Onderstepoort J. vet. Res. (1969), 36 (1), 105–128
Printed in the Repub. of S. Afr. by the Government Printer, Pretoria

STRONGYLIDOSES: DELAFONDIASIS IN THE ZEBRA

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ABSTRACT

McCULLY, R. M., KRUGER, S. P., BASSON, P. A., EBEDES, H. & VAN NIEKERK, J. W. Strongylidoses: Delafondiasis in the zebra. *Onderstepoort J. vet. Res.* 36 (1), 105–128.

Post mortem examinations on 125 zebras [*Equus burchelli* (Gray, 1824)] from the Kruger and Etosha National Parks revealed nodular and cystlike lesions of parasitic thrombophlebitis within the intrahepatic branches of the portal vein of most of the adults. These lesions contained either the larvae of the fifth stage of *Delafondia vulgaris* (Looss, 1900) Skrjabin, 1933. The lesion was usually a combination of thrombosis, its organization and host response to the parasite. The fifth stage specimens were larger than the sexually mature *D. vulgaris* present in the caecum and ventral colon. Though larger, none of the females in the liver contained ova in their uteri.

Somewhat similar lesions due to fifth stage *D. vulgaris* were rarely observed in the pulmonary artery. Fourth stage larvae and the fifth stage of the parasite were found in the anterior mesenteric arteries and their branches of many of the zebras. Though enlarged and having thickened walls, the lumens were narrowed and none of the arteries appeared to have true aneurysms.

These findings in zebras lend support to the contention of a previous investigator that it is the time factor and not the environment of the larva that determines its moults. It was obvious that some of the larvae of *D. vulgaris* migrate into the liver. Whether it is in the course of a normal migratory pattern or an aberrrant one was not determined. Those which become trapped in the lesions are at a dead end. The authors refer to the disease by the derivative from the generic name of the nematode, viz. " Delafondiasis ".

INTRODUCTION

Various strongylidoses of domestic solipeds have been dealt with in a voluminous literature but similar coverage for zebras is lacking. Delafondiasis, a form of strongylidosis, is a disease caused by larval stages of the genus *Delafondia* (Railliet, 1923) Skrjabin, 1933. The observation of developmental stages of *Delafondia vulgaris* (Looss, 1900) Skrjabin, 1933 [syn. *Strongylus vulgaris* (Looss, 1900)] within vascular lesions of the livers of zebra [*Equus burchelli* (Gray, 1924)] which were necropsied in the Kruger National Park led to further research of this entity. Certain differences in the distribution of the developing stages of the parasite in the zebra as compared to domestic equidae appear to be worthy of documentation along with an account of the pathogenesis of the vascular lesions they cause.

Railliet (1923) divided the genus *Strongylus* Mueller, 1780 into the subgenera *Strongylus* (Goeze, 1782), *Decrusia* Lane, 1914, *Alfortia* and *Delafondia*. Subsequently Skrjabin (1933; according to Skrjabin, Shikhobalova, Schulz, Popova, Boev & Delyamure, 1952) divided it into three genera, *Strongylus* Mueller, 1780; *Alfortia* (Railliet, 1923) and *Delafondia* (Railliet, 1923) on the presence, number and position of the teeth in the buccal capsule and on the degree of development of the genital cone. According to this classification the genus *Strongylus* has two subventral and two subdorsal teeth at the base of the buccal capsule and has a well developed genital cone. Consequently *Strongylus vulgaris* (Looss, 1900) and *Strongylus asini* Boulenger, 1920, which have two subdorsal teeth and a poorly developed genital cone, are removed from the genus *Strongylus* and placed in *Delafondia*.

Authors are divided on the validity of these two classifications. Yorke & Maplestone (1926), Baylis & Daubney (1926), Mönnig (1928) and Neveu-Lemaire (1936) followed the subgeneric classification of Railliet (1923). According to Skrjabin *et al.* (1952) both Ershov (1943) and Popova (1952), adopt the generic division proposed by Skrjabin (1933); Yamaguti (1961) also adopts the latter classification.

There has been considerable difference of opinion over the migratory route of the larvae which cause extensive lesions in the anterior mesenteric artery and its branches in domestic equidae. Discussions of the four main theories on the routes of larval migration (Poynter, 1960; Soulsby, 1965) will not be repeated.

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Received for publication on 2 December 1968.—Editor

Farrelly (1954) considered that the larvae moult at specific times, which are related to the age of the larvae, and that it occurs irrespective of their environment.

It is believed that the present account of the findings in zebra contributes to the knowledge of the pathogenic versatility of this worm. This report will also document the pathogenicity of these larvae within the intrahepatic branches of the portal vein and the pulmonary artery, as it appears that previously such lesions have not been associated specifically with the larvae. Evidence that many of the larvae of *D. vulgaris* in zebra enter the liver will be presented.

Materials and Methods

Necropsies were performed on 14 zebra in the Etosha National Park in South West Africa and 11 in the Kruger National Park, Transvaal. Tissues were collected and preserved in 10 per cent buffered formalin. Sections of 5 to 6 microns in thickness for histopathological examination were prepared in a routine manner using paraffin embedding, a sliding microtome and a hematoxylin and eosin staining technique.

Sections of liver were available from all cases and in eight it was the only tissue examined. In some cases, where a variety of macroscopic lesions were present, sections were prepared from as many as twenty blocks of liver. The number of other tissues taken varied considerably from animal to animal. Lung blocks with known or suspected macroscopic changes only were sectioned. The parasitic specimens found in association with the vascular lesions and others from the colon and caecum were either collected and placed directly in 10 per cent formalin or first killed with hot water, then measured in their relaxed state and eventually preserved in 10 per cent formalin. The age of the zebra ranged from a young colt to aged adults.

Further observation at both parks on an additional large number of zebras (in excess of 100) which were shot for one reason or another indicated that, with few exceptions, the lesions in the intrahepatic portion of the portal vein were consistently present in the adult animals. Two young foals were examined but were found to be negative for such lesions.

Results

Macroscopic findings

There was mild ascites in some cases but severe ascites was not observed. Lesions associated with fourth stage larvae and the fifth stage *D. vulgaris* were predominantly in the liver and anterior mesenteric artery and its branches. Because of the greater interest in the hepatic lesions, the collection of tissue and the amount of attention given were heavily biased in favour of this organ.

Liver: Apart from the localized lesions which altered the external appearance, the livers were all of normal size and colour. On the surface *Delafondia* lesions consisted of localized, slightly elevated, opaque, white to yellowish-brown areas which were 2 to 3 cm in diameter [Plate 1 (1)]. The most common, more deeply seated lesions which could not be seen from the surface but which could be palpated as hard areas were widely and unevenly distributed throughout the liver. Those on the surface were the largest, being on cut surface up to 4 cm in diameter and of somewhat cystic nature. Some appeared multilocular [Plate 1 (2)]. The cavities contained a thick reddish-brown or darker chocolate coloured substance in which some fourth stage larvae, but mainly fifth stage *D. vulgaris* were embedded. Such lesions were invariably within the intrahepatic branches of the portal vein, the surface lesions being in branches located more superficially. Their pattern of distribution was most apparent when this vein and its branches were opened with a sharp pointed blade starting at its entrance at the portal fissure. The first and largest portion of the portal vein was not affected in any of the cases but the first branches, the interlobar veins, were sometimes involved. The interlobular veins which were located from 2 to 10 cm from the sharp edges of the liver were most frequently involved [Plate 1 (7)]. The number of lesions varied widely from a solitary nodule to approximately 50 nodules.

The nature of the lesions varied considerably. Most were of a rather chronic nature and affected the lumen and wall of veins to various degrees. Some consisted of a dilated cystlike portion of a branch of the portal vein. On close examination after careful dissection they were often found to be of considerably greater diameter than the immediately proximal and distal portions of the vein [Plate 1 (5 and 6)]. The walls of some were thick and fibrous but others were thin. Many had a single, large, fluid-filled cavity which contained remnants of dead parasites while others consisted of several small cavities filled with similar thick brown fluid and parasites. Some of the less chronic lesions contained viable fifth stage *D. vulgaris* approximately 2·5 cm long. Smaller forms were only rarely found. It was most common to find a single parasite in a nodule but occasionally two were found. There were some live fifth stage worms surrounded by white fibrous tissue which appeared to occlude the lumen of the veins completely [Plate 1 (4)]. Associated with many of the parasitic lesions in the veins there were recognizable thrombi, apparently of various ages and ranging in colour from dark red to slightly yellowish brown. Some extended along the course of the veins for several centimetres. Thrombi in the absence of the parasite were occasionally present in smaller veins of some cases and they were best seen in transverse section on the cut surface of the liver. A reddened inflamed intima was the only change observed in some larger branches.

