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VASCULAR ABNORMALITIES IN EXPERIMENTAL AND HUMAN LYMPHATIC FILARIASIS

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ABSTRACT

Whereas clinical descriptions of grotesque lymphedema and standard light microscopy in human filariasis have elucidated the natural progression of this disease, the link between the nematode and vascular abnormalities including elephantiasis remains poorly understood. Accordingly, we examined the nature and distribution of lymphatic and blood vascular derangements in a variety of tissues and organs from 37 ferrets acutely and chronically infected with Brugia malayi *and in 15 patients with* Wuchereria bancrofti *or* Brugia malayi *infestation (resected skin, subcutaneous tissue, and lymph nodes) using light and transmission electron microscopy, immunohistochemistry, and* in vivo *microscopy. In ferrets, eosinophilic abscesses and epithelioid and giant cell granulomas with fragmented worms in various stages of disintegration were found in multiple organs. Blood microvasculopathy consisted of endothelial hyperplasia, focal thickening and stenosis, vessel obliteration with marked perivascular infiltration of lymphocytes, plasma cells, eosinophils, and numerous large macrophages laden with a coarse golden-brown pigment. Endothelial ballooning and swelling, pavementing, denuding, scarring, and sludge formation were seen along with high endothelium in atypical locations. Dilated lymphatics were most prominent near adult worms and showed plump endothelium, thickened walls and valves, thrombus formation, and often perilymphangitis and adjacent tissue fibrosis.* In vivo *microscopy showed wriggling live adult worms in dilated incompetent sludge-filled groin lymphatics even when microfilaremia and peripheral edema were absent. In human tissues, in addition to "pachyderm" skin changes (keratosis, papillomatosis, acanthosis and collagen deposition), there was blood vessel and lymphatic vasculopathy similar to ferrets (angiocentric inflammation, congestion, vasculitis, thrombosis, thickened vessel walls, dilated lymphatics, lymphangitis, reactive lymph nodal hyperplasia and nodal fibrosis). These changes reflect generalized endothelial damage due to worm products, physical injury to valves and vessel walls from lymphatic-dwelling live worms, and host immune reactivity. Whereas adult worms target the lymphatic apparatus, their offspring and the host immune response primarily affects the blood microvasculature.*

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Filariasis, a parasitic infection afflicting hundreds of millions of people worldwide, is a prototype disorder of the lymphatic system. Adult nematodes primarily target peripheral lymphatics where they reside and reproduce seemingly unaffected by the host's immune defense like "criminals" living in the "police station" of the body and the "cops do nothing about it" (1). Beginning early in their life cycle vigorous thrashing by adult worms directly impacts on the endothelial lining of the lymphatic trunks and at least indirectly also distorts the lymph nodal architecture. In addition, filarial offspring (microfilariae) circulate widely throughout the blood vascular and reticuloendothelial systems triggering regional and systemic immune and inflammatory responses. Despite extensive clinical descriptions and several standard light microscopic studies elucidating the natural progression of filarial diseases in man (2,3) and experimental animal counterparts (4-12), the link between the long-term intralymphatic residence of the nematode, lymphatic dysfunction and the development of lymphedema, remains poorly understood. Furthermore, associated lesions in the blood vascular system have been largely neglected. Accordingly, we examined the nature and distribution of lymphatic and blood vascular abnormalities in a variety of tissues and organs in ferrets acutely, subacutely, and chronically infected with *Brugia malayi*, and compared them to non-infected (control) ferrets using light and scanning electron microscopy and immunohistochemistry. For purposes of comparison, sections of skin, subcutaneous tissue, and lymph nodes removed from several patients with filariasis (from either *Wuchereria bancrofti* or *B. malayi*) were similarly studied.

