دراســات تجريبية على عدوى الأرانب الغينى بيرقات اســكارس العجول

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الملخص

أربعون أرنب غينى استعملوا فى استكشاف التغيرات التى تطرا على الكبـد والرئتين نتيجة العدوى المتكررة لهذه الأرانب بواسـطة أعطائها البيض المخصب بديدان اسـكارس العجول عن طريق الفم .

التغيرات التى لوحظت فى الكبد والرئتين ازدادت حدتها كلما تعرض الحيوان لتكـرار هذه العدوى .

التغيرات التى شوهدت فى الرئتين كانت غالبا موضعية وتتميز بتكاثر الخلايا الالتهابية آكلة الأنسجة الميتة وكذلك الخلايا الناتجة عن اتخاذ هذه الخلايا الآكلة .

التغيرات التى ظهرت فى الكبد كانت على شكل موت خلايا الكبد فى مواضع متفرقة مـع تكاثر الخلايا الالتهابية التى تحتوى على المواد المضادة للهستامين .

الحساسية وجدت أنها تلعب دورا كبيرا فى طريقة حدوث التغيرات المرضية عن هـذه العدوى وكذلك الأجسام المضادة التى وضعت فى الاعتبار .

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STUDIES ON THE EXPERIMENTAL PICTURE OF INFESTING GUINEA PIGS WITH NEOASCARIS VITULORUM

(with 22 figures)

By

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SUMMARY

Fourty guinea pigs were employed to investigate the nature of lesions produced in lugns and liver by repeated infection using larvae of Neoascaris vitulorum per os. The liver and lungs presented progressive lesions with the repeated infection. The pulmonary lesions were manily focal granulomatous, and the hepatic ones were chiefly focal necrotic with eosinophilic infiltration.

Hypersensitivity was concluded to play a major role in pathogenesis of the lesions. Immunity was also considered.

INTRODUCTION

The available literature points out that the work concerning *Neoascaris vitulorum* is generally little inspite of its high incidence in the buffalo calves in Egypt.

SPRENT (1951) showed that guinea pigs infected with larvae of *Ascaris lumbricoides* from pigs, exhibited anaphylactic shock when injected intravenously with materials obtained from larval tissue and larval metabolic product- KAGAN (1958), SPRENT (1961), and SHARP and OLSON (1962) showed that anaphylactogenic substances are located in several organs of adult *Ascaris* worms and in the larvae; and that these substances are either protein or polysaccharide or both.

The present study deals with an investigation of the nature of tissue reaction which results from repeated infection of guinea pigs with *Neoascaris vitulorum* larvae. The study was carried on guinea pigs because the lesions produced in them by the migrating larvae of *Neoascasis vitulorum* were more outstanding than in the other laboratory animals. The organs which persistently showed prominent lesions in guinea pigs were the liver and lungs (ATALLAH *et al.*, 1974), This may explain the emphasis on examining liver and lungs in the present work, in addition to the supposition that a great deal of pulmonary and hepatic affections in the Egyptian farmers could be due to larvae of *Neoascaris vitulorum*.

MATERIAL AND METHODS

Fourty guinea pigs of 50 days old and of 250 to 300 gms. body weight were used and divided into 10 equal groups. The animals were kept under adequate hygienic housing and managment. The diet consisted of barley and green clover, free choice. Five groups received the larvae and the others were used as control.

Fresh mature females of *Neoascaris vitulorum* were collected from the intestine of buffalo calves while the eggs were obtained from the terminal part of their uteri and left in 2.5% potassium dichromate for 5 weeks. Infection of guinea pigs was induced weekly by giving oral dose of about 4,000 embryonated eggs in 2 ml. water where groups, I, II, III, IV, and V took 1, 2, 3, 4, and 5 doses respectively. The animals were sacrificed one week after the last does.

Fresh specimens were collected from lungs and liver, fixed in 10% formalin, embedded in paraffin, serially sectioned and stained with hematoxylin and eosin. Othere stains used were prussian blue and Van Gieson's stain.