Whether some of the larger more chronic lesions [Plate 1 (5)] should be considered as aneurysms (= varices) is debatable. It was obvious that the dilatation was due to the expanding mass of host response in the lumen, which it occluded, and which was gradually but completely replacing the original wall.

Other lesions which contained fifth stage *D. vulgaris* appeared to involve only one part of the vein wall. They had a smooth endothelial surface and it appeared as though the parasite had invaded the wall [Plate 2 (8)]. On section however, it was

apparent that the parasites were in organized thrombi [Plate 2 (9)]. At other sites, fresh thrombi were attached. The protrusion of these lesions into the lumens [Plate 1 (3)] partially obstructed them. Small subintimal tracts were observed in the portal vein of one zebra. Involvement of the hepatic veins and/or arteries by lesions such as described above was never observed.

Many livers of the cases from the Kruger National Park contained hydatid cysts [Plate 4 (19)]. Some were very large, containing as much as 1000 ml of fluid and thousands of scoleces. There were a few, however, which were quite small and their size occasionally fell within the range of the lesions caused by *D. vulgaris*. Since they were sometimes situated near the serosal surface difficulty was experienced initially in differentiating between the hydatid and delafondia lesions. The hydatid cysts, however, were not in the branches of the portal vein. They were usually mineralized and contained moist, chalky material and necrotic remnants of the hyaline membrane.

A few of the livers contained numerous small white foci which were easily seen subserosally and on the cut surfaces. They appeared to be either micro-abscesses, small granulomas or foci of disseminated hepatitis. Histologically, some of them proved to be granulomas due to schistosome ova but some were focal areas of hepatitis frequently involving the portal areas.

Lungs: The exterior of the lungs appeared normal. These organs were carefully palpated in 15 cases and in three of them a solitary lesion of a cyst-like nature was present in a small branch of the pulmonary artery. They were approximately 2 cm in diameter and were located a few centimetres from the sharp border of the organ. When incised they closely resembled lesions in the branches of the portal vein and two of them each contained a single fifth stage *D. vulgaris*. The third lesion was of a similar nature but a parasite was absent.

Anterior mesenteric artery and branches: These were carefully examined in 15 zebras, four in Etosha and 11 in the Kruger National Park. Fourth stage larvae and/or fifth stage *D. vulgaris* and associated lesions were present on and in the intima of the anterior mesenteric artery [Plate 2 (13) and Plate 3 (14)], as well as in one or more of its branches of all the zebras examined in the Kruger National Park. The combination of thrombosis and intimal reaction caused the lumen of these arteries to be narrowed. Most of the parasites, even the fifth stage, were smaller than those seen in the liver. The thrombotic material was in different stages of organization. None of these arteries were extremely enlarged by either bulbous dilatation or aneurysms. The walls of the vessels were somewhat thickened, however, in the vicinity of the thrombi. Minute intimal streaks or tracts presumably due to larval migrations were observed in the aorta of one case near the origin of the anterior mesenteric artery [Plate 2 (12)]. Similar signs were present in some of the anterior mesenteric arteries and their branches. None of the four zebras so examined in the Etosha National Park exhibited arterial lesions.

Many adult strongyles of various species including some *D. vulgaris* were present in the caecum and colon. There were a few reddish, elevated, subserosal lesions identical to *haemomelasma ilei* which have been seen in domestic horses and which are due to strongyle larvae. Similar lesions in the mesentery adjacent to intestinal veins also proved to be caused by strongyle larvae. Larvae were not obtained from either of these types of lesions for the identification of the species. Pieces of strongyle larvae, however, were present in the sections pepared for histopathologic examination.

Findings of helminthological studies

Delafondia vulgaris adults which were found in the caecum and ventral colon varied in length, the females being from 19 to 22 mm and the males from 15 to 16 mm. The measurements of both sexes therefore coincide with those given by G. Theiler (1923). Of these adults 8 per cent were males and the remainder females. The females had eggs in their uteri.

Predominantly fifth stage *D. vulgaris* were found in the parasitic lesions of the liver. In one case there were two fourth stage larvae in addition to numerous fifth stage specimens. The fifth stage male parasites ranged in length from 12 to 38 mm but the majority measured from 30 to 38 mm. The male specimens from the liver measuring less than 20 mm appeared to be rather young, considering the poor sclerotization of the male copulatory organs.

The majority of the fifth stage males in the liver were larger than the adult males and females in the caecum and colon. Fifth stage females in the liver were even larger than the males, the range being from 15 to 42 mm with the majority ranging between 30 to 42 mm. No eggs were present in their uteri.

In the livers the ratio of the male to female fifth stage *D. vulgaris* varied from animal to animal. Either sex predominated in one or other zebra. Though it was more common to find only one specimen in a parasitic nodule, two were sometimes found. In at least one nodule both a male and a female fifth stage *D. vulgaris* specimen were present but in spite of this cohabitation no ova occurred in the uterus of the female. The only two forth stage larvae recovered measured 18 mm and 21 mm for the male and female respectively. They were thus also as large as most of the adult male and female *D. vulgaris* found in the caecum and colon.

Although *D. asini* was described from the liver of a donkey in Zanzibar (Boulenger, 1920), the specimens from the liver of the zebras differed. They had the rounded edge of the teeth of *D. vulgaris* and were not divided into the rounded cusps of *D. asini*. No *D. asini* were found in the caecum or colon of the zebra, nor were there specimens, either mature or immature, which showed the identical morphologic features of those of the liver. The teeth of adult *D. vulgaris* in the caecum and colon were morphologically similar to those described in the literature. The fifth stage specimens in the liver differed in that the teeth were more restricted to the base of the buccal capsule and the worms were considerably longer

than the adults. This abnormal morphology may be due either to the effect of the host or the organ from which the worms were recovered.

Larvae in the fourth stage resembled those illustrated by Soulsby (1965). Mainly fourth stage larvae were found in the anterior mesenteric artery and/or its branches. Some were moulting to the fifth stage [Plate 3 (15)]. The measurements for the fourth stage males were 11 to 12 mm and for the females 11 to 17 mm. The fourth stage moults measured 16 to 20 mm for females and 14 to 19 mm for the males but the enclosed fifth stage specimens measured only 12 to 15 mm for the female and 10 to 12 mm for the male. The majority of fourth stage larvae were female, there being at least ten females to every male. This ratio coincides with that encountered in adults in the caecum and colon.

All the specimens collected from the liver, the anterior mesenteric artery, the caecum and colon are therefore classified under the name *Delafondia vulgaris*. If, however, it is later proved that the specimens from the liver coincide morphologically with fully mature specimens from the caecum or colon from the zebra, the authors will accept that two different species exist in the material they examined.

Microscopic findings

Liver: The response to the parasites was primarily within the branches of the portal vein, but the portal areas and other portions of parenchyma were also occasionally affected. One larva was present in the parenchyma with no host response [Plate 4 (20)]. Within several hepatic lobules of one zebra there were foci which appeared as necrotic areas, presumably migratory tracts, filled with intensely eosinophilic amorphous detritus [Plate 4 (21)]. A zone of epithelioid cells immediately surrounded them. In the centre of some of these tracts structures which were interpreted as the remnants of cast cuticles of migrating fourth stage strongyle larvae [Plate 4 (22)] were also encircled by a zone of granulomatous inflammation. Outside the zone of granulomatous response and extending into the surrounding portions of the liver lobules, an intense though localized hepatitis sometimes completely replaced the hepatic cord cells. The reaction appeared to be subacute, consisting predominantly of eosinophiles and numerous small round cells interspersed between cords of atrophied liver cells [Plate 4 (23)]. Although complete lobules were sometimes replaced by this inflammatory process, others were only partially affected, and the latter most frequently occurred near the portal areas.

In severely affected portal areas bile duct reduplication and proliferation of small blood vessels were present. Adjacent to some of the portal areas lesions of a very chronic nature were noticed. They consisted of circumscribed areas of partially mineralized, necrotic detritus surrounded by a capsule of dense collagenous tissue which in turn was surrounded by a zone of less dense, younger connective tissue with round cells interspersed between strands of immature collagen. These lesions were interpreted as being at a site where a migrating fourth stage larva was previously entrapped. In some portal areas, at a distance from the migratory tracts and adjudged to be of fairly recent origin, indications of involvement of a slightly milder nature occurred. In these areas there were numerous eosinophiles but few round cells. Acute endophlebitis was present in smaller branches of the interlobular veins. The endophlebitis was characterized by an oedematous more cellular, thickened intima [Plate 4 (24)] which in some veins projected into the lumen as villous polypoid proliferations covered by hyperplastic endothelium. The proliferating villi had eosinophiles on their surface as well as within their substance. Eosinophiles were also present in the adventitia of the veins. In sections which did not contain evidence of migratory tracts, the earliest change was an endophlebitis of the portal veins of similar nature.

