

OBSERVATIONS ON THE PATHOLOGY OF BILHARZIASIS AND OTHER PARASITIC INFESTATIONS OF *HIPPOPOTAMUS* *AMPHIBIUS* LINNÆUS, 1758, FROM THE KRUGER NATIONAL PARK

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INTRODUCTION

The effective preservation of wild animals during the last five decades has resulted in a marked increase in the big game population of East and Southern Africa. The proliferating herds of elephants, hippopotami, buffaloes and giraffes have caused overgrazing as well as forest and bush destruction in many game reserves. This devastation has also been promoted by periodic droughts. Game "cropping" has been resorted to in order to preserve the vegetation and to retain adequate numbers of each species in relation to the carrying capacity of the parks.

During 1964 the authorities of the Kruger National Park were faced with a severe drought, which by then, had persisted for three years. A critical shortage of grazing and water was developing in certain regions of the park. The diminishing supplies were also aggravated by certain species of large animals. Biologists of the park concluded that hippopotami, amongst other species, were approaching an undesirable level in the population curve. After due consideration of the many factors involved, a decision was made to kill 100 of the hippopotami that occupied the Letaba river region which had suffered most from the prevailing drought. This decision was accompanied by an offer to scientists to conduct a thorough study of this species in relation to the effect of the environment and diseases. The hope was expressed that such information would serve as a scientific basis for future culling programmes and for precautions that might have to be taken, when animals would have to be transferred to other countries. The proposed number to be culled represented approximately 3 per cent of the total hippopotamus population inhabiting the environs of five perennial and numerous seasonal rivers of the Kruger National Park. For these studies it was planned to select animals that would be fairly representative of various ages of both sexes.

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PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

The general nature of the studies including the *modus operandi*, description and illustrations of the necropsy technique is being prepared for publication. Biometrical records and other extensive biological data formed the basis of a report by Pienaar, Van Wyk & Fairall (1966). A summary of the incidence and distribution of schistosomes harboured by the hippopotami, the associated pathology and other features of the infestation was published by McCully, Van Niekerk & Kruger (1965). The present report is based on a more detailed study of the pathology and parasitological features of hippopotamine bilharziasis and other types of parasitism. The incidence and distribution of histopathological changes in 97 animals and other relevant data are presented in Table 1. This account should be of interest to veterinary and medical pathologists as well as to biologists concerned with game preservation.

MATERIALS AND METHODS

While conducting necropsies at various sites along the banks of the Letaba river, attention was paid to macroscopic changes and to the distribution of helminths. Some of the typical lesions were photographed. Representative tissues from 97 animals were collected and preserved in a 10 per cent formalin solution. These were processed later and embedded in paraffin wax. Three-micron thick sections were cut from each block with a sliding microtome and stained with haematoxylin and eosin (H. and E.). Special staining techniques including periodic acid—Schiff (PAS) (MacManus, 1948) Gomori's methanamine—silver nitrate (GMS) (Grocott, 1955) and others were applied as required to demonstrate various features of affected tissues. Specimens of helminths were collected in a glycerine and alcohol mixture.

PARASITE DISTRIBUTION AND ASSOCIATED PATHOLOGY

Macroscopic Observations

PHYLUM PLATYHELMINTHES

Trematoda

Schistosomatidae

Blood flukes, belonging to the genus *Schistosoma*, were numerous in most animals but less frequent in others. Systematic examination revealed that they had a most unusual distribution in these animals in which they occasionally appeared in any of the major blood vessels. Adult schistosomes were encountered in the aorta, pulmonary arteries and veins, anterior and posterior venae cavae, iliac, uterine, ovarian, testicular, renal, adrenal, intrahepatic portal, hepatic, phrenic, splenic and mesenteric veins, and coronary and gastric arteries and veins. Parasites were also observed in specifically unidentified veins which included those draining the skeletal musculature. A high degree of infestation occurred in the heart. Worms were commonly seen in the right atrium and/or ventricle but less frequently in the left atrium and/or ventricle. They were plentiful in the pulmonary arteries, hepatic veins and posterior venae cavae, particularly in the section of the latter between the entrances of the hepatic veins and the heart. When the thoracic portion of the posterior venae cavae, supported by the diaphragm [Plate 1 (1)], was opened *in situ*, parasites were readily detected at this site [Plate 1 (2)]. Although counts were not made regularly, this section often harboured between 75 and 100 specimens.

Macroscopic lesions, associated with parasites, were most prominent and frequently extensive in veins of the liver, and in the right atrium and ventricle. Cardiac lesions appeared as a "shaggy" proliferative endocarditis [Plate 1 (5, 6)]. In extreme cases the normal features of the myocardium, usually seen through the endocardial surface, were masked by a gray to whitish appearance of the endocardium resulting from the proliferative reaction [Plate 1 (4)]. In some hearts, less extensively involved, there were delicate thread-like villi projecting 5 to 6 mm from the endocardium. They were detected best by watching for their movement after placing clear water in the heart chamber and gently moving the entire organ. In some of the hepatic veins and intrahepatic branches of the portal veins small, white, cauliflower-shaped nodules of lymphoid tissue were attached to the intima [Plate 1 (7)]. They varied in size from 1 to 8 mm in diameter, had a shiny surface, a sessile attachment and protruded for a distance of 1 to 2 mm into the lumen of affected veins. Often several clusters of nodules appeared in close proximity but small isolated ones, 1 mm in diameter, were quite numerous. They were more common in branches of the intrahepatic portal than in hepatic veins.

Another type of lesion, which was observed in a few of the large arteries, consisted of a delicate, lace-like proliferation on the intima. It could be separated from the intima by means of a forceps but was very fragile.

Fasciolidae

Only a single *Fasciola* sp. was found. It was present in large numbers in the bile ducts of all hippopotami except in several of the youngest ones. Flukes were especially numerous in small bile ducts, the walls of which were thickened by fibrosis. The proliferating fibrous tissue extended into the pericholeductal areas, and sometimes also affected the adjacent liver parenchyma. Continuous with the fibrosis, there frequently appeared a similar involvement of the interlobular septa which caused a sharp delineation of the liver lobules [Plate 1 (3)]. As a result of these changes the bile ducts in older animals were 10 to 20 mm in thickness. On cutting they showed great resistance and occasionally it was impossible to do so with a sharp knife due to plaques of metaplastic bone tissue within the wall. In the fresh state, the flukes measured up to 90 mm in length. They were brownish gray in colour and had the typical shape of a liver fluke.

Paramphistomidae

A number of conical flukes, two *Gigantocotyle* spp., one *Nilocotyle* sp. and two related species were found in the stomach. One of the first two mentioned species also infested the anterior duodenum and, in a few instances, the bile ducts. At times, *G. gigantocotyle* was clustered together in patches and attached to the gastric mucosa. After their removal it was observed that their suckers had caused circular impressions which gave the mucosa a "hob-nailed" appearance. The remaining species of flukes apparently caused no visible lesions.

Notocotylidae

A hitherto undescribed *Ogmocotyle* sp. was numerous in the small intestine of some of the young animals. The infestation was accompanied by a severe catarrhal enteritis characterized by intense hyperaemia of the intestinal mucosa, and an accumulation of thick tenacious mucus [Plate 12 (106)].

PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

Polystomatidae

A monogenetic fluke (*Oculotrema hippopotami*), which adhered to the conjunctiva by means of six suckers, infested all animals. Their number was never excessive on any one hippopotamus. As far as could be determined, they did not cause any significant damage at the site of infestation.

Cestoda

Taeniidae

The larval stage (hydatid cyst) of an *Echinococcus* sp. was found in the liver of 17 hippopotami and concomitantly in the lungs of two of them. They were never numerous, and usually not more than two or three groups were seen in a liver [Plate 1 (3)].

PHYLUM ASCHELMINTHES

Nematoda

Dipetalonematidae

A filarial worm (*Dipetalonema* sp.) was encountered frequently in the abdominal cavity. It appeared to be non-pathogenic.

Atractidae

A very large number of an extremely small worm (*Coboldina* sp.), not readily detected with the naked eye, appeared in the stomach of many animals. There was no evidence that this parasite produced any lesions. It thus appears that, in common with other closely related species, it is non-pathogenic.

PHYLUM ANNELIDA

Hirudinea

Blood sucking annelids were often attached to the anus, and, in some cases, they occurred as far forward as 30 cm in the rectum.

Miscellaneous Observations

Necropsies revealed that one of the large cows harboured a 42 lb mummified foetus [Plate 12 (105)] within her abdominal cavity. Pieces of maternal omentum enveloped the fore feet and the abdominal skin on one side of the foetus. The exit wound in the uterus had healed completely but the scar tissue was clearly visible. The maternal skin contained large cicatrices and suggested that this was possibly an example of traumatic intra-abdominal abortion.

In many of the animals there was evidence of a chronic peritonitis which was especially prominent over the diaphragmatic surface of the liver [Plate 1 (6)].

In the gastric diverticula of some animals, there were quite a few small papillomata. No record was kept of how many animals were affected. A tumorous mass, measuring approximately $5 \times 3 \times 2$ cm, was present on the gingiva opposite the angle of the mouth of one hippopotamus.

Microscopic Observations

Heart

Bilharziasis

For these studies one or more sections from different parts of the heart were available from 90 cases. Specimens showing an endocardial surface were present in 77 of these cases and of these 63 (82 per cent) had lesions involving this lining. They were of an inflammatory nature which varied considerably, not only from one heart to another, but also from one site on the endocardium to another of an individual heart. In the descriptive names, applied to the various inflammatory stages, the term "verminous" could have been included regularly to indicate the aetiology. In the paragraphs that follow, this term will be omitted except in such instances where the aetiology has to be stressed to avoid misinterpretations.

The spectral aspects of the lesions, constituting the endocarditis, were quite extensive as evidenced by numerous combinations of various components. At either end of the endocarditis spectrum there was relatively simple evidence of changes. In the acutely affected segment there was an inflammation characterized by the presence of many eosinophiles and a few small round cells, namely an acute endocarditis [Plate 3 (22)]. At the opposite end of the spectrum, the endocardial surface was represented by a thick composite composed of consolidated laminae of dense connective tissue with very few inflammatory cells, namely a chronic pachyendocarditis [Plate 3 (25)]. Between these two extremes the spectrum contained a diverse assortment of lesions.

While the spectrum from the acute endocarditis to the chronic pachyendocarditis was being studied the second distinct type of reaction was recognized. This was designated as an active, subacute, proliferative endocarditis [Plate 2 (10, 11)]. It was characterized by villous proliferations which had a stroma of immature connective tissue containing numerous eosinophiles and other inflammatory cells in the meshwork of fibres [Plate 3 (23)]. Similar cells covered their surfaces. In the absence of the inflammatory cells the third type, a subacute, proliferative endocarditis became apparent. With maturation of the connective tissue in the villous proliferation the fourth type developed, namely a chronic, proliferative endocarditis [Plate 2 (17)]. In some hearts an acute inflammation was superimposed on this chronic type giving rise to an active chronic, proliferative endocarditis [Plate 2 (14, 15); Plate 3 (24)].

Frequently the endocardial proliferations anastomosed, and with some maturation of the connective tissue and reorganization of the structural pattern a new lining or pseudoendocardium was formed [Plate 2 (12, 13)]. This constituted the fifth type of reaction which will be referred to as a chronic, simple laminar or lamellar, proliferative endocarditis [Plate 2 (18)]. Subsequent layers of varying thicknesses were added by a repetition of the steps involved in the formation of the first layer of pseudoendocardium. As the number of layers increased, it became the sixth type, best described as a chronic, multiple laminar or lamellar proliferative endocarditis [Plate 2 (16)]. Lamina is suggested as fitting for the designation of the thick layers and the diminutive lamella for the thin ones. If inflammatory cells were present as well then the proposed expressions would have to be preceded by the term "active". With further re-organization, maturation and compression or contraction of the connective tissue stroma, lamina, which were formed from smaller lamellae, became consolidated into even thicker layers and the chronic type of pachyendocarditis evolved with the composite even more compact [Plate 2 (19, 20,

PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

21)]. The luminal surface could later become activated, and through a series of similar steps form another luminal lamina or laminae of pseudoendocardium [Plate 2 (27)]. There were some areas of the endocardial surface, however, which consisted of such dense collagen that it appeared that the foregoing account was not sufficient to explain the pathogenesis. Such areas of endocardium probably became thickened due to a fibrosis beneath the surface cells without forming a pseudoendocardium. In a few sections there was a consolidation of the endocardial proliferation in spaces between the muscular trabeculae of the walls of the right atrium and ventricle. Such consolidating lesions, in the early stages, consisted of a meshwork of collagenous strands heavily infiltrated with eosinophiles. The surface was also often covered by eosinophiles. Because of the intercommunicating spaces, the tissue proliferating between the cardiac trabeculae somewhat resembled a sponge. In another lesion of a similar nature, the anastomosing bands of connective tissue were separated by small vascular spaces. It was suspected that in this instance, it represented the organization of a thrombus within the tip of the auricle of the right atrium. Such an intimately attached, organized mass of tissue caused the endocardium to appear greatly thickened.

Combinations of active and chronic stages of endocarditis were sometimes carried to the extreme. In such instances there was very active proliferative reaction on the luminal surface resulting in the formation of numerous villous projections associated with an appearance of eosinophiles in the superficial and intrastromal tissues. Between these lesions and the myocardium there was a thick layer of fibrovascular tissue which had been present for a much longer period.

There were a few examples of valvular endocarditis of a proliferative nature, but this was not an outstanding feature. Large verruca were never observed on the valves.

Focal nodules of inflammatory cells, disseminated over the endocardium of some hearts, were also encountered [Plate 2 (8)]. Eosinophiles were especially frequent, and occurred together with lymphocytes in the foci. A number of larger nodules, composed of only lymphoid tissue, protruded inwardly from the endocardium [Plate 2 (9)].

Thirty-nine of the 90 cases, from which heart sections had been prepared, showed segments of coronary veins. Thirty (77 per cent) of these had lesions believed to have been caused by adult schistosomes. There was a proliferative endophlebitis characterized by villous protrusions extending from the intima into the lumen. Earlier stages of these lesions in the intima, were represented by concentrations of inflammatory cells, especially eosinophiles [Plate 3 (26)]. There were no indications of lesions due to adult schistosomes or their ova in other regions of the heart.

Lungs

Bilharziasis

Lung sections, for histological examination, were available from 92 cases. Of these 84 (91 per cent) exhibited within the branches of the pulmonary artery some form of verminous endarteritis believed to have been induced by adult schistosomes. In common with the endocarditis described above, there was a broad spectrum of lesions. The earliest indication of an inflammation was a hyperplasia of the endothelial cells which showed a tendency to pile up and bulge into the lumen [Plate 4 (28, 30)]. The hyperplasia was often accompanied by an accumulation of many eosinophiles on the surface as well as beneath the endothelium. This acute

endarteritis may also be considered as the acute phase of the rather complex reaction since there were, to some extent, stepwise stages progressing from the acute to the chronic end in the spectrum of the arterial lesion. As the sequence of changes continued, the lumina of some of the small arteries were ultimately obstructed by elongated cells which appeared to originate from the endothelium and/or underlying primitive mesenchymal cells [Plate 4 (31)]. At this stage, eosinophiles were plentiful in the lumen and in the intima.

From the elongated cells an indication of some organization eventually became perceptible. This was detected when some of the cells were oriented in a linear fashion to form cords which were covered by endothelial cells, and which sometimes protruded from the intima into the lumen in the form of villous projections. The designation of the latter part as the villous developmental phase is appropriate.

