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HISTOPATHOLOGY OF CATFISH, MUD BARBEL *CLARIAS MOSSAMBICUS* INFECTED WITH *TRYPANOSOMA MARKEWITSCHI* UNDER LABORATORY CONDITIONS

(With 7 Figures)

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هستوباثولوجى أسماك القراميط المعداه بالتريبانوسوما ماركوتشى تحت الظروف المعملية

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تم جمع ١٠٠ سمكة من القراميط لفحصها لوجود أو غياب التريبانوسوما وذلك فى دم الأسماك . وقد أخذت عينات الدم بعد تخدير الأسماك بمادة MS222 وذلك من الوريد الخلفى للأسماك . تم عمل ثلاث أفلام من الدم مصبوغة بصبغة الجيمسا من كل سمكة وذلك للتحرى عن وجود الطفيل فى الدم . تم أخذ قياسات للطفيل وذلك باستخدام الميكروميتر العينى للميكروسكوب الضوئى . تم جمع الدم الموجب من الوريد الخلفى للأسماك ثم حقنت ٤٠ سمكة غير مصابة بالطفيل وذلك بمقدار ٠,٥ ملل من الدم الموجب عن طريق الغشاء البريتونى . أما ضوابط التجربة فكانت تتمثل فى ١٠ سمكات غير مصابة وغير محقونة . إحتفظت الأسماك المحقونة وضوابط التجربة فى أحواض زجاجية ممتلئة بـ ٤٠ لتر من الماء عند ٢٠ درجة مئوية ولمدة خمس أسابيع من الحقن . تم أخذ العينات المختلفة من الأعضاء لدراستها بالميكروسكوب الضوئى بعد خمس أسابيع من الحقن . كانت التغيرات واضحة فى القلب والكبد والطحال والكلية الخلفية . ومن أهم التغيرات هى التهاب عضلات القلب وظهور الأشكال النسيجية للتريبانوسوما بين عضلات القلب وخارج الأوعية الدموية للقلب . وتعتبر هذه التغيرات من العلامات المميزة التى تثبت أن هذا الطفيل مرضى . أما الكبد فكانت هناك تغيرات فى الأوعية الدموية والتهابها . صبغة الهيموسيدرين كانت واضحة فى طحال الأسماك المحقونة مما يشير الى حدوث نزيف وكذلك قلة الخلايا

الليمفاوية فى الكرات الليمفاوية للطحال مما يشير الى تغير الجهاز المناعى أو إحباطه . ظهور صبغة صفراء فى الخلايا المبطنة للجزء العلوى والسفلى من الأنابيب المتعرجة للكلية . قورنت هذه التغيرات بما يحدث لنظيراتها فى الثدييات . واستنتج من هذه الدراسة بأن طفيل التريبانوسوما هو طفيل مرضى فى الأسماك ويحدث تغيرات باثولوجية بالرغم من عدم وجود أعراض مرضية

SUMMARY

One hundred catfish, mud barbel *Clarias mossambicus* were examined for the presence / absence of Trypanosomes in their blood. The organisms in blood films stained with Gimesa were measured (30 - 40 µm in length; 3-5 µm in width) and identified as *Trypanosoma markewitschi.* Fresh blood was obtained from the fish caudal vein. Fourty un-infected fish were inoculated with 0.5 ml whole blood per fish intraperitoneally and kept at 20°C for 5 weeks. By the end of 5 weeks post-inoculation the heart showed myoradial degeneration and interstitial lymphocytic myocarditis. Tissue forms of *T. markewitshi* were observed either single or double or 4 - 6 large basophilic structures within vacuolated myocardial cells. The spleen had hemosidrosis and formation of large hypocellular follicles occurred. Vascular changes in the liver were experssed by damage of the endothelium lining the blood vessels and perivascular cuffing. The posterior kidneys showed marked cellularity of haematopoietic tissue and vacuolation of the tubular epithelium, which were filled with yellow pigment and suggested bilirubin. These findings were consistent and did not observed in non-inoculated fish and confirmed that *T. markewitschi* is pathogenic in catfish, despite the low degree of parasitemia.