In many of the lesions the exudate had not been completely confined by the vessel wall and/or capsule and thus inflammatory cells were present in the surrounding parenchyma. Numerous eosinophiles and small round cells had infiltrated and appeared to be crowding out the liver cord cells which were small and appeared atrophic. Necrosis of liver cord cells in irregular patterns was frequently present in the lobules adjacent to the involved branch of the portal vein. Several of these foci were confluent because they were connected by such an extension of the exudate. In the centre of the primary lesion in the vein from which these encircling lesions arose, remnants of the dead parasite were frequently seen. Such involvement of adjacent parenchyma was relatively rare compared to the constancy with which lesions were present in the veins. Although not common, areas of necrosis which were interpreted as infarction were occasionally seen adjacent to recent thrombi.

In the affected veins thrombosis was the next thing observed following the oedema, endothelial proliferation and infiltration of the intima by eosinophiles. None of the liver sections contained an extremely early vascular lesion with a parasite in the lumen surrounded by a very recently formed thrombus or a larva beneath the endothelium in the absence of an extensive host reaction. The small tracts observed macroscopically in the portal vein proved to be elevations of the intima with accumulations of leucocytes beneath the endothelium [Plate 4 (25)]. Similar cells were present in the underlying media. Although every phase was not available in this material, one can assume that after the initial irritation to the vein wall by the parasite, thrombosis occurred [Plate 5 (26)] and, apparently in most instances, the parasite was entrapped. In many of the more advanced lesions, cross sections of the parasite, either viable or non-viable, were present along with an extensive reaction [Plate 2 (11)]. The changes present from this stage onwards in the pathogenesis of the lesion varied depending upon the age of the lesion. Not only was there an attempt to organize the thrombus, but at the same time the effect of the parasite itself was apparent. The reaction thus was not a simple organization of a thrombus, but the process of organization modified by the toxic effect of, initially the live, and subsequently the dead, disintegrating parasite.

A wide range of changes was therefore observed in certain vessels. Some contained mural thrombi with changes present in only one segment of the circumference of the lumen; others contained occluding thrombi affecting the entire lumen and intima. The changes in the mural thrombi were essentially the same as in the occluding type except for their segmental nature. There was usually an intense proliferation from the wall of the vessel resulting in a fibrovascular tissue with numerous histiocytes, or in other words, a type of granulation tissue. Surrounding the parasite, presumably in response to the parasitic material, were varying numbers of eosinophiles mixed with other types of inflammatory cells. This response changed considerably with the lapse of time, and in the process the original thrombus was organized although a mass of necrotic cells frequently persisted.

Subsequent changes which took place seemed to be more influenced by the parasitic material [Plate 5 (27)] than by the thrombus. The initial process of thrombus organization changed into an inflammatory process aimed at encircling, destroying, encapsulating and removing the parasite. The parasitic material attracted large numbers of eosinophiles, most of which became necrotic, eventually contributing to the enlarging eosinophilic centre of necrotic, amorphous debris within the lumen of the vein. The histiocytic cells in the response from the vessel wall often appeared as macrophages filled with phagocytosed necrotic material which was either foamy-appearing or composed of nuclear debris. Some of the histiocytes became epithelioid in appearance often lining up in pallisades immediately around the eosinophilic necrotic material in the centre. Multinucleated giant cells of foreign body type were sometimes present in such reactions [Plate 5 (30)]. After the death of the entrapped parasites, they stained poorly and gradually lost most of the recognizable characteristics of a worm, the remnants of the cuticle frequently being the last remaining vestige or outline of the parasite to be seen in the centre of the intense host reaction [Plate 5 (28)]. The central portion of these lesions became progressively larger due to necrosis along its periphery [Plate 5 (29)]. Frequently because of this reaction, the wall of the vein was gradually destroyed, the smooth muscle eventually being replaced by the fibrovascular tissue with the exception of odd remnants of muscle here and there. This process obviously accounted for the cystic appearance of the affected veins observed at the post mortem examination [Plate 1 (5)]. In some veins small quantities of the necrotic eosinophilic contents escaped into the wall [Plate 7 (37)]. Subsequently as the pressure and possibly the seepage of toxins from the centre became less pronounced or the supply of the latter became exhausted, a capsule of dense connective tissue was formed by the maturing fibrovascular tissue [Plate 6 (32)]. In and around this cicatrization there were considerable numbers of macrophages containing haemosiderin [Plate 6 (33, 34 and 35)]. The centres became amorphous masses of material which either liquefied [Plate 6 (31)] or became inspissated [Plate 6 (35)]. Cholesterol clefts were sometimes present [Plate 7 (36)]. A few small branches of the portal veins, probably interlobular veins, were occluded by partially mineralized masses. These were interpreted as representing the end result of reactions to entrapped migrating larvae which were dispensed with by a minimum of host response. Others contained organized thrombi but no parasites [Plate 7 (38)].

The degree of changes involving the wall of the affected vein was dependent on the extent to which the luminal surface of the intima was occupied by the base of the primary lesion. Therefore, in some vessels three-fourths of the luminal surface was free of the extensive alterations which affected the entire inner circumference of others [Plate 7 (39)]. However, some mild changes involving the intima at a distance from the largest lesion were present in many veins. These consisted of mild endophlebitis with various degrees of proliferative and infiltrative phenomena [Plate 7 (40)]. A very rare change consisted of a marked intimal proliferation of a villous nature [Plate 7 (41)]. Some of the polypoid projections were of broad clublike type.

Another aspect of the vascular lesions in a few of the cases that showed early stages must be mentioned. There was a proliferative intimal change with protrusions into the lumen. The intimal protrusions were heavily infiltrated with eosinophiles. Unfortunately some of the cases from the Kruger National Park in which this was observed were also lightly infested with *Schistosoma mattheei* Veglia & le Roux, 1929. Other cases from Etosha which did not show evidence of bilharziasis exhibited similar lesions.

Lungs: The lesions in the lung of two zebras in which fifth stage *D. vulgaris* were identified, involved small branches of the pulmonary artery and the surrounding alveoli. The lesions were primarily of a chronic nature being most severe in the artery and destroying the wall to such an extent that it was sometimes difficult to recognize [Plate 2 (10)]. Elastic fibres arranged in an undulating pattern could occasionally be found which were used as a point of identification. From this point in the intima toward the lumen, a granulomatous reaction similar to that in the portal veins of the liver occurred. Numerous epithelioid cells, some with confluency of their cytoplasm forming giant cells and others with foamy cytoplasm, were present. In the lumen eosinophilic proteinaceous fluid was mixed with blood. The parasites were removed for identification and were thus absent from the sections. Some haemosiderin-containing macrophages were within the remains of the vessel wall, and beyond its limits a thick zone of connective tissue was permeated with similar pigment-containing cells. The wall was sometimes very indistinct on one side because complete destruction had occurred resulting in an extension of the reaction into surrounding alveoli. This consisted of eosinophilic fluid freely sprinkled with eosinophiles and epithelioid cells. In a number of the smaller branches of the pulmonary artery there was evidence of an endarteritis [Plate 8 (42)].

Anterior mesenteric artery and branches: Lesions affecting these arteries varied from leucocytic infiltrates of the intima [Plate 8 (43)] and rather minor

intimal, fibrinous tracts [Plate 8 (44)] to the proliferative reactions which, combined with thrombosis, completely filled the lumen. Deeper layers of the wall were also affected to a variable degree.

Early lesions observed also consisted of tiny foci which represented a cross section of the streaks on the intima observed macroscopically [Plate 8 (45)]. Some were composed of eosinophilic fibrinous material and accumulations of leucocytes of mixed types. This lesion progressed as a result of the reaction of the intima which sometimes succeeded in the envelopment of the larvae. Hyperplasia of the endothelium with intensive focal accumulations was observed. From the limited number of sections of these early lesions which were studied, it was not possible to establish if the larvae actually penetrated the endothelium or were incorporated into the intima by the proliferation of the endothelium. Some remnants of the cast sheaths of larvae were present in small subendothelial elevations containing fibrin and a few leucocytes [Plate 8 (46 and 48)]. Larger lesions were of somewhat similar nature, but there were additional changes on the endothelial surface as well as in the underlying intima and media. On the surface, thrombi [Plate 8 (47 and 50)] and frequently larvae or cast sheaths [Plate 8 (49)] were present and a zone of accumulated leucocytes just beneath or upon the endothelium was occasionally seen. The media eventually also became very cellular because of increased vascularization and the accumulation of, predominantly, mononuclear cells from the blood and tissue histiocytes. Nearer the intima there were more leucocytes from the circulating blood; many were eosinophiles. This reaction gradually blended with the intimal reaction.