The cords, which had a tendency to anastomose, were initially composed of undifferentiated cells. This phase was soon followed by a development of very primitive-appearing connective tissue cells, presumably young fibroblasts. The stroma of the cords also contained eosinophiles [Plate 6 (52)]. As time progressed the fibroblasts matured, and formed dense collagenous strands which were quite eosinophilic when stained with H. and E. The described manifestations were considered to be the active, proliferative stage of endarteritis.

Consideration of the successive phases of the abovementioned inflammatory process, suggested that in the early stage elongated cells within the lumen were probably of endothelial as well as of mesenchymal origin even though no distinctive morphological features were evident. This assumption appeared to be supported by the ensuing differentiation into endothelial and fibroblastic cells. In the initial part of the pulmonary artery and in its branches with large lumina, there was a closely corresponding stage. Here the proliferative cells did not completely block the passage but instead protruded, in the form of endothelial projections, for a short distance into the lumen.

The developmental courses taken after the early active, proliferative stage in passing to a chronic phase were numerous. What appeared to be the simplest and very common process was the formation of a new inner lining or pseudo-intima derived from the active proliferative stage within the arterial lumen. The pseudo-intima evolved when the ends of the intimal proliferations, still in the villous developmental phase [Plate 5 (35)], began to intertwine and to anastomose [Plate 5 (36)] to form one or more new layers of tissue superimposed on the true intima [Plate 5 (37, 44)]. The connective tissue eventually reached maturity and appeared as dense collagen. In the simple chronic stage of verminous endarteritis, the pseudo-intima was single-layered, and composed of mature collagen covered by a flat layer of endothelium but lacking an elastica interna [Plate 5 (38)]. Depending on its thickness, the terms lamina or lamella are applicable to it also. By a repetition of the formative process, the pseudo-intima could become a multi-layered formation [Plate 5 (39, 40, 41)] as will be explained later.

The microscopic appearance of the pseudo-intima depended to some extent on the plane of the section through it and the adjacent arterial wall. In an artery transversely sectioned, the simple form of a pseudo-intima appeared a short distance from the true intima [Plate 5 (42)]. It was composed of collagen, and had a luminal and a mural surface corresponding to the direction each faced. Both surfaces were covered by endothelium. The thickness of the pseudo-intima usually varied between 10 and 25 microns with some exceptional ones thicker. On the mural surface there

PATHOLOGY OF BILHARZIASIS OF HIPPOPOTAMUS AMPHIBIUS

were collagenous connections spaced at intervals between the true and pseudo-intima [Plate 5 (46)]. These connections appeared as small bridges or struts across a portion of the lumen which contained blood. When viewed in an artery which was sectioned through the central axis of the lumen, the pseudo-intima appeared as layers of endothelium-covered collagenous tissue superimposed on the luminal surface but separated from the true intima by endothelium-lined spaces containing blood [Plate 6 (49)]. The spaces were interrupted at intervals by small struts of connective tissue which joined the pseudo-intima with the true intima. By observing a section of an arterial branch, which was cut at a tangent to the wall and only passing through a part of the lumen, the erroneous impression could be gained that the pseudo-intima filled much of the lumen [Plate 5 (43)]. In such preparations it appeared as a network of anastomosing, meandering cords of endothelium-covered collagenous tissue. Sections of this nature provided evidence which indicated that the pseudo-intima was perhaps more like a sieve or fishnet, made of collagen meshwork, rather than of a solid uninterrupted layer as often appeared to be the case in other sections.

When the pseudo-intima was being formed during the active proliferative stage, it was composed of fibroblasts. After studying a number of them, it became apparent that the active phase could pass directly to the simple chronic stage with the production of either a lamella or lamina of mature collagen, or alternatively it could continue to proliferate to form multiple lamellae or laminae subsequent to the first [Plate 5 (44)]. In some of the arteries the maturation of the connective tissue of the first layer was interrupted by an immediate resumption of the proliferative process. This formative process could continue with an eventual addition of one or more layers of pseudo-intima even though the first one was of a premature nature. Another possibility for the formation of multiple layers would be for the first to reach maturity and for its surface to become activated [Plate 4 (32)] to form a second layer which matured, and then to have a repetition of the process with the beginning of the formation of a third layer as illustrated [Plate 5 (47)]. Between each successive layer and the preceding one, there were connecting struts. Such combinations of mature and immature layers could be referred to as a compound or multi-layered pseudo-intima depending upon whether two or more would eventually be present.

There were varying degrees of cellular activity in the endothelium of the pseudo-intima. The endothelium of both the luminal and mural surfaces could become very hyperplastic [Plate 4 (32)]. Numerous eosinophiles and other leucocytes were often present. The cells forming the subsequent layers of pseudo-intima appeared to be essentially the same as those which formed the first. They arose either from the hyperplastic endothelium or the underlying primitive or undifferentiated mesenchymal cells of the preceding layer. In some arteries there was pseudo-intima of either a simple or multi-layered structure which had developed into a very chronic stage as evidenced by highly dense, mature fibrous tissue. Superimposed on this, there had been a reactivation of the endothelium followed by an activity of a proliferative nature. On their surfaces there were usually numerous eosinophiles, and while the villous projections began to take shape they also appeared within their stroma. Such a stage, showing evidence of active inflammation as well as chronic changes, could be referred to as an active chronic, proliferative stage of verminous endarteritis [Plate 5 (48)].

Besides the many abovementioned formations, there were some in which there appeared diffuse proliferations of connective tissue with no particular pattern of lamination. On transverse sections such formations might, however, appear somewhat laminated. Even in these formations, the impression was gained that there were spurts of growth with intense proliferation, alternating with periods of quiescence during which there was maturation of the connective tissue component.

Although pseudo-intima lesions occurred in many branches of the pulmonary artery, there were instances where subendothelial fibrosis only caused a thickening of the intima. In addition, fibrosis also occurred beneath the endothelium of the layers of the pseudo-intima. Since the luminal and mural surfaces could respond similarly when reactivated, it was possible that the ensuing subendothelial fibrosis would eventually occupy the spaces of the net-like formation of the multi-layered pseudo-intima. This was apparently a way in which the proliferating tissues became very compact instead of remaining either fenestrated or laminated structures; a consolidating type of reaction which also appeared to have accounted for the formation of the chronic pachyendocarditis previously described.

Further investigations revealed that the wall of one of the arterial branches was very thin, and similar to that seen in an aneurysm. The lumen was markedly dilated, and filled with villous proliferations extending from the intima. In its adventitia, there were prominent vascular channels apparently representative of a collateral circulation. Adjacent to another artery was a structure which resembled a glomus body tumor, but differed in that it was infiltrated with numerous inflammatory cells, especially neutrophils. Angiomatoids, described in human schistosomiasis by Shaw & Ghareeb (1938), were present in some of the arterial branches of 7 (8 per cent) of the animals from which lung sections were examined [Plate 6 (53, 54)]. Schistosome ova were not present in association with the angiomatoids.

The intimal reactions in many of the arteries almost occluded the lumen, particularly those of the small ones, during the active proliferative stage of verminous endarteritis. In very few sections of the small arterial branches, there was thrombosis and/or an organization of thrombi. This manifestation was definitely uncommon. In most of the animals, the principal lesions were only of an inflammatory nature as described above. In view of the endarteritis it would not have been surprising to have found extensive thrombosis.

Sections from the pulmonary artery near the semilunar valve usually showed a pseudo-intima. In most instances, the villous projections of fibrous tissue gave the luminal surface a ragged appearance [Plate 11 (94)]. Although no critical attempt was made to determine to what extent the elastic tissue of the arterial branches was affected, the impression was gained that there was no modification despite the extensive alterations within the rest of the intima.

The contracted state of the arterial branches altered the appearance of the pseudo-intima considerably [Plate 6 (50)]. One could visualize how they would have appeared when fully expanded by systolic pressure. The undulating appearance of the elastica interna in cross-sections of affected arteries indicated the degree to which they had contracted after fixation. In contracted arteries, the simple chronic pseudo-intima was represented by a number of double loops which extended at intervals from the struts into the lumen all around the inner circumference of the vessel [Plate 6 (50)]. A similar looping in the multi-layered structures also showed undulations. In a dilated vessel the loops would presumably disappear and be

PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

stretched taut by the expansion of the intima between the struts connecting the true and the pseudo-intima. Thus some of the small arteries, with alterations approaching endarteritis obliterans in appearance when contracted, would probably be patent when expanded.

Where some viable and other apparently non-viable schistosomes were found in the lumen of arterial branches, there were granulomatous reactions composed of epithelioid and small round cells and occasionally in association with multinucleated giant cells [Plate 4 (33)]. Such granulomas extended from the intima and surrounded the parasites. In other vessels there were disintegrating adults surrounded by an intense cellular reaction in which lymphocytes predominated. The wall of vessels containing such reactions in the lumen also showed a severe inflammation [Plate 4 (34)]. In some small arteries there was thickening of the media but this was exceptional, most of them having a normal-appearing media.

Some of the pulmonary veins were affected in a similar way as the arterial branches, particularly in regard to the proliferations from the intima [Plate 5 (45)]. Twenty-nine (31 per cent) of the 92 cases with lung sections, showed a verminous endophlebitis. In some instances the inflammation was not limited to the intima and resulted in a panphlebitis with numerous eosinophiles in the three histological layers. This was prominent in response to dead adults within the lumen. The degree of cellularity as well as the intensity of the proliferative response of the intima with the resultant pseudo-intima formation was greater in veins than in arteries. The amount of smooth muscle of the veins was definitely increased. This may have been primarily due to hypertrophy but there seemed to be smooth muscle hyperplasia as well. Veins from 9 (10 per cent) cases contained sections of adult schistosomes. In 10 (11 per cent) of the animals, scant schistosome ova were present within the alveolar septa [Plate 12 (108)] and sometimes surrounded by granulomatous reactions. Ova were never present in the vascular lesions.

Besides inflammatory reactions surrounding some veins and extending for a short distance into the adjacent parenchyma, there were no additional significant changes in the lung which could be attributed specifically to bilharziasis. One or two ova were occasionally present in the alveolar septae but they were definitely scarce and evoked only a mild local response.

Liver

Bilharziasis

Lesions referable to adult schistosomes were present in the livers of the majority of animals. Vascular changes were the most significant, being quite severe in the intrahepatic portal and hepatic veins. Nine-one (93 per cent) of the 97 animals from which liver sections were available contained lesions of verminous endophlebitis. Eighty-nine (91 per cent) showed such lesions in branches of the portal vein, and 52 (53 per cent) in tributaries of the hepatic vein. Thirty-seven (38 per cent) thus had lesions in some of both.

Although there were primarily lesions of a chronic nature in the branches of the portal vein, there were also acute ones [Plate 7 (55, 56)], and other types could be classified categorically in between the acute and the chronic forms. The very early stages of acute endophlebitis, consisting of just inflammatory cells and endothelial hyperplasia, were rarely encountered but a relatively frequent finding was an active, subacute proliferative endophlebitis. Cells, arising from the endothelium and/or underlying primitive mesenchymal cells, were hyperchromatic and

hyperplastic, and practically filled the lumen of some vessels. The villous projections were densely cellular. Inflammatory cells, represented mainly by small round ones and eosinophiles, were within the stroma of the projecting villi as well as on the surface [Plate 7 (60); Plate 8 (70)]. The pseudo-intima and its struts, attached to the true intima, sometimes presented an arboral appearance [Plate 7 (61)]. Stages of endophlebitis, extending gradually from an acute to a chronic phase and analogous to those of the spectrum of a pulmonary arteritis, were observed. The chronic proliferative endophlebitis in veins of the liver often consisted of a pseudo-intima that was composed of multiple layers of collagenous tissue with small slit-like spaces lined by endothelial cells [Plate 7 (62, 63)]. It could not be ascertained in all instances whether this net-like structure was formed by deposition of successive layers of pseudo-intima or by re-organization of a profuse sheet or mass of connective tissue cells arising from the true intima. In many of the veins the process was obviously similar to that previously described for some of the pulmonary artery branches in which the formation of layers was somewhat stepwise. The surface of the older mature pseudo-intima could be activated as described for pulmonary vessels. The end results again varied from a single-layered pseudo-intima to laminated structures composed of multiple layers, either of laminar or lamellar nature. Frequently it was a reaction which was not uniform around the lumen; one quadrant would remain unaffected while the adjacent ones would be covered with a pseudo-intima of either simple or laminated types. Proliferative changes were essentially the same in the tributaries of hepatic veins.

In 33 (34 per cent) animals, there was another unusual reaction in many portal vein branches. A similar manifestation was observed in the hepatic veins of 16 (16 per cent) cases. Both afferent and efferent veins of the liver of some animals were affected. The reaction consisted of the formation of lymphoid nodules either in or on the intima where they occurred as isolated foci or in conjunction with a proliferative endophlebitis [Plate 7 (64, 65)]. Usually, they extended into the lumen but sometimes the lymphoid proliferations remained confined within the vascular wall. The lymphoid nodules were often attached to the intima and bulged into the lumen without involving the media. Then again the proliferations would involve the media from which they extended through the intima into the lumen. There was a remarkable degree of organization, and in some nodules there were striking resemblances to specific regions of a mature lymph node [Plate 7 (65)]. These were represented mostly by prominent lymph follicles, while in some instances trabeculae and sinuses were also visible. The presence of small arteries and veins within the nodules suggested that they possessed an independent blood supply, coming from the vessel wall and not from the lumen. Lymphoid formation occurred in some veins without a proliferative endophlebitis of other types but usually they were in conjunction with an endophlebitis characterized by a pseudo-intima [Plate 7 (66)]. In some veins they could be seen in the same transverse section where each type occupied a different sector of the periphery. Lymphoid nodules were attached frequently to the free tips of the villous projections. In some of the larger veins there were masses of lymphoid cells, and granulomatous reactions similarly situated. Such lesions were sometimes necrotic.

In some of the central veins, there were masses of inflammatory cells, primarily round ones, bulging into the lumen. Sublobular veins of a few animals had proliferative changes which filled the lumen [Plate 7 (57)]. Lobules affected by such obstructions, showed rather prominent ectasia of the sinusoids due to chronic passive congestion. This type of lesion was limited to the vicinity of affected central and sublobular veins. In approximately 20 per cent of the animals there were sections

of schistosomes within the veins. They appeared in the larger afferent and efferent veins of the hepatic lobules [Plate 8 (68)] and, in some instances, also within the smaller central veins [Plate 7 (59)]. Viable worms were observed in association with proliferative and lymphoid reactions in a few branches of the portal veins and tributaries of hepatic veins [Plate 8 (71, 68)]. Intense cellular reactions, associated with many eosinophiles, were sometimes present in adjacent liver sinusoids in response to schistosomes [Plate 8 (71)]. Hematin appeared within the digestive tract of the parasites but little of it was phagocytosed by Kupffer cells or reticulo-endothelial cells of other tissues. Several animals suffered from a localized, acute, necrotizing, suppurative hepatitis. Sections of dead schistosomes were seen in one such lesion. Foci of eosinophilic necrotic material, of unknown origin, were sometimes present. The liver of one case showed an area of fatty metamorphosis involving entire lobules. This could have been a sequel to a partial ischaemia, secondary to the vascular lesions.

In the livers of several there were a few schistosome ova. There were usually only two or three and they were very deteriorated in appearance and sometimes near the centre of the liver lobule [Plate 8 (69)].

