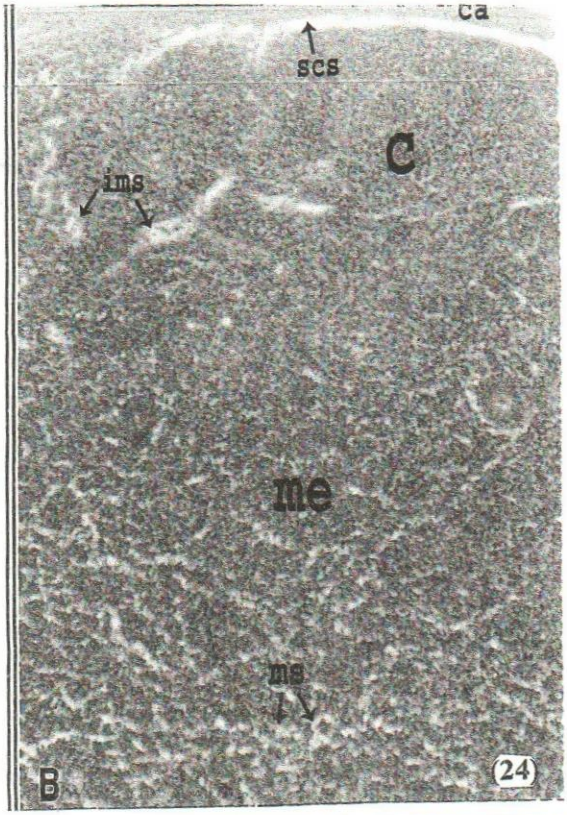

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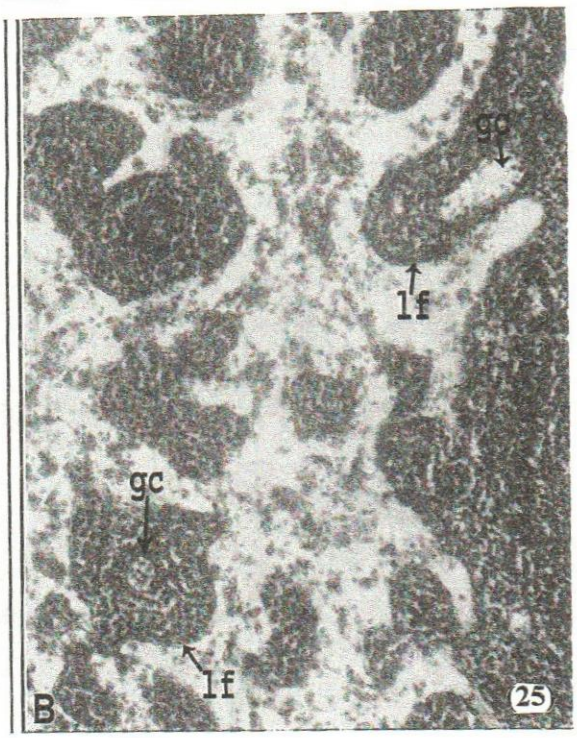

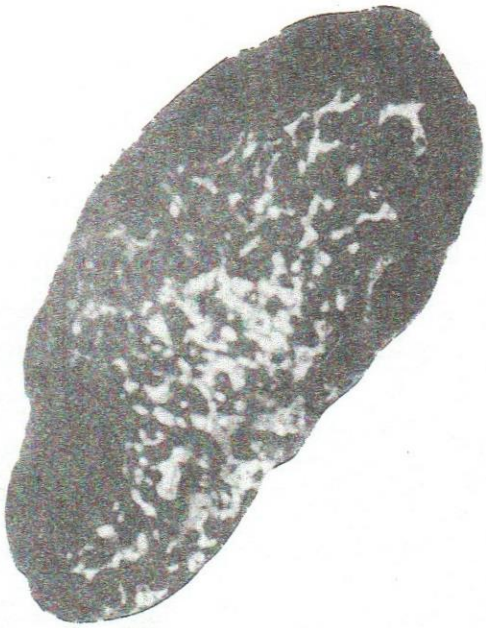

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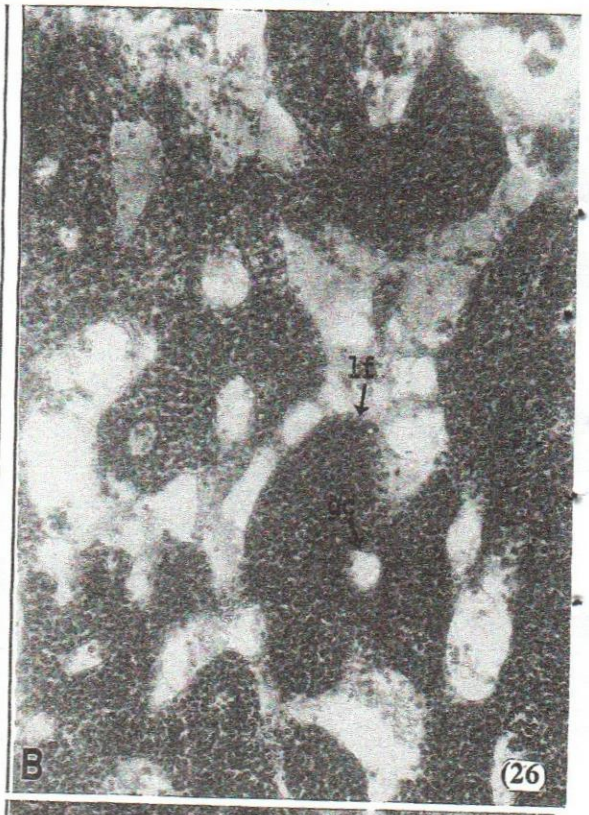

