

Clinical and Patho-anatomical Studies on Cases of Theileriosis in Calves Histopathologically Confusable with Juvenile Leukosis

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SUMMARY

A decade of pathological studies on *Theileria annulata* infection in exotic or cross-bred cattle has revealed that in some instances, due to undetermined factors, the disease assumes a feature untypical of the infection. Although in such cases the proliferative changes in lymphoid tissues were found to be conspicuous, the most prominent changes were the presence of follicles and nodules of proliferative lymphocytes in all organs including skeletal muscles. Cryo-sectioning technique and Giemsa staining revealed a large proportion of the lymphocytes in these nodules containing *T. annulata* schizonts, whereas formalin fixation and H&E staining of the same specimens presented a picture closely resembling that of juvenile bovine leukosis. It has been postulated that such lesions were host-dependent and most probably appeared due to the phenomenon of immunological tolerance.

INTRODUCTION

Tropical theileriosis due to *Theileria annulata* is a disease of major importance in several countries of Asia and some parts of North Africa where there are millions of cattle at the risk of exposure to this disease. In enzootic areas the parasite has been found to be more pathogenic to pure breeds than to native or cross-bred cattle (3, 5, 6, 12). The pathology of experimental and natural *T. annulata* infections has been studied and reported (10, 14, 17). Unusual forms of the disease such as cases with cutaneous lesions and/or with nodule formation in the internal organs and intramuscular fibers have also been described (1, 2, 4, 11, 14, 15, 16, 18). The clinical and patho-anatomical observations on the latter cases were carried out in the present study.

* Paper presented to the 4th joint meeting of veterinary pathologists (Cordoba (Spain) 1986

Materials and Methods

Subject animals:

Since 1975 six hundred cattle (450 sick animals and 150 carcasses) have been received at the Pathology Department of the Razi Institute which were sick or had died of tropical theileriosis. Only 16 calves did not show the clinical signs and necropsy changes ascribed to tropical theileriosis. Three calves were of Holstein breed and the rest of native/Holstein cross-breeds. Their ages ranged from 45 days to 7 months. Attempts to save these animals by symptomatic treatments failed and they either died or were slaughtered when moribund.

Tissue sectionings:

Tissues from various organs were collected for histopathological studies. A set of sectioning were prepared with a rotary microtome after the tissues had been fixed in 10% formalin solution processed and embedded in paraffin wax. These sections were stained by H & E staining method. Another set of sections were prepared with cryostat microtome, by freezing tissues at -35 C. The latter set of sections were stained by Giemsa's stain.

OBSERVATIONS

Clinical signs:

The calves were depressed and anorectic but the extent of the loss of appetite differed in individuals; while food intake remained normal in some, it completely ceased in some others. The latter cases were found to have abomasal ulcers when necropsied. The rectal temperatures ranged from 38.5 to 39 C. Symptoms of pneumonia and dyspnea were noted in all cases. Numerous subcutaneous or interdermal nodules measuring 0.3-1 cm. in diameter were noted throughout the body. In some cases these nodules were quite prominent (Fig. 1). The visible mucosae were slightly yellowish and in some cases one or more pinkish nodules were noted on the conjunctivae, the internal surface of the lips and on the gums (Fig. 2). Exophthalmos was present in some cases (Fig. 3). Superficial lymph glands, particularly the prescapular ones, were enlarged 2-5 times of the normal size (Fig. 4).

Gross pathology findings:

The visible mucous membranes were pale and petechiated. In some cases nodules with haemorrhagic foci were present on the conjunctivæ (Fig. 2). Both parietal and visceral lymph nodes were enlarged, but the enlargement of the prescapular and submaxilar nodes was striking. These lymph nodes were markedly enlarged, their cut surface revealed a good deal of fluid and most of them had lost the prominent nodular-like appearance in the cortex.

Haemorrhagic spots or nodules were noted in the subcutis, pleurae, peritoneum, omentum, serous surface of stomachs, intestines, mucous membrane of lips, the skeletal muscles, myocardium, endocardium and the tongue (Figs 5, 6, 7, 8, 9).

Liver was yellowish brown in colour, slightly enlarged and had haemorrhagic spots on the surface as well as in the parenchyma. Haemorrhagic spots were also noted on the surface of kidneys (Fig. 10). Splenomegally and punched out ulcers with necrotic centres and haemorrhagic borders in the mucosal surface of abomasum, which are the marked changes in typical theileriosis, were also noted but to a lesser extent.