Peculiar cells, associated with the venous lesions, were seen in four animals. They appeared to be macrophages possibly related to the Gaucher cell of man. They had a large, plump and spherical shape, and abundant eosinophilic cytoplasm which was rather acicular in appearance [Plate 9 (79)]. They were strongly positive to the PAS reaction [Plate 8 (83)] and to GMS [Plate 9 (77)] but they were negative for alkaline phosphatase. The nucleus had a spherical to slightly ovoid shape. Eosinophiles regularly occurred in association with these cells [Plate 9 (75)] which were often found in little nests of three to six surrounded by connective tissue. Reactions, consisting of these cellular groups and numerous eosinophiles, were usually adjacent to either afferent or efferent veins of the lobules [Plate 9 (80, 82)]. Similar but smaller cells were observed lining some of the adjacent veins [Plate 9 (76)]. It appeared as if they could possibly migrate into the perivascular tissue and evoke a host response consisting of a localized eosinophilia and fibrosis. Some were present in sinusoids midzonally [Plate 9 (81)]. It was not determined if they arose there or migrated from the periphery or centre of the lobule.

Fasciolasis

Of the 97 animals examined all but three had numerous liver fluke lesions. Some liver sections contained segments of adult parasites. The associated lesions were either within or around the biliary tree, with secondary involvement of the adjacent liver parenchyma. In response to the parasite, the epithelium of the bile ducts in 66 (77 per cent) of the 94 cases was either hyperplastic or showing metaplasia. The proliferation was sometimes quite extensive, approaching an adenomatous hyperplasia. As an aftermath of the pressure, due to the distention of bile ducts by parasites, there was frequently necrosis of the hyperplastic epithelium. Probably also in response to pressure, there was squamous metaplasia of the lining epithelium. Occasionally, reduplication of bile ducts, adenomatous hyperplasia and squamous metaplasia of the biliary epithelium were present within a single section. The smooth muscle of many bile ducts was greatly thickened.

Pericholangitis was established in 65 (67 per cent) cases, and varied from the acute to the chronic stages. In the acute stage there were numerous mononuclear and polymorphonuclear leucocytes. Eosinophiles were frequent. Many of the

macrophages contained various types of pigment which included hematin, hemosiderin and lipofuscin. In the active, chronic stage of pericholangitis, associated with an increased thickness of the wall due to smooth muscle changes, there was an excess of collagenous tissue. The reaction was accompanied by numerous inflammatory cells predominated by plasma and small round cells. Deposits of mineral and plaques of poorly formed bone were detected in the wall of some bile ducts. Liver fluke ova were occasionally seen in the pericholeductal tissue where they provoked a chronic inflammation of a foreign-body type. Bile stasis occurred in some lobules as indicated by an excess of bile pigment in the cytoplasm of hepatic cord cells. This was in sections where the biliary system was partially obstructed by flukes and the associated inflammation.

In some regions of the hippopotamus livers, the individual lobes were more distinct than in those of other species except for the domestic pig. This distinct delineation resulted from an abundance of connective tissue in the interlobular septa. This feature was also observed in hippopotamus calves less than six months old and even in those free from flukes. One of the confusing things about it was that it was not found throughout. In 72 (76 per cent) of the 94 animals, affected by fascioliasis, the degree of delineation was, however, very prominent. This was attributed to a pronounced peripherolobular fibrosis resulting from excessive collagen formation associated with chronic peri-cholangitis due to the liver flukes.

Echinococcosis

Hydatid cysts were encountered in 17 (16 per cent) of the 97 animals. Liver sections were prepared from all except one of those which revealed a readily detectable cyst at autopsy. All reactions to cysts were of a chronic nature and encapsulated by a thick connective tissue layer. Immediately around the capsule were numerous small round cells. Viable appearing scolices, within brood capsules [Plate 10 (92)], were found in groups. Some had as many as seven scolices. The cysts were unilobular and resembled those of *Echinococcus granulosus* (Batsch, 1786) occurring in bovine livers.

Additional hepatic lesions

Specifically unidentified, small, spherical bodies, measuring from 15 to 20 microns in diameter, were encountered in a few livers (five). Similar bodies were also found in 12 (32 per cent) of 38 uteri. Their behaviour in this organ will be described later under the sub-heading "Uterus".

Liver sections, cut through the centre of these bodies, showed that they had a double-contoured wall and an inner membrane. The outer wall had radiating cross-striations inter-spaced at close intervals [Plate 8 (74)]. In thick tissue sections, the objects were occasionally so situated that the surface of their outer wall could be studied. In this position, spines were observed protruding from the surface in a way similar to that seen in a cockle bur. In H. and E. stained sections, the outer wall had an inherent lightly pigmented golden brown colour. Usually the inner membrane was partially wrinkled or collapsed so that the inner structure assumed numerous shapes [Plate 8 (72)]. It contained a large fragment of amorphous, basophilically staining material, possibly of nuclear origin. Some of the H. and E. stained sections revealed that the inner membranes were inherently light golden yellow. These bodies were always in close proximity to the portal areas. They were often present in groups of approximately 200, though smaller numbers were also seen. Several

PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

small groups gave the impression that they were situated within the lumen of small portal vein branches but due to changes one could not be unequivocal. The bodies sometimes appeared to consist of only the inner membrane surrounded by a distinct smooth-lined space. Not even a vestige of the outer wall with its spines was visible. This could have been an artefact produced in sectioning by displacement. In other groups the outer wall was represented merely by a single very thin line.

With GMS stain the outer double-contoured wall was weakly stained by the light green counter stain. The amorphous material, within the confines of the inner membrane, had an affinity for silver. It appeared as a granular substance which was dense in some and more scattered in others. In some of the well-preserved bodies, the inner membrane contained cytoplasm, and a clearly-defined nucleus enveloped by a membrane. Bodies with a rough outer membrane produced more prominent hepatic lesions than those not endowed with the same feature. In some instances, they evoked a foreign body granulomatous response occasionally associated with the bodies within giant cells. Though these objects were not examined in a fresh, unfixed preparation, it is believed that they represent oocysts of a previously unknown coccidium.

Kidneys

Bilharziasis

Kidney sections were examined from 81 of the 97 cases. Forty-nine (60 per cent) had an endophlebitis of a proliferative nature within the renal veins. As some sections did not contain large branches of renal veins it is possible that more were affected than were thus determined to be. Compared to those of other vessels already described, the lesions in renal veins were primarily of a chronic nature. The usual finding was a rather irregular-appearing pseudo-intima lining the vein. It was composed most often of mature collagen with relatively few nuclei. Though single-layered in many veins, it was sometimes multi-layered. A more active, subacute, proliferative endophlebitis, as indicated by many inflammatory cells and villous projections, was occasionally seen. Examples of organized lymphoid nodules in renal veins were lacking but some of the club-like intimal projections were heavily and diffusely infiltrated with lymphocytes. Lesions in the parenchyma secondary to the endophlebitis were not observed.

Additional kidney lesions

In nine (11 per cent) animals, lesions of other types were found. Three revealed localized areas consisting of nodules of only lymphoid cells. Macroscopically, they appeared as white foci in the cortex similar to those in cattle affected by East Coast fever. Two animals had a chronic nephritis characterized by fibrosis of both layers of Bowman's capsule and the cortical interstitium. Four showed lesions equivalent to a chronic interstitial nephritis of domestic animals with no glomerular involvement.

Adrenal Glands

Bilharziasis

Adrenal tissue sections were prepared from 86 of the 97 cases. Twenty-eight (32 per cent) revealed an endophlebitis of the medullary vein, and 15 (17 per cent) contained sections of adult schistosomes [Plate 10 (91)]. The endophlebitis was most

frequently of a chronic nature with either a single or double-layered pseudo-intima similar to that observed in veins of other organs. Several of the lesions showed a tendency toward lymphoid proliferation but lacked the organization of the lymphoid nodules seen in veins of the liver. As in the renal veins, the lymphoid element was a diffuse infiltration from the intima into the villous projections. Occasionally groups of lymphocytes were present in the parenchyma immediately adjacent to the medullary vein.

Schistosome ova were demonstrable in sections of 59 (69 per cent) of the 86 animals from which sections were examined. With two exceptions, all ova were situated in the adrenal cortex. One of the former was located within a granuloma in the medulla, and the other within a club-like projection extending from the intima into the lumen of the medullary vein. Individual or groups of ova appeared to be either viable or in various stages of deterioration. Viable-appearing miracidia were observed in some ova [Plate 10 (86)] while others contained basophilic material possibly of miracidial origin. Intact or fragmented empty shells were also visible. The degree of the host response varied a great deal. It was absent in some instances [Plate 10 (90)], and readily detectable in others. The earliest response consisted of an accumulation of eosinophiles and round cells, with a preponderance of eosinophiles, around the ovum or ova [Plate 10 (87)]. As time progressed the number of eosinophiles diminished while that of plasma and round cells increased. A more chronic reaction was a granulomatous one composed of a few small round cells, epithelioid and multi-nucleated giant cells [Plate 10 (88)]. Encapsulation was not a prominent feature, and fibroplasia was not observed. Some of the granulomas appeared to be somewhat circumscribed but this was apparently due more to compression of pre-existing stromal connective tissue rather than to a production of new tissue. In the reactions necrosis of individual or groups of the inflammatory cells occurred sometimes. Necrosis of eosinophiles resulted in eosinophilic amorphous centres in the lesions [Plate 10 (89)]. Adrenal lesions which could be ascribed to other causes were not observed.

Spleen

Bilharziasis

Spleen specimens were collected from 83 of the 97 cases. Forty-six (55 per cent) showed endophlebitis of the splenic veins. In the splenic capsule and trabeculae the venous branches often contained a pseudo-intima of mature connective tissue. They were either single or multi-layered but rarely consisted of more than two layers [Plate 11 (95)]. These structures lay superimposed on the true intima with an open space between them. Sections of viable schistosomes were present in a few veins. In response to them, there was an active cellular reaction which, in association with the existing intimal proliferation, almost completely obstructed the lumen. A panphlebitis occurred in a few veins. Their histologic layers contained inflammatory cells, including numerous eosinophiles. Lymphoid proliferation was limited to a few veins but it was neither as prominent nor as well-organized as in the veins of the liver [Plate 11 (96)].

Other changes, not regularly seen in the spleen, were of a non-specific nature. They included congestion of the red pulp, and hyperplasia or atrophy of the white pulp.

PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

Testes

Bilharziasis

Sections of testes from 38 males, were examined. Six (16 per cent) of them contained veins showing endophlebitis which varied from a very cellular acute stage to one characterized by a pseudo-intima composed of dense collagen. It is possible that more animals were affected but the available sections contained only a limited number of veins. Areas of disseminated focal, interstitial orchitis, characterized by a large number of small round cells, were seen in one testis. Ova were present in some of these foci [Plate 12 (109)]. In addition, there was a very active, highly cellular endophlebitis in the same testis.

Additional Testicular Findings

Eight (21 per cent) of the 38 animals had inactive testes as judged by their histological appearance and absence of spermatogenesis. Most of the animals weighed less than 1,350 lb, while one weighed 1,760 lb and another one 1,890 lb. In one of the sexually-mature males a focal spermatic granuloma was seen.

Ovaries

Bilharziasis

Sections from ovaries of 24 hippopotami were examined. Sixteen (67 per cent) showed an endophlebitis which varied from a rather acute stage with intense hypercellularity [Plate 11 (99)] to the chronic stage characterized by a pseudo-intima composed of mature collagen. Schistosome adults were present in a small percentage of veins [Plate 11 (101)]. Lymphocytes heavily infiltrated some of the intimal projections but there were no well-organized lymphoid nodules as seen in liver veins. There were no schistosome ova.

An additional ovarian lesion

Of all ovaries, examined histologically, only one contained neoplastic tissue. It was interpreted as a granulosal cell tumor [Plate 12 (110)].

Uterus

Bilharziasis

Sections of uteri of 38 animals were examined. Twenty (52 per cent) revealed an endophlebitis of essentially the same types as those seen in some of the other organs [Plate 11 (98, 100, 102)]. Schistosome ova were not observed.

Additional uterine lesions

In the lamina propria of the endometrium of twelve (32 per cent) animals were specifically unidentified, small, spherical bodies morphologically similar to those described from the liver. They appeared either in large groups [Plate 12 (107)] or as individuals. In some instances single bodies were situated within venules. The endometrium showed very little response. They are also believed to represent oocysts of an unknown coccidium.

Additional interesting observations, which occurred in 25 (60 per cent) of the 38 animals on which uteri were sectioned, were alterations in some of the small branches of the uterine arteries. Lesions appeared in the muscular arteries and were

characterized by a thickening of the wall. This resulted from an increase in the connective tissue most frequently just beneath the endothelium [Plate 11 (103)] but sometimes also within the media. There appeared to be a marked hypertrophy and/or hyperplasia of the longitudinally oriented smooth muscle which lay immediately beneath the intima. These changes were not found in young animals. The smallest one affected weighed 2,000 lb. It is possible that the lesions were in some way related to or were the result of a post-partum change as seen in the bovine (Cohrs, 1967) and called pregnancy sclerosis. There was nothing to suggest that they were due to bilharziasis because the endothelium was invariably quiescent.

Gastro-intestinal Tract

Bilharziasis

Sections from the gastric diverticula were prepared from 78 of the 97 animals. Different types of parasitic endophlebitis, which varied from the acute to the chronic stages [Plate 11 (104)], were encountered in 52 (66 per cent) of these. The features of these lesions resembled those described from other organs. Schistosome adults were present in veins of some animals. Ova were encountered in veins of one case and in the lamina propria of three others. Enderteritis of small gastric artery branches was seldom seen.

Abomasal sections from 62 of the 97 animals were examined, and of these 14 (22 per cent) showed endophlebitis. This type of lesion was found also in intestinal sections of 22 (31 per cent) of 70 animals.

The largest portion of the portal vein just before it entered the liver was observed to contain a well formed chronic type of pseudo-intima [Plate 11 (93)] in the one animal on which this vessel was sectioned.

Miscellaneous Organs

Endophlebitis was observed in additional tissues including the mammary gland [Plate 11 (97)], pancreas and skeletal muscle. Schistosome ova were detected occasionally in other sites, e.g. thyroid gland and pancreas. A tumor found on the gingiva opposite the angle of the mouth, proved to be a malignant melanoma [Plate 12 (111)]. Small tumors, in the gastric diverticula, were identified as benign papillomata. *Hepatocystis hippopotami* Garnham, 1958 schizonts were seen in a single lymph node [Plate 12 (112)].

The urinary bladder was examined on such a limited number of animals that the true picture of what was present in this tissue cannot be given. Of those examined there were no lesions present.

TAXONOMIC ASPECTS OF THE HELMINTHS

PHYLUM PLATYHELMINTHES

Trematoda

Schistosomatidae

Schistosoma hippopotami Thurston, 1963, was the predominant species which was encountered in these hippopotami. As to the validity of this species, Thurston (1963) discussed its affinities with species having terminal, subterminal and lateral

spined eggs. Thurston differentiated *S. rodhaini* Brumpt, 1931, from *S. hippopotami* by the "numerous small uterine eggs" of the former. However, Schwetz (1954), Fain, Thienpont, Herin & Deramee (1953) and Saoud (1966) stated that only a single ovum was observed in the uterus of *S. rodhaini* with the spine subterminal as for *S. hippopotami*. The measurements of the ovum in the uterus were given by Fain *et al.* (1953) as 0.093×0.04 mm. The measurements of the ova of the present material were 0.081 to 0.103 mm \times 0.031 to 0.043 mm fitting into the range given for *S. rodhaini* by Fain *et al.* (1953). Further observations on the adults of *S. hippopotami* were that the overall measurements differed particularly between specimens obtained from young animals and those of older animals. It seemed as if the parasites were more stunted in the older animals. As to the measurements of schistosomes in general, various authors have commented on their unreliability due to various factors, e.g. type, condition, age of host, etc. (Le Roux, 1961; Pitchford, 1955, 1965, 1966).