MATERIALS AND METHODS

Thirty-seven male ferrets (*Mustela putorius furo*) weighing 1-2kg, obtained from Marshall Research Laboratories (North Rose, NY) were singly or multiply infected with third-stage *B. malayi* larvae, an established model of filariasis (13) and later examined acutely (<3 months; n=6), subacutely (3-12 months; n=21), or chronically (>12 months; n=9). The experimental technique is displayed in *Fig. 1*. Thirteen other non-infected ferrets served as controls. Lymphatic imaging findings in some of these ferrets have previously been reported (14-16).

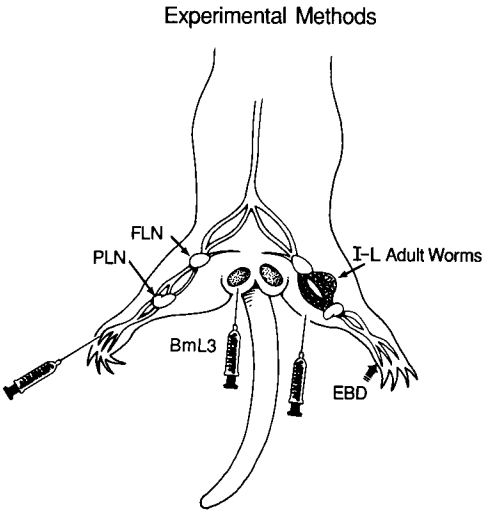

Fig. 1. Ferret model schema depicting the site(s) of subcutaneous Brugia malayi *third stage larvae (BML_3) inoculations, intradermal Evan's blue dye (EBD) injections, and nesting of intralymphatic (IL) live adult worms between the popliteal and femoral lymph nodes (PLN and FLN, respectively).*

Following completion of noninvasive imaging studies, each ferret was anesthetized with pentobarbital (25mg/kg intraperitoneally) and the hindlimb lymphatics were exposed from the paw to the groin after intradermal injection of Evans blue dye (0.5ml) into the dorsum of each hindpaw. This area was then magnified on a Sony video monitor attached to an operating stereomicroscope to view and record lymphatic vasomotion and activity of intralymphatic-dwelling adult worms (17). The ferrets were then sacrificed, a necropsy performed and organs and tissues prepared for light (formalin-fixed paraffin embedded) and electron microscopy

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(Karnovsky's-cacodylate-preserved plastic embedded) as well as immunohistochemistry for endothelial markers (Factor VIII-associated antigen and Ulex europaeus ligand) (as for light, using biotin avidin method or OCT-snap-frozen for immunofluorescence). Special attention was directed to lymphatics, blood vessels and lymph nodes.

Skin, subcutaneous tissue, and lymph nodes from 15 patients infected with either *W. bancrofti* or *B. malayi* were also obtained (courtesy of S. Jamal and S. Radhakrishnan at Thanjavur Medical College in Thanjavur, Tamil Nadu, India) and examined similarly with these techniques.

RESULTS

In filarial infected but not healthy ferrets (which showed only a few hair-like hindlimb lymphatics grossly), increased numbers of markedly dilated, intensely blue-staining peripheral lymphatics were arranged as a collateral network most prominently in the groin and in the thigh between the femoral and popliteal lymph nodes. Videomicroscopy in the acute and subacute groups demonstrated nests of wriggling live adult worms, which typically stretched and dilated the lymphatic truncal walls and occasionally traversed and disrupted intraluminal valves.

Whether acutely, subacutely, or chronically infected, ferrets showed eosinophilic abscesses with epithelioid and giant cell granulomas. Fragmented microfilariae in various stages of disintegration were found in multiple organs, including femoral and popliteal lymph nodes, kidney, spleen, liver, and lung *(Fig. 2)*. When present, adult worms, whether alive or dead, were found

