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Dept. of Pathology,
Fac. Vet. Med., Assiut University, Assiut, Egypt.

LIGHT AND ELECTRON MICROSCOPICAL OBSERVATIONS ON MYXOSPORIDIA IN NILE CATFISH, CLARIAS LAZERA

(With 3 Tables and 30 Figures)

By

S.H. AFIFI; NADIA FARID R.*; AZIZA MARWAN** and A.M. HASSANEIN**

*: Dept. of Zoology, Fac. Science, Ain Shams University, Cairo, Egypt.
**: Dept. of Zoology, Fac. Science, Assiut University, Assiut, Egypt.

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ملاحظات بالميكروسكوب الضوئي والألكتروني على إصابات طفيليات الميكسوسوبريديا في أسماك القراميط النيلية

صلاح حسن عفيفي ، نادية فريد ، عزيزة مروان
عبدالفتاح محمود حسانين

تم عمل دراسات ميدانية على مجموعة من الأوالى التي تصيب أسماك القراميط النيلية بمحافظة أسيوط في الفترة من عام ١٩٩٥ حتى ١٩٩٧. أظهرت الدراسة أن نسبة الأصابة في ٢٠٦ سمكة جمعت من نهر النيل كانت (٨٣,١%) حيث تم التعرف أكثر من نوع من الأوالى التي تتبع مجموعة المكسوسوبريديا. كانت أعلى نسبة للأصابة بهذه الأوالى في الخريف والشتاء (٩٣,٦%) ثم إنخفضت تدريجياً إلى ٦٩,٢%) في فصل الصيف. تم أخذ عينات من الأعضاء التي أظهرت وجود حويصلات لهذه الأوالى في العضو التنفسي الثانوي ، الكلية الخلفية ، الكبد ، الطحال ، والمبيض من ثلاث أسماك مصابة وسمكه أخرى غير مصابة وذلك على مدار فصول السنة في الفترة من ١٩٩٥ حتى ١٩٩٧. تم تثبيت هذه العينات في محلول البوان ثم تمريرها وتقطيعها وصبغها بالهيماتوكسلين والأيوسين ، المالورى والألسين والأزرق وفحصها بالميكروسكوب الضوئي. تم وصف التغيرات الباثولوجية المصاحبة لطفيل HENNEGUYA BRANCHIALIS في العضور التنفسي الثانوي ، وطفيل MYXOBOLUS LAZERI في الكلية الخلفية ، وطفيل MYXOSOMA CLARII في الكبد وطفيل MYXOBOLUS CLARII في الطحال والمبيض. أما بالنسبة للميكرسكوب الألكتروني فتم أخذ العضو التنفسي الثانوي والكلية الخلفية والمبيض وتثبيتها في ٥% جلوترا ألدهايد البارد وتمريرها وصبها في مادة الأيبون (٨١٢) وفحصها

بالميكروسكوب الألكتروني النافذ. تم وصف التغيرات الناتجة عن الأوالى الممتطفلة في هـذه الأعضاء وتحليها ومناقشة أهمية هذه التغيرات بالنسبة للعلاقة بين الطفيل والعائل.

SUMMARY

Parasitological survey on myxosporidia protozoal infestation in Nile Catfish, *Clarias lazera* was conducted during the period of 1995-1997. The study showed that 171 fish out of 206 (83.1%) were infested with more than one species of myxosporidia. The highest prevalence of infestation was observed in Autumn (93.6%) and decreased to 69.2% during summer. The secondary Respiratory Organ (SRO), posterior kidneys, livers spleens and ovaries of infested and non-infested fish were fixed in Bouin's fixative, processed and examined by light microscopy. Histopathological alterations induced by *Henneguya branchilis* in the SRO, *Myxobolus lazeri* in the posterior kidneys, *Myxosoma clarii* in the livers, and *Myxobolus clarii* in the spleens and ovaries were described. Infested SRO, posterior kidneys and ovaries were fixed in 5% cold glutraldehyde, dehydrated, embedded in Epon 812 and examined by Joel CXII 100 transmission electron microscopy. The significanse of these results regarding the host-parasite relationship was discussed.

Key words: Myxospridia, Nile Catfish.

INTRODUCTION

Myxosporidian protozoan are parasites affecting both fresh and marine fishes and found to invade many organs. There are two major families based on the classification described by Lom et al. (1983). The first family is Myxosomatidae Poch, 1913 which represented by the genus Myxosoma and characterized by the absence of iodinophilous vacuole. While, the second family is Myxobolida Thelohan, 1982 including three genera namely are Henneguya, theohanellus and Myxobolous and characterized by the presence of iodinophilous vacuole (Lom et al., 1985 and Kudo, 1966).

These protozoa are transmitted from host to host by the intermediate host triactinomyxon, which peneterate into the skin or gills (Markiw, 1989) or ingestion by the fish host (Garden, 1992) or fish to

fish as in some species affecting marine fishes (Diamant, 1997). There are several reports on the occurrence of these protozoa in the gills, livers, bile ducts, kidneys, heart. Moreover, some species are found either inter or interacellularly (Mitchell, 1978; Lom and Dykova, 1992).

The ultrastructure of early stages and sporogony of different genera have been described (Supamattaya et al., 1993; Martell, 1995; Desser et al., 1983).

The purpose of the present study is to describe alterations induced by the genera identified during the period of 1995-1997 in Nile catfish, Clarias lazera collected from the River Nile, Assiut, Egypt using light and electron microscopy.

MATERIALS and METHODS

Fish:

Nile catfish, *Clarias lazera* were collected from the River Nile, Assiut, Egypt. Seven to twelve fish were obtained monthly during the period of 1995-1997 and brought to the laboratory in a plastic bags supplied with fresh water. A total number of 206 fish was examined for the presence of Myxosporidia.

Methods:

Parasitology:

Fish were dissected and examined for the presence or absence of cysts in different organs by naked eye. Positive samples were recorded, spreaded on slides, airdried, fixed with methyl alcohol and stained with Giemsa stain and examined by light microscopy. For scanning electron microscopy (SEM), cysts detected in the SOR of fish collected from the River Nile were fixed in 4% cold glultraldehyde, dehydrated, coated with gold and examined by JOEL/JSM-T220 A, SEM.

Histopathology:

A) Light microscopy:

Samples of the SRO, posterior kidneys, livers, spleens, and ovaries of three infested and one non-infested fish were taken immediately, fixed in Bouin's fixative, dehydrated, embedded in paraffin, sectioned at 4-6μ and stained with Hematoxylin and Eosin, Mallory's triple stain, and Alcian blue (Bancroft and Stevens, 1982) and examined by light microscopy.

B) Transmission electron microscopy (TEM):

The infested SRO, posterior kidneys, and ovaries were selected, fixed in 5% cold glutraldehyde, washed in cacodylate buffer, post-fixed in 1% osmium tetraoxide, dehydrated, embedded in Epon 812 (Gupta, 1983).

Semithin sections were made and stained with toulidine blue and examined by light microscopy. Ultrathin sections were made, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined at 80 KV Joel CXII 100, TEM.

RESULTS

The prevalence of infestation with myxosporidia in fish collected from the River Nile occurred in order in the posterior kidneys, spleens, ovaries, and SOR (Table 1).

Table 1: The prevalence of infestation as percentage (The number of +ve infested samples divided by the total number examined) in different organs of Nile catfish, *Clarias lazera*.

SRO	Kidneys	Livers	Ovaries	Spleen
27 / 206 = 13.1%	106 / 206 = 51.4%	9 / 206 = 4.3%	45 / 206 = 21.8%	62 / 206 = 30.1%

Table (2) showed that the highest prevalence was Myxobolus followed by Myxosoma and then Henneguya.

Table 2: The prevalence of infestation with myxosporidian as percentage (the number of +ve infested samples divided by the total number examined) in Nile Catfish, *Clarias lazera*.