Thurston (1963) considers *S. hippopotami* to resemble *S. incognitum* Chandler, 1926 with marked differences, however, in the ovary, the ovum especially the spine and the intestinal caeca of the male. Sinha & Srivastava (1956) discussed the synonymy of *S. incognitum* with *S. suis* Rao & Ayyar, 1933.

The present authors feel that a significant morphological characteristic of *S. hippopotami* is the enormous size of the suckers and mouth opening of the male [Plate 10 (85)]. The unrelaxed position of the male in a fixative is also different from other *Schistosoma* spp. It would seem that the large suckers are a special adaptive feature for its mode of life accounting for its wide distribution within the hippopotamus and its ability to crawl around vigorously on the wall of the vena cava.

A few immature specimens were collected from a very young hippopotamus weighing 180 pounds. One of these, a rather stout female, about the size of *S. mattheei* Veglia & Le Roux, 1929, contained a single terminal-spined egg in the uterus, which was much larger than the single egg usually seen in the uteri of *S. hippopotami*. The shape of the egg was similar to that of *S. haematobium* (Bilharz, 1852). In the collection there were also specimens with ova having no trace of a spine. The morphology of the adult, however, was the same as *S. hippopotami*. The other species, *S. edwardiense* Thurston, 1964, which has been reported from hippopotami was not specifically identified in this study.

Fasciolidae

Fasciola nyanzae Leiper, 1910, named after the placename Nyanza in Kenya, was the only species of this family present in the hippopotami examined. Jackson (1921), without giving any reasons altered the species name to *F. nyanzi*. The original name applied to it by Leiper fulfils the "International Code of Zoological Nomenclature" Appendix D; IV (22-b). Moreover taking into consideration the specific locality where it was first found, there is no reason to change it. Jackson (1921) closely related this parasite to *Fascioloides magna* (Bassi, 1875). Dinnik & Dinnik (1961) disagreed and pointed out the affinities with *Fasciola gigantica* Cobbold, 1885, and *Fasciola hepatica* Linnaeus, 1758. The present authors agree with Dinnik & Dinnik (1961).

Paramphistomidae

Paramphistominae

Gigantocotyle gigantocotyle Näsmark, 1937

As the name indicates this species is characterized by an extremely large sucker, which is situated at its posterior end, by which it attaches itself to the wall of the stomach as do the other conical flukes. In some cases they were clustered together in patches where on removal, rings in the stomach mucosa caused by these suckers, gave it a "hobnailed" appearance.

Gigantocotyle duplicitestorum Näsmark, 1937

This species was distributed in the glandular portion of the stomach and first part of the duodenum. It is about the same size as *G. gigantocotyle* but differs from it in having a somewhat smaller sucker.

Nilocotyle (Nilocotyle) praesphincteris (Näsmark, 1937)

This small conical fluke with a very small sucker at the posterior end, also occurred in the stomach. This species was redescribed by Swart (1966) from material collected during this investigation.

Gastrothylicinae

Two species of this subfamily were present in the stomach. As the general appearance of this parasite is much the same as *N. (N.) praesphincteris*, little material was collected. Therefore a specific diagnosis could not be made. These two parasites are a little larger than *N. (N.) praesphincteris* but differ from it in having a hollow sack ventrally.

Polystomatidae

This family of flukes was represented by a 100 per cent infestation of the monogenetic fluke *Oculotrema hippopotami* Stunkard, 1924, attached to the conjunctiva by six suckers. In contradistinction to other flukes mentioned, this family has a direct life cycle, there being no intermediate snail host. This is of interest in that the hippopotamus is the only known terrestrial mammalian host for a monogenetic trematode. This is the second published report of the species in the hippopotamus. The first report was of five specimens which were presumably collected by Prof. A. Looss from a Nile hippopotamus. The specimens were examined by Stunkard (1924) who identified them as belonging to a new genus and species and thus a unique case of a monogenetic trematode on a warmblooded animal. Yamaguti (1961) created a new subfamily Polystomatinae for this very interesting parasite.

Notocotylidae

Species of the above four families of flukes have been reported as occurring in the hippopotamus from other parts of Africa as well as from South Africa. In addition during this operation a new species of the genus *Ogmocotyle* was discovered. This is the first record of a species of the Notocotylidae to be found in the hippopotamus.

PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

Cestoda

Taeniidae

The larval stage (hydatid) seen in some of the animals was of *Echinococcus granulosus africanus* Verster, 1965. The adults probably occur in one or more of the wild carnivores of the park. Some of the cysts were taken from one of the livers and fed to a domestic cat. They developed into rather poor adult specimens upon which the specific identification was based.

PHYLUM ASCHELMINTHES

Nematoda

Dipetalonematidae

The filarial worm in the abdomen was *Dipetalonema hippopotami* Leiper, 1910. They were not very numerous and very little is known about their life cycle.

Atractidae

Large numbers of a very small parasite were found in the stomach, viz. *Coboldina vivipara* Leiper, 1910. The life cycle is continuous in the host and since closely related species in other animals are not considered of any importance in causing disease, this is probably also the case with this species.

DISCUSSION ON THE PATHOLOGY AND PATHOGENESIS

The survey on the incidence of parasites, harboured by 100 hippopotami, revealed the presence of 12 helminth species belonging to the classes Trematoda, Cestoda and Nematoda, a protozoon of the class Sporozoa and a specifically unidentified unicellular organism probably a coccidium. Consideration of their pathogenicity made it apparent that the outstanding lesions were produced by *S. hippopotami* and *F. nyanzae*, while infestations or infections by the remaining parasites caused a relatively mild or an insignificant effect on the hosts. As mixed infestations were the rule, it was impossible to determine to what extent one pathogen could have influenced another. In the discussion that follows attention will be paid to the pathogenesis and pathology resulting from the *S. hippopotami* infestation. This will be accompanied by a brief comparison between animal and human bilharziasis to illustrate the significant differences in the pathogenicity of certain *Schistosoma* spp. on their respective hosts.

It is generally accepted that adult *Schistosoma* spp. exert much of their pathogenicity by chemical action on the hosts. Metabolic products or secretions of schistosomes and their ova as well as katabolic products of dead worms and ova have a cytotoxic action while antigens sensitize the host tissues. Changes brought about by adult worms as seen in laboratory animals, exclusively infested by male schistosomes, are relatively insignificant. The resulting lesions consist of perivascular infiltration and deposition of dark brown pigment in the reticuloendothelial cells of the liver and spleen. Dead schistosomes from the intestinal and mesenteric veins reach the branches of portal veins of the liver as emboli and are then gradually phagocytosed within the thrombophlebitic foci. In human schistosomiasis, ova are the chief pathogenic agents. They exert a cytotoxic effect mainly in small blood vessels of the intestinal and urogenital tracts and in certain circumstances also in the liver, lungs and other organs (Nauck, 1956).

Consideration of the extraordinary distribution of the adult schistosomes, their regular appearance in large and small veins of the viscera and other tissues, and the absence of evidence of any other pathogens in the blood circulation, permits the conclusion that constant changes within the cardiovascular system are caused by *S. hippopotami*. The infrequent appearance of granulomatous reactions or fibrous encapsulations of ova in the intestinal tract, liver and lungs, is a clear indication that the pathogenicity of this developmental stage is of minor significance in hippopotami.

The mechanical irritation by schistosome adults, at sites of contact between parasites and endothelial lining, appeared to have caused minute injuries. The impression was gained that the focal cellular reactions on the intima and endocardium were caused by the large suckers of male parasites. At a site where one or more dead parasites occurred, there was usually a granulomatous response within the vascular lumen. This was sometimes superimposed on a proliferative endovasculitis which obviously had been present before the arrival of dead parasites at this site. Close scrutiny of the striking responses within the heart and various vessels, where proliferations were accompanied by a massive eosinophilic infiltration, made it apparent that mechanical irritation was not the sole source for the lesions. It appeared that metabolic and katabolic products contributed towards the inflammatory process. Although it was not possible to determine which of these agents was the most active stimulus, it was nevertheless assumed that parasitic derivatives played an important role. Although early lesions were sometimes the most prominent, it was usual to find older lesions somewhere in other vessels. It thus appeared as if most animals had been affected previously at other sites, and that acute lesions in one vessel probably followed in response to a local challenge of a particular vessel after it had been sensitized. Whether the somatic response resulted from the mechanical action by suckers of male schistosomes after repeated attachment onto the intima, cuticular excretions or expelled metabolic substances could not be determined. Should the latter have served as a stimulus it would probably have been composed of haematin and one or more associated enzymes from the gut of the parasite. The quantity of haematin, harboured by *S. hippopotami*, was negligible in comparison with that held by *S. mattheei* in infested sheep. As the precise nature of the harmful substances is obscure, they will be referred to collectively as an irritant.

The spectral aspects of the lesions in the heart, pulmonary artery, hepatic, portal and other veins had a common trend in the development of various stages of endocarditis, endarteritis and endophlebitis. This tendency appeared to be a host response which could develop from an active to a simple chronic stage, possibly as the result of a single stimulus by the irritant or alternatively it could undergo numerous spectral stages in response to repeated exposures to the irritant at various intervals. Thus it was observed that final formations consisted of multilayers of pseudoendocardium or pseudo-intima in branches of the pulmonary artery and various veins. It appeared as if the host was attempting to place barriers between the irritant and the cardiovascular lining by laying down pseudo-formations. The laminated formations were effective barriers against the schistosomes and where present usually prevented direct contact with the true endocardium and vascular intima. It was nevertheless observed that an activation of the surfaces appeared on the surface of some of the small fenestra in the network. As they were usually too small for direct contact by schistosomes with the underlying layers, the impression was gained that at least part of the irritant consisted of a soluble component or of extremely fine granules in suspension capable of passing through the minute apertures

PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

as small as or even smaller than the diameter of capillaries. This would imply that the latter two components could have been derived from exudates, excretions or secretions of parasites. The localized lesions suggest that the strongest action was of a focal nature. The possibility that sensitization could have encouraged the parasitic effect must not be lost sight of.

Although several mammalian species can be infested with different *Schistosoma* spp. they are not necessarily normal hosts as reflected by the relatively small size of adult parasites and by a low degree of ovigenesis. The agouti (*Dasyprocta aguti*) can be infested experimentally with *S. mansoni* but shedding of ova in the faeces is scanty and irregular (Price, 1953). There is evidence that in this host although quite a few ova are produced they are often diverted to the liver and hence are not evacuated regularly with the faeces. In naturally infested hippopotami the manifestation differs considerably in that ovigenesis by *S. hippopotami* is extremely poor except by females lodged in the adrenal veins. The host response appears to have had a remarkable suppressive effect on *S. hippopotami*, and even in adrenal veins few ova were produced. The relatively higher degree of ovigenesis at this site is attributed to the greater concentration of glucocorticoids and other hormones. This deduction is justified as these hormones are known to decrease the host resistance as demonstrated in animals harbouring one or other pathogen. It is self-evident that the concentration of these hormones would be higher in adrenal veins than in other regions of the body. The relatively few ova encountered in lungs in all probability originated from the adrenal veins.

The dwarfed appearance of adult schistosomes in old hippopotami is attributed to the suppressive action on their growth by immune bodies. A similar phenomenon has also been recorded by Horak (1967) who encountered stunted paramphistomes in cattle rendered immune by repeated infestations in contradistinction to well developed parasites harboured by cattle after their primary infestation.

The microscopic appearance of the elaborate formation of lesions within the heart and blood vessels is not only interesting but also stimulates speculation on the pathogenesis of bilharziasis in hippopotami. Consideration of the formations makes it apparent that the general process of development is based on the same scheme at all sites in the cardiovascular system. The progressive growth of lymphoid nodules within the portal and hepatic veins is on the other hand of a different design.

The first perceptible change was hyperplasia of the endothelial cells which gradually protruded into the lumen of a blood vessel. This process was often accompanied by an infiltration of eosinophiles and other inflammatory cells including lymphocytes. It was assumed that the eosinophilic infiltration was an indication that on a previous occasion the host had been sensitized by the parasite. Examination revealed that endothelium hyperplasias of small vessels grew into solid masses which eventually filled the vessel lumen. In other instances the hyperplasias remained as pendulous extensions within the lumen. In some vessels, filled by proliferating masses of cells, organization occurred resulting in the formation of a network of cords composed of connective tissue covered by endothelium. Between the cords were vascular spaces. Eosinophiles frequently lined the endothelial surface and in addition were interspersed in the connective tissue of the cords. Growth of the villous projections sometimes persisted and eventually became interwoven. Subsequently it became evident that consolidation of the connective tissue occurred when the acute phase subsided. Depending upon the site of the lesion, the consolidation of the stroma led to the formation of a pseudo-intima or pseudo-endocardium. Examinations of many sections disclosed that the basic inflammatory process could

be repeated several times resulting in an ever increasing complexity of the final reactive formation. These cardiovascular lesions are considered by the authors to be pathognomonic for hippopotamine bilharziasis.

Since the nature of the reactions varied considerably in an individual animal, it is difficult to rationalize on this basis that this manifestation could have been induced by repeated exposures to different groups of cercariae. On the contrary, it would be more feasible to assume that lesions resulted from movements of adult schistosomes from one to another site of attachment within the cardiovascular system. If the irritant, released by the parasite, had been capable of exerting an effective local influence then this would support the hypothesis that parasitic movement within the vascular system caused the disparity seen in lesions at different sites. Whatever the nature of the irritant might have been, the easily detectable marred sites remained as evidence of their most recent effect.

Shaw & Ghareeb (1938) state that in human bilharziasis the living worm is harmless but that products derived from dead parasites are extremely toxic. The writers concur with the latter statement but emphasize that the former is not applicable to the behaviour displayed by living schistosomes parasitizing hippopotami and domestic ruminants. In contradistinction to what has been reported by Shaw & Ghareeb (1938) about the absence of lesions in the pulmonary arterial wall of infested human beings, the morbid effects by adult worms in the cardio-vascular system of hippopotami were extensive. They also stated that in man thrombosis never developed in response to living worms. Absence of this response was also observed in hippopotamine bilharziasis.

Shaw & Ghareeb (1938) considered that angiomatoids, first described by them in human bilharziasis in Egypt, were due to schistosome ova. The relatively few ova found in lungs of hippopotami in relation to the extensive lesions produced by adult schistosomes, caused the writers to conclude that these angiomatoids were unequivocally due to the effects of mature worms and not to the ova. The absence of any evidence of Ayerza's disease and cor pulmonale may be an indication of a better developed collateral circulation of the pulmonary artery of the hippopotamus.

Vascular changes similar to angiomatoids were rarely observed in small intra-hepatic branches of the portal vein but proliferative endophlebitis was common. Somewhat similar manifestations have been observed in portal veins of domestic ruminants suffering from bilharziasis. The initial or acute as well as more advanced stages of endophlebitis have been seen in cattle (McCully, 1966). Although the formation of a pseudo-intima is different, some lesions of these ruminants attest to the severity of the endophlebitis resulting from bilharziasis. As in the case of hippopotami, these were found in affected vessels in association with adult schistosomes.