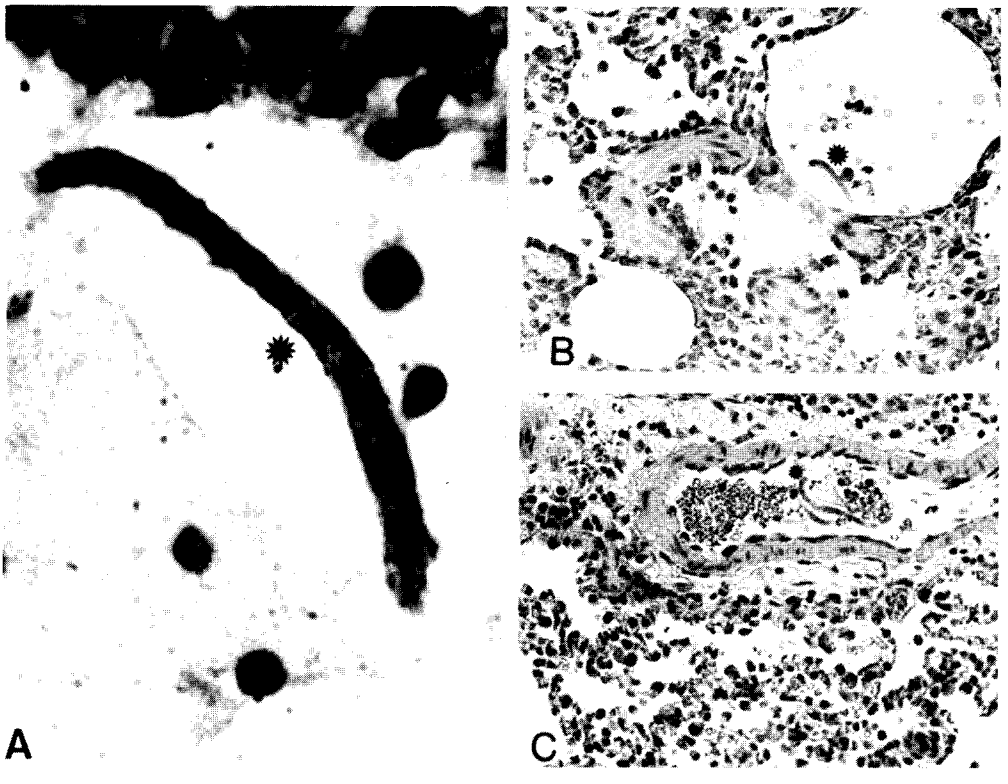

Fig. 2. Microfilariae (*) within multiple organs, including A) dilated spaces of a femoral lymph node, B) lung alveolus and C) pulmonary blood vessel. Hematoxylin and eosin; original magnification A, x1700; B, x387; C, x375.

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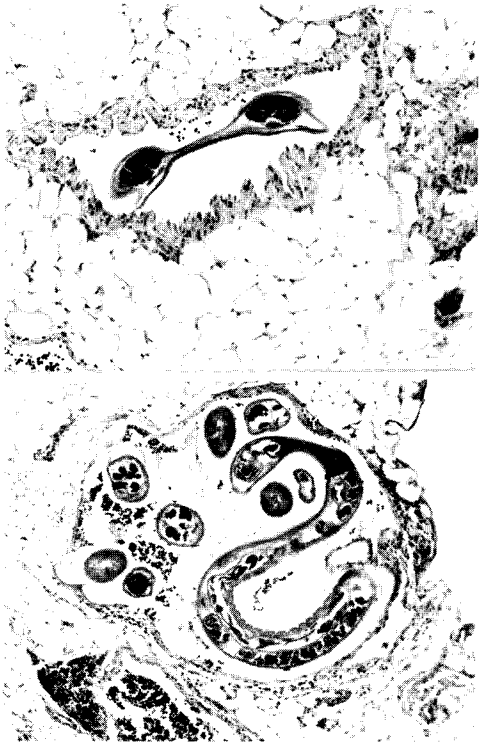


Fig. 3. Calcified adult worm within a markedly dilated lymphatic located between the popliteal and femoral lymph nodes, with a thickened lymphatic wall (above) and numerous inflammatory cells infiltrating from an adjacent venule (below). Hematoxylin and eosin; original magnification above, x160; below, x110.