Henneguya	Myxobolus	Myxosoma
27 / 206 = 13.11%	104 / 206 = 50.48%	64 / 206 = 31.06%

The infestation with myxosporidia showed to be higher during Autumn (93.6%) and decreased to 69.2% during Summer (Table 3).

Table 3: The prevalence of infestation with myxosporidia as percentage (the number of +ve samples divided by the total number examined) in Nile Catfish, *Clarias lazera*.

Winter	Spring	Summer	Autumn
52	39	36	44
----- =88.1%	----- = 81.2%	---- = 69.2%	---- = 93.6%
59	59	52	47

Histopathology:

1 - The SRO:

Gross lesions: The infested SRO was swollen and had whitish cysts of an average size of 1-3 mm on its tips and released milky material on cut-section.

Light microscopy:

Fig. 1 showed the spores of *Henneguya branchialis* Ashmawy *et al.*, 1989, identified in the SRO, which had polar capsule and posterior process. These mature spores showed the iodinophilus vacuole (Fig. 2).

Non-infested SRO were consisted of three layers: a) lamellar-like structure lined with one raw of cuboidal epithelium with scattered mucous cells b), connective tissue layer, and c) Chondrocytes embedded in matrix (Fig. 3 a,b,c,d).

Infested SRO showed variable degrees of alterations and divided into three stages. The first stage described as early infestation and was dominated by the presence of sporoplasms of *Henneguya branchialis*. Fig. 4 showed the intracellular localization of sporoplasms embedded in the chondrocytes. The chondrocytes, which showed signs of degeneration expressed by Karyorhexis. In some cases examined pronounced damage occurred and expressed by cleavage of the cartilage. This cleavage resulted in the formation of cleft-like, which was infilterated with RBCs, macrophages, remnants of necrotic chondrocytes and few lymphocytes (Fig. 5 a,b).

The second stage was dominated by polysporic plasmodium inside the cartilage. This polysporic stage was demarkated by a thin layer of connective tissue. The chondrocytes were irregular in shape and lost their matrix homogenity (Fig. 6).

The third stage was dominated by the presence of large plasmodium. Fig. 7 showed disappearance of the lamellar-like layer, area of hemorrhage, and proteinicous acidophilic exudate and mature spores,

leaving a thin layer of degenerated chondrocytes. Semithin sections stained with toulidine blue showed either uninucleated or binucleated developing stages in between the necrotic chondrocytes (Fig. 8).

Electron microscopy:

SEM:

Fig. 9 showed the surface structure of the mature spores of *Henneguya branchialis*. These spores were elongated in shape with two parallel edge and provided with anterior and posterior grooves.

TEM:

Infested SRO showed dissolution of the chromatin of the chondrocytes nuclei and disappearance of their nucleoli. Marked cystic dilatation of the rough endoplasmic reticulum (Fig. 10). Vesiculation of the rough endoplasmic reticulum (RER) into discrete vesicles, which filled with few dense matrical bodies was observed. Wavy longitudnal bundles of electron dense laminated fibrils of collagen were deposited on the RER (Fig. 11).

2 - Posterior kidneys:

Cross lesions:

No visible gross lesions except darkness was associated with heavy infestations.

Light microscopy:

Fig. 12 showed the characterstics of the mature spores of *Myxobolus lazeri* Marwan, 1980. The spores appeared as oval-shaped or elongated, but rarely circular. The spores had polar capsule, coiled polar filaments and rounded posterior iodinophilous vacuole.

Non-infested kidneys showed the normal appearance with no evidence for any spores (Fig. 13). The infested samples had early developing stages of the spores in the epithelium linning the collecting duct (Fig. 14), which resulted in vacuolation of the epithelium. Moreover, these spores were detected in the Melano-macrophage centers (MMC) and appeared yellow in colour by Mallory stain (Fig. 15). Degenerative changes were expressed by granular proteineous dystrophy of the proximal convoluted tubules.

TEM:

The renal epithelium showed vacuolation of the cytoplasm, swollen nuclei, margination of their chromatin and disappearance of the nucleoli (Fig. 16). The mitochondria were disoriented and had disintegrated cristae. Moreover, attenuation and break of the outer mitochondrial wall

was also observed. Formation of whorled membranous bodies, which appeared as osmiophilic laminated membranes was evident (Fig. 17).

3 - The livers:

Gross lesions:

There were no lesions observed in the infested livers.

Light microscopy:

Myxosoma clarii Marwan, 1980 appeared as ovoid to ellipsoid in shape with two rounded end. Absence of iodinophilous vacuole was evident.

The control livers had the normal architecture without any evidence for the presence of spores (Fig. 19). While, infested livers showed aggregation of mature spores surrounded by a thin layer of fibroblastic tissue and attached to the wall of blood vessels (Fig. 20). Moreover, hyperemia of blood vessels and vacuolation of hepatocytes were observed. Fig. 21 showed thicknening of the bileduct wall with periductal accumulation of lymphocytes.

4 - The ovaries:

Gross lesions:

Small white cysts with an average size of 0.25 - 1.5 mm were found embedded in the ovary.

Light microscopy:

Mature spores of *Myxobolus clarii* Mandour et al., 1993 were oval to rounded in shape. Polar filaments, polar capsule and one or two iodino philous vacuoles were observed (Fig. 22).

Non-infested ovary showed the normal appearance without any evidence for the presence of spores (Fig. 23). Infested ovaries showed the spores of *Myxobolus clarii* in the connective tissues supporting the ovarian oocytes. Disquamation of the epithelial lining the oocytes and margination of their nuclei were observed (Fig. 24). Semithin sections of infested ovaries showed the presence of spores in the tunica albuginea (Fig. 25) and between the oocytes (Fig. 26).

TEM:

Infested ovaries showed marked loss of yolk material, condensation of chromatin of the oocytes nuclei at the periphery and pyknosis were observed (Fig. 27).

5 - Spleens:

Gross lesions:

There were no lesions observed grossly.

Light microscopy:

Myxobolus clarii spores were identified (Fig. 22). Non-infested spleens showed the normal appearance of the white follicles, red pulp and haemopoietic tissues, which contain the MMC-with no evidence for the presence of spores (Fig. 28). While, infested spleens showed the presence of refractile spores in the MMC (Fig. 29) and in the blood sinusoids beneath the outer capsule (Fig. 30). Hypocellularity of the splenic follicles were also observed (Fig. 31).

DISCUSSION

Myxosporidian protozoa with its broad classification can live with the host together with minimal damage to each other or severe consequences may result. This host-parasite relationship is mointored by several factors such as prevalence of infestation, temperature, location of parasite, water quality and immune-status of the host (Lom and Dykow, 1992; Masoumian et al., 1996; Diggles and Lester, 1996; Dykova, 1982). The present study showed the highest prevalence of infestation occurred in the posterior kidneys followed by the spleens, ovaries, SRO, and livers. This result suggests that parasitaemia occurred, which was confirmed by the intravascular localization of the spores in the liver. Moreover, the highest prevalence observed in the kidneys and livers in this study was found to be correlated with the severity of atterations in these organs.

Temperature plays an important role in the pathophysiology of fishes. Fore example, the virulence of organisms, the rate of inflammation, and immune response were shown to be affected with temperature fluctations (Robert's, 1989; Diggles and Lester, 1996). In this study, the infestation was higher in Autumen (93.63%) and Winter (88.1%), where the water temperature ranged from 10-25°C. This pattern of infestation was decreased in Summer (69.2%), where the water temperature ranged from 18-40°C. Cold temperature proved to decrease both the immune response and the rate of inflammation (MacMillan, 1985). This fact could explain the highest infestation rate during Winter and Autumn.

The SRO or air-breathing organ is a unique organ in catfish and has the same characterstics like the main respiratory organ of fishes (gills). There are common features of the gills and SRO that both have large blood supply, large surface area, moist surface and similar histological

features. Moreover, the main function of the SRO is to be used when oxygen concentration in water is not sufficient for gill respiration (Grizzle and Rogers, 1976).