In the more advanced lesions the intima was prominently affected. The lumen of the vessel was greatly stenosed by a combination of the thickened proliferative intima and the accumulation of thrombus on the intimal surface [Plate 3 (16, 17 and 18)]. Remnants of larvae, some of which were mineralized, were found in the thrombus or within the intimal proliferation [Plate 3 (18)]. Cross sections of viable larvae were observed in more recent thrombi. Immediately surrounding the larvae that were not disintegrated, thick collars of eosinophiles, neutrophiles and other leucocytes were also found. Some small round cells frequently accumulated in the media, especially along the *vasa vasora* penetrating to the intima, but the media was never as severely affected as the adventitia. The adventitia was thickened due to an increase in fibro-vascular tissue and a heavy infiltration of mononuclear cells, most of which were of histiocytic origin.

Discussion

The reaction in the branches of the portal vein in the liver is a combination of the response of the vessel wall to the presence of the entrapped parasite and the organization of the thrombus. The response to the parasite is no doubt influenced by a number of factors including the sensitivity of the host because of previous infestation and subsequent periodic reinfestations. Toxic substances from the parasite must also be considered in view of the extensive necrosis present around the parasite and extending into the liver lobules from some of the severely involved portal veins.

Comparisons of these vascular changes with those due to some intravascular parasites are interesting. Although this study was somewhat deficient in examples of the very early stages and consisted primarily of cases with chronic changes, it gave some insight into the pathogenesis of the lesions. The impression was gained that the early proliferative intimal changes were perhaps of a very transient nature soon surplanted by thrombosis, entrapment of, and response to the parasite together with organization of the thrombus. Somewhat similar stages of proliferative endovasculitis, but of far greater magnitude, are of considerable prominence in certain intravascular parasitisms, i.e. dirofilariasis as affecting the pulmonary artery of the dog (Adcock, 1961) and fox (Hirth & Nielsen, 1966); bilharziasis of the hippopotamus (McCully, Van Niekerk & Kruger, 1967); cordophilosis of the pulmonary artery of various antelope (McCully, Van Niekerk & Basson, 1967); and to a lesser extent bilharziasis of domestic ruminants (McCully, 1966). Furthermore, whereas thrombosis is rare in response to the live parasites in the diseases just mentioned, it is very common with delafondiasis. This seems to indicate that damage to the intima in delafondiasis is considerably more severe than in the abovementioned diseases. This may be readily explained by the fact that these other parasites do not attempt to penetrate the intima, quite obviously because they are vascular parasites in the true sense. *D. vulgaris* attempts to penetrate at certain stages because it is an intestinal parasite and it must penetrate in order to leave the vessel and reach the lumen of the intestine. One can therefore assume that the attempt to penetrate the intima triggers the formation of the thrombi in which so many times the parasite is entrapped.

Some of the lesions in the liver parenchyma, which appeared to be caused by migrating larvae may have been due to those of other species of strongyles since specific identification was not possible.

It is apparent that the controversy over the possible migratory routes of *D. vulgaris* larvae in the horse will continue until more critical experiments have solved the problem. Our conclusions and discussion therefore must be prefaced by placing emphasis on the fact that the present observations refer only to *D. vulgaris* in the zebra and not to other equidae as we have not yet studied lesions due to this parasite in horses, mules or donkeys.

Based on the evidence presented here, it can be stated unequivocally that in virtually 100 per cent of the adult zebra in both Etosha and Kruger National Parks the branches of the portal veins of the liver are frequent sites of fifth stage *D. vulgaris;* that fourth stage larvae may also be found and that fifth stage specimens may occasionally be present in the lung. There was no way of determining how many, if any, pass through but it is obvious that larvae of this parasite frequently enter the liver in the zebra. The question arises as to whether or not it is an aberrant route. This is no simple problem and it will not be answered by conjecture alone so perhaps it will

suffice to state that if it is an aberrant route it is quite common, being used to some extent in all the zebras examined. The high incidence of these lesions in zebra and the apparently low incidence of similar lesions in the horse are interesting. In future such lesions should be looked for by those pathologists who do large numbers of equine necropsies. The possibility that some of the parasitic nodules which have previously been seen in the liver and described in precise detail (A. Theiler, 1920) were actually *Delafondia* lesions affecting the branches of portal veins must not be ignored. The description by Cohrs (1967) of a nematodal parasitic nodule in the liver likewise appears to be essentially of the same process observed in this study except that it contains no mention of the involvement either of the walls or of the lumens of veins. The possibility that such lesions affecting the intrahepatic branches of the portal veins were frequently overlooked in the past seems to be most improbable.

One may therefore assume that if the vascular lesion occurs in the horse at all it must be either very small or quite rare. This permits some interesting speculation as to the reasons for the large lesions in branches of the portal vein of the zebra and their apparent absence in the horse. The degree of parasitism in zebras was judged to be extreme and more severe than in an average horse. It follows that the larger the number of migrating larvae, the greater the probability that various routes, either the usual or aberrant ones, would be used. In support of this view the finding of fewer lesions per liver in the zebra of the Etosha National Park stands out prominently. This is probably related to a lower level of infestation in this area because of less favourable microclimatic conditions. The Kruger National Park is known to have a higher humidity, more open waters including rivers, a more dense vegetation and a better aerated topsoil with lower salinity. Another interesting feature about the heavily infested zebras in the Kruger National Park was that most of them had lesions in the anterior mesenteric artery and/or its branches. Of those four specifically examined in Etosha for such lesions, none were positive. This may also be related to the low level of parasitism by *D. vulgaris* as compared to those in the Kruger National Park.

Besides the level of infestation, other factors which could play a role should be considered. One of these is the host-parasite relationship. This worm appears to be better adapted to the horse than it is to the zebra. It can be postulated that zebras are not the normal hosts and their first experience of the parasite was when horses were introduced into Africa. This of course cannot be proved although comparative life cycle studies in the two equine species would support or reject this postulate.

The present findings in the zebra seem to lend support to the contention of Farrelly (1954) that it is the time factor and not the environment of the larvae that determines its moults.

With the immobilization, capture and relocation of the zebra from various parks in Africa, together with a concentration of them in holding pens before translocation and the stress associated with such operations, it is anticipated that in some of the animals with heavy infestations of *D. vulgaris* there may be a loss of condition and even deaths. This disease may also be expected as an incidental finding, or in severe cases the cause of death in zebras in zoological gardens over the world. In most instances the source of the animal is known and good data as to the distribution of delafondiasis of zebra can thus be obtained.

Summary and Conclusions

1. An account of the vascular lesions due to fourth stage larvae and fifth stage *Delafondia vulgaris* is given. It is based on observations made on the viscera of 125 zebras, 25 of which were necropsied, the tissues collected and then studied in detail microscopically.

2. The distribution of fourth stage larvae and fifth stage *D. vulgaris* with a detailed description of the associated pathology is included. The results of helminthological studies form a part of the report.

3. The primary lesions in the liver were nodules which were the result of a type of parasitic thrombophlebitis in the intrahepatic branches of the portal vein. These contained fifth stage *D. vulgaris* primarily, but fourth stage larvae were present in one case. A combination of the organization of thrombi and host response to the entrapped parasites formed the basis of these lesions with the result that the wall of the vein was often destroyed and culminated in large defects wider than the diameter of the vein. Fifth stage specimens which were found in these lesions were larger than the sexually mature *D. vulgaris* present in the caecum and ventral colon. In no instance did the females from the liver contain ova in their uteri.

4. Fifth stage *D. vulgaris* were collected from lesions in the lungs from two zebras. Histologically it was seen that they involved branches of the pulmonary artery with destruction of the wall and resulted in a host response similar to that in the intrahepatic branches of the portal vein.

5. Both fourth stage larvae and fifth stage *D. vulgaris* were present in the anterior mesenteric arteries and/or their branches of many zebras. These vessels, though having thickened walls, narrowed lumens and being enlarged, did not appear as having true aneurysms.

6. Virtually all zebras in which the branches of the portal veins were examined, contained the parasitic lesions. From this it is apparent that larvae of *D. vulgaris* frequently enter the liver in this equine species. Whether it represents a normal or an aberrant route was not determined.

7. Attention is drawn to a number of other vascular lesions due to helminths of domestic and wild animals. They are briefly discussed in regard to comparative aspects of the lesions.