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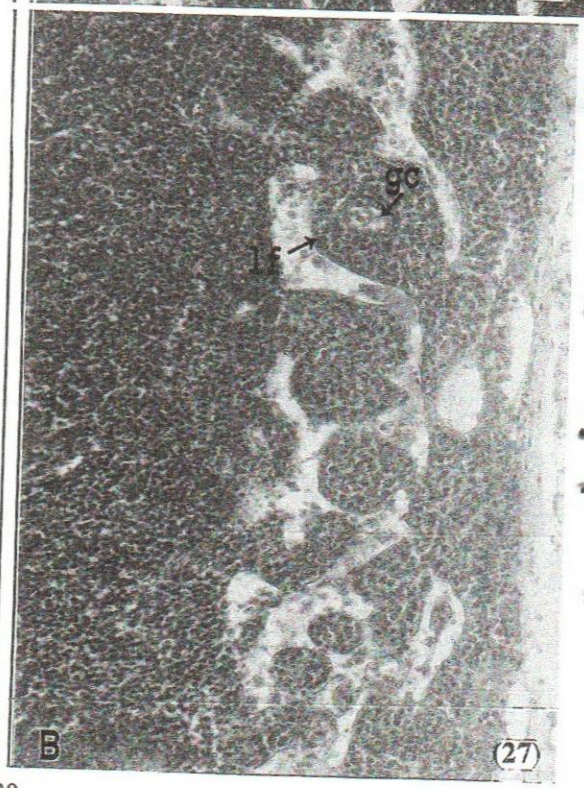