The present study showed three degrees of infestation with variable alterations. In the first stage sporoplasms of *Henneguya branchialis* were found infilterated the chondrocytes without connective tissue capsule. Moreover, cleft-like which filled with remnants of necrotic chondrocytes, Rbcs, macrophages and few lymphocytes, were observed. Myxosporidia parasites showed to induce dystrophic changes, necrosis, and circulatory disorders (Lom and Molnar, 1983). Molnar (1982) reported fragmentation of the cartilaginous fins rays with the plasmodium of thelohanellus sp. Moreover, *Henneguya shaharini* was found to cause disintegration of the gill cartilaginous support and detected within or near the cartilage (Shariff, 1982). Afifi, (1992) described irregularity of chondrocytes, loss of matrix homogeneity, and cleft-formation with inflammatory cellular reaction in the cartilaginous support of the gills of Nile catfish invaded with unidentified encysted metacercaria and suggested branchitis occurred. In the present study, the dystrophic chondrocytes may result from the direct action and/or by-products of the accumulated sporoplasms withint he cartilage. The second stage of infested SRO in this study was characterized by the absence of inflammatory cellular reaction, but the alterations of chondrocytes was still observed. Absence of inflammatory response could be due to the presence of connective tissue capsule, which act as barrier preventing the escape of spores and thus no provoked inflammatory response was observed.

In the third stage of infestation, disappearance of the lamellar-like structure and hemorrhage and proteinicous exudate were observed suggested the severity of infestation induced by the large.

Plasmodium cyst. El-Matobuli et al. (1992) reported that the alterations induced by myxosporidian cysts were due to mechanical damage. Moreover, considerable hyperemia of the afferent artery of the gills infested with *Henneguya psorospermica* was described (McCraren et al., 1975). The physiological consequences due to structural alterations induced by myxosporidian would cause impairment of respiration (Lom and Dykoa, 1981).

In the present study, scanning electron microscopy confirmed the identification of *Henneguya branchialis*, Ashmawy 1989. (Ashmawy et al., 1989; Abdel Ghaffar et al., 1995).

TEM observation of the infested SOR indicated irreversible injury had occurred (Ghadially, 1975). While, the vesiculation of RER of the chondrocytes with its dense matrical bodies observed in this study may suggest calcification in the degenerated chondrocytes started to occur.

The chondrocytes, fibroblasts, and synovial cells are sites for production nad storage of collagen and chondromuco protein (Bloom and Fawcett, 1975; Ghadially, 1975). Bloom and Fawcett (1975) stated that the site where cartilage matrix undergo calcification arise from buds of chondrocytes and appeared as small membrane limited structure known as matrix vesicles.

In this study, the degenerative changes in the posterior kidneys expressed by granular proteineous dystrophy, and vaculation of epithelium lining the collecting duct were similar to the previous reports in other fish species infested with myxosporidea (El-Matbouli et al., 1992; Wlasow et al., 1995).

The presence of refractile spores in the MMC of the posterior kidneys suggested that the immune cascades had started to occur. The MMC are widely distributed in the hemopoietic tissues of spleen and kidneys as spherical aggregates of pigment containing cells (Roberts, 1989). The role of these centers in the uptake of antigen and its digestion has been confirmed i.e. Aeromans hydrophila; Myxosporidia (Roberst's 1989; Walsow et al., 1995). MMC_S have been reported to increase in number, pigmentation and encapsulation in the hematopoietic renal tissue (Dykova, 1982).

TEM in this study indicated vaculated appearance fo the infested renal epithelium, swollen nuclei margination of their chromatin and loss of their nucleoli. These results are indices for irreversible cell injury (Ghadially, 1975). While, the mitochondrial changes and the break of its outer layer were considered pathological alteration due to the protozoan rather than an adaptive response or fixation artifacts. This suggestion is supported by the presence of whorld membranous bodies observed in this study. Ghadially (1975) stated that whorled - membranous bodies is a finding indicates that degeneration and elimination of mitochondria occurred.

Myxosoma clarii Marwan, 1980 spores were detected and observed as intravascular aggregation within the blood vessels of liver. This result suggested that parasitaemia had occurred. While, thickening of the bile duct wall with periductal accumulation of lymphocytes suggested that pericholangitis had occurred. Similar results were reported by Lom and Dykova (1981, 1984).

The ovarian changes observed in this study expressed by damage of the theca layers and dissolution of yolk material may suggest altered spwaning function had occurred. Similar observations were reported by Lom and Dykova, 1992). While, the TEM observations confirmed that degeneration and necrosis of oocytes had occurred as a result of the invading protozoan.

The splenic changes observed in this study confirmed previous suggestions for the activation of MMC and its role in antigen trapping. While, the exhausion of lymphocytes from the splenic follicles suggested an altered immune response. Parasitemia also was supported by the presence of spores in the blood sinusoids.

FIGURES LEGENDS

Fig. 1: Mature spores of *Hennguya branchilis* of the infested SRO showing polar capsule (PC), Posterior process (PP), and iodinophilus vacuole (V). Giemsa stain. X 320.

Fig. 2: Mature spores of *Hennguya branchilis* of the infested SRO treated with iodine and showed positive iodinophilus vacuole (V), which appeared brown in colour. Lugol's iodine. X 320.

Fig. 3: Non-infested SRO: A) lamellar-like layer (L) followed by connective tissue layer (C) and chondrocytes (d) embedded in bluish matrix. H. & E. X 32. B) Higher magnification of a showed the lamallae lined by one raw of cuboidal epithelium (e) and few mucous cells (m). H. & E. X 128. C) Chondro cytes (d) embedded in bluish matrix. H. & E. X 128. D) Mucous cells (m) in between the lamallae. Alcian-blue X 128.

Fig. 4: Infested SRO showing the intra and intercellular localization of sporoplasm, (arrows). mallory's stain. X 128.

Fig. 5: Infested SRO (the early stage). a) Cleft-like formation (F). H. & E. X 12.8. b) higher magnification of a) showing infilterated Rbcs (r) macrophage (mc) within the cleft. H. & E. X 128.

Fig. 6: Infested SRO showing the polysporic plasmodium (second stage) embedded in the middle of cartilage (p) and surrounded by a thin layer of connective tissue (c). H. & E. X 32.

Fig. 7: Infested SRO (third stage) showing mature spores (m), proteinicous acidophilic exudate (PX) and degenerated chondrocytes (d). H. & E. X 32.

Fig. 8: Semitin section showing either uninucleated or binucleated developing stages (arrows) in between the degenerated chondrocytes. Toluidine blue. X 320.

Fig. 9: The surface structure of *Henneguya branchilis* showing anterior groove (AV), posterior groove (PV) and suture (s). SEM preparation. X 7.500.

Fig. 10: Infested SRO showing dissolution of the chromatin of chondrocytes nuclei, disappearance of the nucleoli, cystic dilatation of RER (d) and abundant interlacing homogenous matrix separating two chondrocytes (hg). uranyl acetate and lead citrate. X 5400.

Fig. 11: Infested SRO showing marked vesiculation of the RER (V) and wavy laminated electron dense fibrils of collagen were deposited on the RER (cg). uranyl acetate and lead citrate. X 5400.

Fig. 12: Mature spores of *Myxobolus lazeri* appeared as oval-shaped or elongated and provided with two polar capsules and polar filaments. Giemsa stain. X 320.

Fig. 13: Non-infested posterior kidneys showing the normal appearance with no evidence of for spors. Mallory's stain. X 320.

Fig. 14: Infested posterior kidney had developing stages (sporoplasms) in the epithelium of collecting duct (arrows). X 320.

Fig. 15: Infested posterior kidney had yellow spores in the MMC. Mallory's stain. X 320.

Fig. 16: Infested renal epithelium showing vacuolated cytoplasm (VC), and several whorled membranous bodies (WM). uranyl acetate and lead nitrate. X 8,000.

Fig. 17: Higher magnification of Fig. 16 showing disintegrated mitochondrial cristae, break in its outer wall (arrows), and whorled-membranous bodies (wm). uranyl acetate and lead nitrate. X 40,000.