RESULTS

Liver and lungs were the organs considered in the present findings as follows:

Liver:

The microscopic picture showed normal larvae among the hepatic cells in most instances (Figs. 1 & 2). Some larvae were present inside the protal vein and were surrounded with eosinophils (Fig. 3), and also inside the bile duct (Fig. 4) during the fourth infection. Eosinophils were the main inflammatory cells present inside the portal tracts, portal veins and among hepatic cells, or focally replacing liver cells. The number of eosionphils increased progressively with repeated infection (Figs. 5 & 6). Fibroblasts were scattered among eosinophils from the third infection upwards, and the Kupffer cells were hypertrophic.

Bile ducts were hyperplastic and frequently contained larvae (Figs. 7 & 8).

Retrogressive changes and necrosis were present in the liver and their severity increased with repeated infection. Cloudy swelling was present during the first and second infections, and progressed to vacualor and hydropic degeneration later (Fig. 9). The hepatic necrosis was coagulative and prominent in animals given two or more infections (Fig. 10). The center of the necrotic areas frequently contained eosinophilic debris and larvae particularly during the fourth infection (Fig. 11). Some hepatic cells presented cytoplasmolysis. Their nuclei were either collapsed or vesicular and about two times the normal size (Fig. 12). Frequently the necrotic hepatic cells disappeared and lef empty reticulum.

The macroscopic lesions were persistent in the liver of animals which received two or more infections. They were represented by small yellowish foci of 2-3 mm. diameter scattered throughout the hepatic parenchyma.

Lungs:

The microscopic picture in most affected animals showed hypertrophic peribronchial smooth muscles, desquamated epithelial cells and granular eosinophilic debris inside the lumina of bronchioles. There were peribronchiolar, perivascular and interstitial aggregations of eosinophils. Focal granulomatous reaction was evident.

Congestion and exudation were clear during the first and second infections. The eosinophilic infiltration was diffuse with focal granulomas (Fig. 13). Larvae were present among necrotic debris, eosinophils, macrophages and foreign body giant cells. Few fibroblasts were recognized.

Focal granulomas with eosinophils were more frequent and of larger size during the third than during the first and second infections. Degenerated larvae (Fig. 14) were present among eosinophils. Numerous yellowish brown pigments, which were prussian blue-negative, were seen inside macrophages and chondrocytes of bronchiolar cartilage (Fig. 15). A few fibrous tissue which was Van Gieson's - positive was slightly scattered among pulmonary tissue, and was relatively more than during the first and second infections.

Coiled larvae were seen inside a thin hyalinized oval capsule in lungs of animals which received four infections (Fig. 16). Such lesion was associated with congested pulmonary tissue, and air sacs filled with acidophilic material containing eosinophils. Other larvae were present among desentigrated eosinophils and surrounded with a thick hyalinized wall (Fig. 17). Granulomas were greatly mixed with fibroblasts, and the fibrous tissue proliferation was somewhat evident (Fig. 18). Areas of hemorrhage were also seen. Yellowish brown pigments which were prussian blue-negative were seen inside macrophages.

Large focal granulomas which consisted almost entirely of macrophages and giant cells mixed with fibroblasts were seen in animals which received five infections (Fig. 19). The remaining few intact pulmonary air vesicles were emphysematous. Other areas were congested and presented coiled larvae inside a thin oval hyalinized capsule (Fig. 20). Radiating hyperplasia of adventitia of arterioles was seen (Figs. 21 & 22). The type of leuckocytes inside the lumina of most blood vessels was almost entirely eosinophils.

The macroscopic lesions were represented by numerous petechial hemorrhages allover the pulmonary tissue in all the experimentally infected animals Brownish patches started to appear during the fourth and fifth infections. Greyish yellow foci of 1 mm. diameter were present in lungs of guinea pigs during the fifth infection.

DISCUSSION

Lesions were progressive in the present study with the repeated infections which suggest the presence of hypersensitivity. Immunity was also considered by the presence of the eosinophilic thin oval wall around the larvae in the lungs. This wall or capsule is suspected to be the result of an antigen-antibody complex, where the antigen is the larvae of *Neoascaris vitulorum* or its produdts.