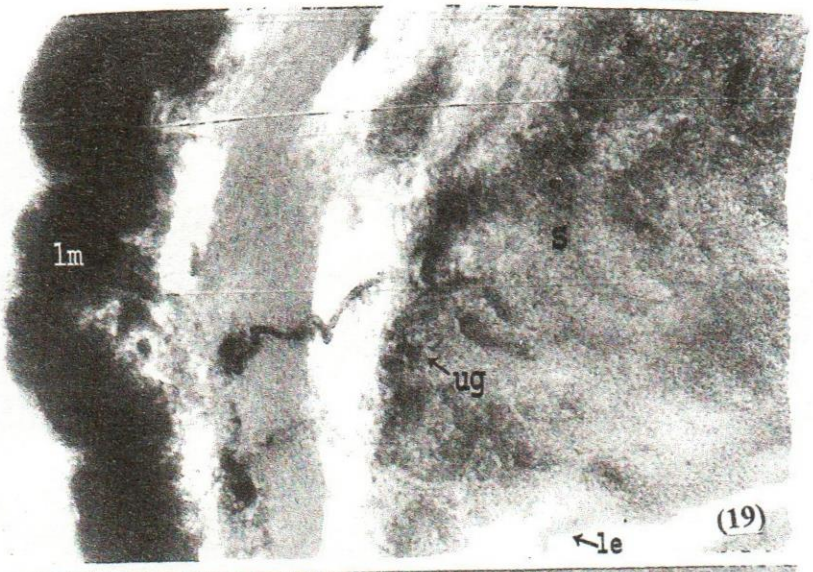
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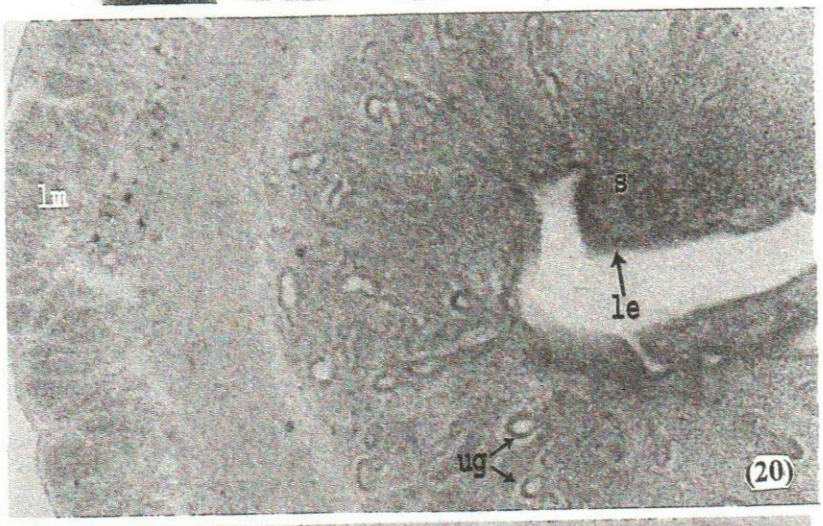
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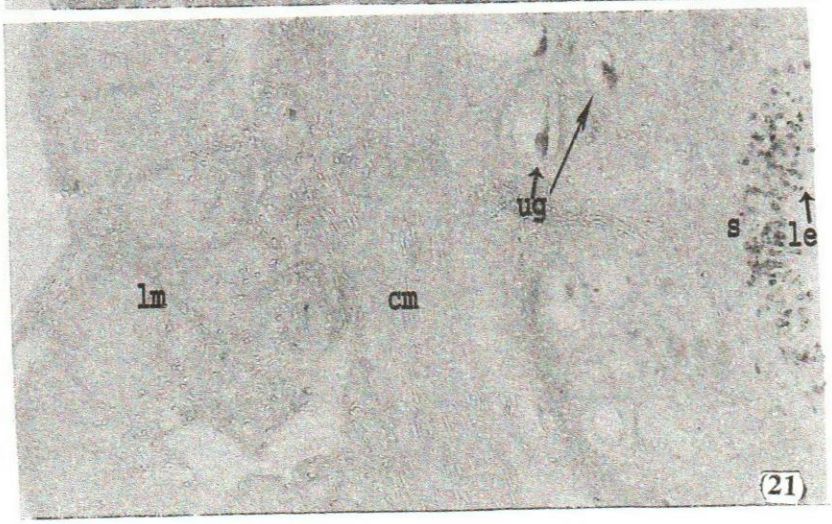
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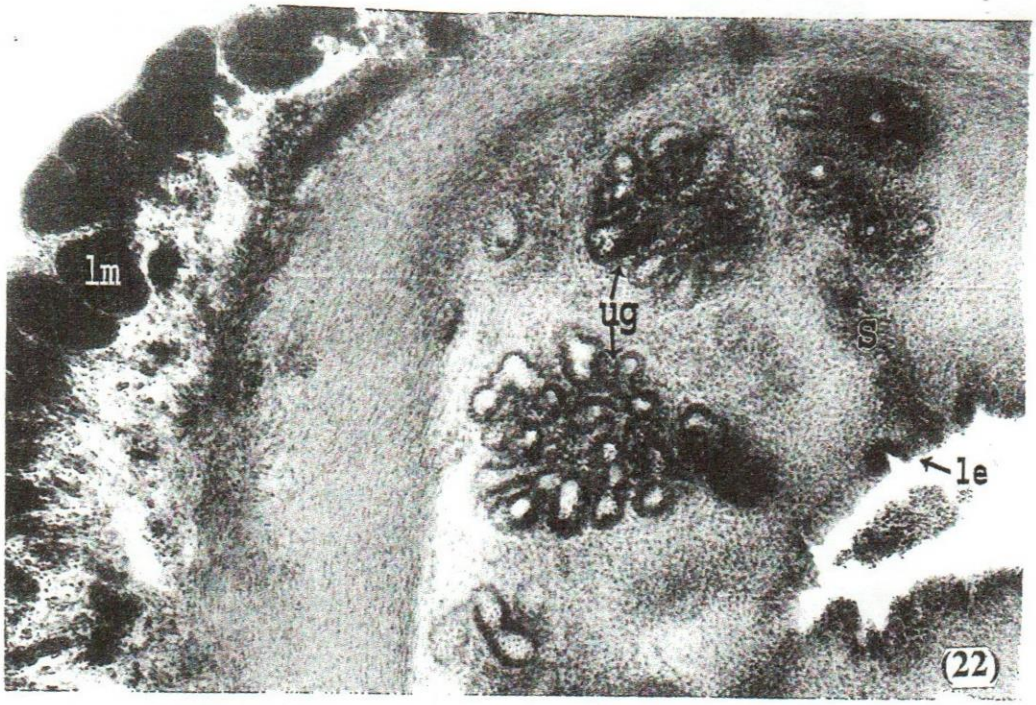
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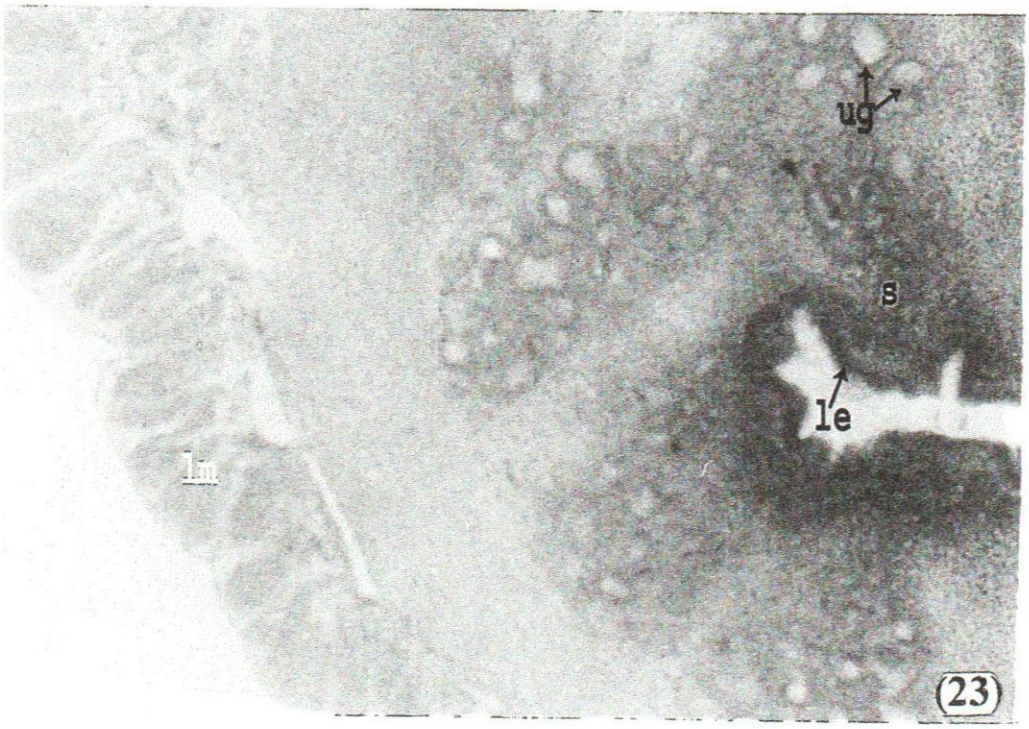
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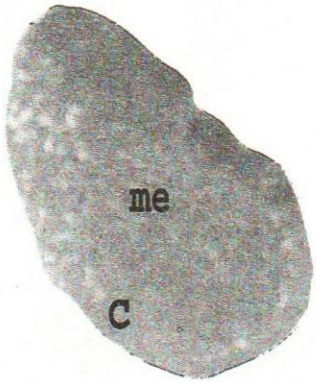

