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A Chronological Lymphographic Study of Cats Experimentally Infected with Brugia Filariasis from 5 Days to 5 Years

B.W.M. Gooneratne

Beit Memorial Research Fellow, London School of Hygiene and Tropical Medicine. 1967-1970 –

Visiting Research Worker, The Nuffield Institute of Comparative Medicine, London

Summary and Conclusions

1. Chronological investigations on the pathogenesis of filariasis were conducted in an experimental model using lymphography with supporting histopathology.

2. The model chosen was *B. pahangi* and *B. patei* infections in cats. In order to delineate the changes in the filarial infected lymphatic vessels and nodes, the technique of direct visual lymphography perfected by *Kinmonth* in 1955 was employed in both uninfected and filarial infected cats.

3. Eight cats varying in age from a few weeks to 2 years and weighing 1 kg to 5 kgs were used in investigating normal lymphographic patterns in the hind limbs.

4. Thirteen cats with pre-patent infections of *B. pahangi*, nine cats with patent *B. pahangi* infections and three with patent *B. patei* were investigated. In addition five long-standing *B. patei* infections ranging from 3 years 10 months to 5 years 9 months were also investigated.

5. Lymphographic changes were visible in the lymphatic vessels and nodes of the infected limbs as early as the 15th day after inoculation. In 19 of the cats changes were confined to the limb receiving the inoculation of larvae while in 4 of these cats changes were additionally visible in the nodes and afferent lymphatic vessels of the opposite side.

In the five cats inoculated with larvae into both hind limbs or into all 4 limbs, lymphatic changes were always visible in the limbs receiving the larvae.

The main changes seen on lymphography and supported by histopathology were as follows:

A. Dilatation of the lymphatics.
B. Enlargement of the nodes.
C. Tortuosity of the lymphatics with simultaneous dilatation.
D. Leakage of contrast medium.
E. Stasis of contrast medium.
F. Collateral formation and lymphatic anastomoses.

6. The lymphographic observations in *Brugia* infected cats provide several criteria distinguishing filarial from non-filarial lymphatic diseases, the stage of the infection in experimentally infected cats, and the degree of lymphatic involvement.

Introduction

Filariasis caused by either *Wuchereria bancrofti* or *Brugia malayi* or both, is indigenous throughout practically all of the warm regions of the tropical and sub-tropical world with highly endemic areas being found in the Pacific region, India, Burma and Ceylon.

In 1947 *Stoll* (1) estimated that nearly 200 million people were infected with this disease. The epidemiological patterns observed since then suggest that the numbers infected are probably more, though the distressing sequelae of lymphoedema and elephantiasis are now less frequently found. *Cahill* (personal communication) has estimated the infection rate in 1972 at 300 million.

Wuchereria bancrofti (*Cobbold* 1877) and *Brugia malayi* (*Brug* 1927) are found as natural infections in man, while *Brugia malayi, Brugia patei Nelson and Heish*, 1957, and *Brugia pahangi Buckley and Edeson* 1956, occur in the lymphatic system of animals. However, man has been experimentally infected with *B. pahangi* and in view of its occurrence in domestic cats and dogs and the fact that the microfilariae are indistinguishable from *B. malayi* the possibility of human infections occurring naturally in endemic areas cannot be ruled out.

The problem of elucidating the nature of the disease process in filariasis has not been fully resolved as yet, and the subject is still beset with many theories and hypotheses. Until 1938, when *Poynton and Hodgkin* (2) discovered animal adapted strains of *Brugia* in Malaya and 1956 when *Buckley and Edeson* (3) also working in Malaya finally isolated *Brugia malayi* (including a human sub-periodic strain) and subsequently *Brugia pahangi*, which were transmitted to laboratory cats and produced patent infections, and 1958, when *Buckley, Nelson and Heisch* (4) isolated *Brugia patei* from naturally infected animals in East Africa (which also readily infected laboratory cats), planned experimental studies in order to determine the disease process of filariasis were not possible.

With an experimental model such as *B. pahangi* infection in laboratory cats one has the advantage of inoculating known numbers of infective larvae at predetermined sites and investigating the infected animals both lymphographically and histologically at planned intervals of time, thereby visualising the chronological changes in the lymphatic system of this deforming disease in man which produces the irreversible sequelae of lymphoedema, chyluria and elephantiasis.