Microscopic pathology findings:

The histopathological changes in different organs were: Lymph nodes: In majority of the cases proliferative changes of varying degrees were observed. The proliferative changes caused an increase in the number and the size of follicles. The intermediate as well as marginal sinuses were distended and packed with enormous amount of lymphocytes and macrophages. In some cases the involved lymph nodes seemed to have completely lost their normal structure. In such cases a fibrinoid material had infiltrated into the reticular framework of the cortex.

Spleen: Changes noted in the spleen more or less resembled those of lymph nodes. Moreover, various degrees of congestion and haemorrhages in red pulp were observed. Cryo-sectioning revealed the presence of lymphoid cells which contained schizonts of *T. annulata* when these sections were Giemsa stained. Extracellular schizonts were also discernible in such sections.

Thymus, tonsils and bone marrow: Thymus and tonsils showed lesser changes whereas sections from bone marrow revealed hyperplastic changes of reticular cells.

Liver: The hepatic cells showed moderate fatty change. Diffuse

mild to moderate congestion was present. Periportal areas were infiltrated by a large number of young lymphocytes in some of which (cryosections) schizonts could be demonstrated.

Kidneys: The general pathological picture was one of acute nonpurulent interstitial nephritis with focal aggregation of lymphocytic cells in the medulla and the cortex.

Lung: There were scattered haemorrhages. Alveolar septae showed thickening and focal infiltration of lymphocytes were the changes noticeable with different degrees of severity. In cases complicated with secondary infections, lesions of suppurative changes were also present.

Skin: Focal haemorrhages and aggregates of parasitised lymphocytes were the prominent findings in sections prepared from haemorrhagic spots of the skin, conjunctivae, mucosal areas and adipose tissues.

Brain: Blood vessels were engorged. The Virchow-Robin's spaces were distended and filled with a homogeneous pinkish material, indicating severe oedema. Some of capillaries showed mild perivascular cuffings and the meninges were moderately infiltrated with cells which were mainly lymphocytes.

Abomasum: Haemorrhagic ulcerations extending to the underlined muscularis mucosa was seen in some cases. Moderate to severe inflammatory cell infiltration was present throughout.

Intestines: Marked infiltration of immature lymphocytes was present in the glandular of the intestinal wall. Cell infiltration was deep down to the submucosa, muscularis mucosa and in some instances to the ring muscles. Occasionally these lymphocytes were accompanied by focal haemorrhages in muscular areas.

Heart, tongue and skeletal muscles: The most striking changes were noted in these organs. Sections prepared from the nodules and affected areas revealed mild to severe degeneration and/or loss of muscle fibers, moderate haemorrhages with a prominent invasion of the lymphocytic cells including immature and mitotic figures. The whole picture resembled that of juvenile leukaemia when H & E stained sections were examined but cryosections that were stained by Giemsa revealed schizonts in almost all the invading cells (Figs. 11-14).

Discussion

Different forms of theileriosis due to *T. annulata* were described by Neitz (12), Barnett (3) and others. The factors which determine the clinical manifestations and the pathological features have been defined as the degree of host susceptibility and virulence of the strain

involved. In a case, reported by Baharsefat **et. al.**, with muscular nodules and characters similar to those subject of this discussion, it was shown that the involving strain did not possess a certain peculiarity and the experimental infection produced did not differ in character from those of the classical forms. The strain isolated from one of the cases under this study and cultivated in vitro did not show any diversity from the common characters attributed to this parasite in cell culture. Therefore, we believe that the host response, or in a better word the lack of response, has been the prominent factor responsible for the pathological changes observed. It was postulated that these animals, which had mostly acquired the infection at the early stages of calthood, had some degree of immunological tolerance. This hypothesis has been based on some parasitological, pathological and epidemiological data:

The number of erythrocytic forms of the parasite, which are the means of survival and are perhaps produced under adverse conditions (8), have been very low in almost all cases, whereas macroschizonts were profusely abundant to the extent that they formed nodules. Lymphoid-cell aggregates contained many mitotic forms indicating the multiplication of these cells on the spot, resembling what is seen in tissue culture of **T. annulata** schizonts. Such form of propagation for schizonts of **Theileria** had been proposed by Hulliger (7) but did not meet the popular acceptance. The argument against it being that towards the end of the infection one observes lymphoid-cell depletion and hypoplasia rather than hyperplasia. However, our inference was that these cells had been transformed and, without being recognised as unself by the body, continued propagation and formed nodules.