Key words: *Catfish-Trypanosoma-Histopathology.*

INTRODUCTION

Trypanosomiasis in both marine and freshwater fishes is caused by small colorless, unicellular flagellate with undulating membrane, and considered to be non-pathogenic to fish (Schäperclaus, 1991). Biniary or

multiple fission occurs in the intestine of the vector (leeches of the genera Piscicola and Hemiclepsis), and in the blood of fish (Schäperclaus, 1991).

Trypanosomes are classified as species based upon the host. For example, *T. danilewskyi* of common carp and goldfish, *T. markewitshi* of common catfish, and *T. choudhuryi* of tilapia mosambique (Schäperclaus, 1991 and Mandal, 1977). The host specificity, susceptability, immunity, and effect of temperature on *T. danilewskyi* of goldfish have been investigated (Woo, 1981; Woo et al., 1983; Woo and Black, 1984). Moreover, Wang and Belosevic (1994) developed an *in vitro* cultivation procedure for *T. danilewskyi* using TDL-15 culture media.

The pathology of mammalian trypanosomiasis has been described (Sollod, 1976; Jubb et al., 1993; Moulton and Damayanti *et al.*, 1994). But, little is known about the pathology of trypanosomes in fish. Consequently, this study was conducted to describe the histopathological changes associated with *T. markewitschi* in catfish, mud barbel *Clarias mossambicus* kept in the laboratory after being inoculated with whole blood for 5 weeks at 20°C.

MATERIAL and METHODS

Fish:

One hundered catfish, mud barbel *Clarias mossambicus* were collected from the River Nile at Assiut vicinity, Egypt. Fish (50 - 100 g), were kept in a glass aquaria, each containing 40 L municipal water.

Examination and inoculation:

Fish were screened for the presence / absence of trypanosomes in their blood. Fish were anaesthetized with tricaine methanesulphonate (MS-222, Sigma Chemical Co., St. Louis, MO, USA). Blood was obtained from the caudal vein using a 1 ml heparinized syringe. Blood films were air-dried, fixed in absolute methyl alcohol and buffered formalin before they were stained in Giemsa (Woo, 1981). The organisms were measured in micrometers using an occular micrometer. The body length excluding the free flagellum and the body width excluding the undulating membrane were taken (Woo, 1981).

Inoculation:

Pooled blood from naturally infected fish was taken from the caudal vein as described. Fourty un-infected fish were inoculated with the whole

blood containing the trypanosomes. Each fish was inoculated with 0.5 ml whole blood once a week for three times intraperitoneally. These fish were kept in glass aquaria at 20°C ± 1 for 5 weeks.

Histopathology:

Fourty inoculated and ten non-inoculated fish served as control were t buffered formalin, embedded in paraffin, sectioned at 4 - 6 μ, and stained with H & E, and Giemsa (Bancroft and Stevens, 1982).

RESULTS

Blood films obtained from inoculated fish by the end of 5 weeks post-inoculation showed very few number (1 - 4) trypanosomes per blood film. The trypanosomes had parabasal body, nucleus, undulating membrane, kinetoplast, and free flagellum (Fig. 1). They were measured 30 - 40 μm in length and 3 - 5 μm in width.

There were no gross lesions observed in inoculated fish. Fig. 2 shows interstitial lymphocytic myocarditis and myocardial degeneration. Tissue forms of *Trypanosoma markewitschi* were observed in the hearts of inoculated fish as single or double or 4-6 large basophilic structures within vacuolated myocardial cells (Fig. 3 a,b,c,d).

The liver shows dilatation and engorgement of blood vessels, damage of endothelium, and perivascular lymphocytic cuffing (Fig. 4).

The posterior kidneys had marked cellularity of haematopoietic tissue and partial obliteration of both parital and visceral layers of Bowman's capsule. Vacuoles filled with yellow pigment were observed in the epithelium lining both proximal and distal convoluted tubules (Fig. 5).

The spleen had marked increase in hemosidrin content (Fig. 6). Formation of large hypocellular follicles was also observed (Fig. 7).