It was therefore decided to investigate on a chronological basis, the disease process in cats experimentally infected with *Brugia pahangi, Buckley and Edeson,* 1956, and *Brugia patei, Buckley, Nelson and Heisch,* 1958.

The technique of direct visual lymphography perfected by *Kinmonth* in human work, was employed in the investigation of both uninfected and filarial-infected cats. In addition, a new technique of histopathology of the lymphatic system was devised which maintained the integrity of the lymphatic vessels at autopsy, and provided extremely good cellular differentiation of the lymphatic system (*Gooneratne* [5]).

Materials and Methods

Origin of the infection:

Susceptible *Aedes aegypti* mosquitoes were allowed to feed on an anaesthetised *B. pahangi* infected cat having an optimal microfilarial count, and the fed mosquitoes were kept in tropicalised insectaries until collection two days later.

Ten to twelve days after the infected blood meal, the mosquitoes were sucked into a test tube, stunned by hard tapping on the upper arm, and the wings clipped off with dissecting (cataract) needles (Weiss, London). Each mosquito was then dissected on a glass side in normal saline – the head, thorax and abdomen in separate drops. The infective stage larvae were found swimming in the saline. They were then picked up under the dissecting microscope on the point of a fine needle 1/2" long which was mounted on a wooden handle, and transferred to a cavity block containing normal saline. At the end of the dissection more saline was added to the glass slides which were then left aside for another 15 minutes - 1/2 hour when more infective larvae could be picked up. The larvae were counted as they were being transferred to the cavity block. They were sucked up, in known numbers, into a 23 gauge No. 12 B-D plastic disposable syringe and inoculated subcutane-

ously in known numbers into kittens. The number of infective larvae inoculated, the site/sites of inoculation and other details are given in Table 2.

The long standing *Brugia patei* infections used in these experiments were in cats infected from the strain of *B. patei* originally isolated in Keya by *Buckley, Nelson and Heish* (4).

Animals used: (Table 1)

Laboratory (domestic) kittens of varying ages, sizes and body weight, were used in the experiments both as normal controls and as infected animals. Most of them were young but a few cats with long standing *B. patei* infections were also used.

The cats were anaesthetised with "Nembutal" (0.5 ml/2.3 kg) when infective larvae were inoculated subcutaneously into animals and care was taken to draw the plunger of the syringe outwards, once the needle was under the skin to prevent accidental intravenous inoculation of larvae.

Lymphographic procedure:

Lymphographic procedure and complications, X-ray details and the new method of measuring lymphatic vessels and nodes, have been published elsewhere (*Gooneratne* [6]).

Histopathology:

Selected animals representative of a particular time interval after inoculation of infective larvae were autopsied. A new technique was devised to study the lymphatic system (*Gooneratne* [5]).

The material, after fixation, was blocked in paraffin after dehydration in the usual way and 4 μ sections were cut. As a rule the lymphatics and nodes in the left side of the body were sectioned longitudinally while the right side was sectioned transversely. The sections were stained routinely in Haematoxylin and Eosin and some sections were specially stained by Dominici's method for mast cells, Lendrum's stain for Eosinophils and the Picro Mallory Trichrome stain.

Table 1. Lymphographic and histopathological observations on the lymphatic system in cats

	No. of animals used	No. having lymphographic investigation	No. autopsied for histology
Uninfected cats	8	8	2
B. pahangi infected cats	22	22	8
B. patei	8	8	5

Results

Lymphatics in normal uninfected cats (Figures 1, 2, 3, 4)

Connell and Whittaker (1959) published the only known report of a single lymphographic investigation in the cat but their method of injecting a water soluble contrast medium directly into the node did not outline the afferent vessels in the limb.

Eight cats varying in age from a few weeks to two years and weighing 1 kg to 5 kgs were used in investigating normal lymphographic patterns (*Gooneratne* [7]).