Other workers reported similar findings in paratenic and definitive hosts using *Ascaris lumbricoides* or *Dictyocaulus viviparus*. AREAN and CRANDALL (1962) reported a more intense inflammation in rabbits which were sensitized by the injection of heat-killed eggs of *Ascaris lumbricoides* than the unsensitized animals. The same authors found acidophilic material associated with the cuticle of the degenerated larvae of *Ascaris lumbricoides* in rabbits. They suggested that material was an antigen-antibody complex. MICHEL (1945), WEBER and LUCKER (1959) and JARRETT *et al.* (1959 a) reported a sort of immunity in calves repeatedly infected with *Dictyocaulus viviparus* represented by the absence of patent infection. JARRETT *et al.* (1959) measured the antibody in immunized calves against *Dictyocaulus viviparus*. The presence of humoral antibody suggests the sharing of immediate hypersensititivity in pathogenesis of such lesions.

The granulomatous reaction in the lungs of guinaee pigs was apparently an attempt by the host to sequester antigens of low diffusability, or their break down products, and to localize them at their sites of deposition in accordance with the suggestion of LICHTENBLRG and MEKPEL (1962). It seems that some kinds of antibodies are born inside those macrophages which suggest the presence of delayed hypersensitivity.

Hepatic lesions due to repeated infection were almost in accordance with the findings of SPRENT and ENGLISH (1958) who reported that marked local eosinophilia and granuloma were suggestive of repeated infection. FERNEX (1963) showed that helminth toxins cause hyperplasia of mast cells which contain histamine. The latter possess an eosinotactic power. Eosinophils contain antihistamine which detoxifies histamine produced by the injured mast cells (VAUGHN, 1953 and VERCAUTEREN, 1953). Exhausion of mast cells delays the formation of healthy scar tissue, since NAHAMARA (1960 a & b) stated that sensitized rabbits injected with embryonated eggs of *Ascaris* presented fibrosis after two months, while the non-sensitized ones developed fibrosis after 10 days of infection. It is evident in the present work that no extensive fibrosis was seen.

NAHAMARA (1959) found nodules of mononuclear cells and histiocytes in connective tissue of sensitized rabbits with eggs of *Ascaris lumbricoides*, and only eosinophils in the nonsensitized ones. This is in accordance with the present findings of a progressive increase in macrophages by repeated infection.

The reaction of pulmonary tissue to the migrating larvae was mainly focal granulomatous, but the liver presented focal necrosis and eosinophilic infiltration. Explanation of such variance may be based upon hypersensitivity which could be of the delayed type in lungs and the immediate one in liver. The presence of numerous eosinophils and necrosis in the liver may suggest an immediate hypersensitivity beside the parasitic affection. The presence of both immediate and delayed hypersensitivities may co-exist in response to the same antigenic substance in the same animal (RAFFEL, 1961).

In conclusion, the pathogenesis of lesions caused by larvae of *Neoascaris vitulorum* in guinea pigs, is mostly based upon hypersensitivity, thus it may be worthwhile to carry out a further work using an extract of these larvae intraperitoneally to find whether the pulmonary and hepatic lesions would be of similar nature in the absence of the mechanical and toxic injuries of the migrating larvae.

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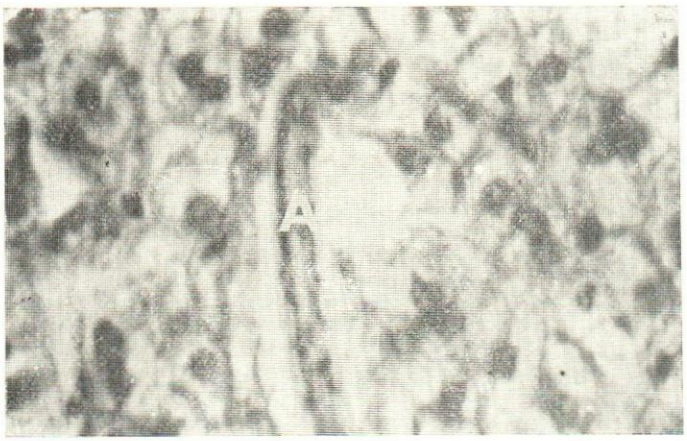

Fig. 1. Showing normal larva "A" among hepatic cells. Two infections H. and E. × 600.

Fig. 2. Showing normal larva "A" among hepatic cells. Five infections H. and E. × 600.

Fig. 3. Showing larva "A" surrounded with eosinophils "B" inside portal veins. Four infections H. and E. X 600.

Fig. 4. Showing larva "A" inside bile duct Four infections. H. and E. X 1000.