Development of lymphoid nodules in portal and hepatic veins is believed to be in response to adult parasites and/or their metabolic and katabolic products. This assumption finds support from similar but not identical nodules appearing within portal veins of sheep and cattle in response to dead schistosomes (McCully, 1966).

The liver and intestinal tract of hippopotami showed a low degree of infestation by schistosome ova; very few of them were encapsulated in the lamina propria of the stomach. The relatively few ova and their situation in localities from which they could not be evacuated for the continuation of the developmental cycle,

PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

suggest that the hippopotamus could be an aberrant host. Studies on the taxonomy and comparisons between known *Schistosoma* spp. revealed that specimens recovered from South African hippopotami are morphologically indistinguishable from *S. hippopotami* Thurston, 1963, described from East Africa. The description of the East African strain was not accompanied by a detailed account on the pathology and ovigenesis. This unfortunately precluded comparative studies which would have added more to our knowledge on the efficacy of the hippopotamus as a host.

Should no other mammalian host be involved in adequately maintaining the life-cycle of *S. hippopotami*, it would be necessary to determine whether or not the hippopotamus can be induced to function as a normal host. Consideration of the pronounced parasitosis seen in hippopotami, suggests that cercaria-production by the invertebrate host is highly efficient in order to maintain a continual high level of the infective stage, for the infection of the mammalian host. The present observations show that the weak link in the vertebrate-invertebrate life-cycle is the limited evacuation of ova to ensure that sufficient miracidia become available for the infestation of snails. Although ova were not found in adequate numbers in either the gastrointestinal or urinary tract to fulfil this requirement, it should be remembered that all animals were killed in winter. It has not yet been possible to determine whether or not the female parasite could be more ovigenic during the ensuing seasons influenced by the effect on the host by lush grazing. Together with an increased ovigenesis though, there would have to be coupled a movement of females from the posterior vena cava and venae hepaticae into veins of organs from which eggs could be expelled regularly to the exterior. An escape by way of the lung appears to be unlikely as only a very limited number of encapsulated ova were found at this site. Should eggs have escaped by way of the alimentary or urinary tract, it would have been expected that a large number of trapped and degenerated ova would have been present at these sites as seen in infested human beings.

From histological sections it was impossible to find evidence for a seasonal periodicity of ovigenesis which could have disclosed that enough ova had been expelled for miracidial production. From this observation it could be assumed that an alternative host exists for the effective maintenance of the lifecycle of *S. hippopotami*. This could be possible if it is considered that the blue wildebeest and impala can serve as hosts of *S. mattheei* which also parasitizes domestic ruminants. Surveys on the incidence of *Schistosoma* spp. within the Letaba and other regions of the Kruger National Park may yet reveal the presence of an alternative host of *S. hippopotami*.

It would be of great advantage to establish the true status of hippopotamus as host. Should it be an aberrant host then the pathological changes could serve as a basis for determining the status of other hosts suffering from bilharziasis. Should the hippopotamus prove to be a normal host then it could be expected to find even a wider spectrum of lesions of the cardiovascular system due to the adult worm than has been recognized up to the present time.

The minimal infestation of ova did not alter the effect that schistosomes and their metabolic and katabolic products had on the liver of the hippopotamus, e.g. proliferative and nodular lymphoid endophlebitis. On the other hand the concurrent infestation of schistosomes and liver flukes, both of which cause periportal cirrhosis, may have cloaked the range of the activity of either worm in this regard. This dual infestation precluded determining whether cirrhosis could have been promoted by lesions in the portal vein as suggested by Gillman (1957) in the case of other animal species infested by schistosomes.

It was not possible to assess the specific effect of bilharziasis on hippopotami, because, as far as could be determined, they appeared clinically healthy. Among the many unknown traits of hippopotamine bilharziasis is whether more severely affected animals succumbed. It is a well known fact that sick wild animals go into seclusion and may eventually become victims of predators.

Liver flukes caused fibrosis of the portal area but in no instance was it judged to be so extensive as to cause any appreciable decrease in the functional activities of the liver. The absence of liver flukes in infant animals probably indicates that they had not yet been infested or alternatively that parasites had not reached the bile ducts.

It is anticipated that careful studies of the other forms of parasitosis, encountered in many animals, would have revealed more significant features. The significance of the specifically unidentified unicellular organisms found in the liver and uterus of some hippopotami and which are believed to be oocysts of coccidia is obscure. The hope is expressed that future investigations may reveal their specific identity. Examination of fresh material may assist the identification. In addition unidentified cellular elements were encountered in the liver. Although their presence was regarded to have been in response to bilharziasis, it is possible that their appearance could have resulted from another stimulus.

SUMMARY

Necropsies were conducted on 100 hippopotami during the winter of 1964 in an area of the Kruger National Park where the continuous drought, over a period of three years, had reduced food supplies to a very low level.

Systematic observations revealed the presence of 12 helminth species belonging to the classes Trematoda, Cestoda and Nematoda, a blood sucking annalid, a single case of *Hepaticystis hippopotami* infection as well as a specifically unidentified unicellular organism in the liver and uterus. The latter is believed to have been a coccidium, the bodies observed being unsporulated oocysts having a spinous and striated wall.

A summary of the significant pathological changes produced by *Schistosoma hippopotami*, *Fasciola nyanzae* and *Echinococcus granulosa africanus* is presented in Table 1.

The pathogenicity of the remaining parasites appears to have been mild or insignificant.

The most striking observations were the high incidence and unusual distribution of *S. hippopotami*, and the pathognomonic cardiovascular lesions in response to adult parasites. These were characterized by the formation of a pseudo-intima in branches of the pulmonary artery and various veins and a pseudoendocardium within the heart.

Significant numbers of *S. hippopotami* were found in the heart and all major blood vessels.

Lesions, encountered in the systemic and visceral circulation, were most prevalent in the heart, pulmonary artery branches, posterior vena cava, venae hepaticae and portal veins.

Taxonomic affinities of *S. hippopotami* are presented.

PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

The unusual distribution of the adults, the very low degree of ovigenesis and the relatively high number of free and encapsulated schistosome ova in the adrenal cortex, medulla and vein as compared to those of the lung, liver, alimentary tract and pancreas, as observed during winter, cause doubt whether the hippopotamus is a normal host of *S. hippopotami*.

Suggestions made that the hippopotamus could be an aberrant host would require proof that ovigenesis is not subject to a seasonal periodicity, and evidence for the existence of an alternative normal host.

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* Approximate age-group

Group	Weight (cwt) Range	Approx. Age
1	0-7	Birth-6 months.
2	6-12	6 months-18 months.
3	12-17	18 months-30 months.
4	17-21	30 months-42 months.
5	21-30	Adults
6	30-40	Older Adults

14426

PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

PLATE I

- 1.—View of thorax and anterior abdomen from dorsal aspect. Liver (LV), post. vena cava (PVC), heart (H) and lung (L)
- 2.—Photograph of adult *S. hippopotami* in an opened post. vena cava
- 3.—Hydatid cyst in liver. Notice the distinct delineation of the liver lobules
- 4.—Endocardium of right ventricle. Whiteness due to “shaggy” proliferative endocarditis
- 5.—Endocardium of right ventricle. Less advanced endocarditis
- 6.—Liver, diaphragmatic surface. Capsule covered by diffuse fibrous connective tissue and adhesions
- 7.—Open portal vein. Notice lymphoid nodules attached to intima (arrows)

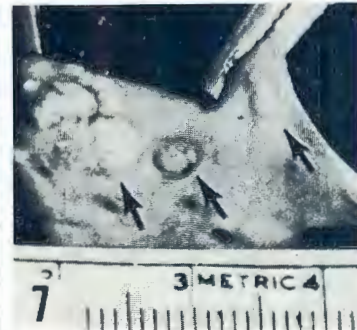
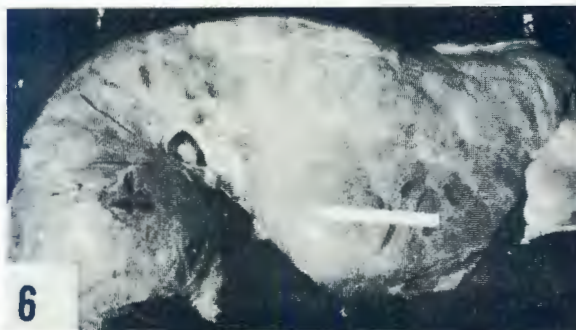
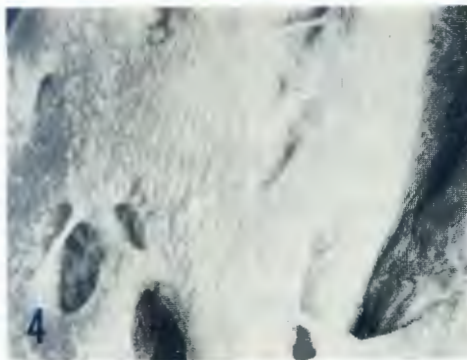
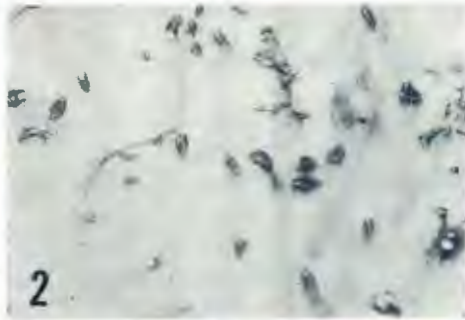
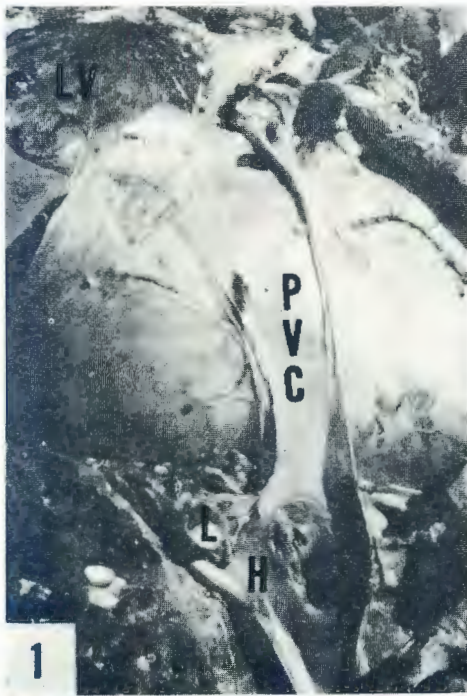


PLATE 2*

- 8.—Focal nodular endocarditis. Endocardium (E). × 125
- 9.—Nodules of lymphoid cells bulging from endocardium (E) into heart cavity. × 125
- 10.—Active, subacute proliferative endocarditis. Endocardium (E). Eosinophiles and other leucocytes within and on surface of villous projections. × 125
- 11.—Active, subacute proliferative endocarditis. More advanced than in 10. Villous projections still appear somewhat immature and many eosinophiles are present. Endocardium (E) and pseudoendocardium (PE, arrows). × 48
- 12.—Very cellular proliferative endocarditis in early stage of pseudo-endocardium (PE) formation. Endocardium (E). × 125
- 13.—Active, chronic proliferative endocarditis. Pattern more coarse or burlap in nature than above in 11. Endocardium (E) and pseudoendocardium (PE). × 48
- 14 & 15.—Considerably thickened endocardium beneath villous projections from surface. Endocardium (E). × 125 & × 48
- 16.—Several distinct layers of the pseudoendocardium (PE) have formed. × 125
- 17.—Chronic proliferative endocarditis. Endocardium (E). × 48
- 18.—Chronic, simple laminar proliferative endocarditis. Pseudoendocardium (PE). × 125
- 19.—Chronic thickened endocarditis. Notice that endothelium of some layers is still quite active while that of others is quiescent. Myocardium (M), endocardium (E) & pseudoendocardium (PE). × 125
- 20.—Active, chronic thickened endocarditis. Note endocardium (E) and pseudoendocardium (PE). × 48
- 21.—Chronic thickened endocarditis. Lamellae becoming rather dense but still fairly distinct. Endocardium (E) and pseudoendocardium (PE). × 48

* Sections for photomicrographs on this and subsequent plates were stained with HE unless otherwise stated in captions.

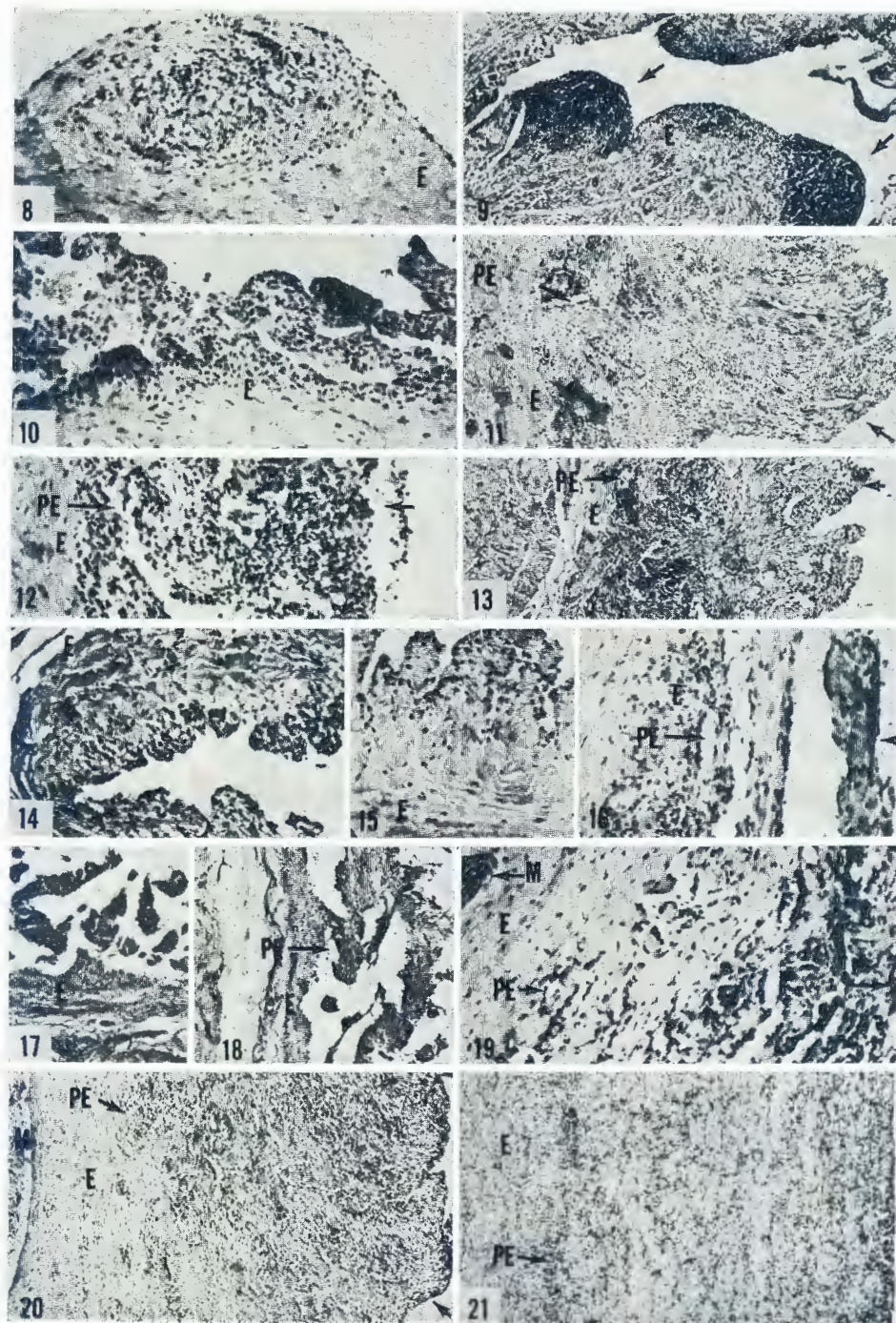


PLATE 3

- 22.—Acute endocarditis. Endocardium (E) with infiltrate of inflammatory cells which extend to underlying Purkinje fibers (P). $\times 500$
- 23.—Active, subacute proliferative endocarditis. Endocardium (E). Many cells within the villous projections are eosinophiles. $\times 200$
- 24.—Active, chronic proliferative endophlebitis. Affected endocardium (E) of cavity between muscular trabeculae with Purkinje fibers (P). Pseudoendocardium (between arrows). $\times 75$
- 25.—Chronic thickened endocarditis. Consolidation of lamellae of pseudoendocardium (PE) almost complete over the true endocardium (E). Myocardium (M). $\times 30$
- 26.—Proliferative endophlebitis of coronary vein. Intima (I), myocardium (M). $\times 100$
- 27.—Reactivation of lamella next to heart cavity (arrow). Notice mature collagen of other lamellae in pseudoendocardium. $\times 150$