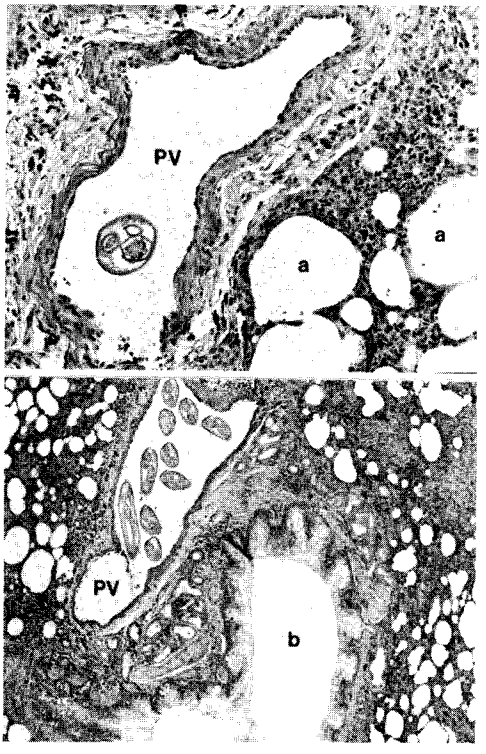
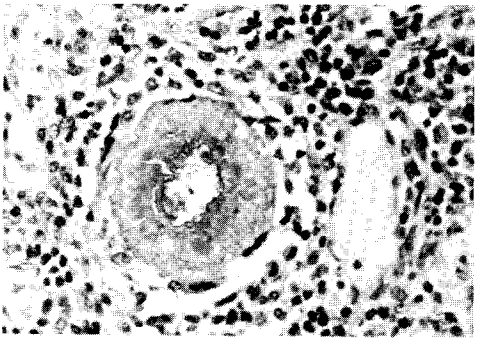


Fig. 4. Cross-sections of an adult worm within a pulmonary blood vessel (PV). Above–alveoli (a), below–collapsed alveoli (a), bronchiole (b). Hematoxylin and eosin; original magnification above, x160; below, x64.

in dilated lymphatics predominantly between the popliteal and femoral lymph nodes, independent of the site of inoculation(s) *(Fig. 3)*. A non-calcified adult worm was also found in a pulmonary blood vessel *(Fig. 4)*.

Splendore-Hoeppli deposits (eosinophilic reaction that surrounds helminths, bacteria, fungi, and foreign bodies) (18) and Meyers-Kouwenaar bodies (a microfilaria remnant covered by acidophilic deposits) (18) characteristic of human filariasis were found in various locations *(Fig. 5)*. Along with these and other lymph nodal changes *(Fig. 6)*, dilated lymphatics, most prominent near living or calcified dead adult worms, exhibited "plump" endothelium, thickened



SPLENDORE-HOEPPLI DEPOSITS	MEYERS-KOUWENAAR BODIES
Mesenteric Lymph Node	Mesenteric Lymph Node
Liver	Liver
Lung	Spleen
Spleen	?Kidney
Kidney	

Fig. 5. Splendore-Hoeppli deposit (left) and Meyers-Kouwenaar body (right) and the organs where each were observed.