Fig. 18: Non-infested liver had the normal architexture with no evidence of spores in the blood vessels. H. & E. X 80.

Fig. 19: Infested liver showing the intravascular localization of spores (SP) H. & E. X 320.

Fig. 20: Infested liver showing periductal lymphocytic accumulation (L). H. & E. X 80.

Fig. 21: Mature spores of *Myxobolus clarii* appeared as oval to rounded in shape, two polar capsule, polar filaments and iodinophilous vacuole. Giemsa stain. X 320.

Fig. 22: Non-infested ovaries showing the normal apearance with no evidence for the presence of spores. H. & E. X 32.

Fig. 23: Infested ovaries showing refractile spores infilterating the connective tissue between the oocytes (arrows). H. & E. X 320.

Fig. 24: Semithin section of infested ovaries showing the presence of *Myxobolus clarii* spores in the tunica albuginea. Toluidine blue. X 320.

Fig. 25: Semithin section of infested ovaries showing the presence of *Myxobolus clarii* spores between the oocytes. Toluidine blue. X 320.

Fig. 26: Infested ovaries showing marked loss of yolk material (Y) and pyknotic eccentric nuclei (arrow). uranyl acetate and lead citrate. X 5400.

Fig. 27: Non-infested spleen showing the normal architecture. H.& E. X 32.

Fig. 28: Infested spleen showing the presence of *Myxobolus clarii* in the MMC. Mallory stain. X 320.

Fig. 29: Infested spleen showing also the presencr of spores int he blood sinusoids benath the outer capsule (arrow). H. & E. X 320.

Fig. 30: Infested spleen showing hypocellularity of the follicles (f). H. & E. X 32.

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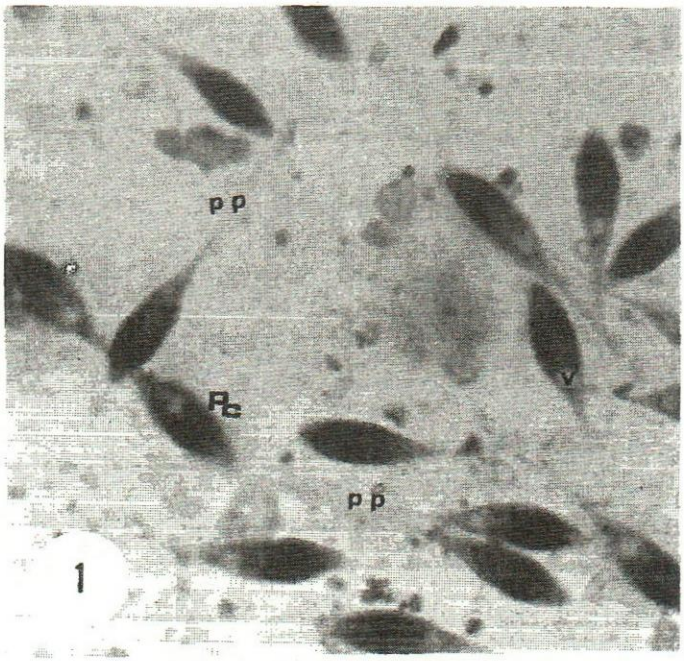

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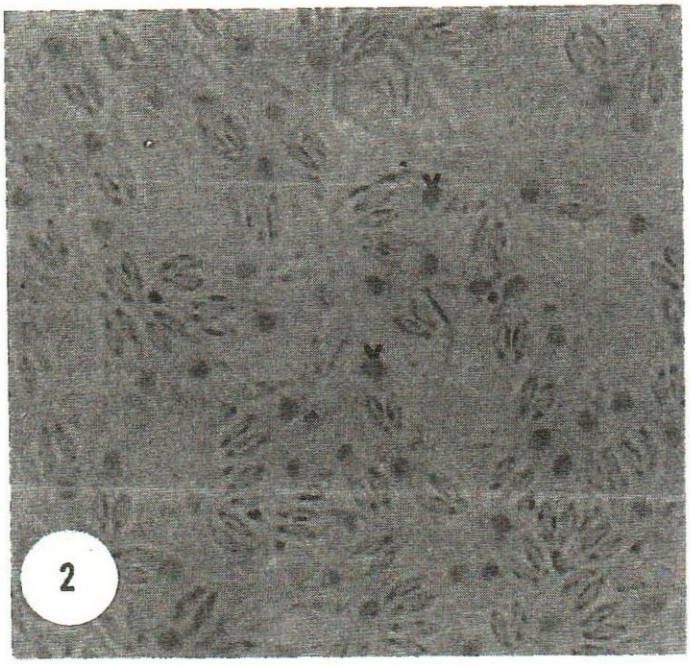