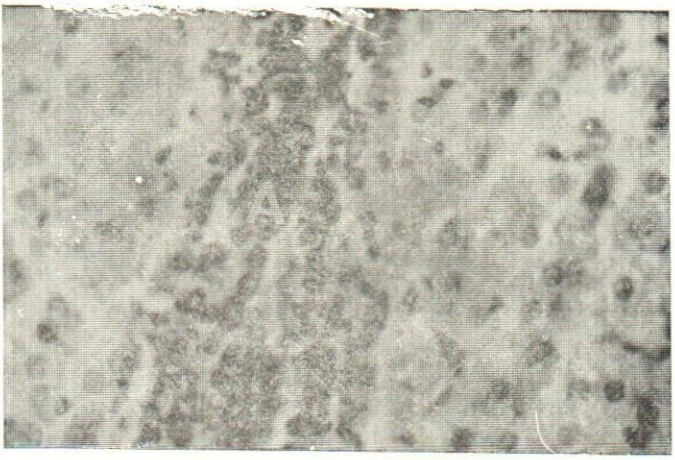

Fig. 5. Showing few eosinophils "A" inside porta tract. One infection. H. and E. × 600

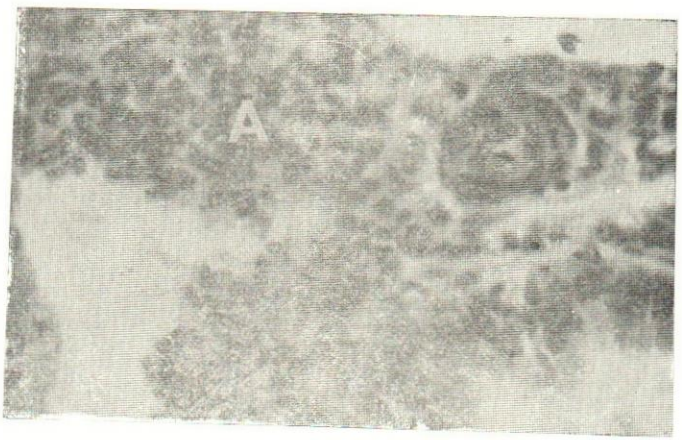

Fig 6. Showing numerous eosinophils "A" inside portal tract. Three infections. H. and E. × 600.

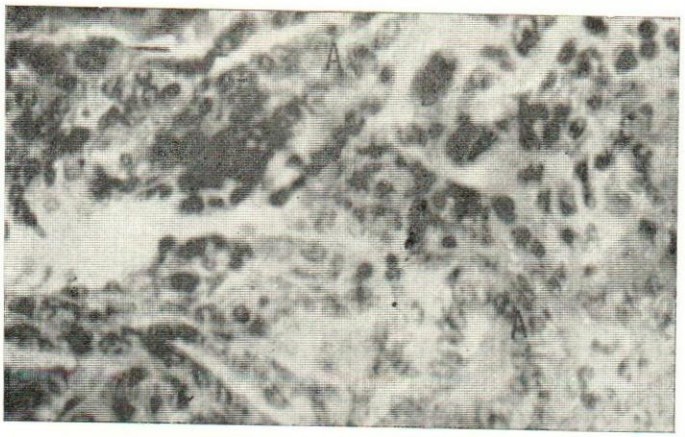

Fig. 7. Showing hyperplasis of bile canaliculi "A" which contain larvae "B" inside their lumina. Five infections. H. and E. × 600.

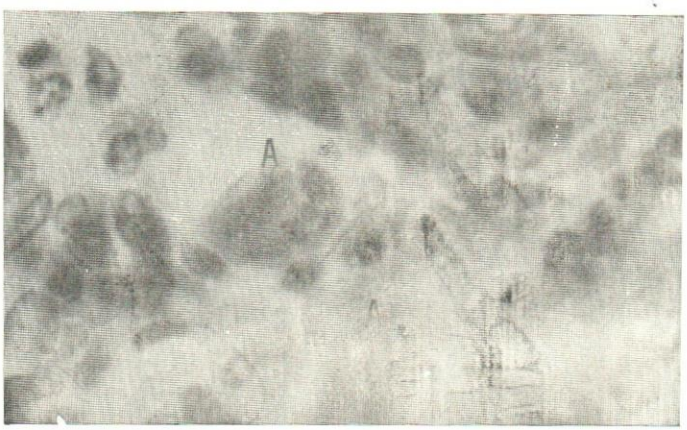

Fig. 8. Showing larvae "A" inside lumen of bile canaliculus. H. and E. × 1000. (Fig. 7 magnified).