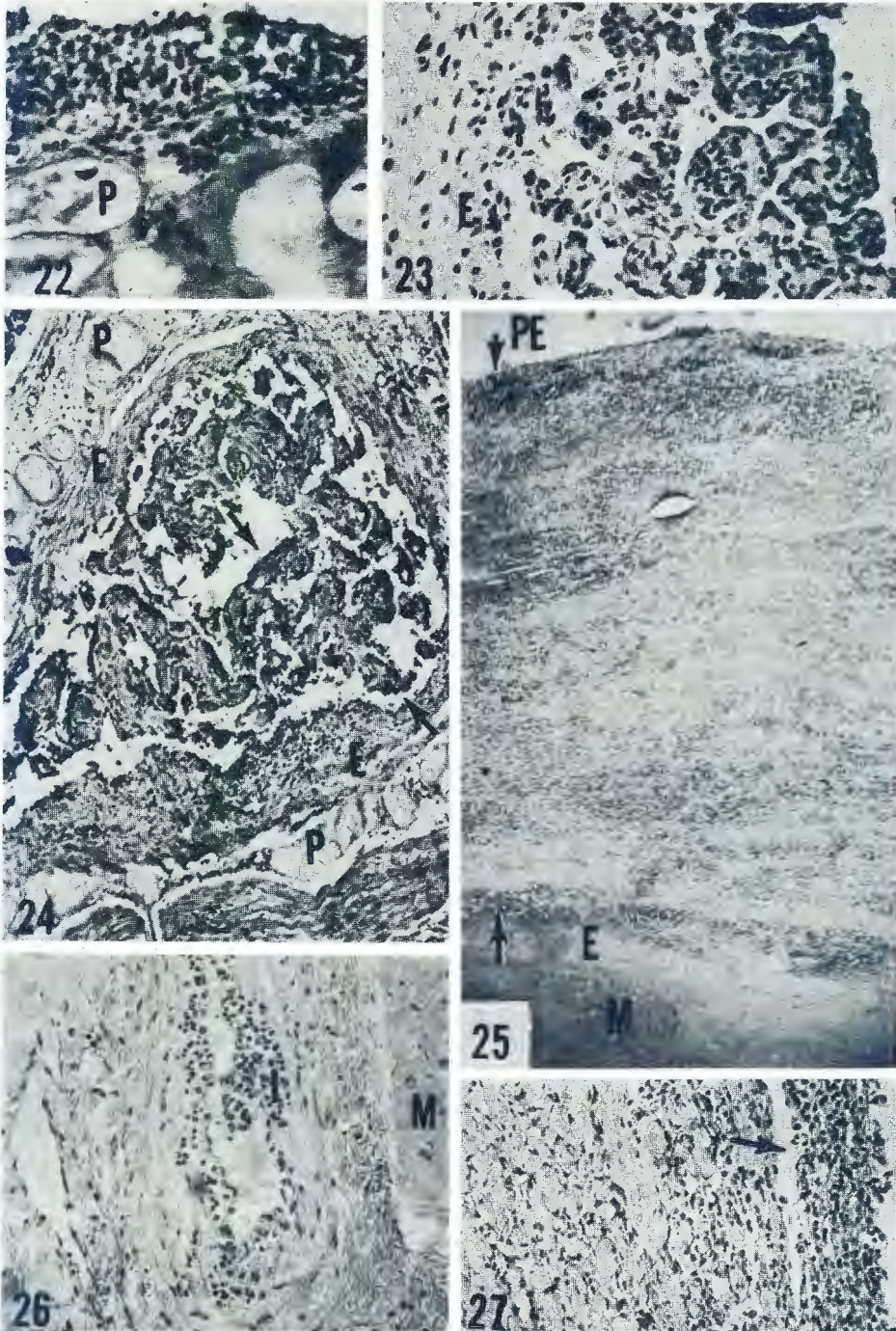


PLATE 4

- 28.—Pulmonary arteriole. Early indication of acute endarteritis, namely hyperplasia of endothelium. × 300
- 29.—Pulmonary venule. Lumen practically filled with spindle-shaped cells. × 200
- 30.—Longitudinal section of small branch of pulmonary arterial tree. Lumen between the true intima (I) on either side filled with undifferentiated cells. × 200
- 31.—Small artery of arterial tree, lung. Streaming elongated cells filling lumen between true intima (I) of either side. × 200
- 32.—Cellular activity on mural and luminal surfaces of a simple, chronic pseudo-intima seen above true intima (I). × 300
- 33.—Viable-appearing adult male and female schistosomes surrounded by granulomatous reaction within pulmonary vessel. × 75
- 34.—Dead adult schistosomes (arrow) in pulmonary vessel. Notice intense inflammatory response in vessel wall. × 75

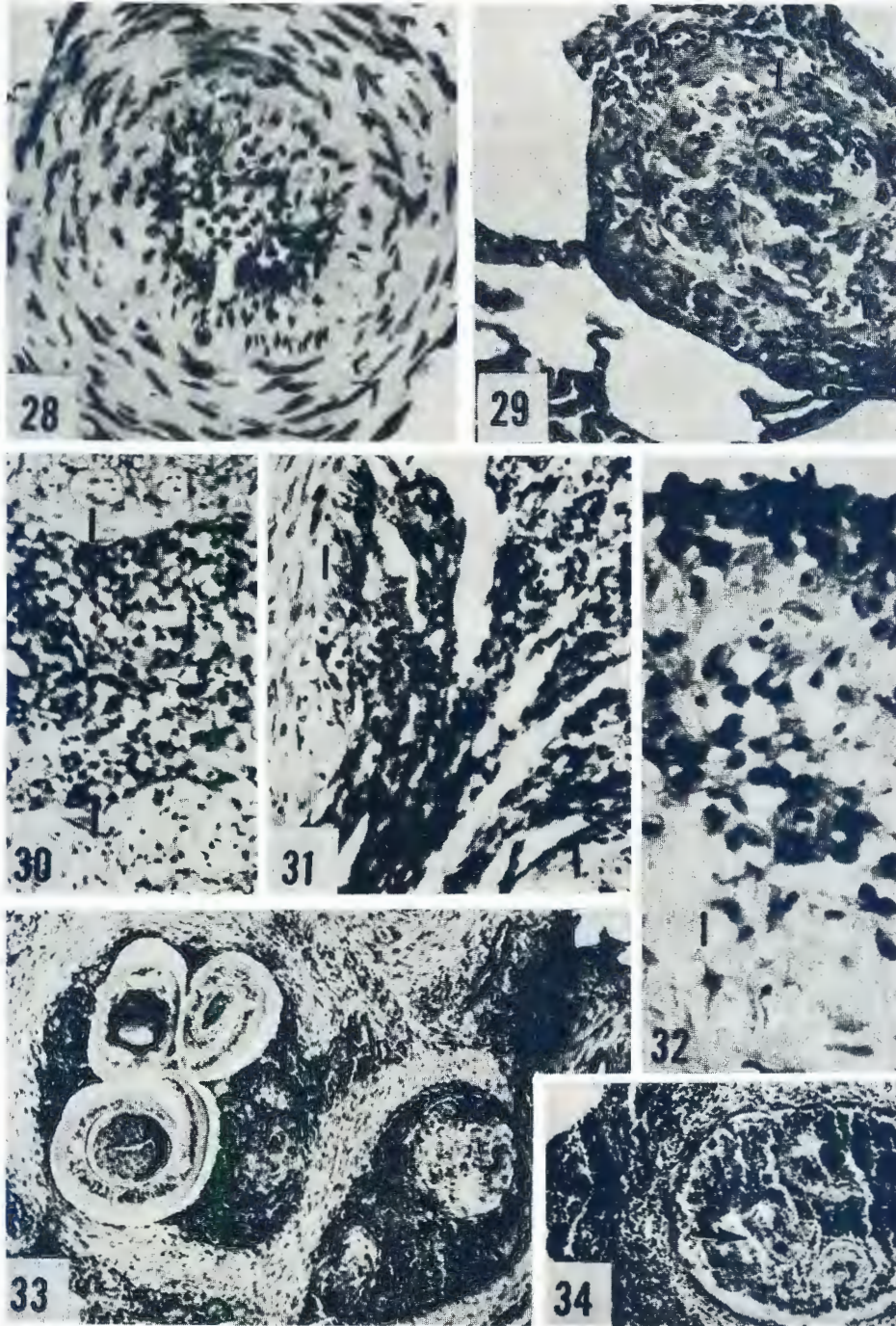
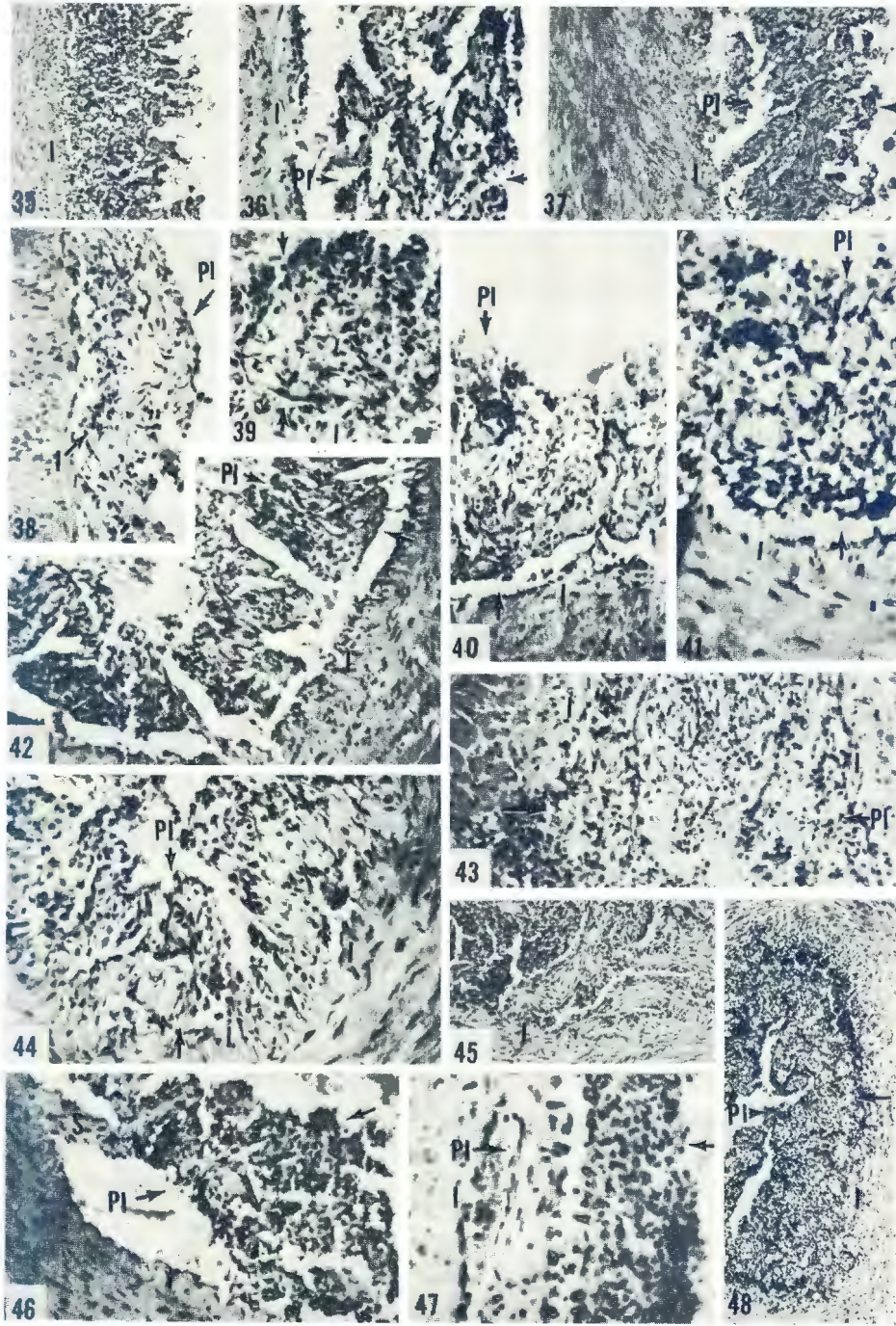