Histological findings in normal uninfected cats following lymphography

Cats were autopsied at 1 week, 3 weeks, 2 months and 6 months immediately after lymphography to determine its effect on the lymphatic system. The lymph channels and sinuses in the nodes were dilated and filled with oily Lipiodol Ultrafluid. No marked cellular response was seen and the architectural integrity of the nodes was maintained with the lymphoid tissue appearing normal (Fig. 5). By one week the sinuses

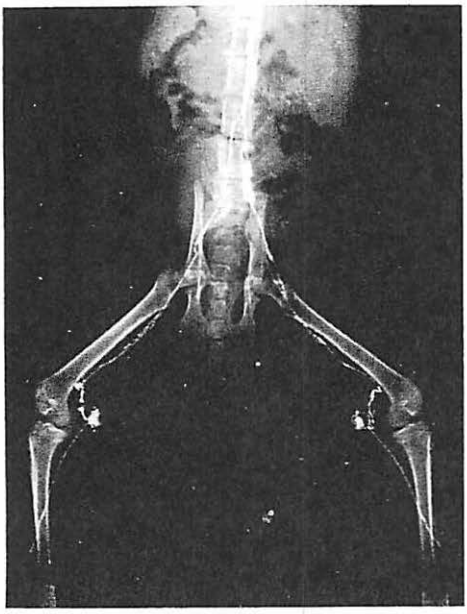


Fig. 1. Normal bilateral hind limb lymphogram (Anterior-Posterior view)

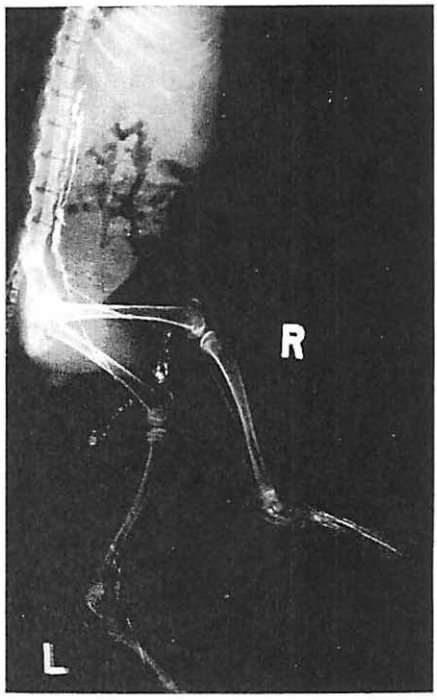


Fig. 2. Normal bilateral hind limb lymphogram (lateral view)

and lymphatic channels were still dilated and polymorpho-nuclear leucocytes were found around the wound caused by the incision which had been made to effect cannulation. At this stage occasional foreign body giant cells made their appearance. The polymorphs had disappeared by the 3rd week while an occasional giant cell persisted even up to the 2nd month. The lymphatic channels and sinuses remained slightly dilated, even in the 6th month, by which time the lymphatic system was otherwise normal.

Lymphographic changes in cats with B. pahangi and B. patei

All the infected cats showed changes in the lymphatic vessels and nodes even as early as the 15th day after inoculation of infective larvae. In 19 of the cats changes were confined to the limb receiving the inoculation of larvae while in 4 of these cats changes were additionally visible in the nodes and afferent lymphatics of the opposite side. In one of the cats receiving larvae into both hind limbs changes were seen in the abdominal part of the thoracic duct and at autopsy worms were found in this situation. In the 5 cats inoculated with larvae into both hind limbs or into all 4 limbs, lymphatic changes were always visible in the limbs receiving the larvae. In the old *B. patei* infected cats changes were seen as well, but as the sites of larval inoculation were not known, interpretation of the findings was difficult. Details of the case histories and lymphatic measurements of each individual animal are given in Table 2 and Histogram 1

The main changes seen on lymphography and supported by histopathology were as follows:

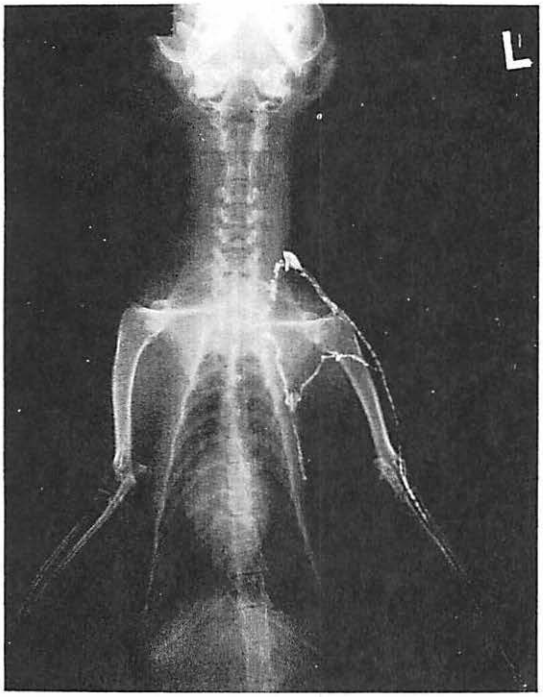


Fig. 3. Normal upper limb lymphogram showing prescapular and axillary nodes

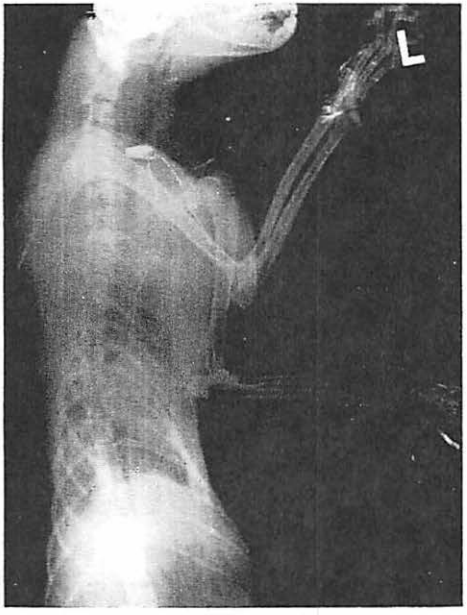


Fig. 4. Lateral view of Fig. 3

1. *Dilatation of the Lymphatics*
(Figs. 6, 7, 8 and Histogram 1)

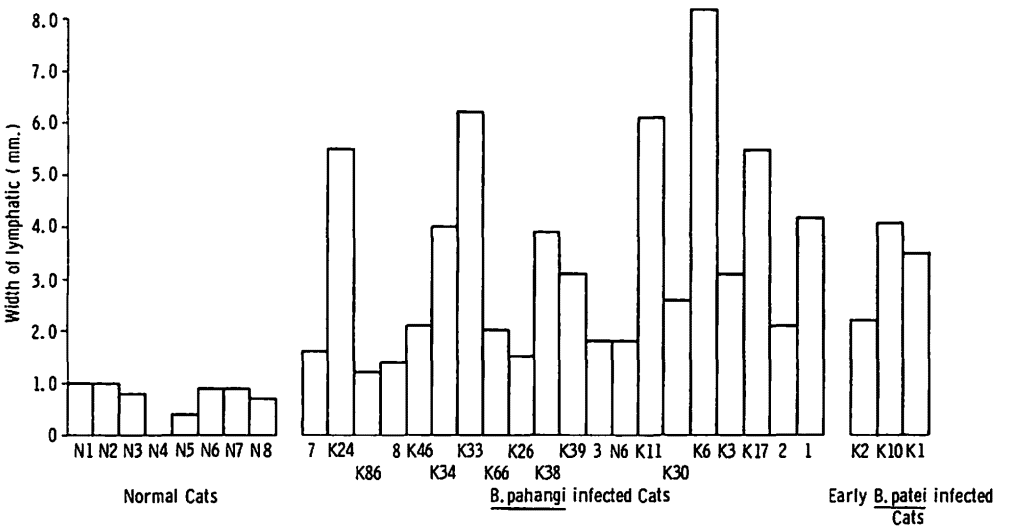
The afferent lymphatics were sometimes as much as 5 times as wide as the corresponding lymphatics in uninfected cats. The dilatation in the afferent popliteal vessels was characteristically segmental in location and usually subcapsular and infra-nodal (Fig. 6). Generalised dilatation along the length of the lymphatic was seen more often in the efferent popliteal lymphatics involved.

Worms were found in histological sections in 7 of the 8 cats autopsied (Fig. 9). The site of occurrence was usually in the lymphatics themselves, in the perilymphatic fat or in the subcapsular lymphatic afferents coinciding with the segmental cystic dilatations visible on lymphography. Rarely, the worms were found in sections of lymph nodes.