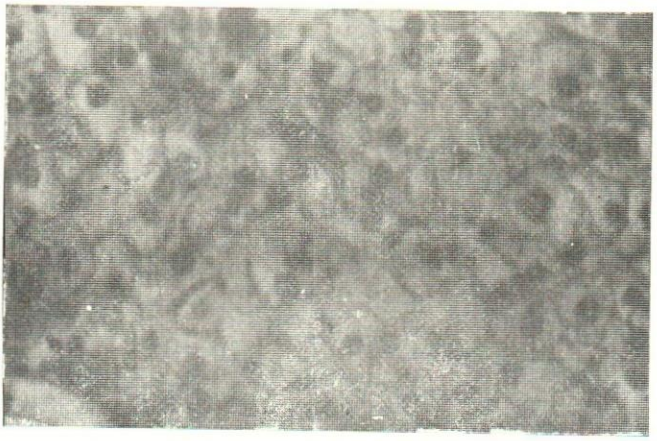

Fig. 9. Showing vacuolar and hydropic degeneration of hepatic cells. Three infections H. and E. × 600.

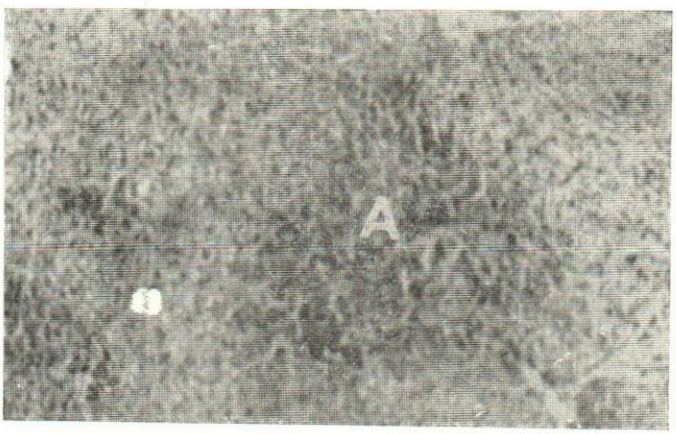

Fig. 10. Showing focal coagulative necrosis "A" of hepatic cells. Two infections. H and E. × 150.

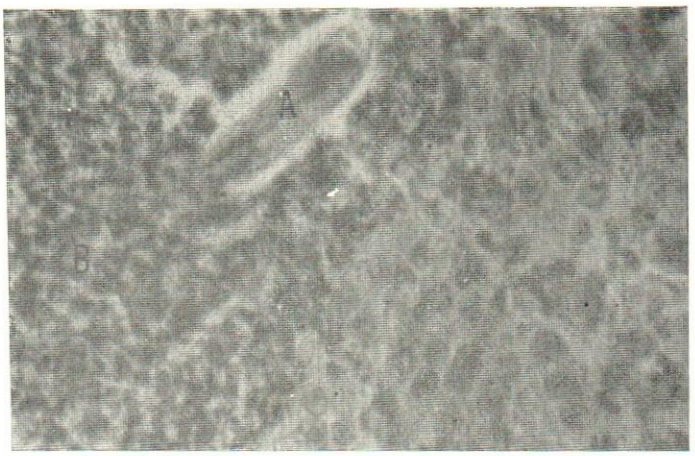

Fig. 11. Showing larva "A" among eosinophilic debris "B" in liver four infections. H. and E. 0× 80.