PLATE 5

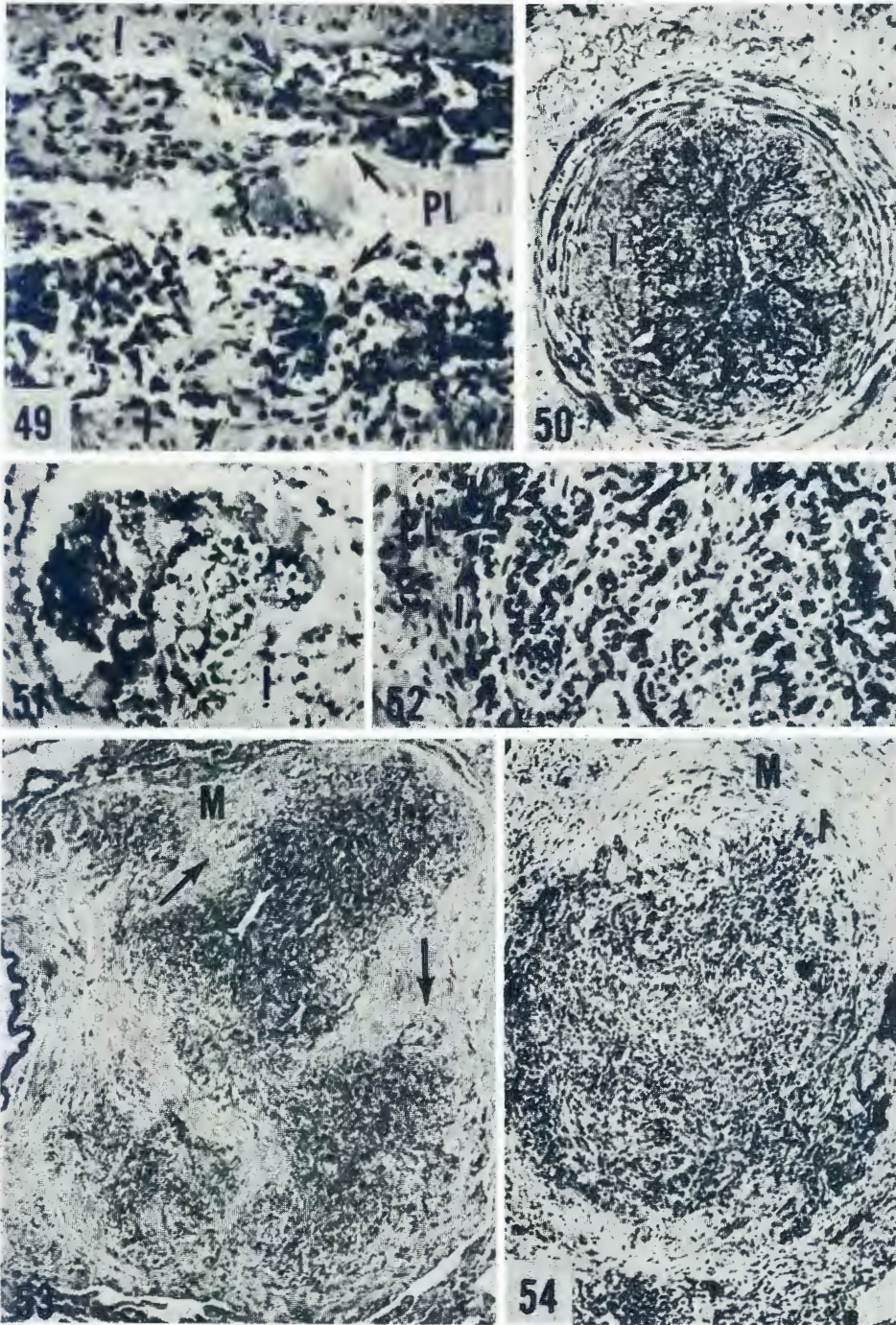
- 35.—Active proliferative stage of endarteritis of a small branch of the pulmonary arterial tree. True intima (I). × 48
- 36.—Pseudo-intima (PI) between arrows forming by anastomosis of villous intimal projections. × 125
- 37.—Pseudo-intima (PI) becoming thicker by consolidation of several lamellae overlying the true intima (I). × 125
- 38.—Simple chronic, proliferative verminous endarteritis. Pseudo-intima (PI) roughly single layered. True intima (I). × 48
- 39.—Active chronic, proliferative endarteritis. Notice intense cellular activity of luminal surface of pseudo-intima (arrows) opposite true intima (I). × 155
- 40–41.—Double layered pseudo-intima (PI) of branches of pulmonary arterial tree. Notice endothelial hyperplasia on luminal surface in 40 and especially of the mural surface in 41. × 125
- 42.—Double layered pseudo-intima (PI) to the left and single and double-layered segments to the right. Notice the struts or attachments between the true intima and the pseudo-intima. × 155
- 43.—Tangential section through branch of pulmonary arterial tree. Notice between the true intima (I) of each of the sides that the pseudo-intima (PI) appears like a sieve or netlike structure. × 125
- 44.—Typical single layered pseudo-intima (PI) within a branch of the pulmonary arterial tree. Notice that with contracture of the vessel wall the pseudo-intima bulges into the lumen except where struts are attached to the true intima (I). × 125
- 45.—Small pulmonary vein with intimal proliferation into lumen arising from true intima (I). × 95
- 46.—Larger branch of pulmonary artery with multi-layered pseudo-intima (PI). Interna elastica of intima (I) appears as corrugated line. Notice well-formed strut (S). × 125
- 47.—Double layered pseudo-intima (PI) with first layer of very mature type and second layer which has become reactivated. True intima (I). × 185
- 48.—Active chronic, multilayered pseudo-intima (PI). Cellularity especially pronounced along luminal surface. × 48



PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

PLATE 6

- 49.—Longitudinal section of small branch of pulmonary artery. Notice simple, chronic pseudo-intima (PI) just above and below the true intima (I) of either side of lumen. $\times 200$
- 50.—Overall view of small pulmonary arteriole in contracted state. Notice that the rather cellular pseudo-intima bulges in from the true intima (I) filling the lumen. $\times 75$
- 51.—Proliferation from intima (I) into lumen of small artery illustrating that the reaction was not always uniform. $\times 200$
- 52.—Higher magnification of proliferations of active proliferative or subacute villous stage to show inflammatory cells within the core of the projections. True intima (I) and pseudo-intima (PI). $\times 200$
- 53-54.—Angiomatoids in branches of pulmonary arteries. Notice vascular spaces (arrows) in media (M) of 53. Inflammatory cells fill the vascular spaces in 54. Media (M) and true intima (I). $\times 50$ & $\times 75$



PATHOLOGY OF BILHARZIASIS OF *HIPPOTAMUS AMPHIBIUS*

PLATE 7

- 55.—Focal endophlebitis of small vein. Intima (I). × 125
- 56.—Active, subacute villous endophlebitis of small vein in liver. Intima (I). Notice cells occupying much of lumen. × 125
- 57.—Sublobular vein with proliferating mass of cells from intima (I) occupying much of lumen. × 125
- 58.—Efferent vein of liver lobules with villous projections from intima. × 125
- 59.—Cross-section of a male schistosome in central vein. × 200
- 60.—Adult schistosome (S) within large branch of portal vein. Notice the severe active proliferative reaction from the intima (I) of both parts of the wall shown. Opposite portion of vessel wall (W). × 48
- 61.—Higher magnification from another segment of the vessel in 60. Notice the strut (S) and the arboreal effect of the pseudo-intima above the true intima (I). × 125
- 62.—Adult female schistosome (arrow) between layers of a pseudo-intima. × 125
- 63.—Intrahepatic branch of portal vein. Pseudo-intima (PI) between arrows might well be called a fluke net. Intima (I). × 125
- 64.—Lymphoid nodule (LN) on pseudo-intima (PI) of intrahepatic branch of portal vein. Intima (I). × 75
- 65.—Lymphoid nodules (LN) with well-formed follicles on either side of the lumen of an intrahepatic branch of the portal vein. Notice thin pseudo-intima (PI) and true intima (I). × 20
- 66.—Large intrahepatic branch of portal vein. Notice juncture of lymphoid nodule (LN) with the pseudo-intima (PI) on which former sits. True intima (I). × 125
- 67.—Large branch of portal vein. Notice several lymphoid nodules (LN) of various sizes superimposed on luminal surface of multiple layered pseudo-intima (PI) with similar cells in wall. True intima (I). × 48

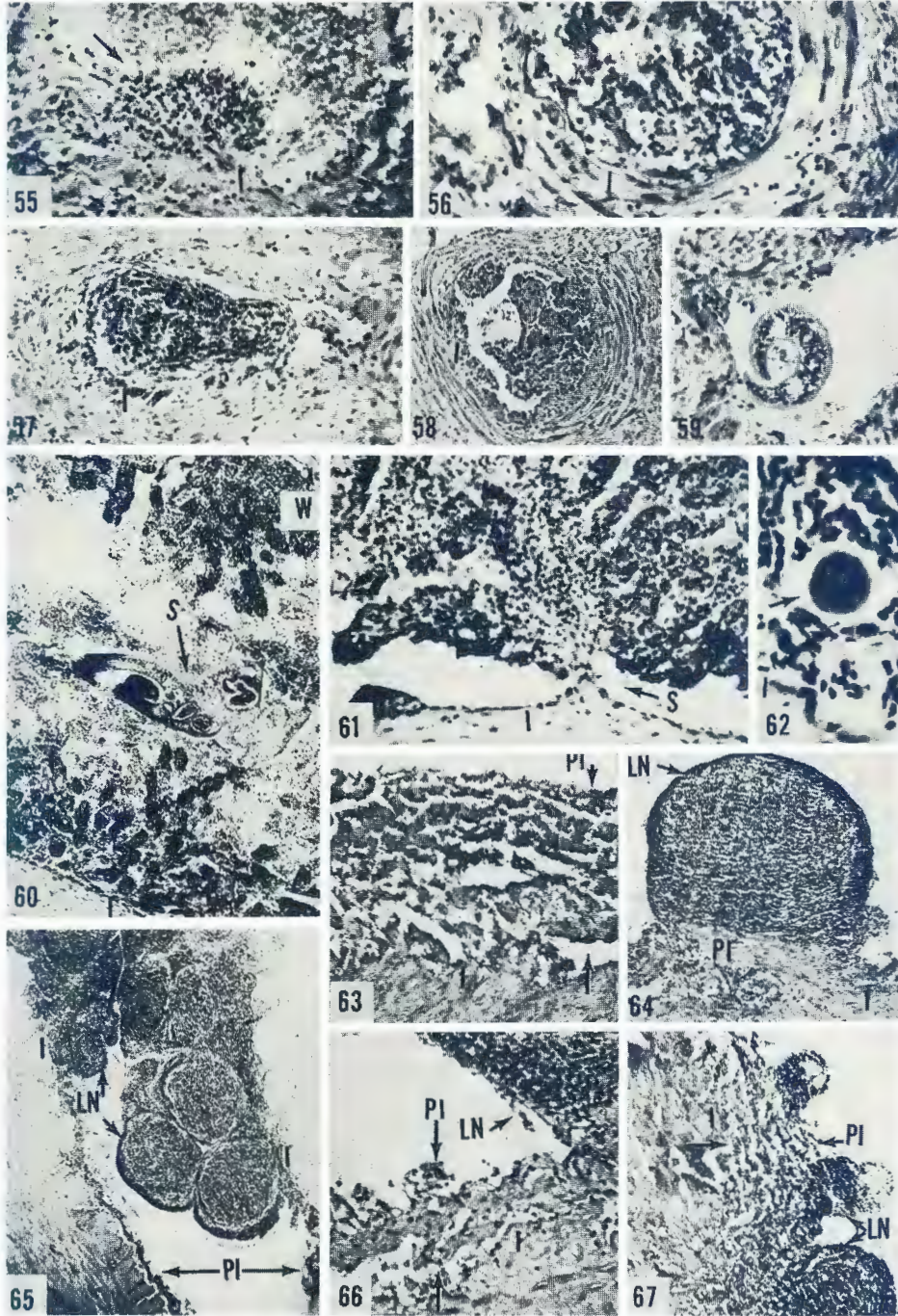


PLATE 8

- 68.—Adult male schistosome (S) in efferent vein. Notice intense inflammatory reaction replacing hepatic cord cells (H). × 200
- 69.—A very rare find of schistosome ova in this instance in central portion of liver lobule (arrow). × 75
- 70.—Higher magnification to show eosinophiles in villous projections from intima of vein shown in 60. × 200
- 71.—Adult schistosome (arrow) in lumen of small vein with many lymphocytes in its wall. Intima (1). × 200
- 72.—High magnification of clutch of specifically unidentified objects thought to be coccidial oocysts in portal area of the liver. × 1200
- 73.—Dead schistosome (arrow) in central vein which is surrounded by acute inflammation. × 200
- 74.—Higher magnification of one of the objects like those shown in 72. Notice outer doubly-contoured wall with serrated appearance (arrow) and the collapsed inner membrane and nuclear mass. × 1500

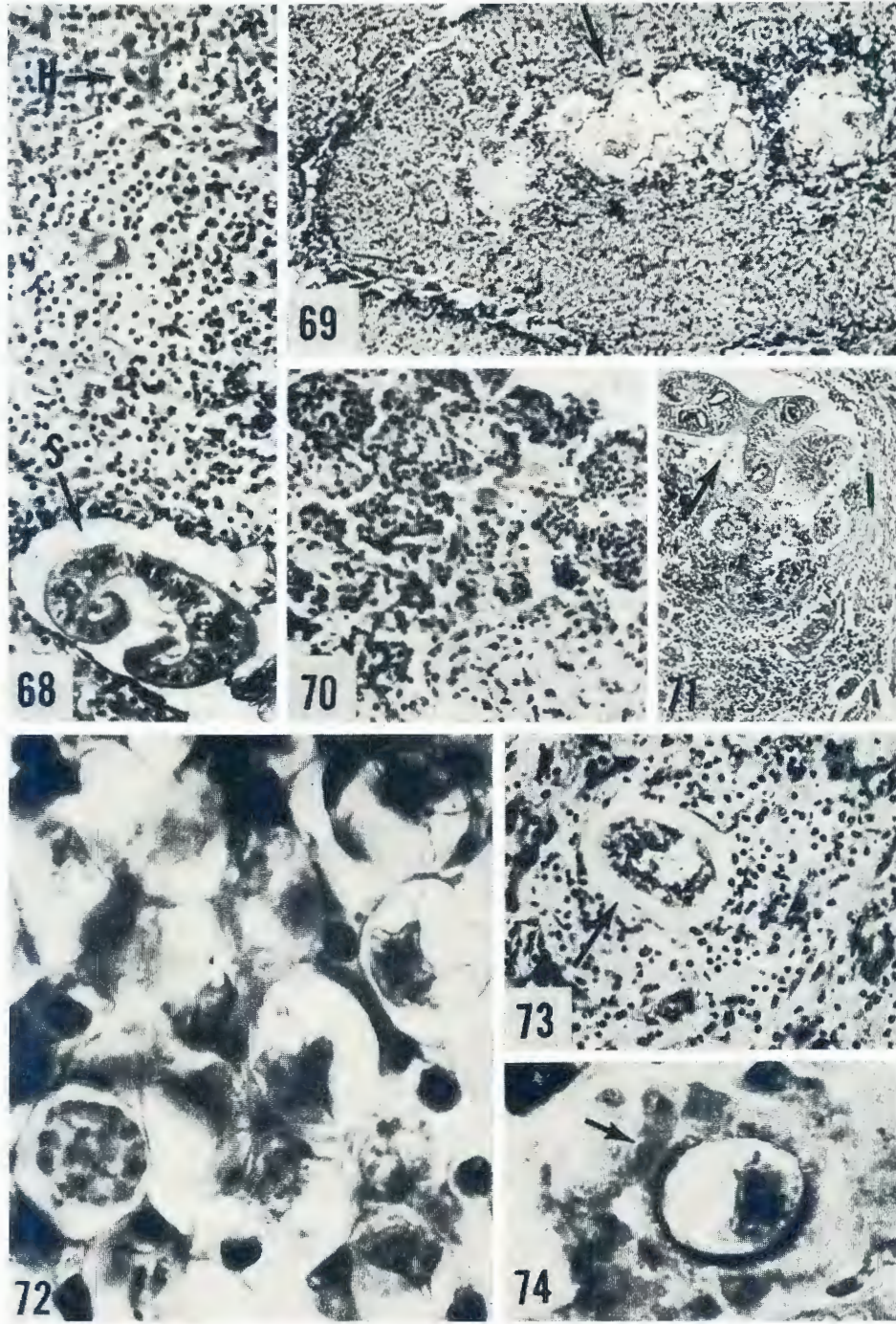


PLATE 9

- 75.—Inflammatory nodule adjacent to central vein (CV). Notice clusters of the odd cells (arrows) between connective tissues and eosinophiles. × 300, Masson trichrome stain
- 76.—Nodule similar to 75 but stained with silver. Notice affinity of the cytoplasm of the cells for silver. Some are seen (arrows) within the central vein (CV) and others outside in clusters. × 300, GMS stain
- 77.—Higher magnification of the silver positive cells seen in clusters of three or more. × 300, GMS stain
- 78.—Clusters of the odd macrophages surrounded by collagenous tissue and numerous eosinophiles. × 300
- 79.—High magnification of the odd cells which somewhat resembled Gaucher cells. × 750
- 80.—Silver positive macrophages were often found in portal areas like this one. × 125, GMS stain
- 81.—Clusters of the odd cells (arrows) occasionally present in hepatic sinusoids. × 300
- 82.—Centrolobular area containing the odd cells adjacent to central vein (CV). × 125, GMS stain
- 83.—Odd cells had cytoplasm which was strongly positive to periodic-acid-Schiff. × 300, PAS stain
- 84.—Clusters of the cells were encapsulated by collagenous fibers (arrow). × 300, Masson trichrome stain