Table 2. Tabulated case histories of cats infected with *Brugia pahangi*

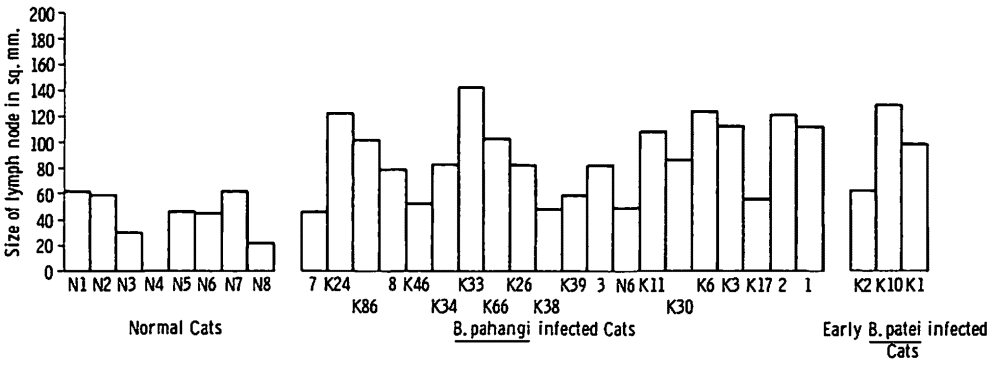
						C	A	T			
	4	5	7	K24	K86	8	K46	K34	K33	K66	K26
Sex	M	F	M		F	F	F	F	M	M	M
Weight (kgms.)	1.1	1.1	1.8	1.6	1.6	2.2	0.9	1.5	1.6	1.6	1.5
Number of inoculated larvae	200	300	100	200	200	100	100	100	100	200	200
Site of larval inoculation	LHL* and RHL	LHL* and RHL	LHL	LHL	LHL	LHL	LHL	LHL	LHL	LHL	LHL
Prepatent period (days)	58	54	–	–	–	–	54	54	54	71	68
Highest microfilariae count/20 mm^3 of blood	161	11	–	–	–	–	54/50mm^3	38	41	?	4
Clinical Features	N.A.D. at time of initial lymphography. On repeat lymphography the nodes were clinically very enlarged	N.A.D.	N.A.D.	L.Pop. node++	L.Pop. node++	L.Pop. node++	L.Pop. node+	L.Pop. node++	L.Pop. node++	L.Pop. node+	L.Pop. node++ R.Pop. node+

*All 4 limbs inoculated subsequently
N.A.D. = Nothing abnormal detected



Histogram 1: Showing dilatation of the afferent popliteal lymphatics in cats with *B. pahangi* and early *B. patei* infections.

K38	K39	3	N6	K11	K30	K6	K3	K17	2	1
F	F	M	M	M	F	M	M	F	M	M
0.6	0.7	1.8	2.5	1.6	1.9	1.1	1.0	0.9	1.1	1.2
100	100	235	200	100	200	100	250	200	180	40
LHL	LHL	LHL and RHL	LHL and RHL	LHL	LHL	LHL	LHL	RHL	All 4 limbs	RHL
–	–	54	68	76	68	75	100	75	81	95
–	–	17	96	30	216	290	17	29	147	93
L.Pop. node+	L.Pop. node+	L.Pop. node	Both Pop. node++	L.Pop. node++	L & R Pop. node++	L.Pop. node++	L.Pop. node++	R.Pop. node++	R.Pop. node++	L.Pop node+



Histogram 2: Showing enlargement of affected popliteal lymph node in cats infected with *B. pahangi* infections and early *B. patei* infections.