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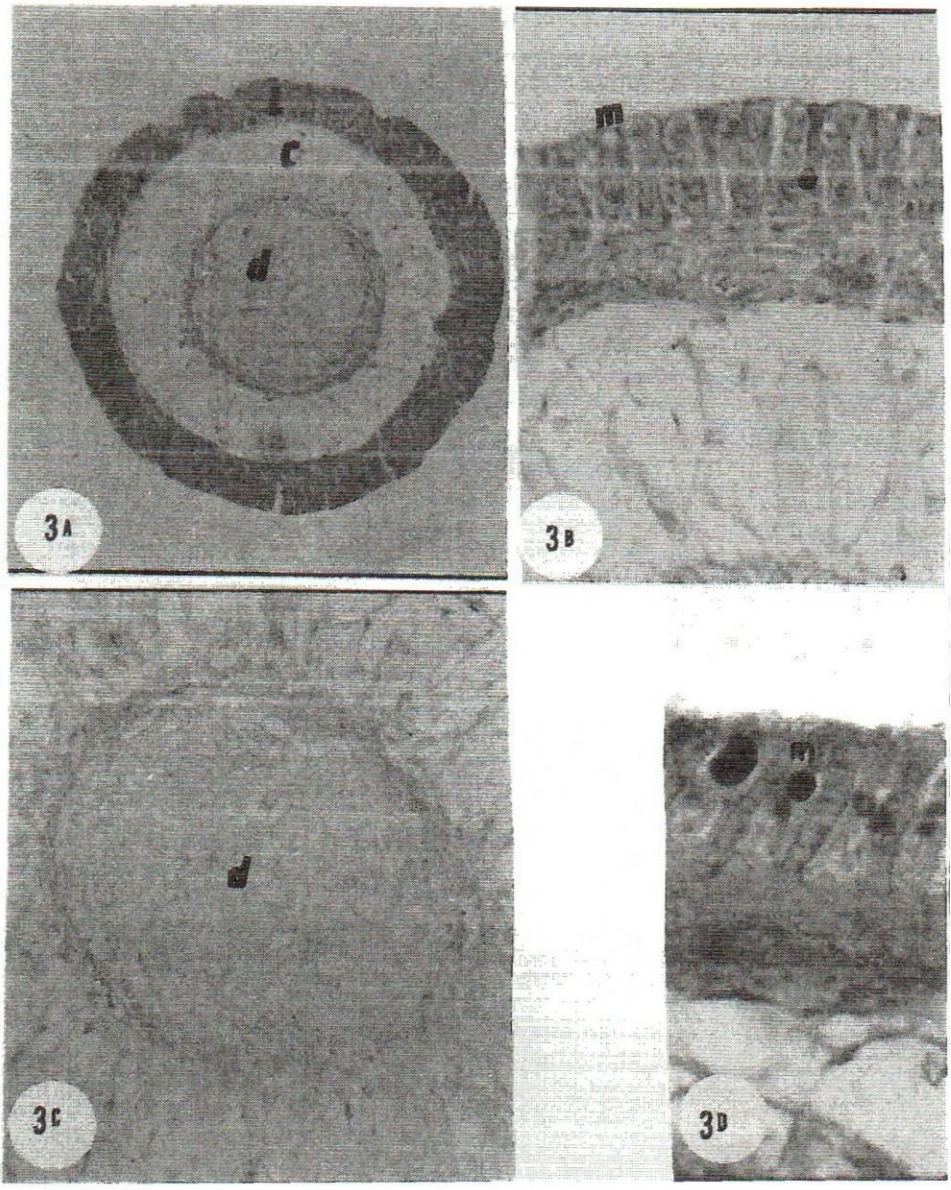
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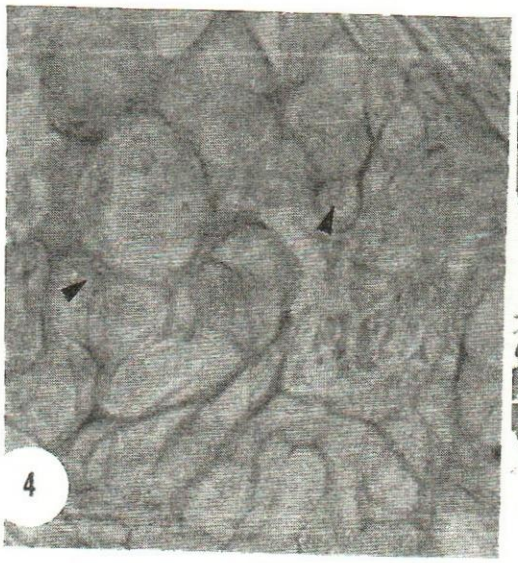
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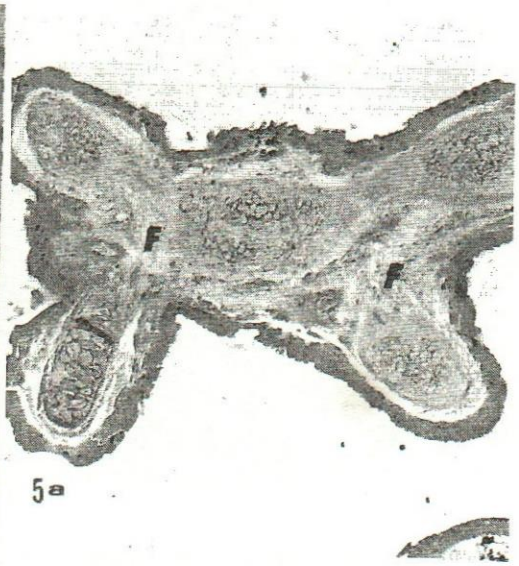
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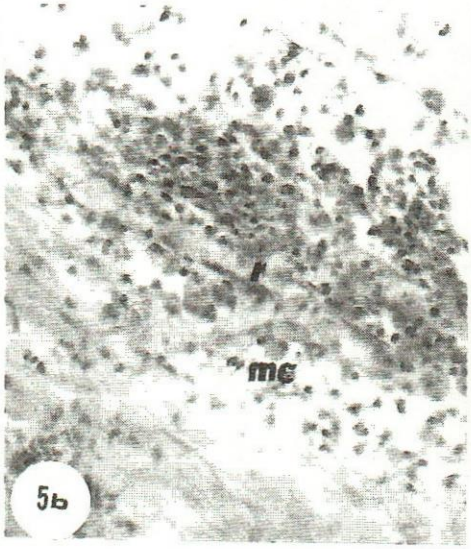
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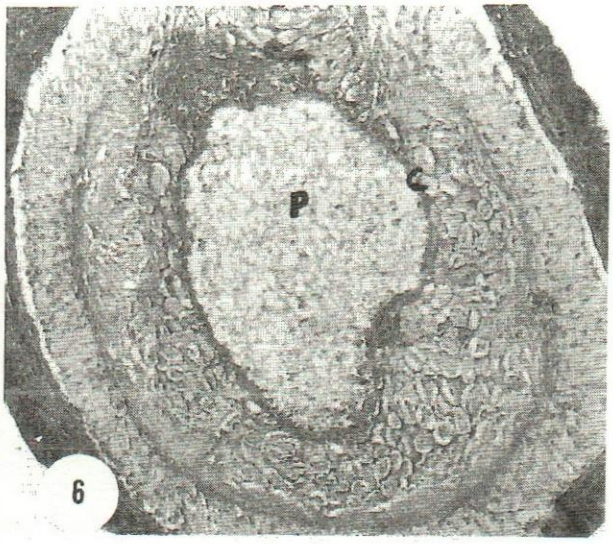
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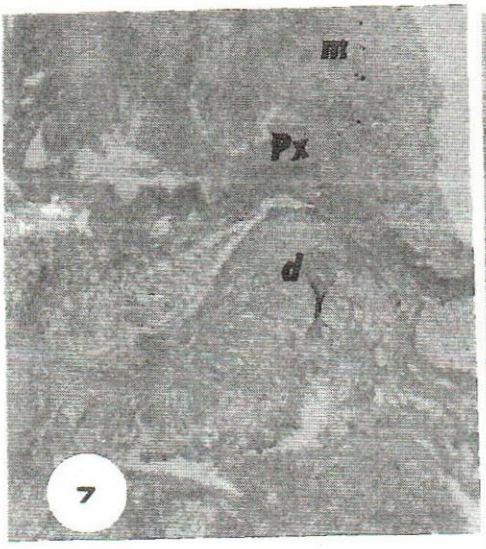
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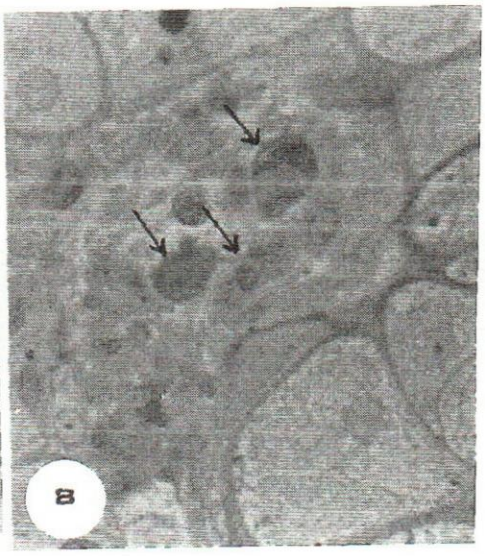
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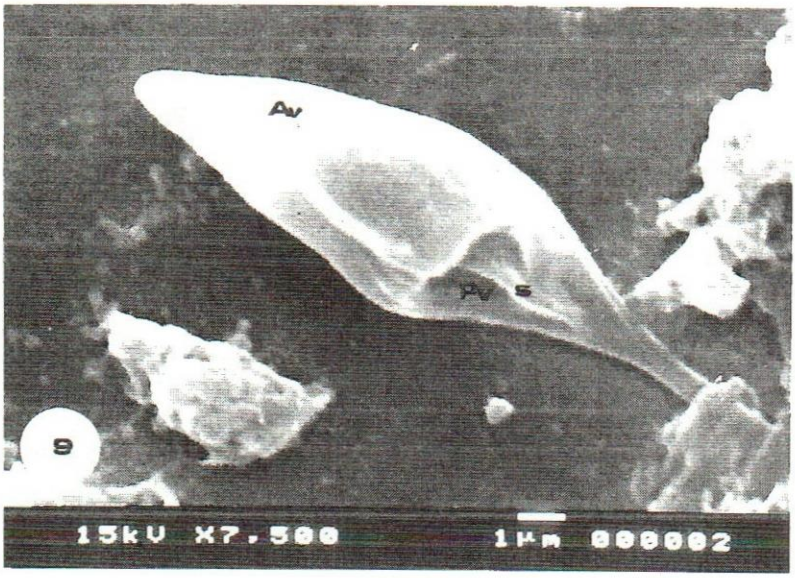
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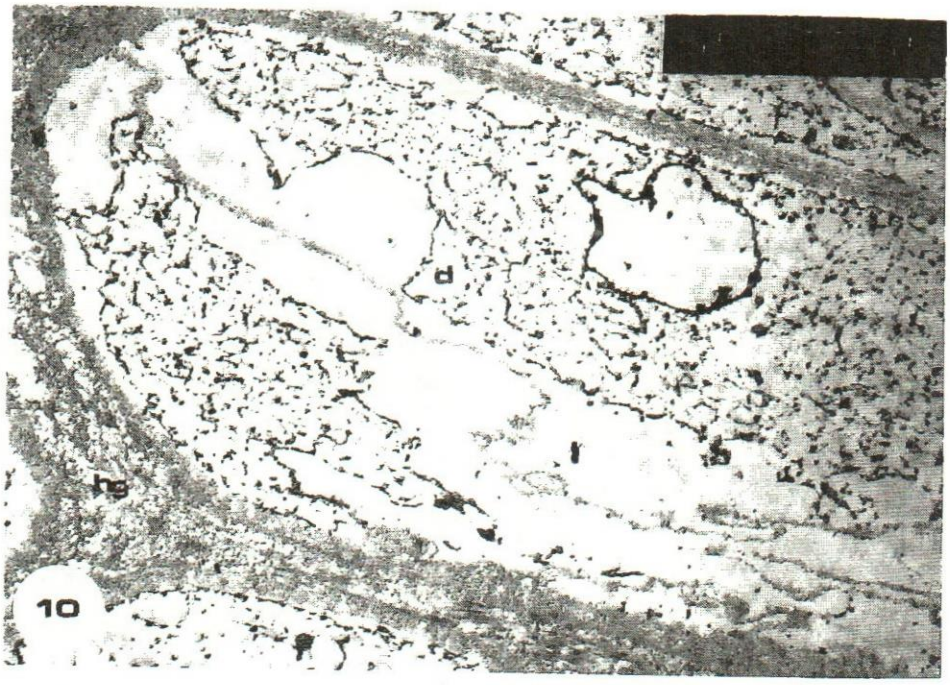
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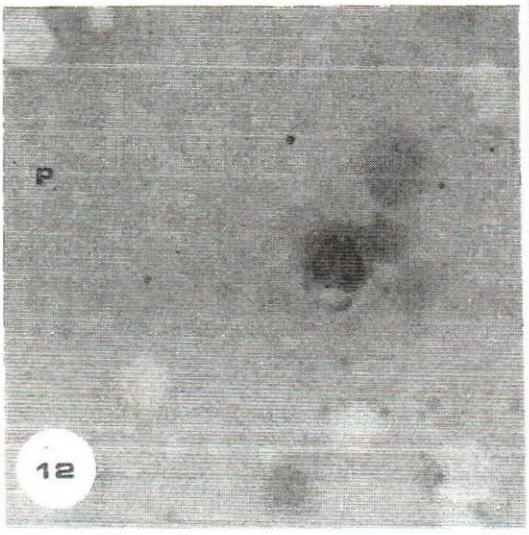
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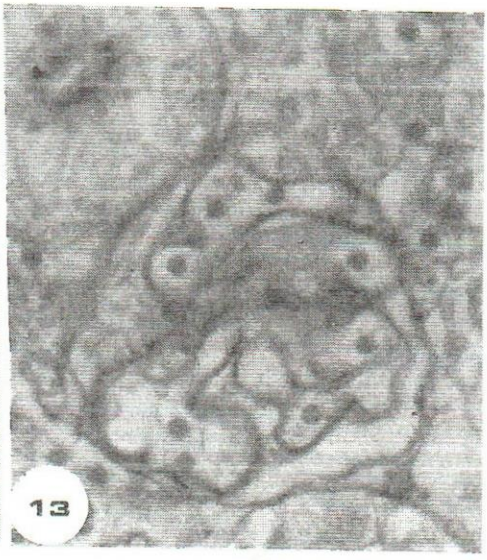
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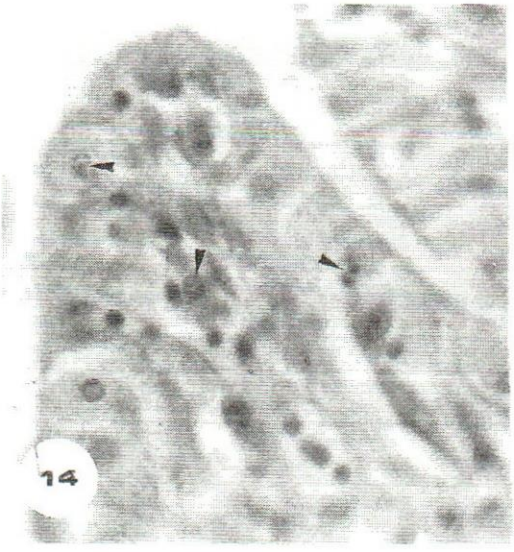