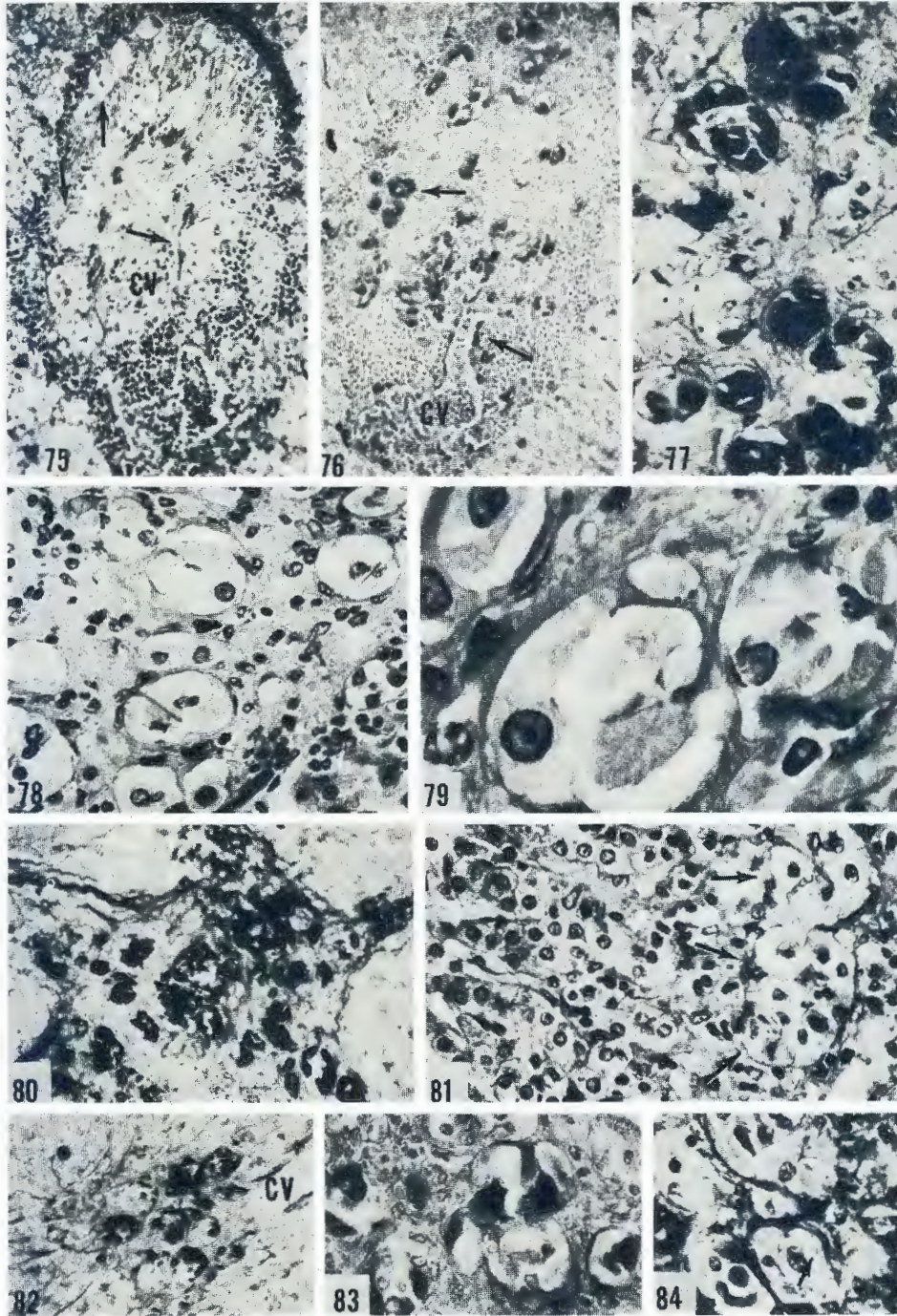
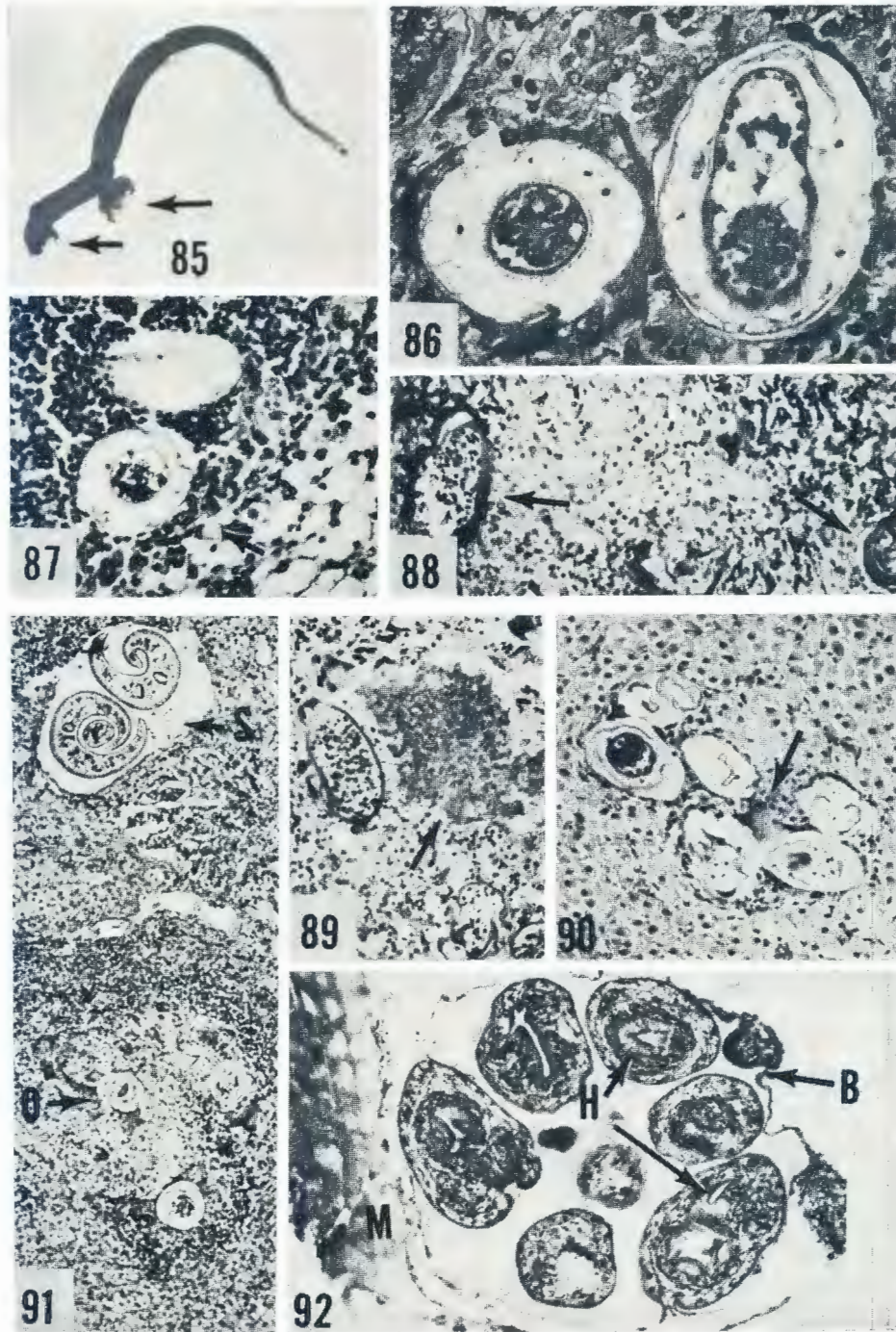


PLATE 10

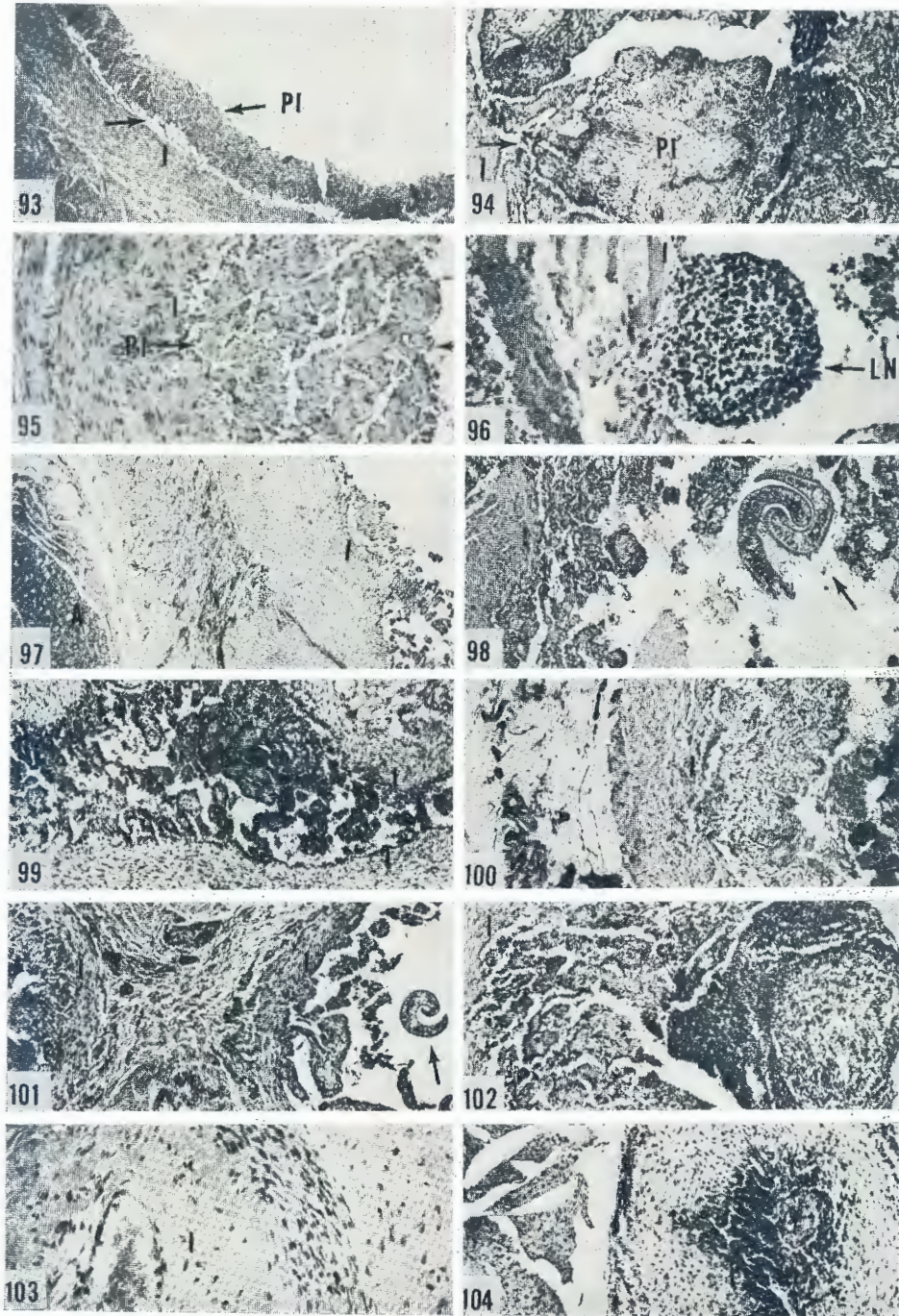
- 85.—Adult male *S. hippopotami*. Notice the size of the suckers (arrows) relative to overall length of specimen
- 86.—Two *S. hippopotami* ova. Left, transverse section through miracidium. Right, longitudinal section through miracidium showing cilia. × 320
- 87.—Early reaction to *Schistosoma* ova (arrow) adrenal cortex. Eosinophiles and round cells with former predominating. × 130
- 88.—Granuloma in response to *Schistosoma* ova, adrenal cortex. Notice heavy layer of material deposited on shell of ova (arrows). × 130
- 89.—Response to *Schistosoma* ova, adrenal cortex. Amorphous area due to necrosis of eosinophiles (arrow). × 130
- 90.—*Schistosoma* ova, adrenal cortex. Notice mildness of host response. Giant cell (arrow). × 130
- 91.—*Schistosoma* adults (S) in medullary vein and ova (O) in adjacent adrenal cortex. × 200
- 92.—Hydatid cyst, liver. Laminated hyaline membrane (M), brood capsule with germinal epithelium (B) and number of scolices with hooklets (H). × 175



PATHOLOGY OF BILHARZIASIS OF *HIPPOPOTAMUS AMPHIBIUS*

PLATE 11

- 93.—Extrahepatic segment of portal vein in transverse section. Notice the very thick pseudo-intima (PI) lying in the lumen beside the true intima (I). × 20
- 94.—Large pulmonary artery near heart. Pseudo-intima (PI) between arrows tended to be rather coarse and composed of fibrous tissue infiltrated with round cells. True intima (I). × 48
- 95.—Multilayered pseudo-intima (PI) of splenic vein. True intima (I). Notice maturity of the connective tissue in the pseudo-intima × 78
- 96.—Lymphoid nodule (LN) on intima (I) of splenic vein. × 125
- 97.—Mammary gland (A) with villous endophlebitis of mammary vein. True intima (I). × 20
- 98.—Proliferative villous endophlebitis, uterine vein. Adult schistosome (arrow). True intima (I). × 75
- 99.—Active subacute villous endophlebitis, small ovarian vein. True intima (I). × 48
- 100.—Uterine vein. Multilayered pseudo-intima, to right of the true intima (I). × 48
- 101.—Two affected ovarian veins. Left, intima (I) with villous proliferation. Right, true intima (I), irregular pseudo-intima and adult schistosome (arrow). × 48
- 102.—Uterine vein. Lymphoid nodule attached to tips of villous proliferations. True intima (I). × 48
- 103.—Subendothelial fibrinoid or hyaline material in the intima (I) of small uterine artery. × 125
- 104.—Villous endophlebitis, gastric vein. × 60



PATHOLOGY OF BILHARZIASIS OF *HIPPOTAMUS AMPHIBIUS*

PLATE 12

- 105.—Mummified foetus in abdomen of a hippopotamus cow
- 106.—Small intestinal flukes (arrows) with accompanying severe catarrhal enteritis
- 107.—Group of the specifically unidentified objects thought to be oocysts (arrows), uterine mucosa.
× 200
- 108.—Appearance of one of the few *Schistosoma* ova (arrow) in an alveolar septum, lung. × 480
- 109.—*Schistosoma* ova (arrow) immature testes. × 75
- 110.—Granulosal cell tumor, ovary × 200
- 111.—Malignant melanoma, gingiva. × 200
- 112.—Schizont of *Hepatocystis hippopotami*, lymph node. The dark material (A) is an artefact.
× 1250