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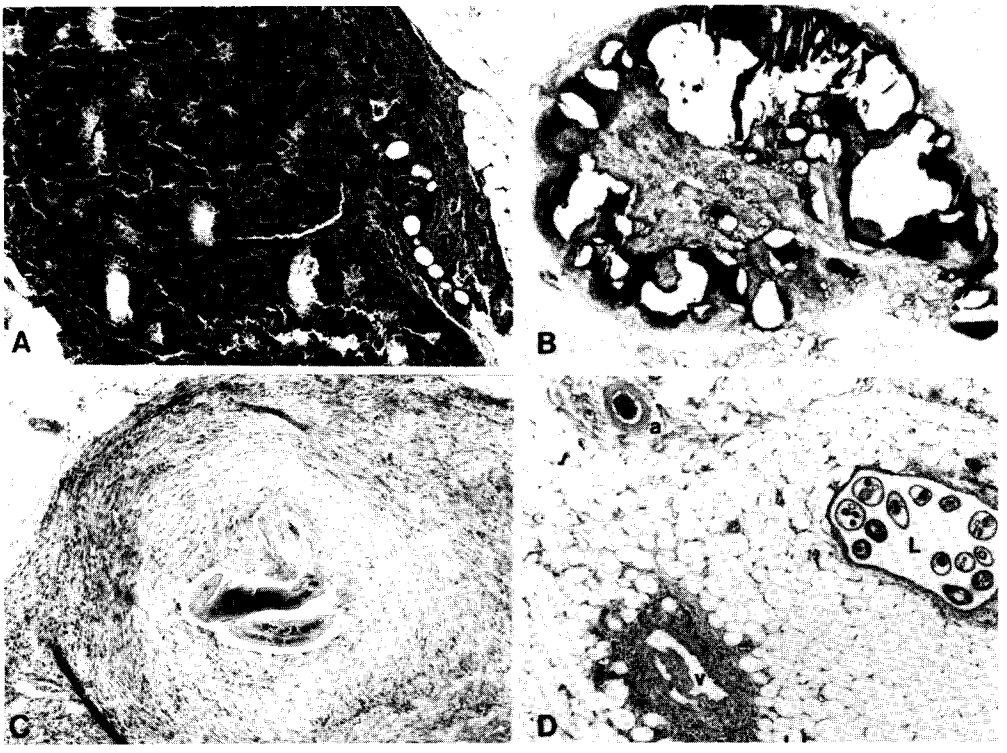

Fig. 6. Lymph nodal changes associated with filariasis include A) "moth-eaten" or fluffy appearance indicative of lymphocyte drop-out, B) dilated cystic spaces, C) granuloma formation around calcified adult worms and D) dilated lymphatic vessel with cross-sections of adult worm associated with hilar perivenular inflammatory infiltrate. [arteriole (a), venule (v), lymphatic (L)]. Hematoxylin and eosin; original magnification A, x150; B, x40; C, x130; D, x74.

truncal walls and valves, and sometimes thrombus formation *(Fig. 7)*. Frequently, perilymphangitis and adjacent tissue fibrosis were also present. Scanning electron microscopy of markedly dilated lymphatics confirmed the presence of bulbous endothelium with a corrugated intraluminal surface corresponding to the periodic cross-striations of the cuticular surface of the intralymphatic-dwelling adult worms *(Fig. 8)*.

In addition to the lymphatic changes, widespread blood vascular abnormalities were also found. Typically these consisted of endothelial hyperplasia, focal thickening, stenosis, and extensive obliteration with perivascular infiltration with lymphocytes, plasma cells, eosinophils, and numerous large macrophages laden with a coarse golden-brown pigment. Endothelial ballooning and swelling were associated with high venous endothelium in atypical locations. These areas were also characterized by intraluminal sludge formation associated with pavementing of neutrophils, denuding, and intimal thickening (? fibroplasia) of the endothelium *(Fig. 9)*. No consistent differences were noted between singly and multiply infected ferrets regarding either lymphatic or blood vessel abnormalities.

In tissues from humans with filariasis, blood and lymphatic vascular lesions paralleled those seen in infected ferrets. Congestion, angiocentric inflammation, vasculitis, thrombosis and thickened blood

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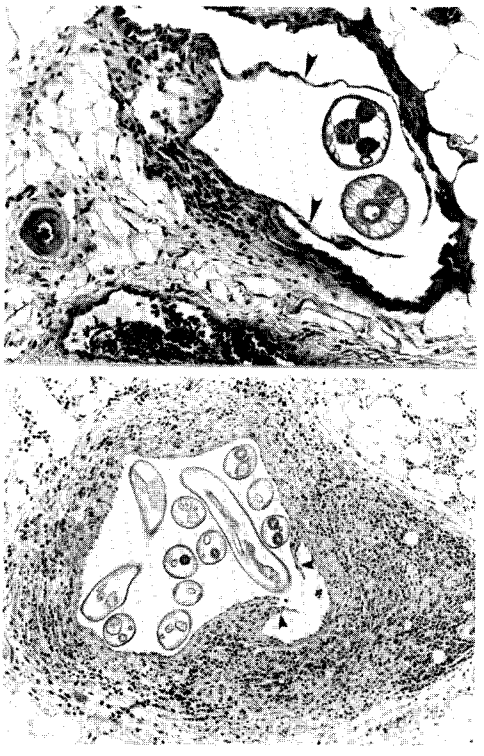