Acknowledgements

The authors wish to express their thanks to the following: the National Parks Board for providing the opportunity to do the autopsies in the Kruger National Park; the board of Nature Conservation

in South West Africa for a similar opportunity in the Etosha National Park and to the staff of both parks for their considerate co-operation and assistance; Prof. J. D. Smit, head of the Department of Pathology for his never-swerving support and encouragement to pursue problems related to disease common to game and domestic animals; Dr. Roy Tustin for assistance with the manuscript; Mr. A. M. du Bruyn and his staff for the unrelenting support in the production of photographs; Mr. J. L. de B. van der Merwe and his technicians for the preparation of the microscopic sections.

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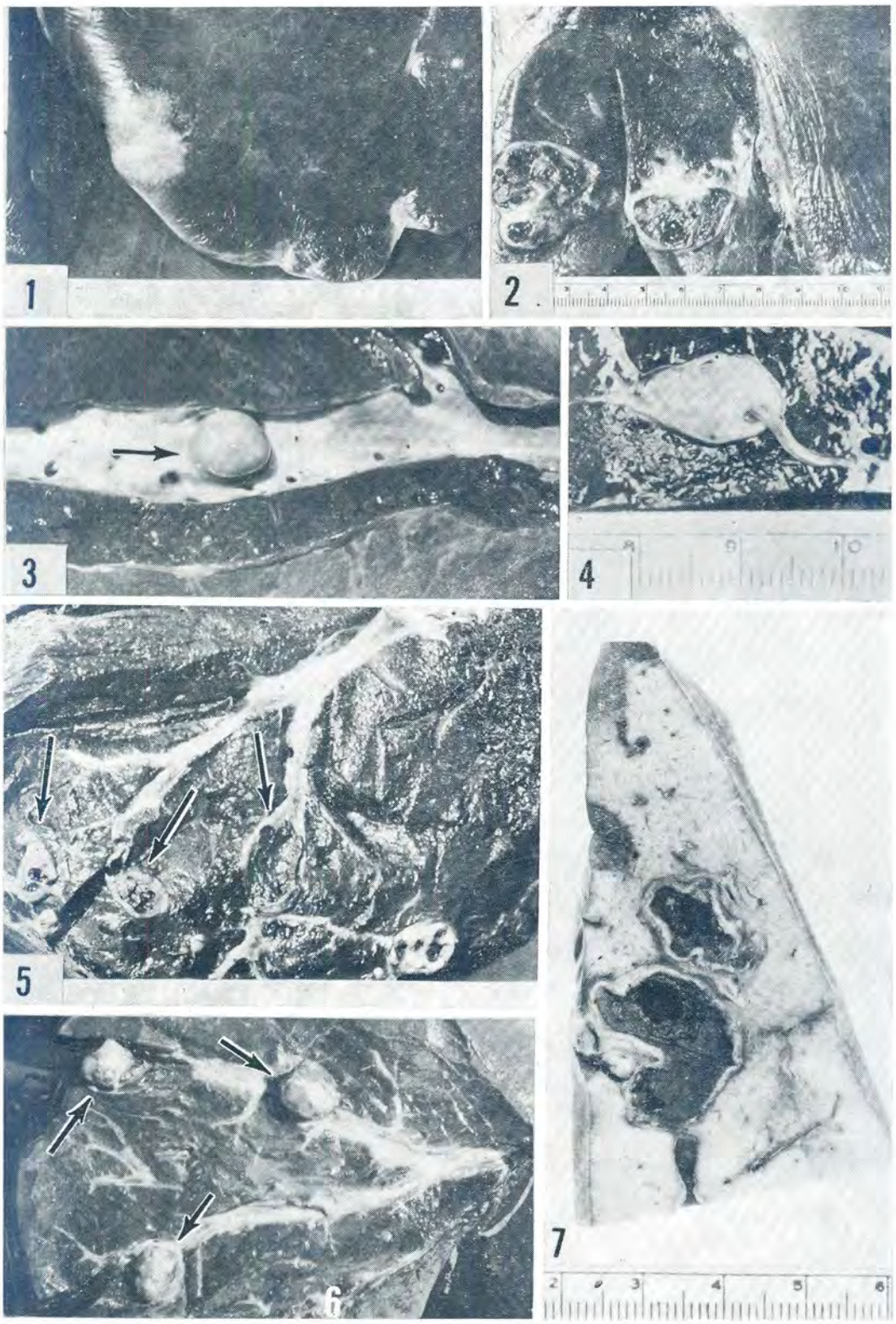

PLATE 1.—Macroscopic lesions of intrahepatic branches of portal veins
1. Surface appearance of one of the lesions due to larvae of *D. vulgaris*. 2. Cut surface of lesion in (1). 3. Endothelial-covered thrombus (arrow) from which a fifth stage *D. vulgaris* was recovered. 4. Fifth stage *D. vulgaris* in a small vein, the lumen of which is filled with fibrous tissue. 5 and 6. Lesions within branches of an opened portal vein (arrows). Notice the large size of several compared to the normal diameter of the vessels. 7. Cut surface of formalin fixed liver showing lesions which could be palpated from the serosal surface

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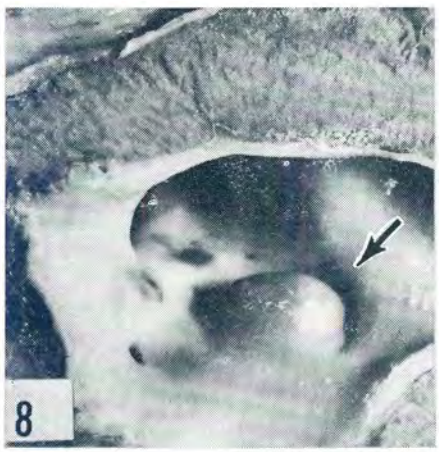
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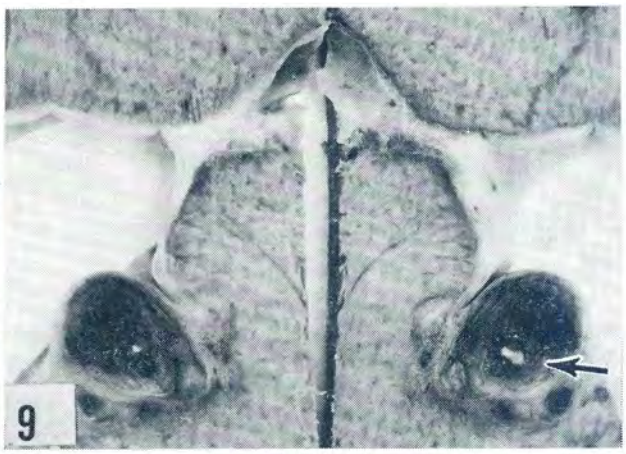
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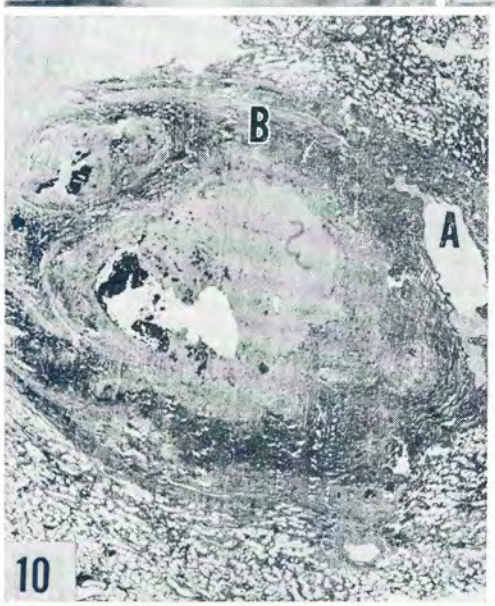
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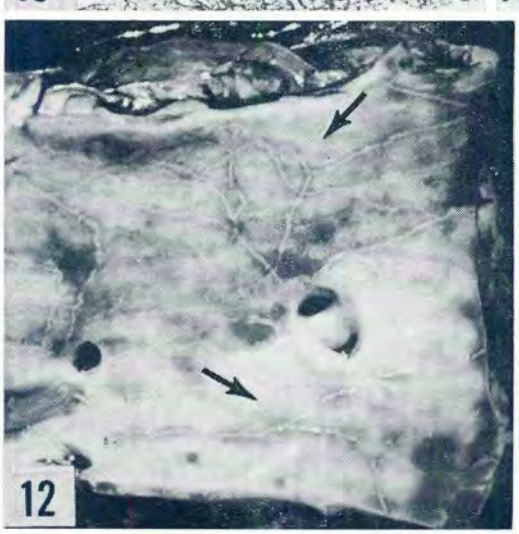
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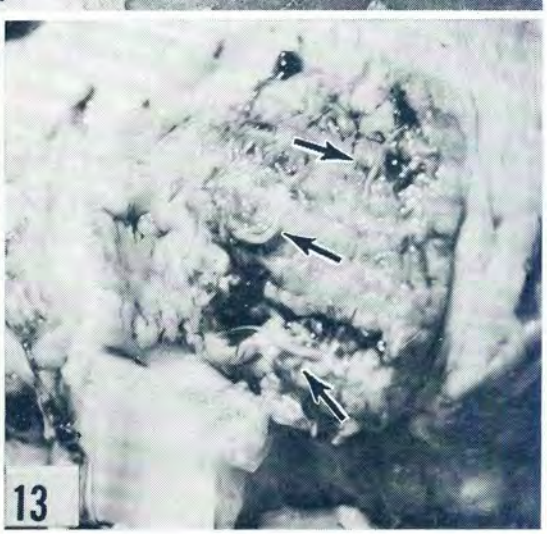
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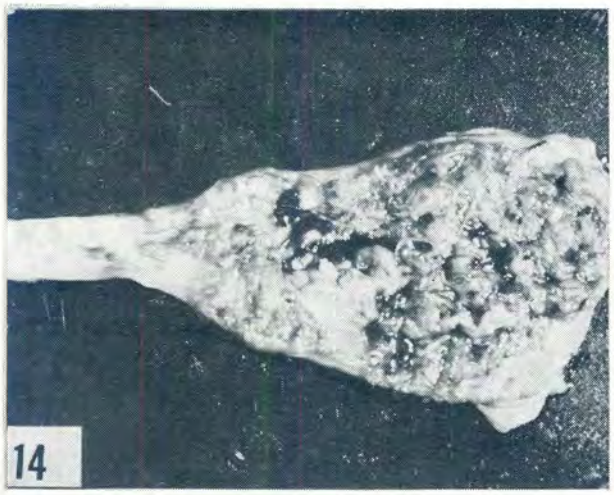
PLATE 2.—Lesions in intrahepatic branches of portal veins, pulmonary artery, aorta and anterior mesenteric artery