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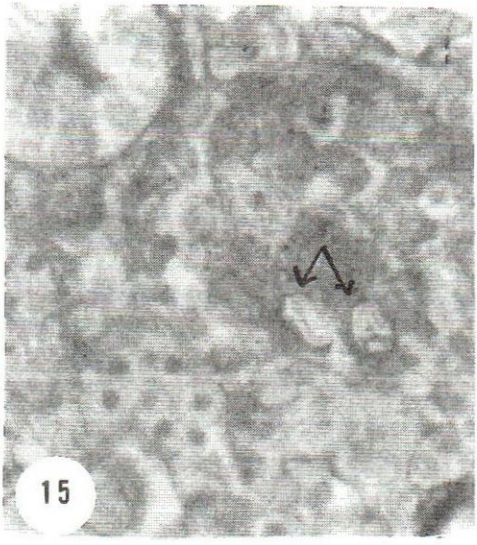
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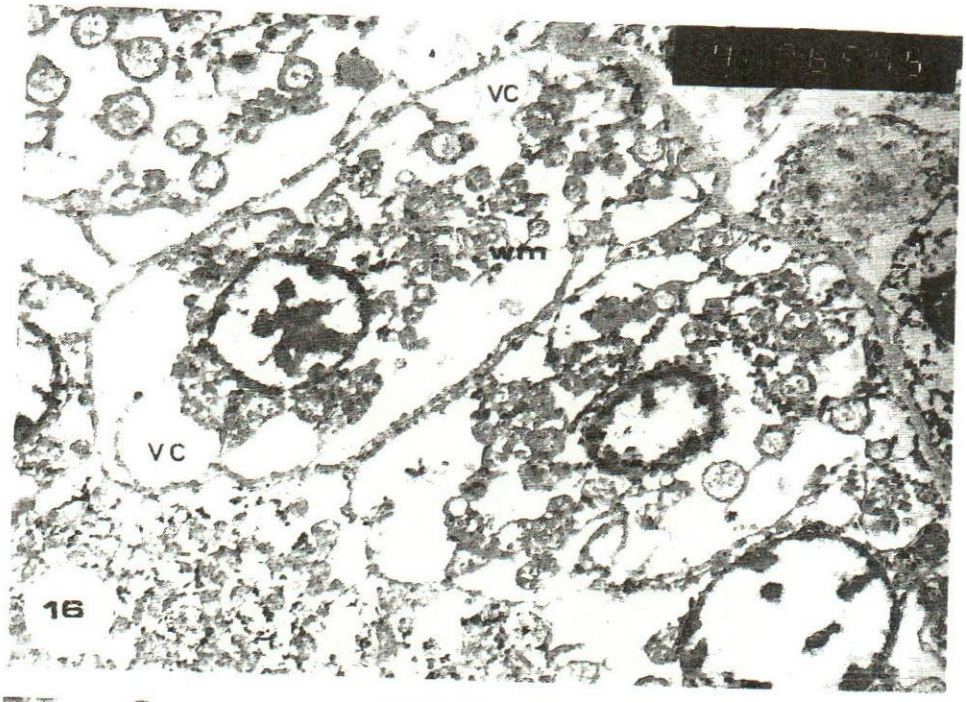
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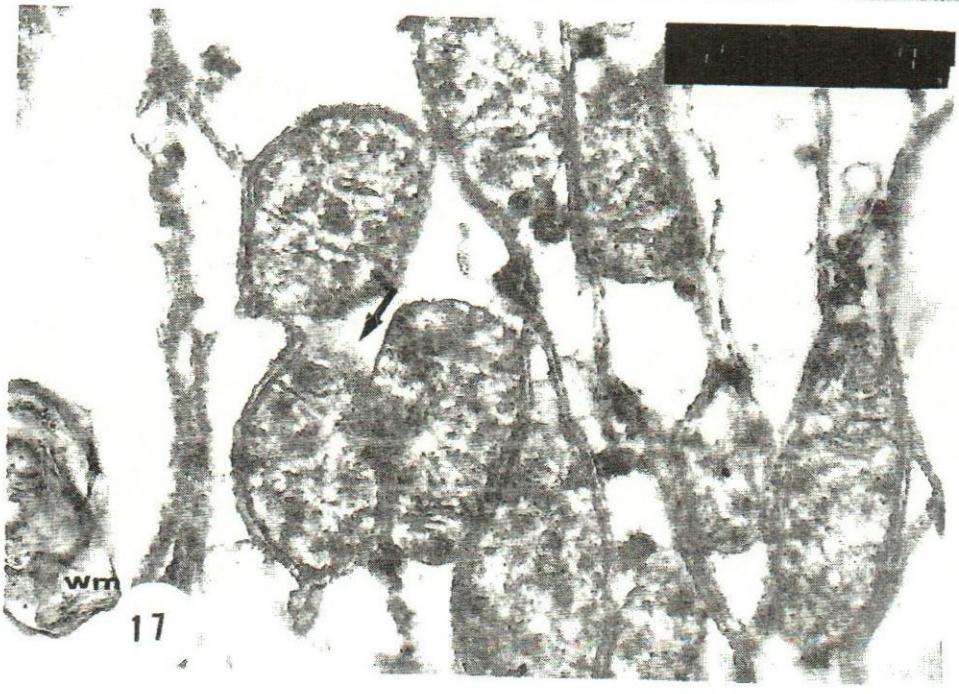
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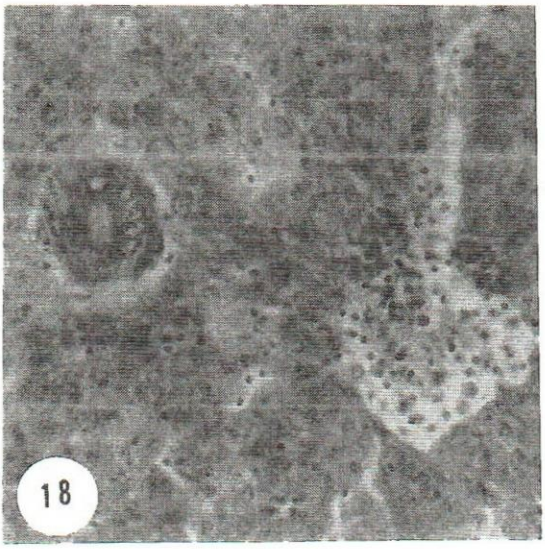
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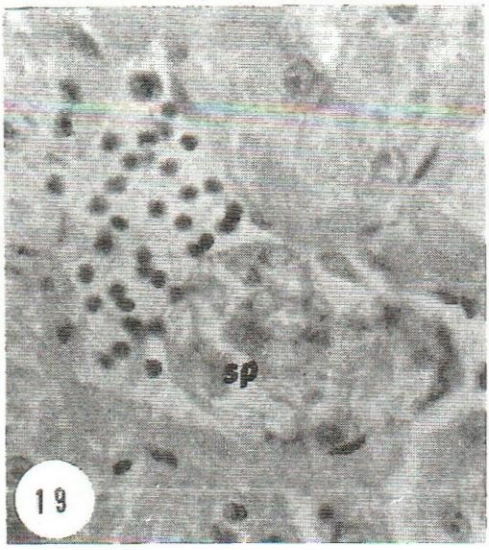
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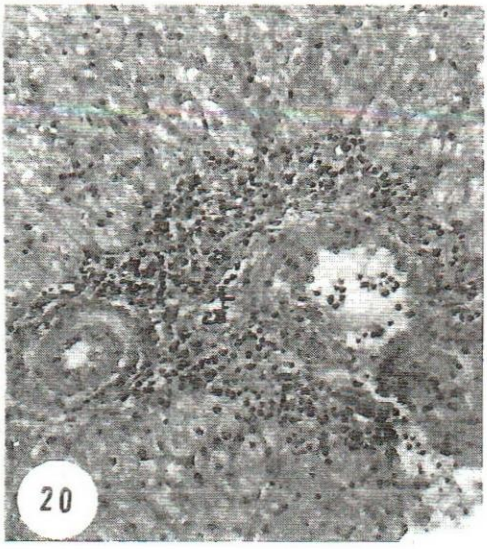
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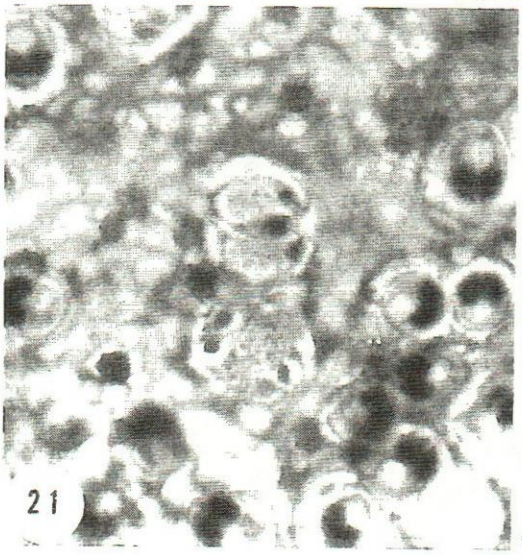
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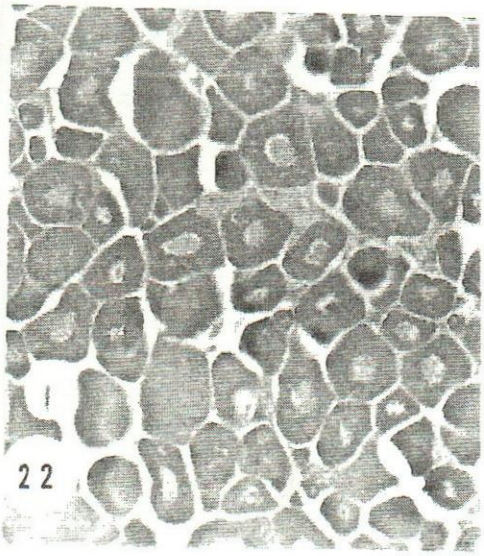
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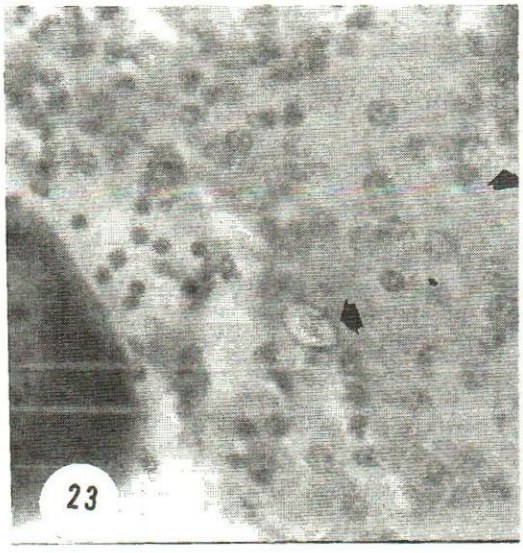
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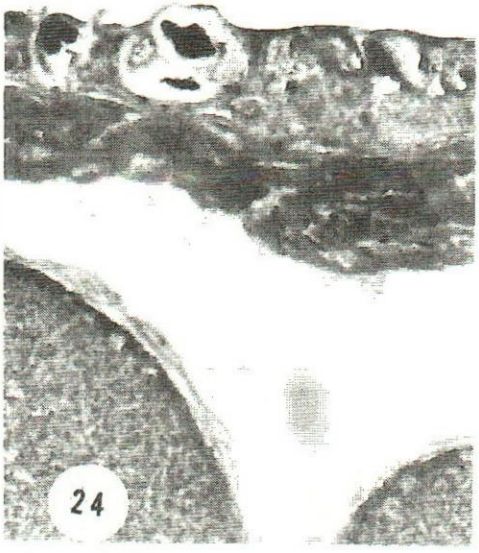
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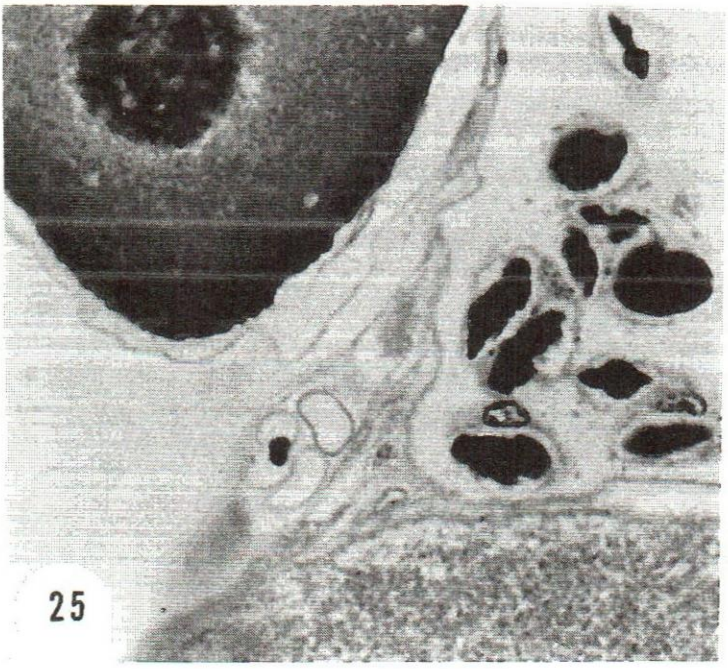