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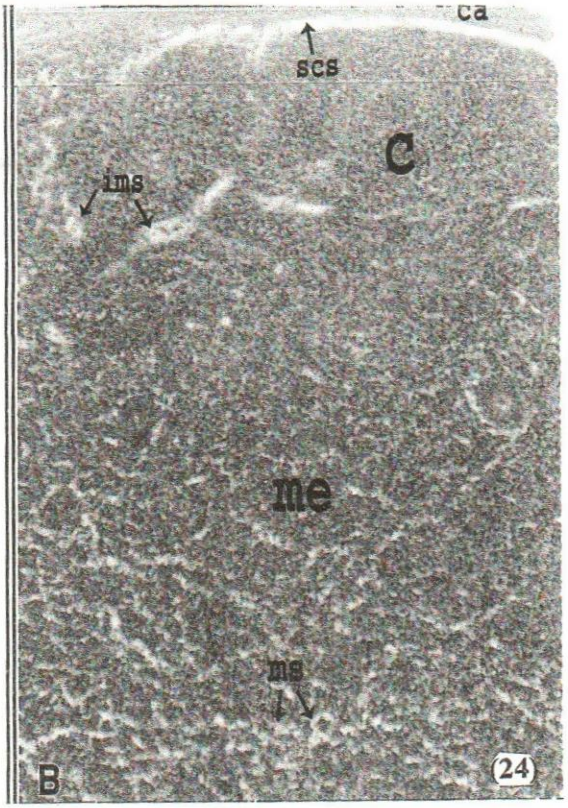

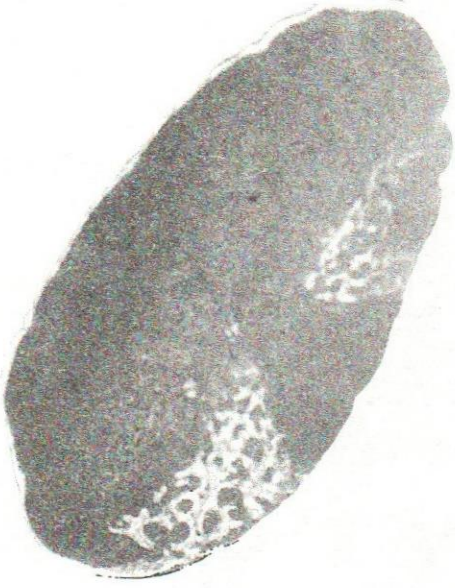

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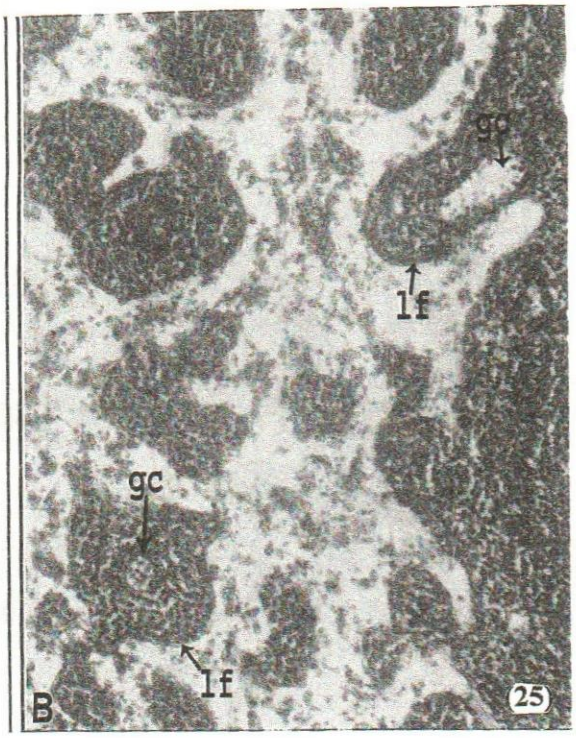