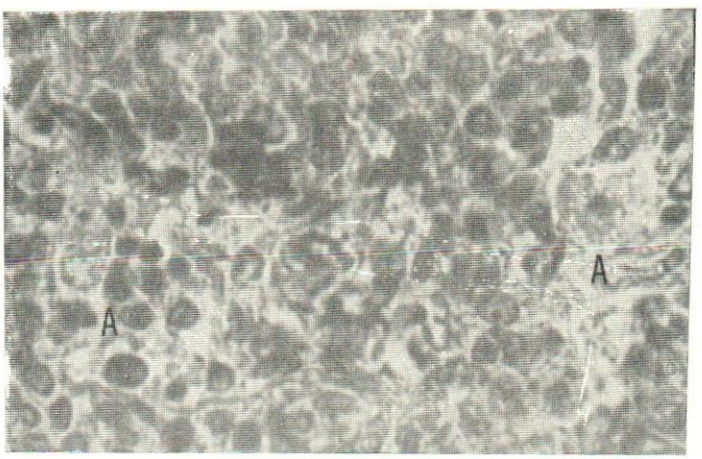

Fig. 12. Showing cytoplasmolysis "A" of hepatic cells. Two infections H. and E. × 800

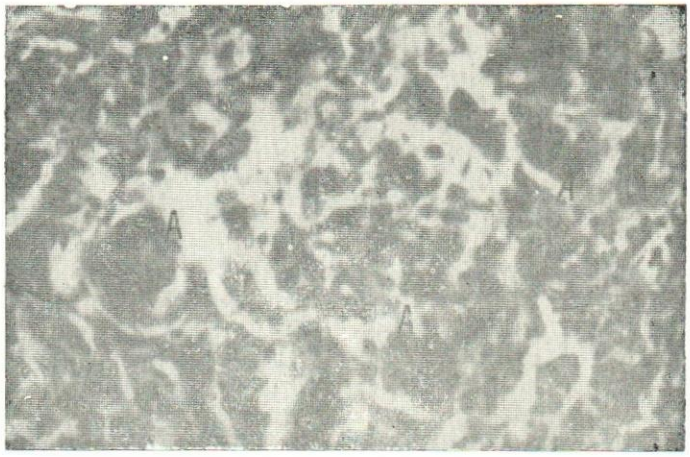

Fig. 13. Showing lungs with diffuse eosinophilic infiltration and a focal granuloma "A". Three infections H. and E. × 150.

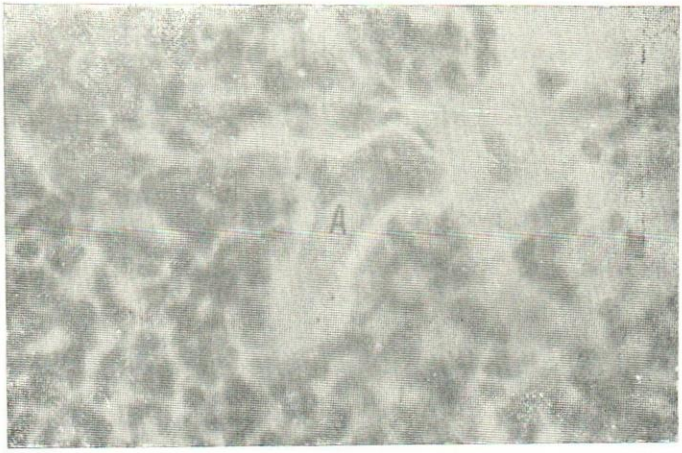

Fig. 14. Showing lungs with degenerated larvae "A" among eosinophils. Three infection H. and E. × 800.

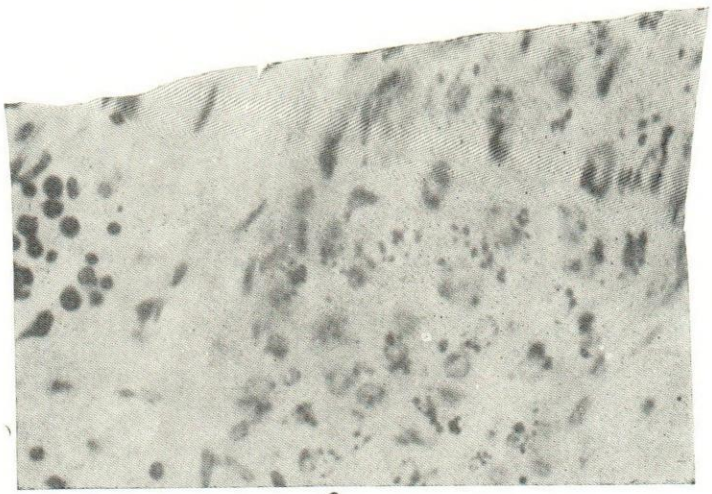

Fig. 15. Showing numerous fine brown pigments inside the chondrocytes of bronchioles. Three infections. H. and E. × 600.