8. Endothelial-covered thrombo-verminous lesion (arrow) bulging into lumen of vein. 9. Cut surface of the lesion shown in (8). Notice the dead parasite (arrow) in the endothelial-covered thrombus. 10. Lesion in a small branch of pulmonary artery from which a fifth stage *D. vulgaris* was recovered. A. Small bronchiole. B. Distorted wall of artery. ×5. H. & E stain. 11. Low power photomicrograph of a section cut from the block pictured in (9). Notice the smooth endothelial-covered thrombus with remains of dead fifth stage *D. vulgaris* (arrows). Necrotic central portion is partially calcified and surrounded by granulation tissue and fibrous wall. ×5. H & E stain. 12. Piece of abdominal aorta taken near diaphragm. Notice the multiple fibrinous tracts of the intimal surface (arrows). 13. Typical thrombo-parasitic lesion in the anterior mesenteric artery. Notice various stages of *D. vulgaris* (arrows)



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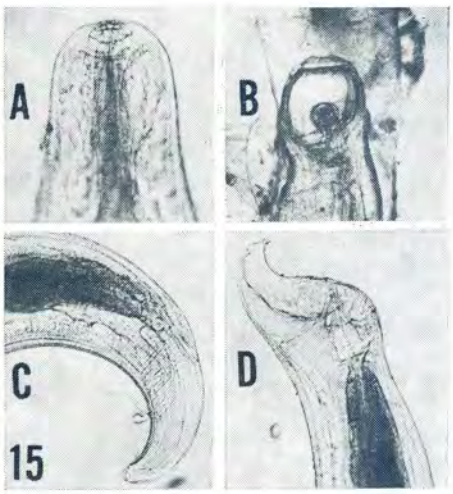

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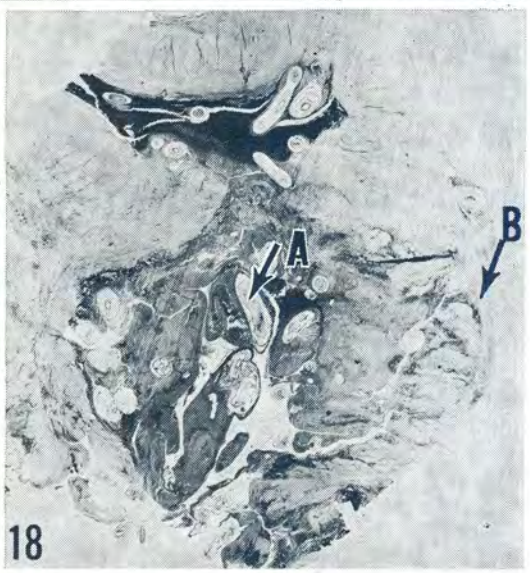

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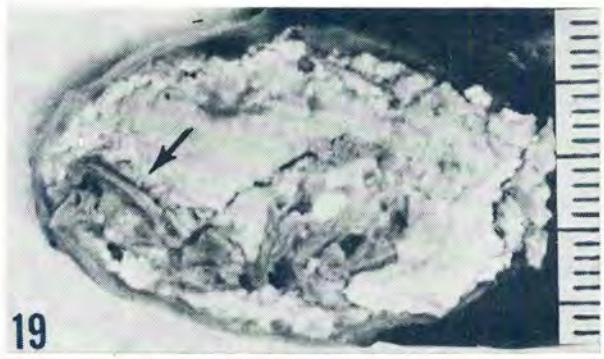
PLATE 3.—Arterial lesions due to fourth stage larvae and fifth stage *D. vulgaris*
14. Anterior mesenteric artery. Fourth and fifth stage *D. vulgaris* specimens were obtained either on or partially embedded in the roughened surface of the luminal portion of the arterial wall. 15. Mounted specimens of *D. vulgaris* from the anterior mesenteric artery. Unstained. A. 4th stage larva: head ×40. B. 5th stage specimen: head with remains of 4th stage cuticle. ×15. C. 4th stage larva: tail, female. ×40. D. 4th stage larva: tail, male. ×40. 16. Low power photomicrograph of a longitudinal section from the gross specimen of the anterior mesenteric artery illustrated in (14). Notice the extensive active chronic inflammatory lesions involving especially the intima and media. ×6. H & E stain. 17. Low power photomicrograph of a cross section of one of the main branches of the anterior mesenteric artery. Notice that the lumen is narrowed mainly by the extensive intimal reaction and thrombus on its surface. ×5. H & E stain. 18. Low power photomicrograph of a cross section from one of the arterial branches at its origin from the anterior mesenteric. Notice organized thrombotic material surrounding the parasites (arrow A) and the weakened arterial wall (arrow B). ×4. H & E stain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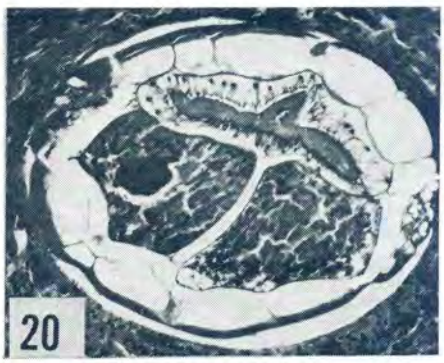
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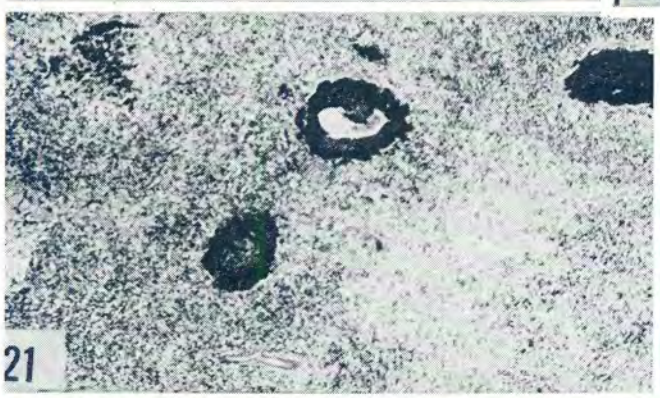
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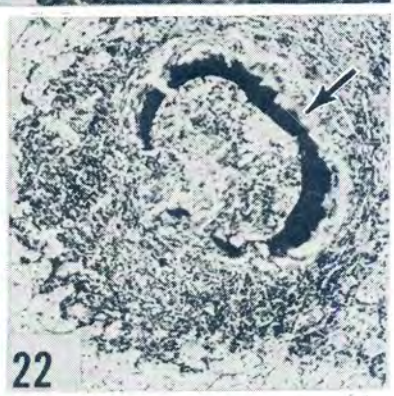
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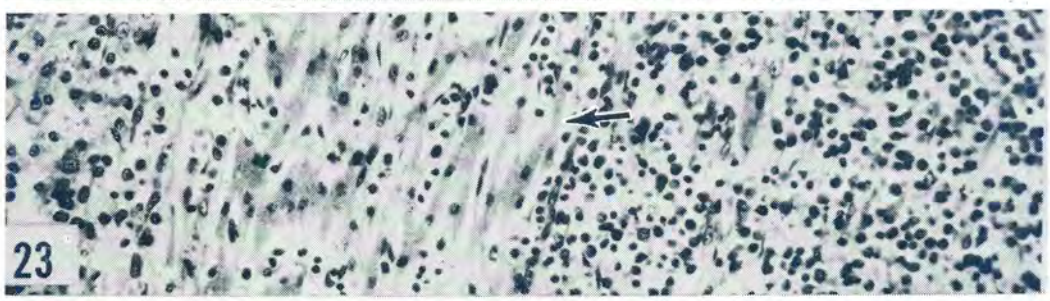
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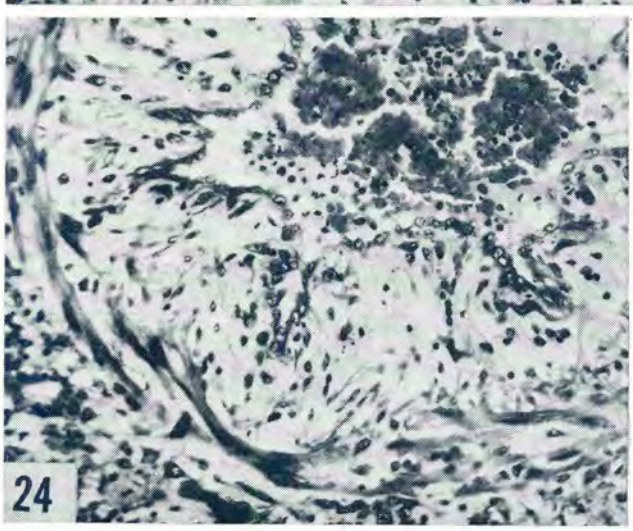
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PLATE 4.—Liver lesions