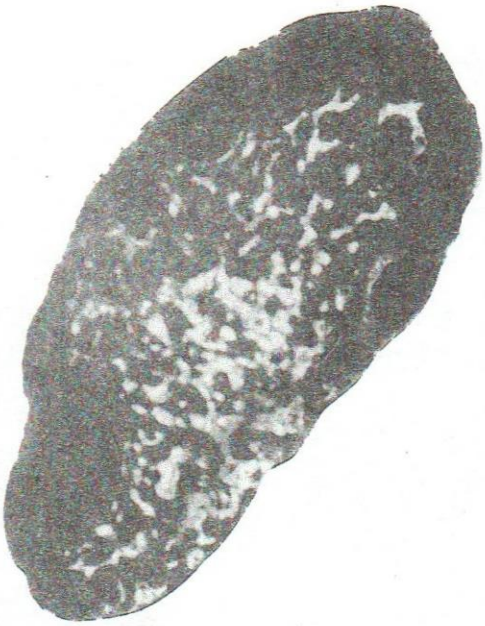

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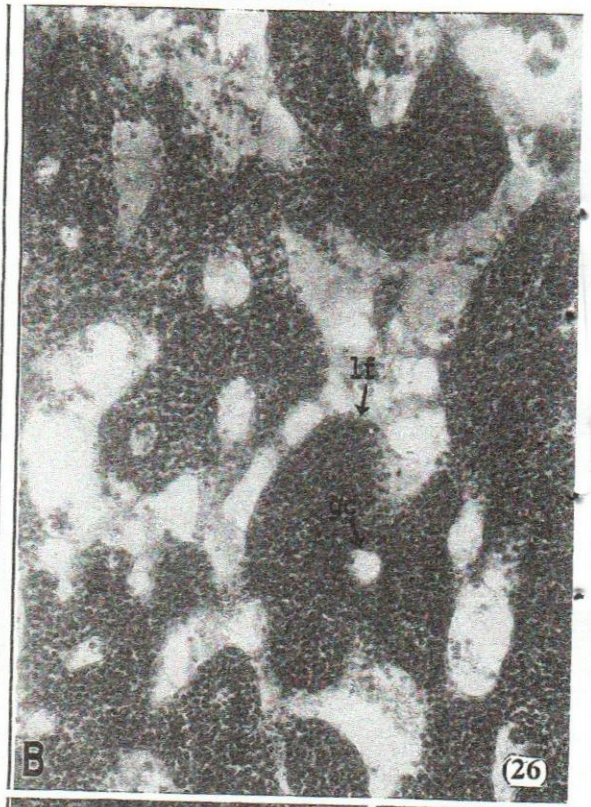

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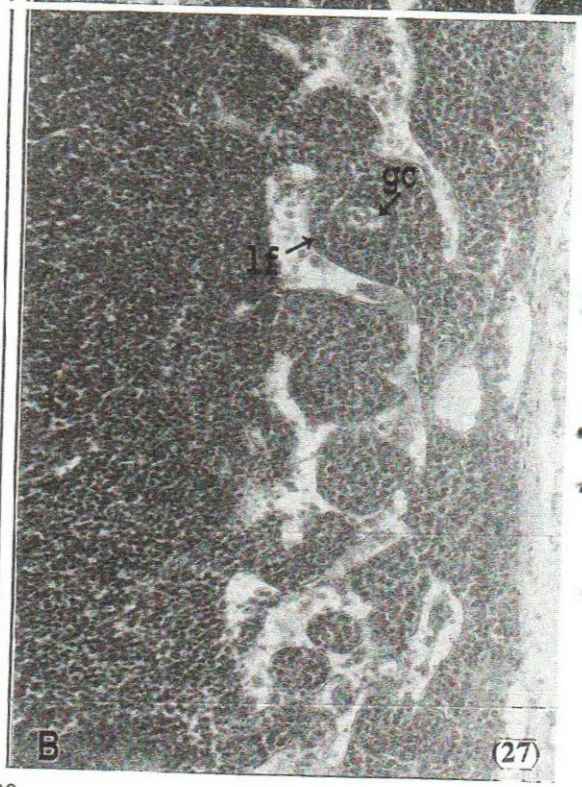

LEGENDS OF FIGURES

Fig. 1: [A] A photomicrograph of a transverse section in the normal mouse uterus at oestrus phase showing; the histological structure of the uterine wall, endometrium (endo), luminal epithelium (le), uterine glands (ug), stroma (s), myometrium (myo), circular muscle (cm), longitudinal muscle (lm), blood vessels, (bv) and perimetrium (p). (H_x & E. X 100). [B] Higher magnification of the box in [A] showing; the stromal cells. Original magnification, X 1000.

Fig. 2: A photomicrograph of a transverse section in the mouse uterus at 4.5 day of pregnancy showing; the blastocyst (b) in close apposition with the luminal epithelium in the antimesometrial end of the uterine lumen (ul), and mesometrium pole (M), and antimesometerial pole (AM). (H_x & E. X 32).

Fig. 3: [A] A magnified part of the previous section showing; the blastocyst (b) is attached to the luminal epithelium (le), the decidual cells (dc) around the blastocyst, and edema (e) in the endometrial stroma. (H_x & E. X 200). [B] Higher magnification of the box in [A] showing; the decidual cells. Original magnification, X 1000.

Fig. 4: A photomicrograph of a transverse section in the mouse uterus at 3.5 day of pregnancy showing; the implantation site (IS) becomes elongated in a mesometrial (M) – antimesometrial (AM) direction, the uterine lumen is closed around the blastocyst (b), the blastocyst occupies almost the entire uterine chamber (uc), and remnant of the uterine lumen (ul). (H_x& E. X 32).

Fig. 5: A magnified part of the previous section showing; tubular elongated blastocyst (b), the luminal epithelium around the blastocyst is completely destroyed, the primary decidual zone (pdz), the secondary decidual zone (sdz), and the dilated blood vessels (bv). (H_x & E. X 100).

Fig. 6: A magnified part of the same section showing; the decidual cells of the primary decidual zone (dc). (H_x & E. X 400).