Fig. 7. Peripheral lymphatic vessels containing adult worms, exhibit "upper"–lymphatic wall thickening and normal valve cusps (arrowheads), and "lower"–thickened valve cusps.

vessel walls were noted in association with dilated lymphatics, lymphangitis, reactive nodal hyperplasia of both the sinuses and follicles along with fibrosis. Unlike infected ferrets, human skin also showed pachyderm-like skin changes of keratosis, acanthosis, papillomatosis, and collagen deposition *(Fig. 10)*. In a femoral lymph node, which exhibited loss of nodal architecture and fibrosis, immunohistochemical staining with Ulex europaeus lectin, an endothelial marker, highlighted dilated capsular lymphatics *(Fig. 11)*. In addition to staining positive with Ulex, arteries and veins exhibited fibrous intimal thickening forming folds and clefts impinging on the lumen as well as intensely eosinophilic perivascular connective tissue.

DISCUSSION

Whole-body lymphangioscintigraphy in ferrets chronically infected with filariasis, including those with or without peripheral edema and with or without microfilaremia, displayed delayed or absent transport of radiolabeled protein tracer from the hindpaw to the groin in conjunction with dilated lymphatic trunks (14). Other imaging (by magnetic and paramagnetic resonance, xeroradiography and conventional lymphography) and microscopy confirmed similar pathologic responses by ferrets, patas monkeys, cats, dogs, and humans to filarial infection (4,15,16,19-25) including the formation of dilated superficial collateral lymphatic channels (21,26).

Abnormalities in the lymphatic apparatus occur in a variety of hosts with experimental filariasis as early as two weeks after larval inoculation with *Brugia* species (8,10,27). The pathogenesis of increased dilated and incompetent lymphatics has been attributed to obstructed afferent lymph flow with truncal back pressure from nodal congestion, inflammatory or granulomatous responses to released ova or calcified filaria (13,28), and reactive thrombosis and sclerosis of lymph nodes in response to indwelling adult worms. Perilymphatic inflammation, such as that resulting from reaction to a calcifying adult worm (a phenomenon apparently rare or non-existent around viable worms) (29) may generate fibrosis and narrowing of lymphatics with gradual distal dilatation (28). These changes are compounded by direct damage to intralymphatic valves by wriggling and migrating adult worms, well depicted by videomicroscopy, with development of thickened and incompetent valves (5,8,10,21,27,30) and subsequent truncal ectasia and varix formation (8,31). Lymphatic dilatation has been described in other animal counterparts of human filariasis (5,10,21,26). For example, in cats infected with *B. pahangi*, lymphatics begin to dilate as early as 10 days and increase 3-4 times by 1 month (7, 23), and up to 10x thereafter (8).

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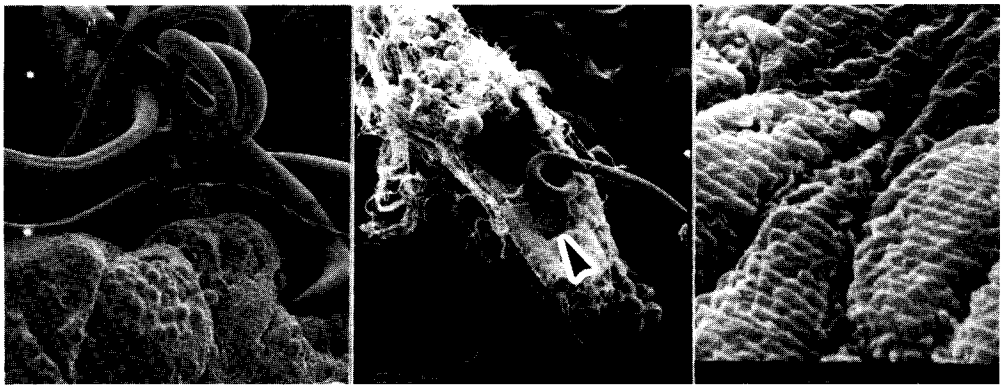