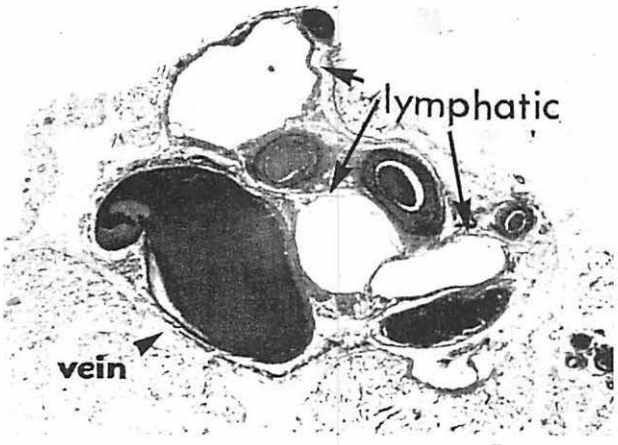


Fig. 5. Normal lymphatic in section

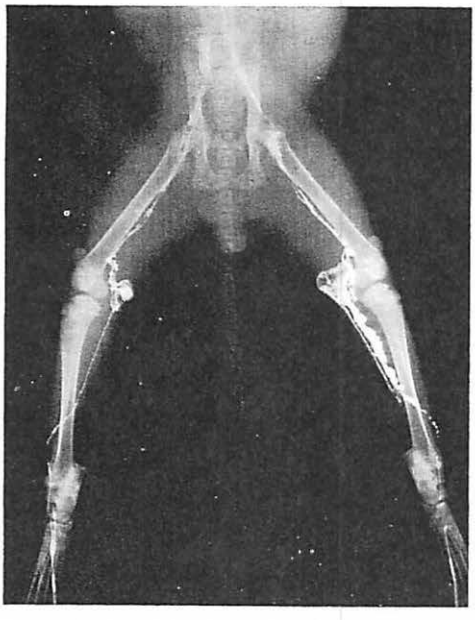


Fig. 6. Bilateral hind limb lymphogram of a 36 days old *B. Pahangi* infection showing a dilated left popliteal afferent lymphatic and an enlarged left popliteal node with a large filling defect

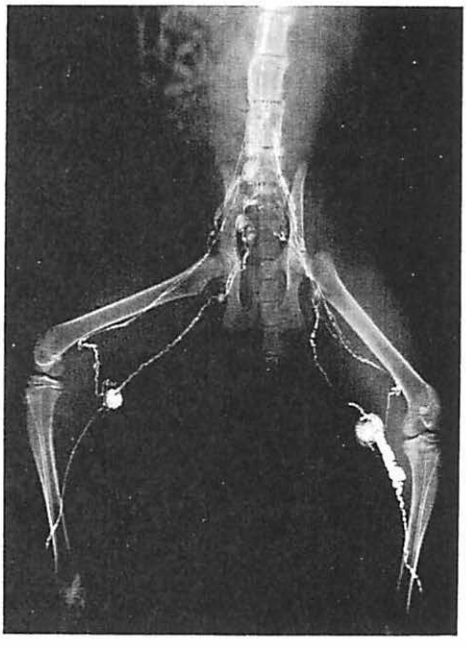


Fig. 7. Bilateral hind limb lymphogram showing a dilated left afferent popliteal node in a 82 days old *B. pahangi* infected cat

Histogram 1 depicts dilatation of the affected lymphatics in the infected cats.

By 136 days the reaction to the parasites within the lymphatics was most intense and thrombus formation more frequent. In addition to the female worms (containing microfilariae) found partially occluding the lymphatic lumen, mushroom-shaped ingrowths attached to the lining wall of the lymphatic together with detached and free lymph throm-

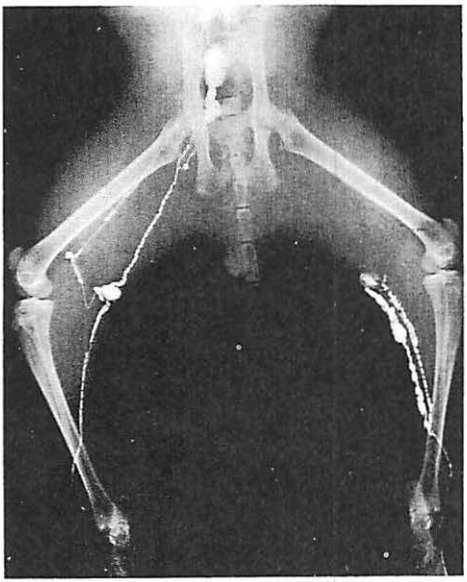


Fig. 8. Lymphogram showing 2 dilated popliteal afferents, an enlarged popliteal lymph node and slow flow of contrast medium. The left popliteal efferents have not been outlined though cannulation was first effected in this limb.

bi were a characteristic feature. The thrombi were seen to be comprised of disintegrating lymphocytes and macrophages and nuclear debris. Dead microfilariae were also seen in the debris. The lymphatic valves were found to be thickened and infiltrated by macrophages, lymphocytes and plasma cells (Fig. 10). Numerous plasma cells with fewer eosinophils and macrophages constituted the perilymphatic reaction. New blood vessel formation was most prominent in granulomatous reaction.

The picture of obliterative endolymphangitis seen in the afferent popliteal lymphatics of a cat with a 317 days old infection (Figs. 11, 12) was similar to the condition graphically described by *O'Connor* (8) and *Bahr* (9) in human patients with elephantiasis resident in the Pacific region.

Enlargement of the nodes (Fig. 6 and 7 and Histogram 2)

The nodes in the infected limbs were enlarged and firm on palpation, and sometimes the nodes were quite hard. These changes were evident before initial lymphography. The infected nodes were usually twice or three times as large as the nodes in the uninfected cats and in the uninfected limbs of the infected cats.