Fig. 7: A photomicrograph of a transverse section in the normal mouse uterus at oestrus phase showing; a strong PAS-positive plugs in the lumina of the uterine glands (ug), a mild reaction in the basement membrane of the luminal epithelium and the blood capillaries (bc), a negative reaction in the luminal epithelium (le) and the stromal cells (s), and a moderate reaction in the longitudinal muscle (lm) more than the circular muscle (cm) (PAS. X 100).

Fig. 8: A photomicrograph of a transverse section in the normal mouse uterus at oestrus phase showing; a strong reaction of alkaline phosphatase activity in some blood capillaries (bc) and the longitudinal muscle layer (lm), a mild reaction in the luminal epithelium (le) and the uterine glands (ug), and a negative reaction in the stroma (s) (Gomori technique for alkaline phosphatase, X 100).

Fig. 9: A photomicrograph of a transverse section in the normal mouse uterus at oestrus phase showing; a mild reaction of acid phosphatase activity in the luminal epithelium (le), uterine glands (ug), and the longitudinal muscle layer (lm), and a faint reaction in some stromal cells (S) (Gomori technique for acid phosphatase, X 100).

Fig. 10 A&B: Photomicrographs of a transverse section in the mouse uterus at 4.5 day pregnancy showing; a mild PAS-positive reaction in the antimesometrial luminal epithelium (le) adjacent to the blastocyst (b) and the subepithelial decidual cells (dc),

and a moderate reaction in the longitudinal muscle layer (lm) (PAS. [A] X 100, [B] X 200).

Fig. 11 A&B: Photomicrographs of a transverse section in the mouse uterus at 4.5 day of pregnancy showing; a moderate reaction of alkaline phosphatase activity in the middle of the stroma (s) and the uterine gland (ug), and strong reaction in the longitudinal muscle layer (lm) (Gomori technique of alkaline phosphatase, [A] X 100, [B] X 200).

Fig. 12: A photomicrograph of a transverse section in the mouse uterus at 4.5 day of pregnancy showing; a moderate reaction of acid phosphatase activity in the endometrial stroma (s), in the lumina of uterine glands (ug) and in the antimesometrial luminal epithelium (le), and a mild reaction in the longitudinal muscle layer (lm) (Gomori technique for acid phosphatase, X 100).

Fig. 13: A photomicrograph of a transverse section in the mouse uterus at 6.5 day of pregnancy showing; embryo (glycogen wings), a mild reaction in the myometrium (my), and a negative reaction in the antimesometrial (AM) decidua (PAS. X 32).

Fig. 14: A photomicrograph of a transverse section in the mouse uterus at 6.5 day of pregnancy showing; a strong reaction of alkaline phosphatase activity throughout most of the endometrial stroma (S), and a mild reaction in the myometrium (my) (Gomori technique for alkaline phosphatase, X 32).

Fig. 15: A photomicrograph of a transverse section in the mouse uterus at 6.5 day of pregnancy showing; a strong reaction of acid phosphatase activity in the antimesometrial (AM) decidual zone, and a mild reaction in the mesometrial (M) decidual zone and the longitudinal muscle layer (lm) (Gomori technique for acid phosphatase, X 32).

Fig. 16: A photomicrograph of a transverse section in the treated mouse uterus at 4.5 day post-coitus showing; the luminal epithelium (le) is intact, the uterine lumen without blastocyst, small number of decidual cells, and inhibition of the stromal edema (H_x & E. X 200).

Fig. 17: A photomicrograph of a transverse section in the treated mouse uterus with at 6.5 day post-coitus showing; absence of the blastocyst, degeneration of the antimesometrial luminal epithelium (le), cellular infiltration inside the uterine lumen, and presence of the uterine glands (ug) (H_x & E. X 200).

Fig. 18: A photomicrograph of a transverse section in the treated mouse uterus at 4.5 day post-coitus showing; a mild PAS-positive reaction in the basement membrane of the luminal epithelium (le) and the lumina of some uterine glands (ug), and a negative reaction in the endometrial stroma (s) (PAS. X 200).

Fig. 19: A photomicrograph of a transverse section in the treated mouse uterus at 4.5 day post-coitus showing; a mild reaction of alkaline phosphatase activitiy in the endometrial stroma (s) and the uterine glands (ug) and a negative reaction in the luminal epithelium (le) (Gomori technique for alkaline phosphatase. X 100).

Fig. 20: A photomicrograph of a transverse section in the treated mouse uterus at 4.5 day post-coitus showing; a mild reaction of acid phosphatase activity in the luminal epithelium (le), the sub-epithelial stromal cells (s) and the uterine glands (ug) (Gomori technique for acid phosphatase, X100).

Fig. 21: A photomicrograph of a transverse section in the treated mouse uterus at 6.5 day post-coitus showing; a strong PAS-positive reaction in the degenerated luminal epithelium (le) and the sub-epithelial stromal cells (s), and small quantities of the PAS positive materials in the lumina of some uterine glands (ug) (PAS. X 200).

Fig. 22: A photomicrograph of a transverse section in the treated mouse uterus at 6.5 day post-coitus showing; a strong reaction of alkaline phosphatase activitiy in the degenerated luminal epithelium (le) and the uterine glands (ug) and a mild reaction in the stroma (s) (Gomori technique for alkaline phosphatase, X 100).

Fig. 23: A photomicrograph of a transverse section in the treated mouse uterus at 6.5 day post-coitus showing; a moderate reaction of acid phosphatase activity in the degenerated luminal epithelium (le) and sub-epithelial stromal cells(s) and a mild reaction in the uterine glands (ug) (Gomori technique for acid phosphatase, X 100).