DISCUSSION

This study confirmed that *T. markewitschi* in catfish, mud barbel collected from a feral population is pathogenic to catfish. However, Schäperclaus (1991) considered trypanosomes to be non-pathogenic to fish, in contrast to the species infecting warm - blooded animals. In this report, interstitial lymphocytic myocarditis, myocardial degeneration, and tissue

forms of *T. markewitschi* observed in the hearts of fish inoculated with infested blood were consistent findings. While, those fish with no trypanosomes in their blood did not show any changes. These changes are pathognomonic lesions of pathogenic mammalian trypanosomes (Jubb et al., 1993). Trypanosomes can be found either extravascular or intravascular (Masake, 1980). In this study, tissue forms were observed extravascular and suggested that *T. markewitschi* is less serious pathogen.

In this report, the livers had vascular changes expressed by damage of endothelium lining the blood vessels and perivascular lymphocytic cuffing. These changes were nearly similar to mammalian trypanosomes tissue damage (Jubb et al., 1993).

The posterior kidneys of inoculated fish in this study had marked cellularity of haematopoietic tissue (homologue to interstitial tissue of mammals), and pigmentation of tubules with yellow pigment suggested bilirubin. These changes were similar to kidney changes reported in mammalian trypanosomiasis (Jubb et al., 1993).

In this study, the spleen showed marked hemosidrin content, which may suggest that haemorrhage had occurred. While, the large hypocellular follicles suggested altered immune mechanisms and/or immunosuppression.

In conclusion, the present study confirmed that even low number of *T. markewitschi* in catfish, mud barbel *Clarias mosambicus* could induce pathognomonic tissue alterations. The sequential pathology is recommended for studying the pathogenesis of these lesions in fish.

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FIGURE LEGENDS

Fig. 1: Trypanosoma showing parabasal body (p), undulating membrane (μ), kinetoplast (k), and free flagellum (f). Giemsa's stain x 320.

Fig. 2: Heart of catfish, mud barbel at 5 weeks post - inoculation. Interstitial lymphocytic myocarditis (L). H & E x 80.

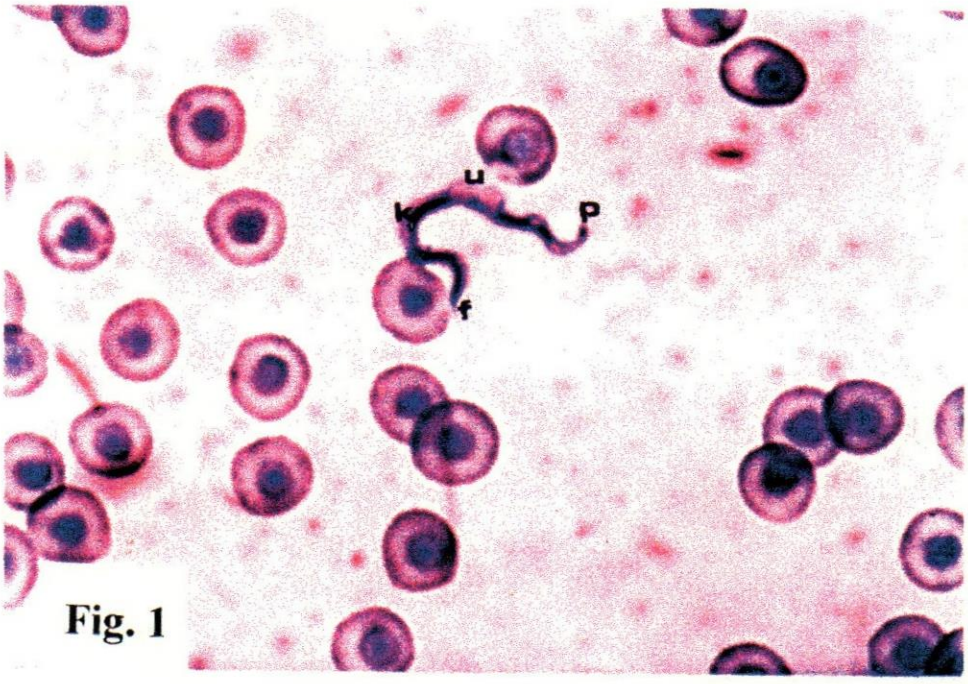
Fig. 3: Heart of catfish, mudbarbel at 5 weeks post - inoculation. Tissue forms of T. markewitschi appeared as large extravascular, (a) single basophilic structures, (b) double, (c) 4, (d) 6. H & E x 320.