19. Hydatid cyst with calcified contents. Notice remnants of the hyaline membrane (arrow). 20. Larva of *D. vulgaris* migrating through liver parenchyma. Note absence of reaction around the parasite. ×175. H & E stain. 21. Migratory tracts through liver parenchyma. ×37. H & E stain. 22. Intense reaction to a cast sheath (arrow) in one of the migratory tracts in liver parenchyma. ×75. H & E stain. 23. Exudate consisting of eosinophiles and round cells crowding and causing atrophy of liver cord cells (arrow). ×255. H & E stain. 24. Early acute endophlebitis of small branch of portal vein. Notice the oedematous thickening of the intima which projects into the lumen. ×55. H & E stain. 25. Photomicrograph of one of the small tracts observed macroscopically on the intimal surface of a branch of the portal vein. Notice the elevation of the intima and the accumulation of leucocytes beneath and extending into the underlying media. ×75. H & E stain

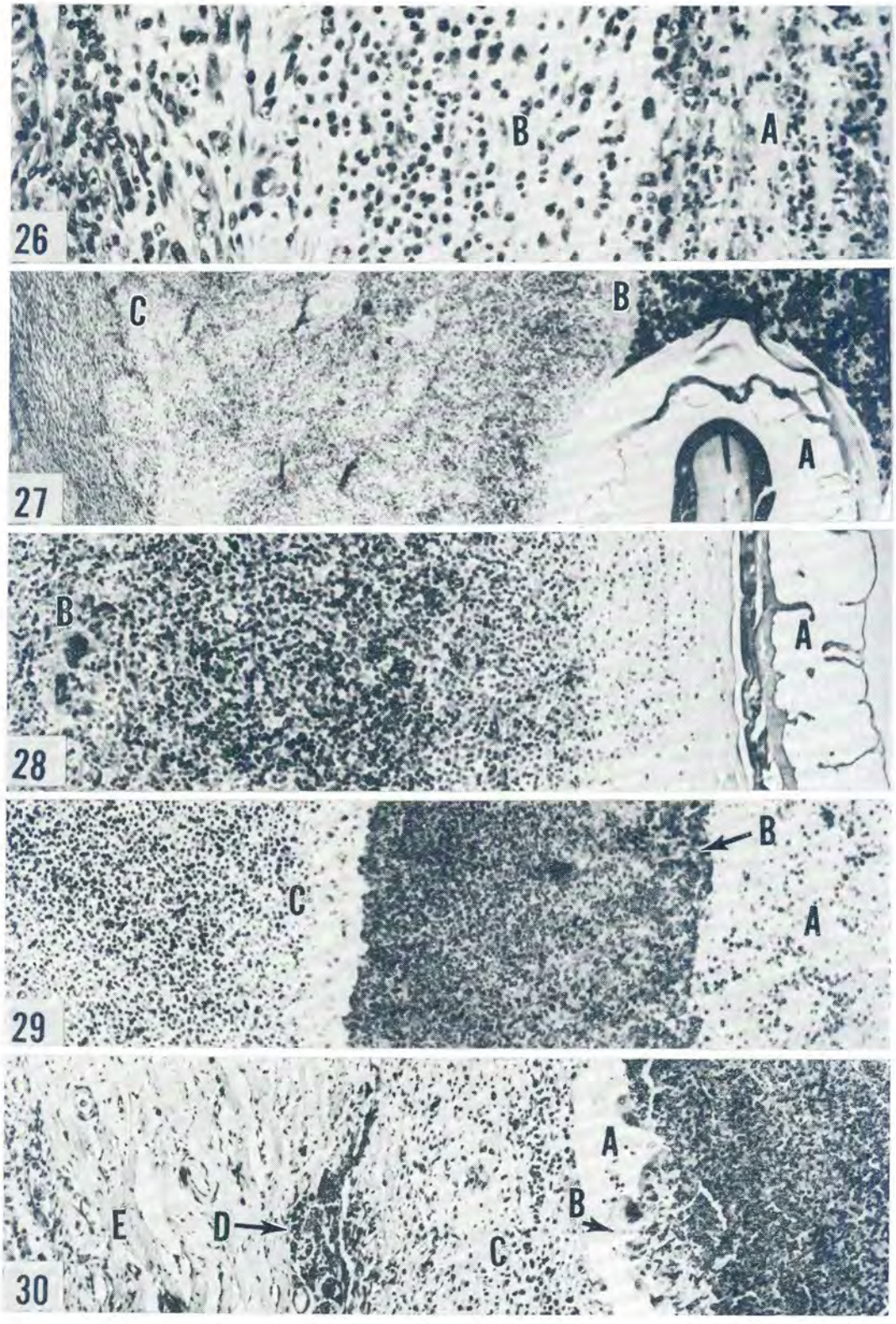
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PLATE 5.—Photomicrographs of various stages of the lesions in the intrahepatic branches of the portal vein. (Lumen always towards the right)

26. Early endophlebitis with laminated thrombus. A. Thrombus. B. Thickened intima infiltrated with leucocytes. ×255. H & E stain. 27. A. Fifth stage *D. vulgaris*. B–C. Highly reactive thickened intima. ×33. H & E stain. 28. Dead *D. vulgaris* (A) in lumen and thickened reactive intima. Notice giant cells (B). ×255. H & E stain. 29. A. Liquefaction of central portion of necrotic cells in lumen. B. Eosinophilic necrotic mass in lumen. C. Portion of intima with extensive reaction. ×95. H & E stain. 30. A. Eosinophilic necrotic luminal mass. B. Epithelioid and multinucleated giant cells around edge of necrotic material. C. Thickened intima with round cells. D. Macrophages containing haemosiderin. E. Remainder of wall composed of fibrous tissue. ×120. H & E stain