Fig. 8. Scanning electron microscopy of intralymphatic adult worms associated with (left) bulbous endothelium, (middle) an indentation in the endothelial surface (arrowhead), and (right) endothelial cross-striations from impressions made by the intralymphatic-dwelling worm.

The presence of a bulbous endothelial cell lining in lymphatics of infected ferrets as noted by electron microscopy coincides with earlier histologic descriptions of intimal ingrowth and infolding (5,28). Similar changes in small arterioles and venules have been demonstrated immunohistochemically. Ash and Spitz note marked hypertrophy of the media of a vein in a patient with bancroftian filariasis, which may have resulted from an inflammatory response to the invasion of the venous wall by microfilariae (29). The direct interaction between adult worms and adjacent endothelium, as depicted by electron microscopy, contributes to endothelial cell damage with formation of fibrocoagulum (10,27,29,30), most commonly next to the intraluminal valves (5). Blood vascular thrombosis with or without an associated inflammatory infiltrative process noted in our study agrees with findings in humans (29).

Lymph nodal enlargement as has been observed before (9,12,13) was verified at necropsy. Nodal architecture was extensively deranged with large intranodal cystic spaces presumably due to migration of filarial larvae and live adult worms through the inguinal and/or popliteal lymph nodes into hindlimb lymphatics (5,7). Other sections showed swollen lymph nodes with hyperplasia of both the sinuses and follicles. Transnodal migration of adult worms into

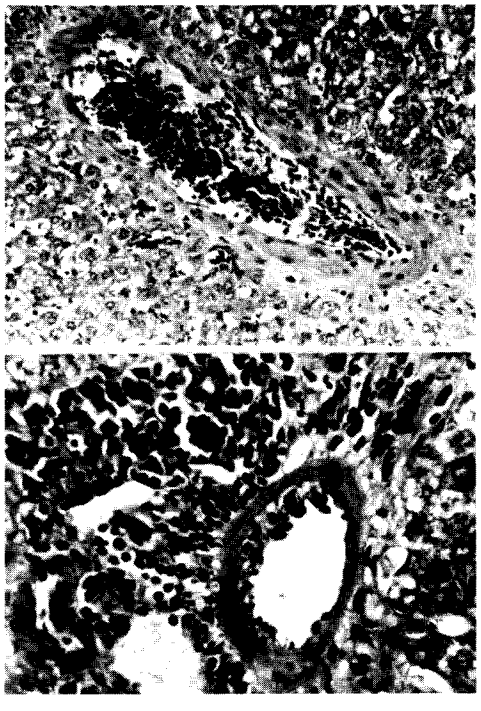

Fig. 9. Ferret liver depicting "upper"– a distended, congested and fibrotic central vein associated with increased collagen and an inflammatory infiltrate extending into the surrounding parenchyma and "lower"– portal tracts with intense fibrosis, denuding of the endothelium, chronic inflammatory cells and extension of acellular fibrosis into the parenchyma. Hematoxylin and eosin; original magnification x400.