Pyrexia is accepted as a means of body defence and is triggered off at the time of invasion by a germ. As it can be seen (Section on clinical signs) the animals referred to in this study did not show a significant rise in the body temperature, another sign of the lack of setting up resistance.

Jaundice that follows red blood cells destruction has been shown to be mainly due to an autoimmune reaction of the host rather than to physical or chemical effects of the erythrocytic forms of the parasite on the red cells, and is triggered off mainly by macroschizonts (9). In the studied cases while the tissues were rife with schizonts, the degree of icterus was comparatively very slight. This again was taken as a sign that immune systems had disregarded the infection. Unfortunately the haematological parametres were not studied in the present work and that is one of its short-comings.

The passage of this pathogen through the placenta barrier has

been claimed by many authors (12). The dams of the subject animals came from enzootic areas and were carriers of the parasite. Though the acquired infection could not accurately be determined, it could have been during pregnancies. Therefore, it is not far from reality if one suggests that the passage of the pathogen had occurred sometimes during the pregnancies, rendering the fetuses immunologically tolerant.

Had it not been for cryostat sectionings, one could have easily mistaken the nodules for those of juvenile leukosis, because the microscopic features of formalin-fixed and H & E stained sections closely resembled those of lymphosarcoma. The sections cut by cryostat and stained by Giemsa stain clearly showed the lymphoid cells carrying **T. annulata** schizonts. If means were available to test these cells for major histocompatibility, lymphoid cells carrying **T. annulata** schizonts could have been differentiated from lymphosarcoma, as Pinder and Roelants (13) have shown that the kind of receptors in Theileria-transformed lymphocytes differ from those of lymphosarcoma.

References

- 1 Arap Kimeto, B. (1978),
Amer. J. Vet. Res, vol. 39, No. 7: 1117.
- 2- Baharsefat M., Amjadi A.R., Hashemi-fesharaki R., Ahourai P. and Arbabi I (1977),
Arch. Inst. Razi 29: 47.
- 3- Barnett S. F. (1968),
In infectious blood disease of man and animals, vol. 2. P. 113, Ed. D. Weinman and M. Ristic, Academic Press Inc, New York, U.S.A.
- 4- Deer, P.A., Sabnis M. G. and Bendre V.U. (1979),
Indian Vet. J., 56: 794.
- 5- Guatam O.P., Sharma R.D. and Kalra D.S. (1970),
Indian Vet. J., 47: 78.
- 6- Gill B.S., Bhattacharyulu, Y. and Kaur D. (1977),
Annal de Parasitologie (Paris), 52, No. 6: 1597.
- 7- Hulliger, L., (1965),
J. Protozoology, 12: 649.
- 8- Hulliger, L., Wilde, J.K.H., Brown C.G.D. and Turner, L. (1964),
Nature, 203: 728.
- 9- Hooshmand-Rad P. (1976),
Research in Veterinary Science, 20: 324.
- 10- Khanna B.M., Kharole, M.U., Dhar S. and Guatam O.P. (1982),
Indian Journal of Parasitology, 6 (1): 91.
- 11- Manickam R., Dhar S., Singh R.P. and Kharole M.U. (1984),
Indian Vet. J. 61: 13.
- 12- Neitz W.O. (1957)
- 13- Pinder M. and Roelant G.c. (1981), Onder, J, Vet. Res. 27, (3): 275.

Advance in the control of theileriosis. 384, eds. Irvin A. et al Martinum Nijhoff Publisher, London.

- 14- Prasad M.C., Ansari S.A. and Knppuswany P.B. (1970),
Indian J. Anim. Sci., 40: 622.
- 15- Sergent E., Donatien A., Parrot L., and Lestoquard F. (1982),
Arch. Inst. Pastour. Alger. 10: 294.
- 16- Shastri U.V., Deshpande P.D., Awab K.B. and Weissenberg I. (1960),
Indian Vet. J. 59: 188.
- 17- Srivastava P.S., Iyer P.K.R., Sharma N.N., and Prasad M.C. (1976).
Acta, Vet. BRNO, 45: 245.
- 18- Tsur-Tchernomoretz I., Davidson M. and Weissenberg I. (1960),
Refuah, Vet. 17: 100.

Fig. 1- The subcutaneous nodules in the perineum are quite prominent.

Fig. 2- The pinkish nodule with hemorrhagic foci in the conjunctiva.

Fig. 3- The quite prominent exophthalmus.

Fig. 4- The prescapular lymph node enlarged 3 times of normal size.