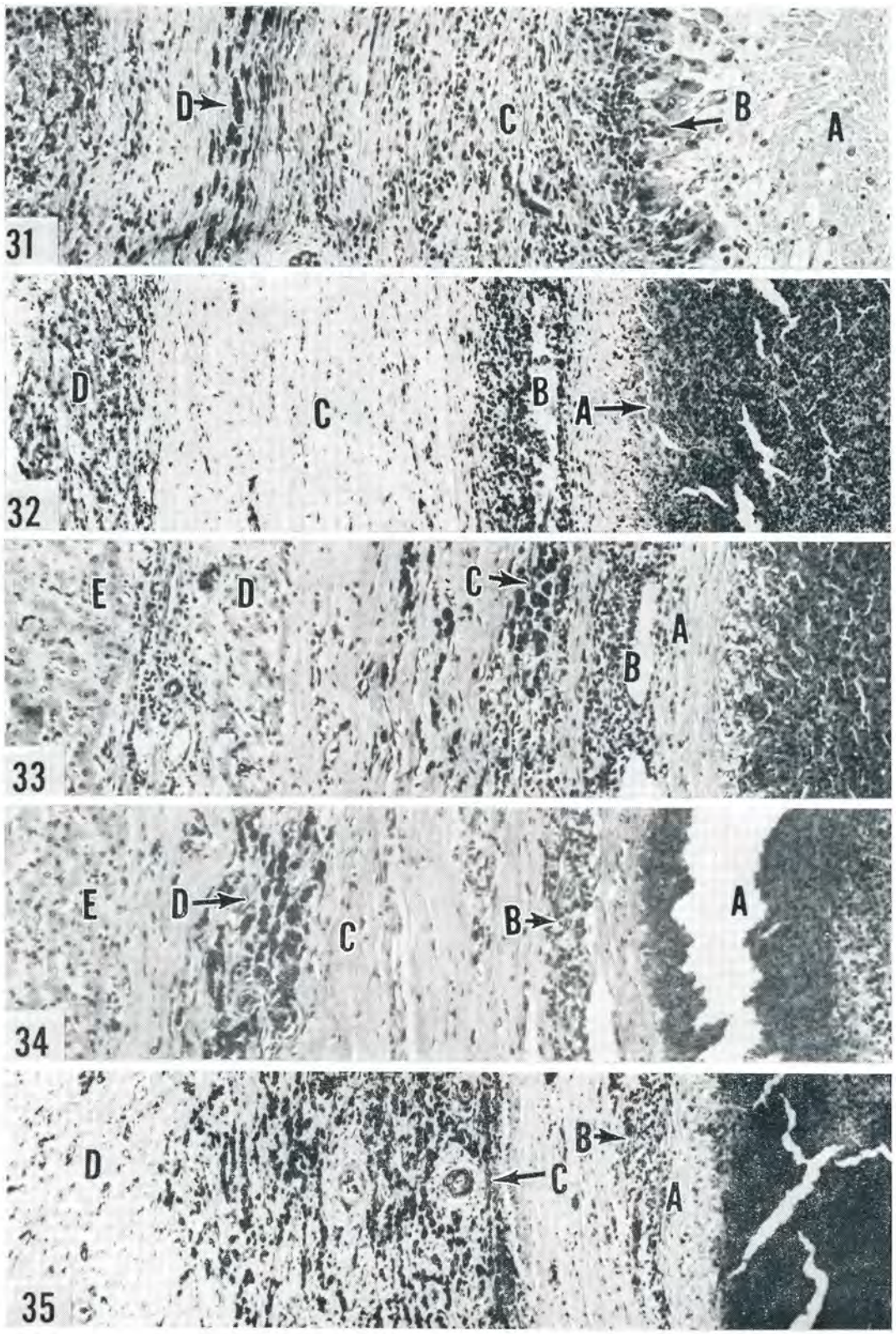
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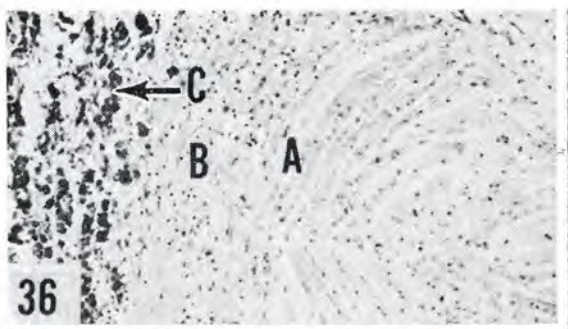
PLATE 6.—Photomicrographs showing further stages of the lesions in intrahepatic branches of the portal vein. (Lumen of each vessel towards the right)

31. A. Complete liquefaction of necrotic material in lumen. B. Zone of epithelioid and foreign body type giant cells. C. Zone of leucocytes in fibrous tissue. D. Haemosiderocytes and other macrophages with lipofuscin. ×120. H & E stain. 32. A. Necrotic eosinophilic material surrounded by a fibrous layer having few round cells. B. Zone of round cells and macrophages. C. Outer zone of fibrous tissue having replaced smooth muscle of vein wall. D. Liver parenchyma. ×120. H & E stain. 33. A and B.—as for 32. C. Macrophages containing haemosiderin and lipofuscin. D. Outer zone of fibrous tissue. E. Liver parenchyma. ×120. H & E stain. 34. A, B and C—as for 32. D. Macrophages containing haemosiderin and lipofuscin. E Liver parenchyma. 35. A and B—as for 32. C and D—as for 33. ×95. H & E stain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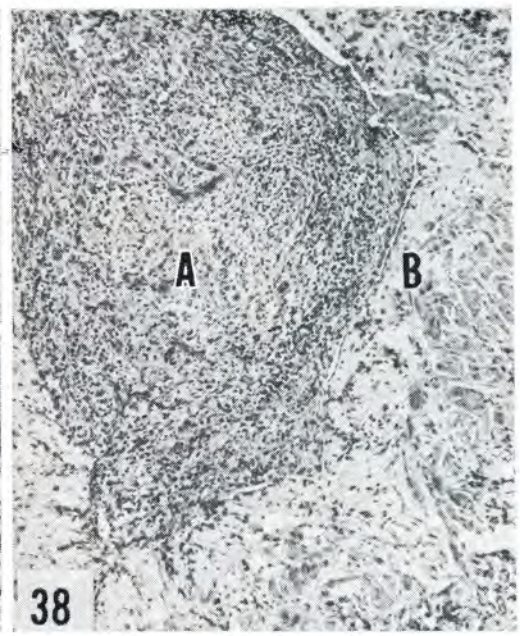
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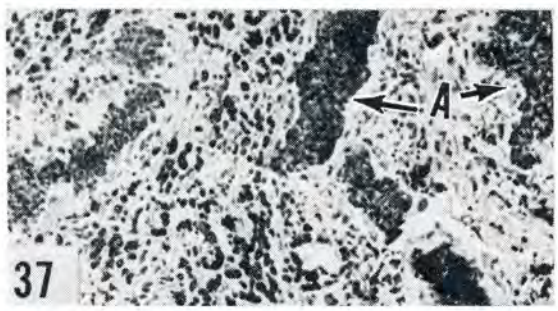
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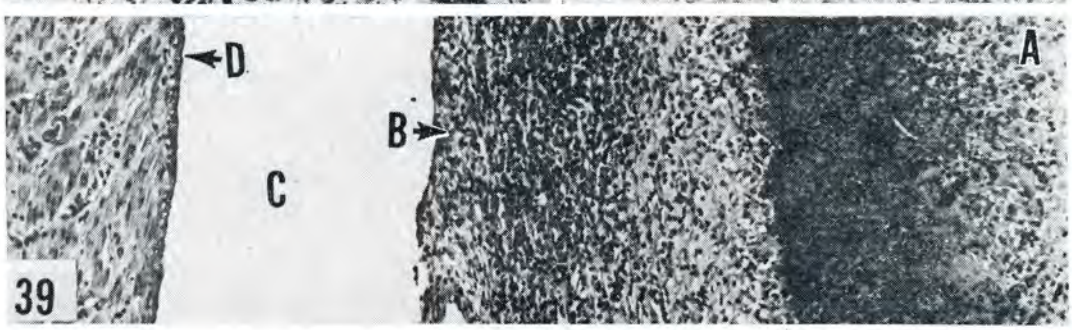
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PLATE 7.—Photomicrographs of lesions in intrahepatic branches of the portal vein
36. A. Cholesterol clefts within lumen. B. Zone of fibrous tissue. C. Zone of macrophages containing haemosiderin and lipofuscin. ×95. H & E stain. 37. Wall of a lesion permeated by the eosinophilic necrotic material (A) from the central area. ×95. H & E stain. 38. Organized thrombus (A) in a small vein. Wall of vein (B). ×75. H & E stain. 39. A. Necrotic eosinophilic centre of the thrombo-parasitic lesion. B. Highly cellular wall of granulation tissue on luminal surface of the same. C. Lumen of vein. D. Uninvolved wall of the vein opposite to the base of the thrombo-parasitic lesion. ×120. H & E stain. 40. Similar situation to that shown in (39) except that in this one there is a mild, early proliferative endophlebitis across the lumen from the base of the main lesion (A). ×95. H & E stain. 41. Another situation similar to (39) but here is a rare finding of villous projections from the intima (A). ×95. H & E stain