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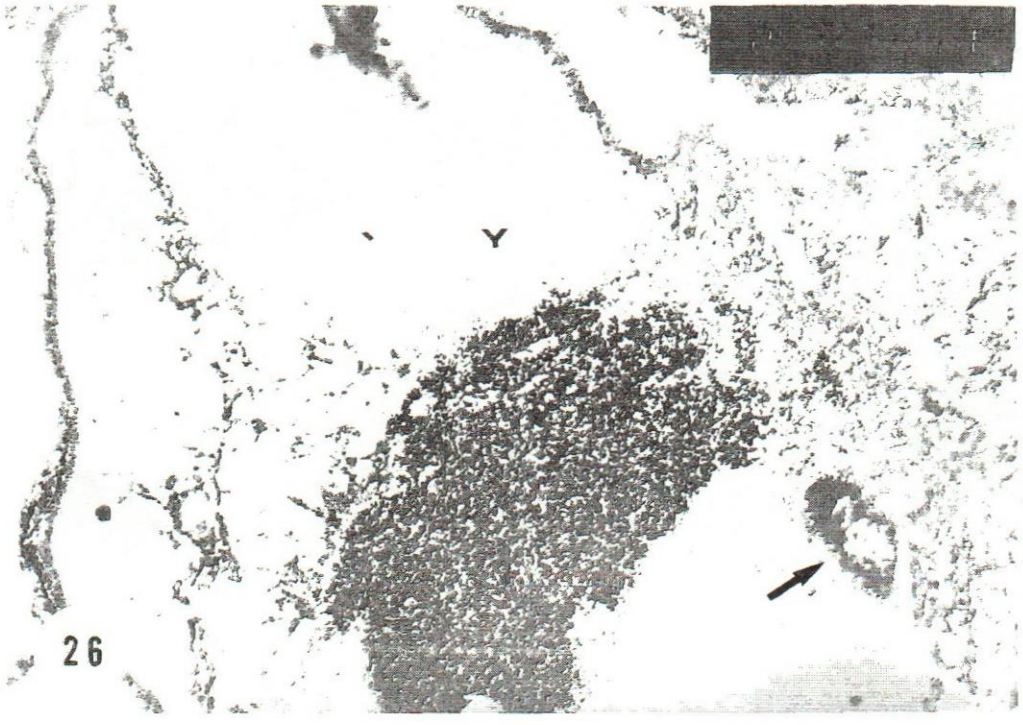