Fig. 24 A &B: Photomicrographs of the uterine drainage (pelvic) lymph node at oestrus phase showing; the normal histological structure, capsule (ca), sub-capsular sinus (scs), cortex (C), intermediate sinus (ims), medulla (me), and medullary sinus (ms) (H_x & E. [A] X32, [B] X 200).

Fig. 25 A &B: Photomicrographs of the uterine drainage (pelvic) lymph node at 4.5 day of pregnancy showing; an increased in the size compared to that of the non-pregnant mouse and the secondary lymphatic follicle (lf) with germinal centres (gc) (H_X & E. [A] X32, [B] X 200).

Fig. 26 A &B: Photomicrographs of the uterine drainage (pelvic) lymph node at 6.5 day of pregnancy showing; an increased in the size of the lymph node and its lymphatic follicle (lf) compared to those of the previous stage (H_x & E. [A] X32, [B] X 200).

Fig. 27 A &B: Photomicrographs of the uterine drainage (pelvic) lymph node of the treated mouse at 4.5day post-coitus showing; a decrease in the size of the lymph node and its lymphatic follicle (lf) compared to those at 4.5 day of pregnancy (H_x & E. [A] X32, [B] X 200).

Fig. 28 A &B: Photomicrographs of the uterine drainage (pelvic) lymph node of the treated mouse at 6.5 day post-coitus showing; a decrease in the size of the lymph node and its lymphatic follicle (lf) compared to those at 6.5 day of pregnancy (H_X & E. [A] X32, [B] X 200).

DISCUSSION

In the present work, the morphological organization of the uterine wall of the non-pregnant mouse at oestrus phase is generally in accordance with those reported by previous authors Hafez (1970) *and* Huang *et al*, (1999).

A strong PAS-positive plugs were observed in the lumina of the uterine glands at oestrus phase. This finding is supported by Hafez (1970) who reported that, the oestrus phase is the major secretory period of the uterine glands, and their secretions (glycoproteins) represent the major source of embryo nutrition before implantation. A negative PAS-reaction in the lumina of the uterine glands which was observed on day 4.5 of pregnancy and the study by Hall (1973) confirm this suggestion. Since the negative PAS-reaction may refer to the consumption of these secretions by embryo, however, Smith (1970) reported that, at oestrus phase no PAS-positive plugs were observed in the lumina of the uterine glands. These conflicting results may be due to the study of Smith was done at late period of oestrus phase.

A strong activity of alkaline phosphatase was also observed at oestrus phase in some blood capillaries of the endometrium and myometrium. Similar result was noticed by Lobel *et al* (1965) who

attributed this activity in the wall of blood capillaries to the increase in their permeability, since the cellular infiltration becomes maximal during this phase.

During pregnancy, the mouse uterus undergoes a series of programmed morphological changes which are essential for successful implantation and establishment of pregnancy. From the present work, on day 4.5 of pregnancy, the blastocyst is attached to the luminal epithelium and the stromal cells around the blastocyst are proliferated and differentiated into decidual cells. This structural activity was revealed by Sreenivasulu *et al.* (1993) Das *et al.* (1999), Paria *et al.* (2000), Correia-da-Silva *et al.* (2004) and Vanden-Heuvel *et al. (2005)* Who found that, after the attachment of the blastocyst to the luminal epithelium, the endometrial stromal cells (fibroblasts) increase in size and become polygonal in shape (decidual cells). Christie (1967) suggested that, the function of these cells is energy production via anaerobic and aerobic glycolysis. On the other hand, Ying & Zhao (2000) reported that, the decidual cells produce hormones, growth factors, and matrix components to support implantation and post-implantation embryo.

Also, at this stage the endometrial stroma around the blastocyst shows marked edema. This finding was also observed by Finn & McLaren (1967) Finn & Bredl (1973) and Rockwell *et al* (2002). This edema is believed to create an environmental optimal for the growth and remodeling of the endometrium preparing it for implantation (Rockwell *et al.*, 2002).

The first appearance of alkaline phosphatase activity in the middle of endometrial stroma which was observed in the present study and by Finn & Mclaren (1967) may refer to the beginning of decidualization process. This suggestion is supported by Parathasarthy *et al.*, (1979) who reported that, alkaline phosphatase plays an important role in cellular differenation and influence the transport rate of nutrients across the cell membrane during the blastocyst development.

On day 6.5 of pregnancy, the decidualization of the stroma is intense, forming the primary and secondary decidual zones. This result is in agreement with that observed by Das *et al.* (1999), Tan *et al.* (2002), and Liu *et al.* (2004) who reported that, the stromal cells immediately adjacent to the blastocyst cease to proliferate and form the primary decidual zone, and the cells outside the primary decidual zone continue to proliferate and form the secondary decidual zone. Also, the luminal epithelium around the blastocyst at this stage is completely disappeared.

This result is similar to that observed by Finn & Bredl (1973), and Li *et al.* (2003).

The disappearance of the luminal epithelium around the blastocyst at 6.5 day of pregnancy exhibits the role of acid phosphatase in this process. This suggestion is supported by El-Shershaby & Hinchliffe (1975) and Parathasarathy *et al.* (1979) who reported that, acid phosphatase, which has been shown to increase at 6 day of pregnancy, is associated with breakdown and phagocytosis of the luminal epithelium and the decidualized cells at the site of implantation. Katz (1998) suggested that, the breakdown and phagocytosis of the luminal epithelium and the decidualized cells allow the expansion of the growing embryo. However, it is also possible that, in the region of mature decidual cells, the breakdown of the matrix constituents is a mechanism to provide nutrients for the growing embryo.