Fig. 4: Liver of catfish, mud barbel at 5 weeks post - inoculation. Damage of endothelium of blood vessel (d) and perivascular lymphocytic cuffing (c). H & E x 80.

Fig. 5: Posterior kidneys of catfish, mud barbel at 5 weeks post - inoculation. Vacuoles filled with yellow pigment (v) within proximal and distal convoluted tubules, and increased haemopoietic tissue (H). H & E x 128.

Fig. 6: Spleen of catfish, mud barbel at 5 weeks post - inoculation. Hemosidrosis appeared as brown granules. H & E x 51.

Fig. 7: spleen of catfish, mud barbel at 5 weeks post - inoculation. Formation of large hypocellular follicles (F). H & E x 128.



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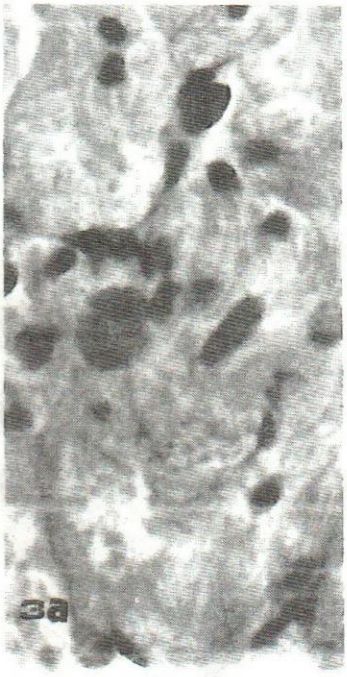
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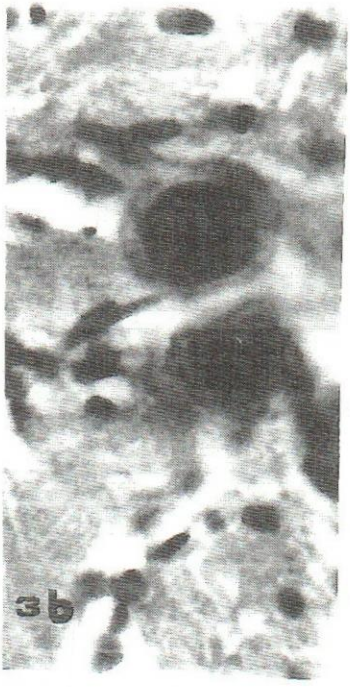
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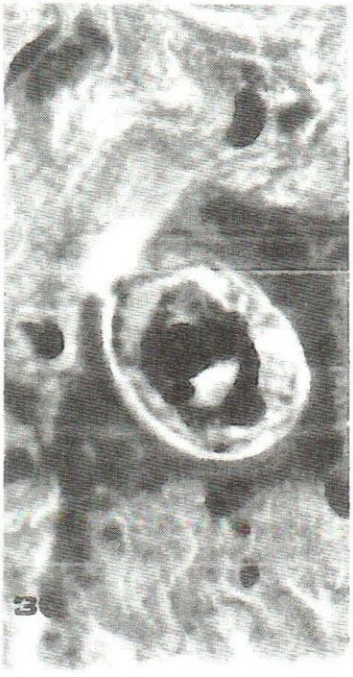