Histogram 2 depicts nodal enlargement in the infected cats.

Old B. patei infections

On previous clinical examination the popliteal nodes were palpable in only one cat but lymphography revealed 5 popliteal nodes outlined in the 10 hind limbs of the infected cats investigated. The nodes were not significantly enlarged on X-ray and some were even seen to be smaller than the corresponding nodes in the uninfected cats. In the old *B. patei* infections ranging from nearly 3 years 10 months to 5 years 9 months, the following histological changes were seen: marked loss of lymphoid tissue and absent germinal centres, with dilated empty lymph sinuses; zones of residual lymph tissue with very few recognizable follicles.

The most striking and significant finding was the lack of scarring and fibrosis in the nodes, suggesting a sweeping away of the cellular content leaving the framework behind without residual scarring. This picture could be described as a lymphangiomatous transformation. This picture is characteristically similar to the lymph nodes of post-surgical lymphoedematous dogs recently described by *Olszewski* (10). The predominant cell type was the plasma cell while macrophages were seen in fewer numbers.

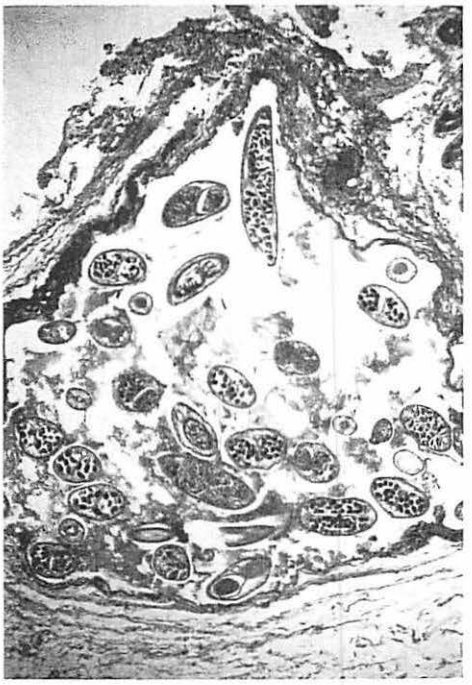


Fig. 9. Section of a lymphatic showing adult filarial worms

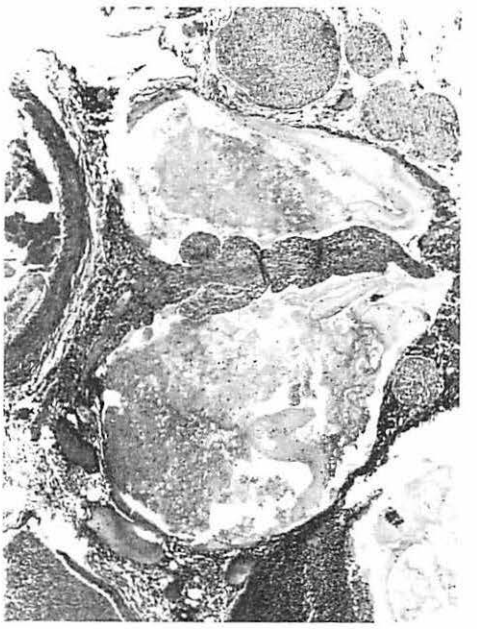


Fig. 10. Transverse section of a lymphatic showing a thickened valve protruding into the lumen.