At the same time, the PAS-positive material disappears from the antimesometrial decidua, and accumulates in the cells mesometrial and lateral to the embryo to form the glycogen wings. The absence of the PAS-positive material from the anti -mesometrial decidua, may refer to the role of the PAS-positive material as a source of the nutrition for the embryo. This suggestion is supported by Christie (1967) who reported that, the decidual cells contain a glucose -6- phosphatase, which can be associated with glycogen breakdown into glucose.

Also accumulation of the PAS-positive material in the cells mesometrial and lateral to the embryo to form the glycogen wings, indicates that, this is an area of the glycogen storage.

It is clear from the present work that, the oral administration of Diphenhydramine from the first day of pregnancy, causes both pre and post-implantation miscarriages.

The pre-implantation miscarriage (before day 4.5) is characterized by intact luminal epithelium, no blastocyst in uterine lumen, no cellular differentiation towards decidualization, and no stromal edema. These findings were observed by Sreenivasulu *et al.* (1993) Duran-Reyes and Hicks (1997) and Rockwell *et al.* (2002) in mated rats treated with antiestrogen, in mated rats treated with colchicine and in mated mice treated with vascular endothelial growth factor antiserum respectively. On the other hand, the great reduction of the stromal edema by a particular administration of histamine antagonists without damage of the uterus was the only finding observed by Brandon and Wallis (1977).

Histochemical changes which were associated with pre-implantation miscarriage showed the absence of PAS-positive material from stromal cells and inhibition of alkaline and acid phosphatases activities in comparison with the corresponding period of control pregnant mice. These changes confirm the inhibition of endometrial sensitivity by antihistaminic drug and may refer to the relation between them and the decidual cell reaction (Parathasarathy *et al.*, 1979). Another histochemical changes associated with pre-implantation miscarriage showed that,the lumina of some uterine glands contain PAS-positive material, while in pregnant mice, the uterine glands show a negative PAS-reaction. Similar results was obtained by Sreenivasulu *et al.* (1993) in treated rats with antiestrogen (antiimplantation), there is a little variation in glycogen content during the same period. This result may be attributed to non-utilization of this energy substrate.

The post-implantation miscarriage (after day 4.5) is characterized by absence of the blastocyst, degeneration of the antimesometrial luminal epithelium, and cellular infiltration in the uterine lumen. This result is in agreement with that observed by Zhang *et al.* (2000) in the mouse uterus injected by anti-implantation 32/67 KDa lamini binding protein antibody.

The post-implantation miscarriage is confirmed by histochemical studies which show an increase in PAS-positive reaction and the activities of alkaline and acid phosphatase in endometrium in comparison to those associated with pre-implantation miscarriage.

From the present work it may be suggested that, the inhibition of implantation and decidualization by using Diphenhydramine may refer to the role of histamine in both processes. This suggestion is supported by Hicks-Gomes (1990), Barkai andKraicer (1996), Paria *et al* (1998 and 2000) and Wood *et al.* (2000) who reported that, histamine is involved in both processes of implantation and decidualization.

The present work shows that the histological structure of uterine drainage lymph node at oestrus phase of the oestrous cycle is in agreement with that reported by Junqueira *et al.* (1995).

At 4.5 and 6.5 days of pregnancy there are marked enlargement and cellular proliferation with germinal centres of the uterine drainage lymph nodes compared to those of the non-pregnant mouse. This result confirms that obtained by Beer & Billingham (1974) and Mattsson & Mattsson (1984) who reported that, at the time of implantation in rodents there is a marked enlargement and increase in cellularity of the uterine drainage lymph nodes compared to those of virgin animals. This finding

is also, supported by Chambers & Clarke (1979) who reported that, the uterine drainage lymph nodes in pregnant mice weighs more than those in the pseudopregnant and non-pregnant mice. They suggested that, the increase in the uterine drainage lymph nodes weighs may be attributed to an immunological response to embryonic antigens. Also, Hoversland & Beaman (1990), and Robertson *et al.* (2003) attributed this phenomenon to the production of cytokine (T cell suppressor factor) by T-suppressor cells that profoundly suppresses maternal reactivity to paternal antigens.

Reviewing literature as far as can be ascertained; the effect of antihistaminic drug on the uterine drainage lymph nodes, during days of pregnancy described here has not been previously described. In treated mice with post-coital diphenhydramine hydrochloride, there is a marked decrease in the size of the uterine drainage lymph nodes and their lymphatic follicles with absence of germinal center compared to those of the normal pregnant mice after implantation. These results indicate that, there is correlation between histamine and the changes of the uterine drainage lymph nodes. This suggestion is supported by Jin *et al.* (1986) who reported that, the suppressor cells possess histamine receptors that may be involved in suppressor cell activation and cimetidine (an H2 histamine receptor antagonist) may inhibit the functioning of these receptors.

In conclusion, the oral administration of antihistaminic drug (Diphenhydramine) from the first day of pregnancy till the sacrificed day, causes inhibition of implantation. This inhibition could be due to the blockade of histamine receptors in the uterus locally. So, this study warns the women from using this drug during pregnancy.

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