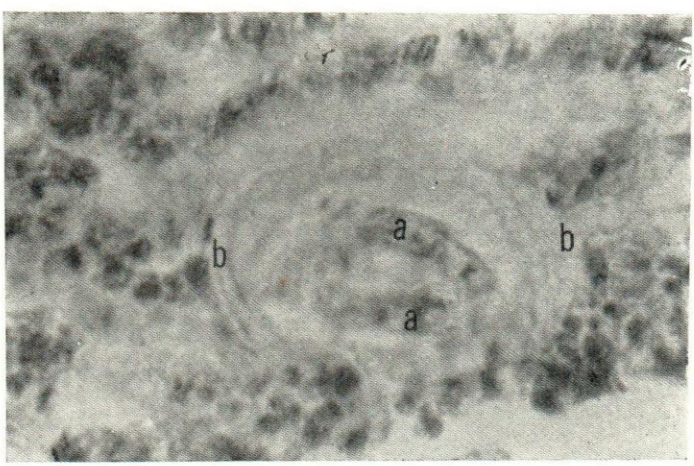

Fig. 16. Showing lungs with coiled larva "a" inside a thin hyalinized oval capsule "b" Four infections. H. and E. × 800.

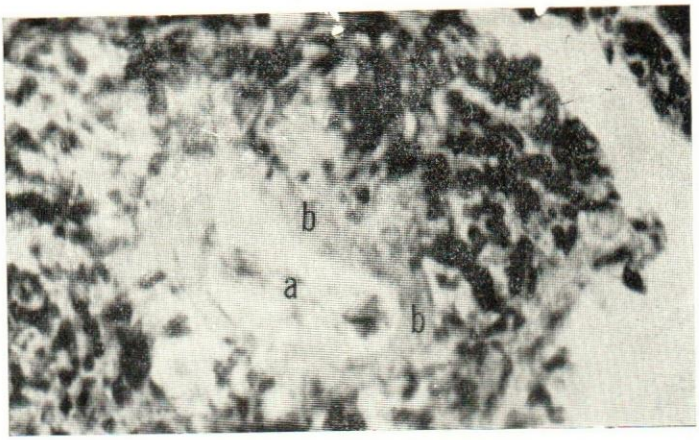

Fig. 17. Showing lungs with larva "a" surrounded by a thick hyalinized capsule "b" in the center of a granuloma. Four infections. H. and E. × 600.

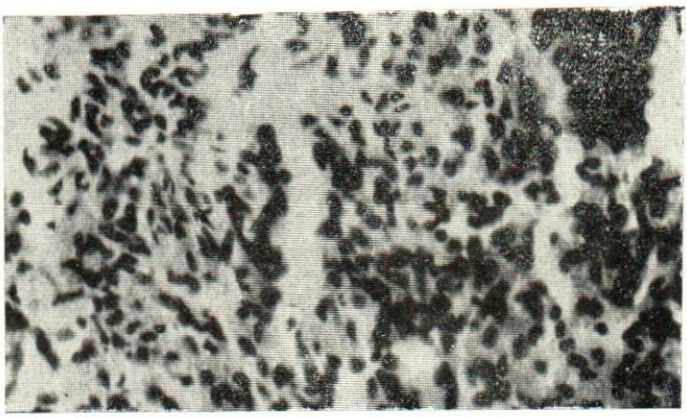

Fig. 18. Showing pulmonary tissue replaced with fibroblasts mixed with macrophages. Four infections. H. and E. × 600.