Fig. 5 & 6- The hemorrhagic nodules in the subcutis, aponeurosis of the skeletal muscles and cut surface of thigh muscles.

Fig. 7- Hemorrhagic and proliferative nodules in the myocardium, endocardium and tongue muscles.

Fig. 8- Hemorrhagic nodules in the myocardium.

Fig. 9- Hemorrhagic nodules in the serous surface of the intestine.

Fig. 10- Note the yellowish brown color of the liver. Hemorrhagic spots are present on the surface of liver and kidney.

Fig. 11- H & E stained section from thigh muscle. Note the leukosis like lesions. x45.

Fig. 12- A higher magnification of Fig. 11, x125.

Fig. 13- Giemsa stained of Cryo-section of thigh muscle. Note the Schizonts in the cytoplasm of the lymphocytes. x45.

Fig. 14- A higher magnification of Fig. 13, x1250.



Fig. 1



Fig. 2



Fig. 3



Fig. 4

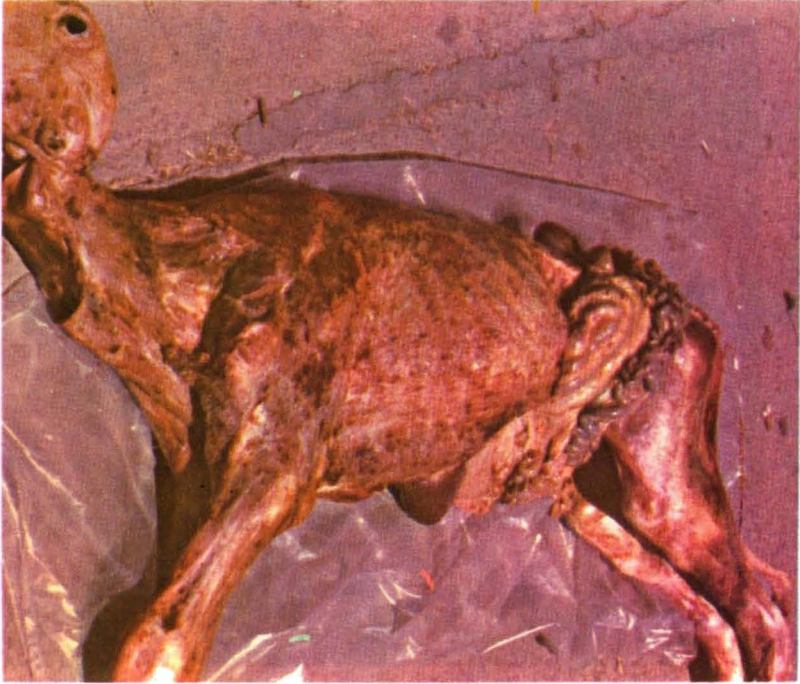


Fig. 5

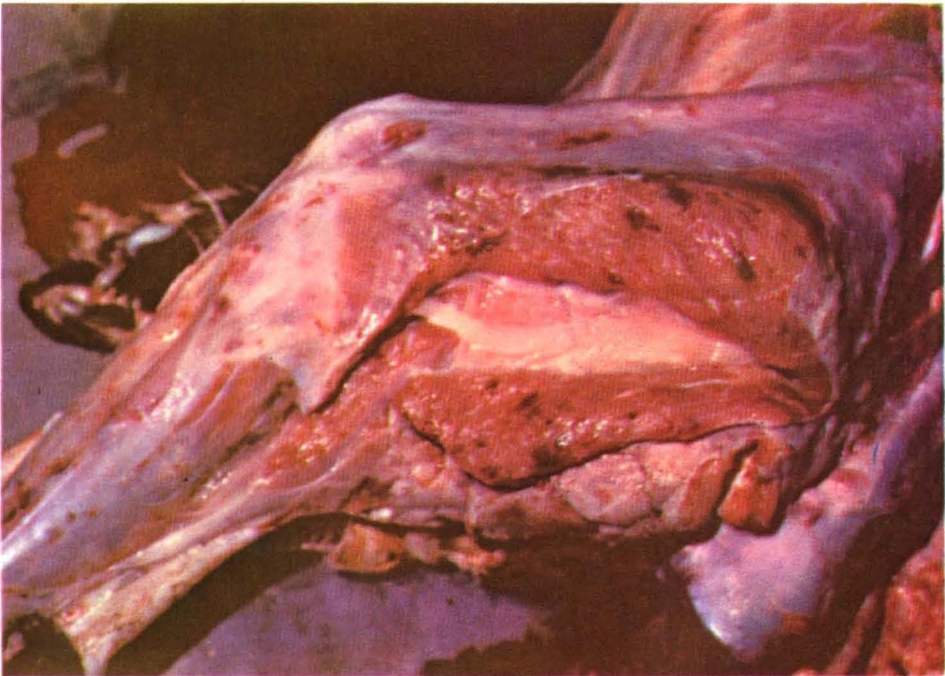




Fig. 7



Fig. 8

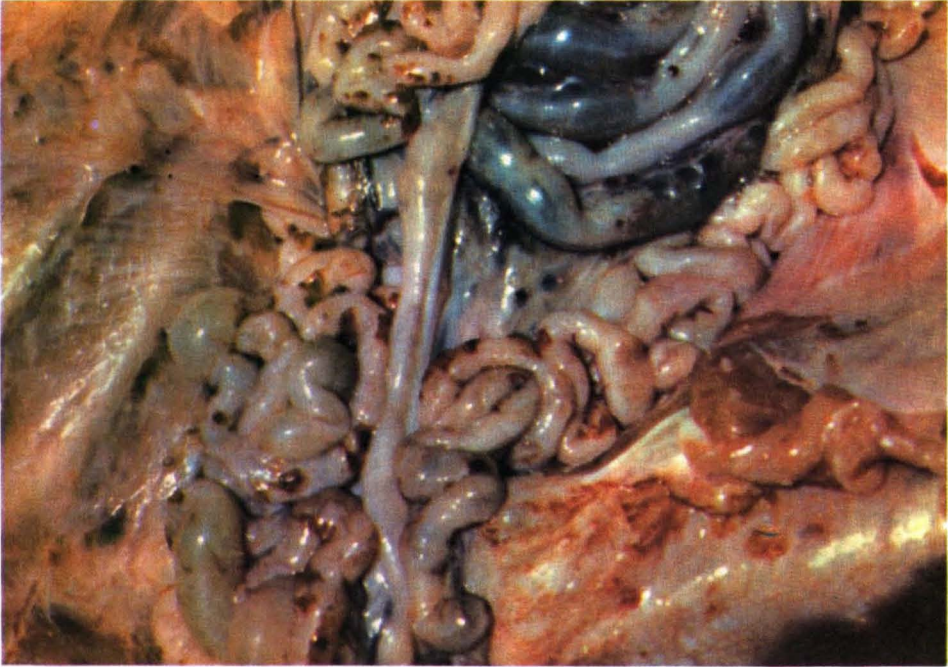


Fig. 9



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Fig. 10

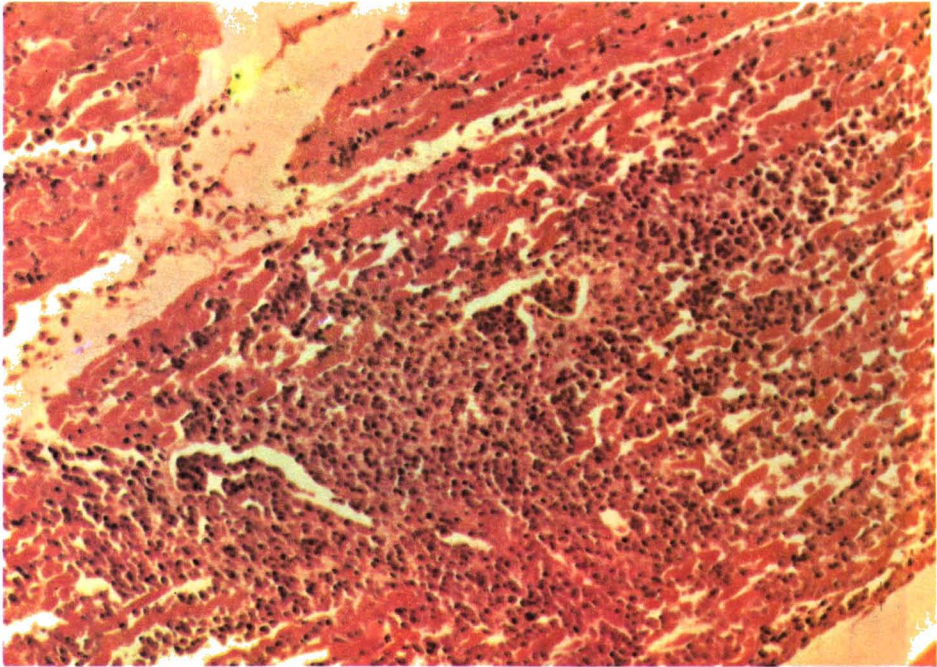


Fig. 11

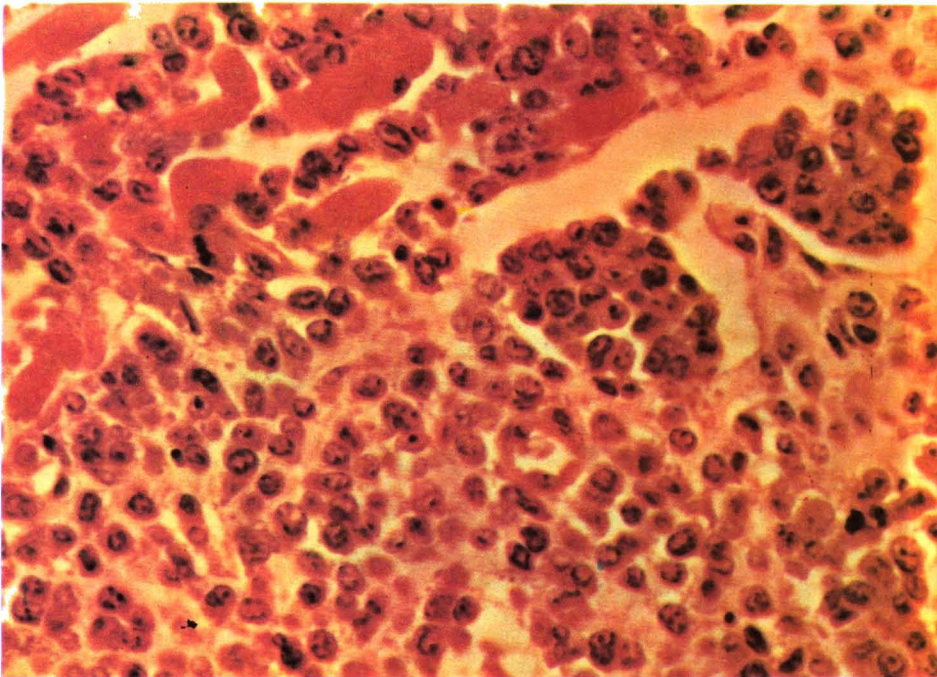


Fig. 12

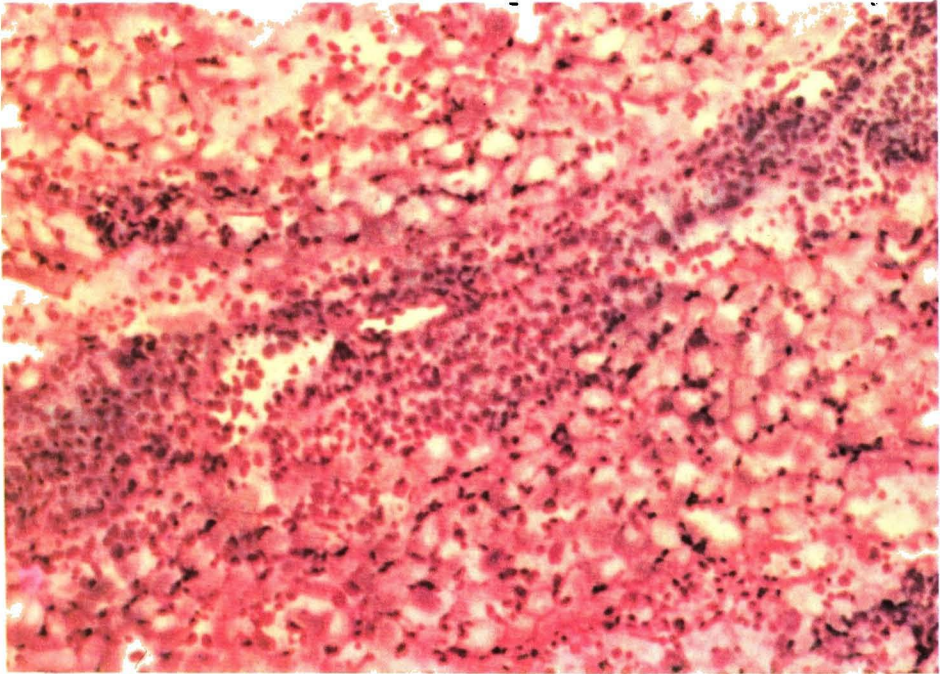


Fig. 13