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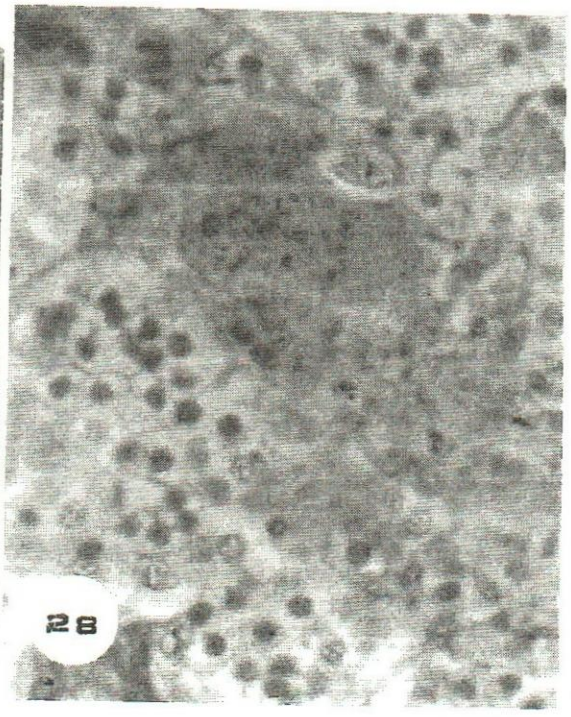
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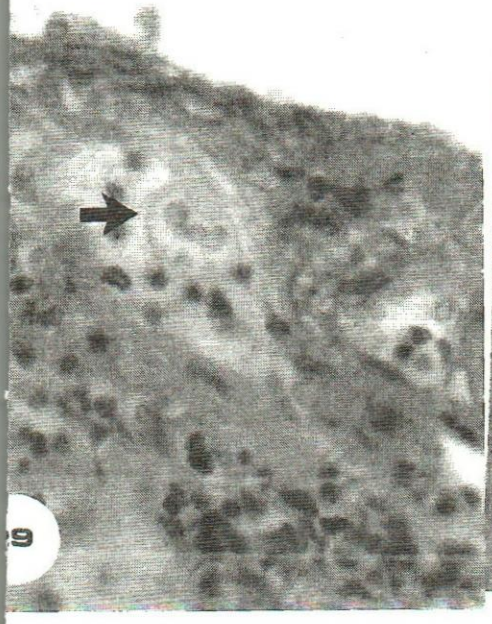
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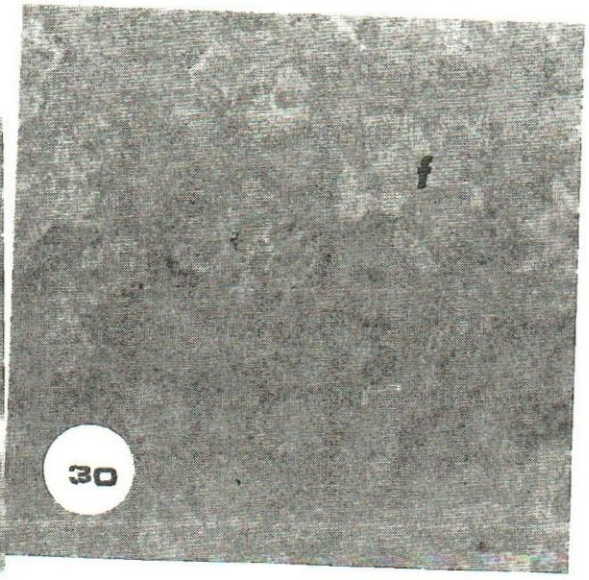
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