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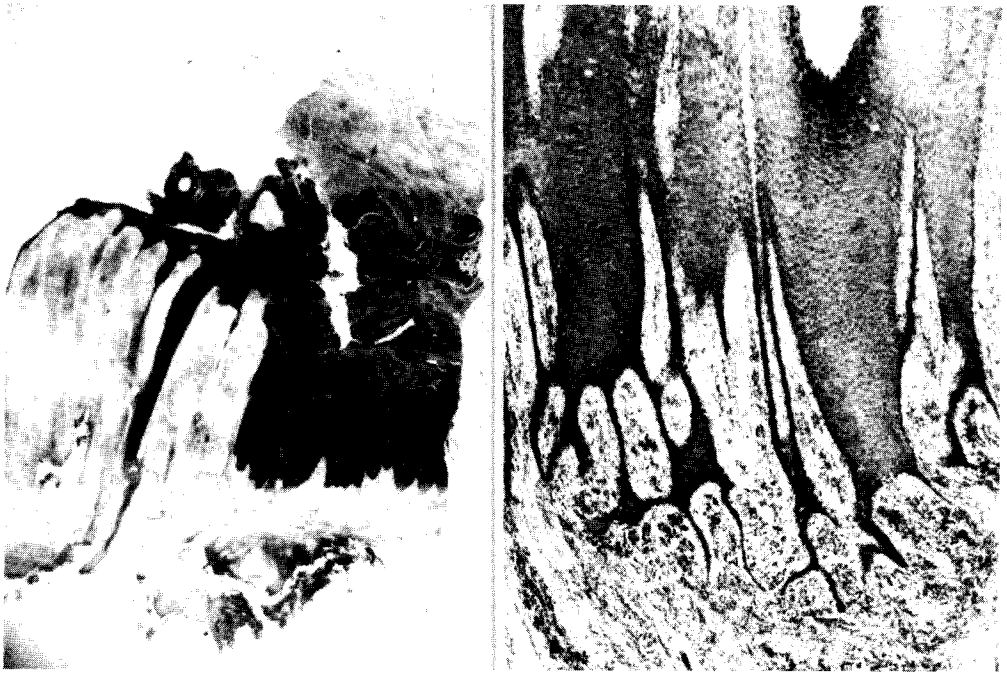

Fig. 10. Photomicrograph of human skin from a patient with filarial lymphedema exhibiting left – acanthosis and hyperkeratosis and right – papillomatosis. Hematoxylin and eosin; original magnification A, x9; B, x68.

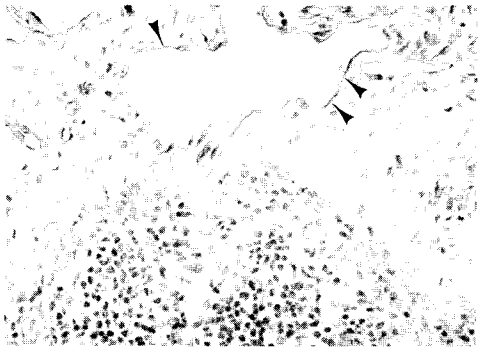

Fig. 11. Positive immunohistochemical staining for Ulex europeaus ligand of dilated lymphatics of a human femoral lymph node in a patient with filariasis which exhibits fibrosis and loss of nodal architecture. In color, the endothelium stained brown, here shown as blackened endothelial lining (arrowheads). Adjacent arterial and venular endothelium stained even more intensely (not shown).

the blood vascular system was supported by the finding of non-calcified adult filarial worms in the pulmonary vasculature *(Fig. 4)* (2,27). Adult worms have also been found in a variety of extralymphatic tissues in man and experimental animals, including skin nodules (32), spermatic cord (27), breast, epididymis, testes, eyes, heart, and aortic wall (2). Microfilariae have also been described within lymphatics or blood vessels of a variety of human organs including brain, renal glomeruli, and heart (28). Lack of microfilaremia in chronically, and some acutely and subacutely, infected ferrets was consistent with the findings of Crandall et al (13,19) with fewer than 15 percent of infected ferrets exhibiting sustained microfilaremia for more than 6-8 months.

Not only is there great variability in histomorphology of animal filariasis whether the host is singly or multiply infected,

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but so too are the clinical manifestations including the variable development of chronic lymphedema (8,20), phenomena corroborated by these ferret studies.

ACKNOWLEDGEMENTS

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