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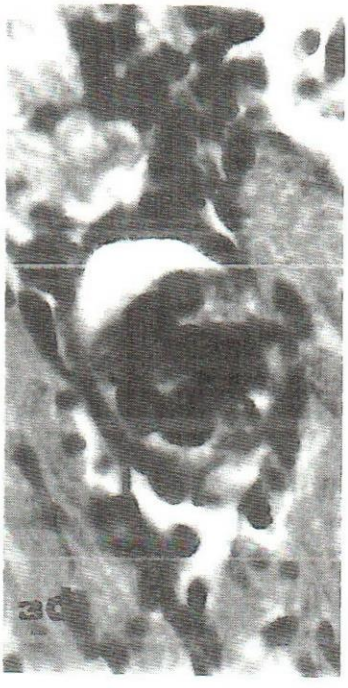
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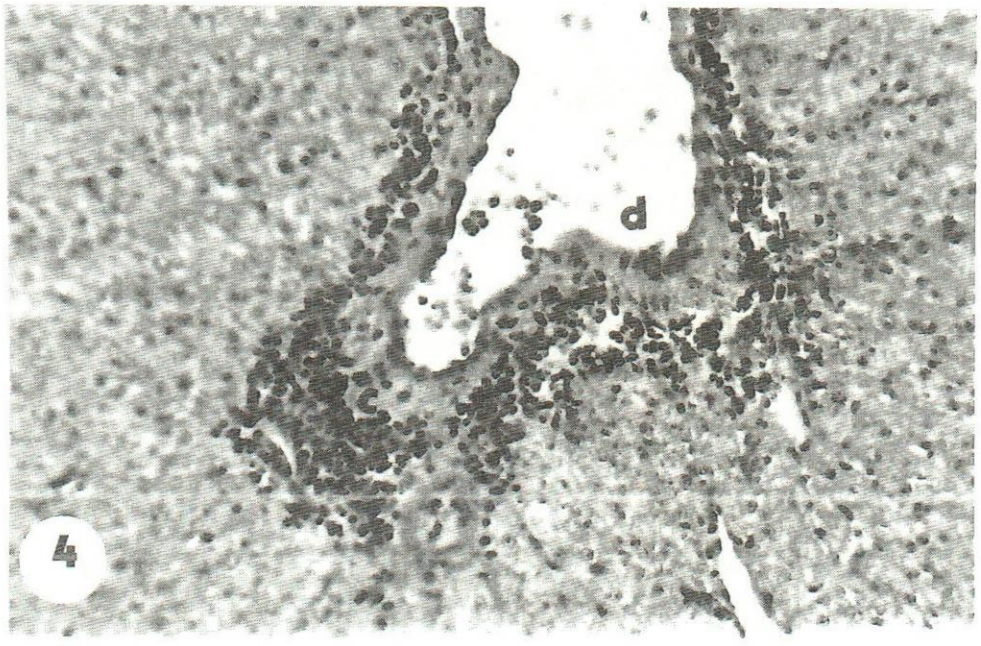
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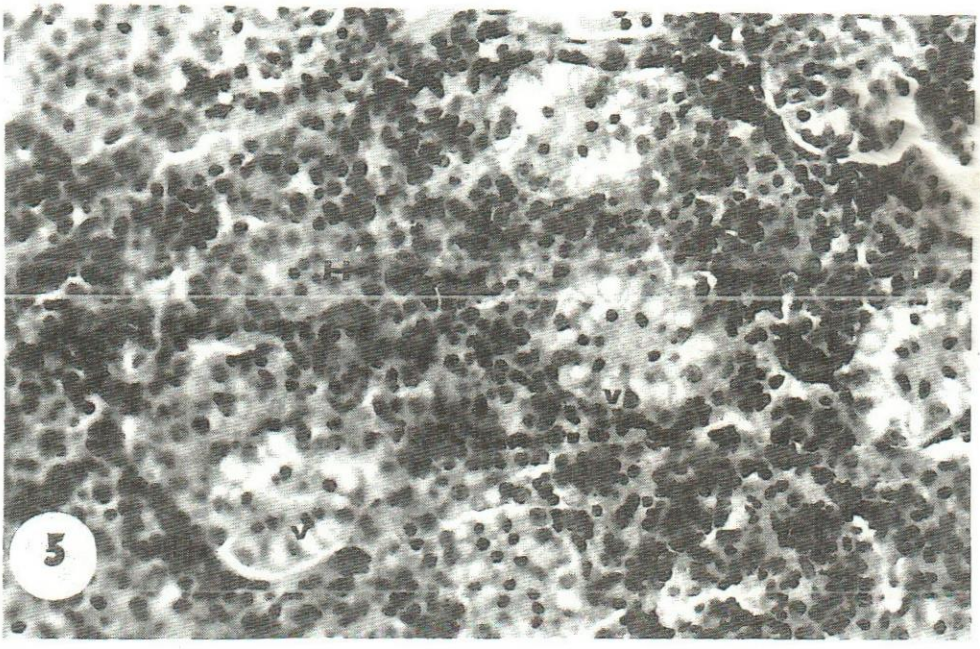
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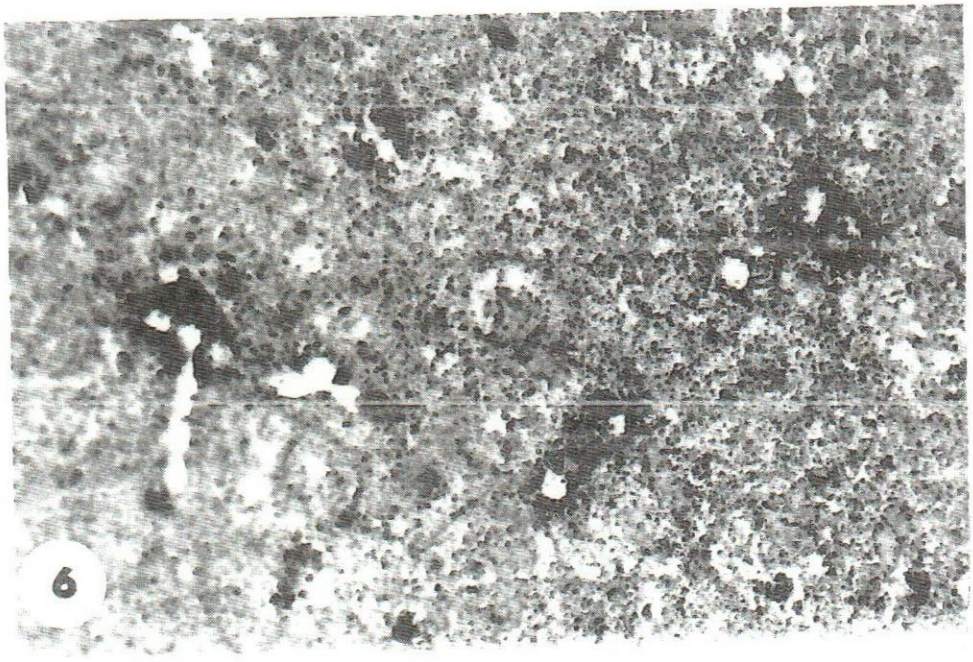
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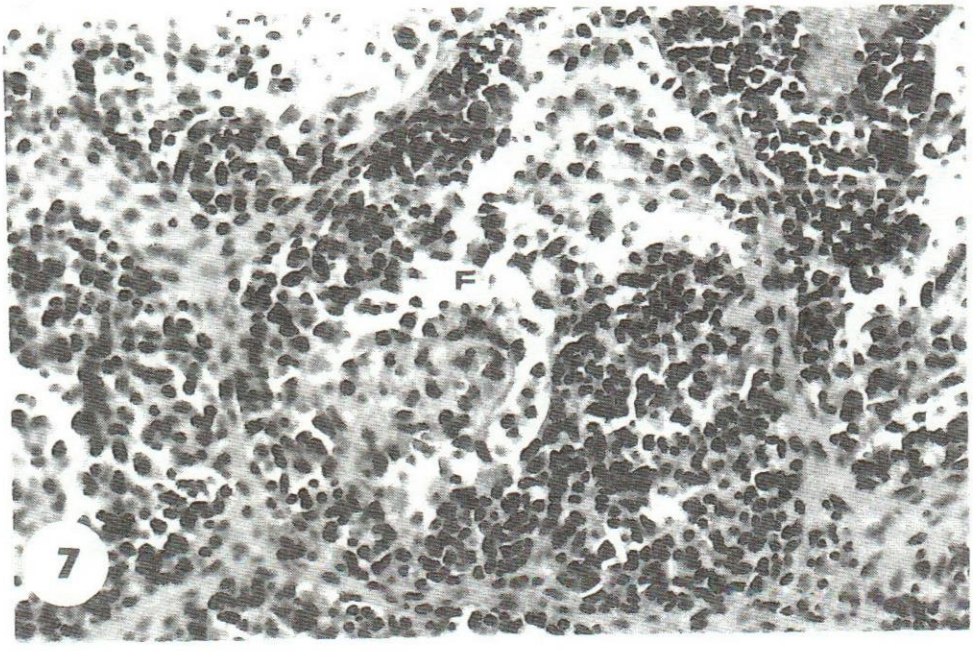
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