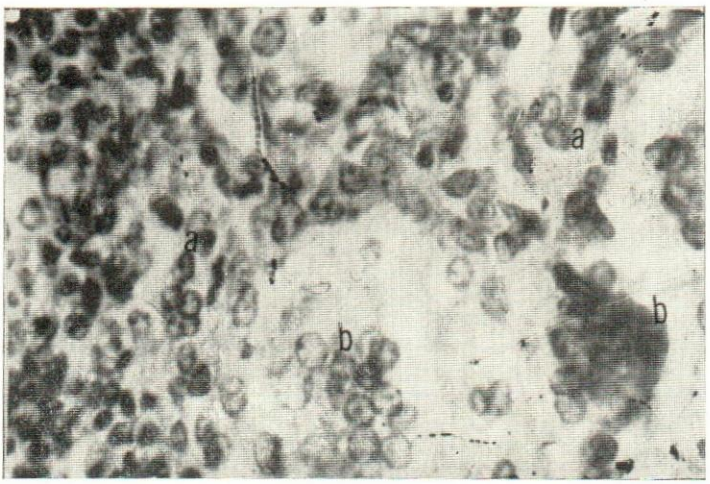

Fig. 19· Showing lungs containing granulomas which consits almost entirely of macrophages "a" and giant cells "b". Five infections. H.E. × 600.

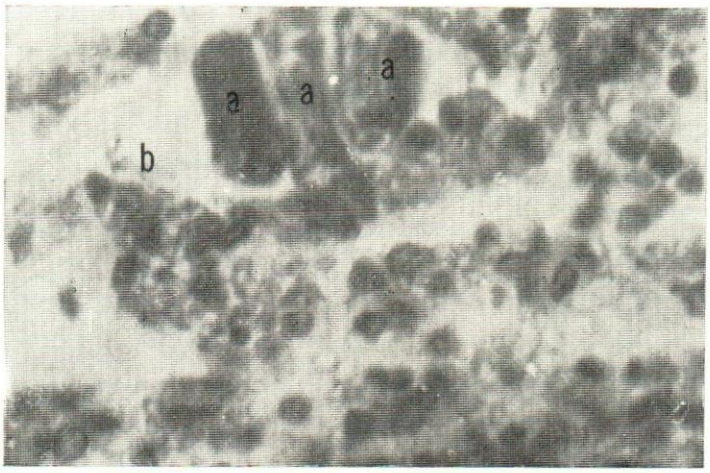

Fig. 20. Showing congested pulmonary tissue containing a coiled larva "a" inside an oval thin hyaline capsule "b". Five infections. H. and E. × 800.

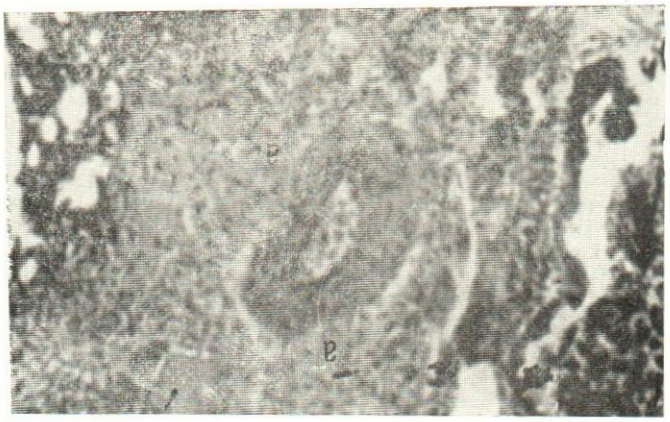

Fig. 21. Showing lungs with radiating hyperplasitc "a" adventitia of arterioles· Five infection. H. and E. × 150.

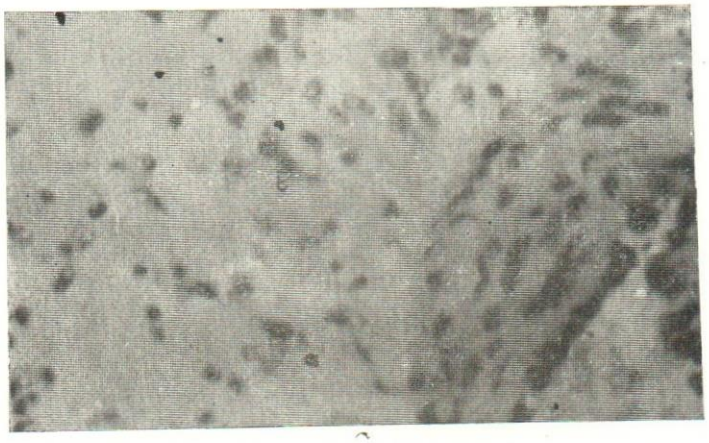

Fig. 22. Showing a higher magnification of Fig. 21 with hyperplastic adventitia "a" × 600.