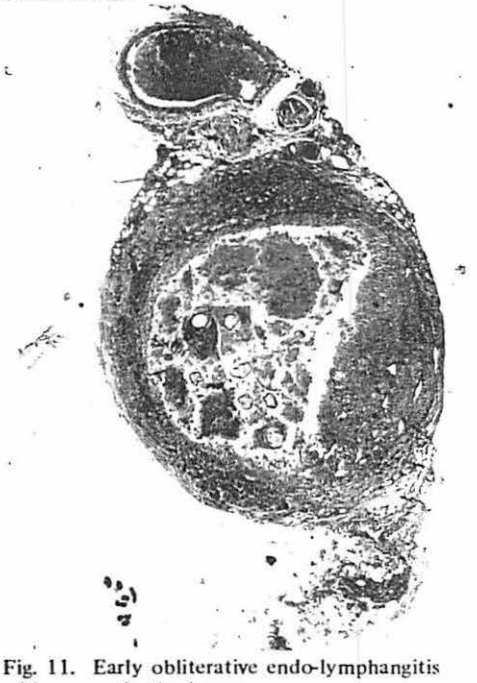


Fig. 11. Early obliterative endo-lymphangitis with worms in the lumen

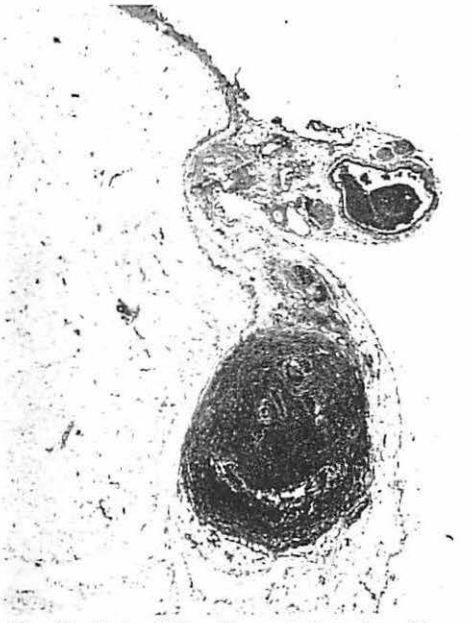


Fig. 12. Late obliterative endo-lymphangitis with nearly total occlusion of the lumen. Disintegrating worms also seen in what was the lumen. From a 317 days old *B. pahangi* infection.

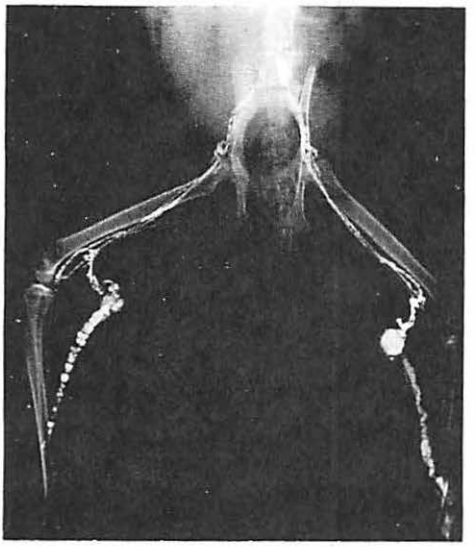


Fig. 13. Bilateral hind limb lymphogram showing tortuosity and dilatation of the left afferent popliteal lymphatic and the right efferent popliteal lymphatic with leakage of contrast medium.

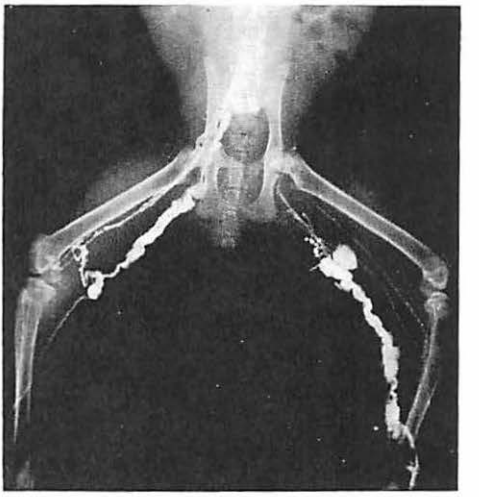


Fig. 14. Leakage of contrast medium in a tortuous lymphatic.

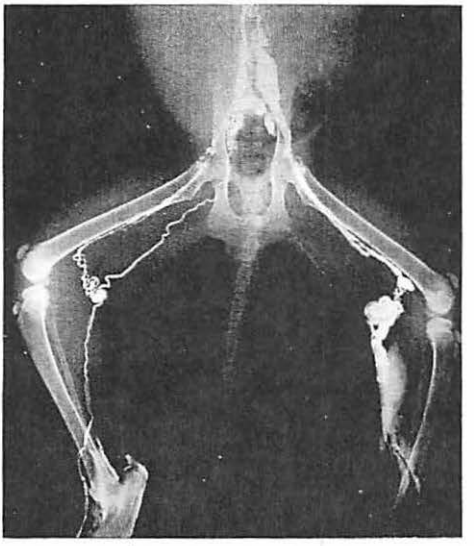


Fig. 15. Infra-nodal segmental dilatation of the right popliteal afferent lymphatic in a 192 days old *B. pahangi* infection.

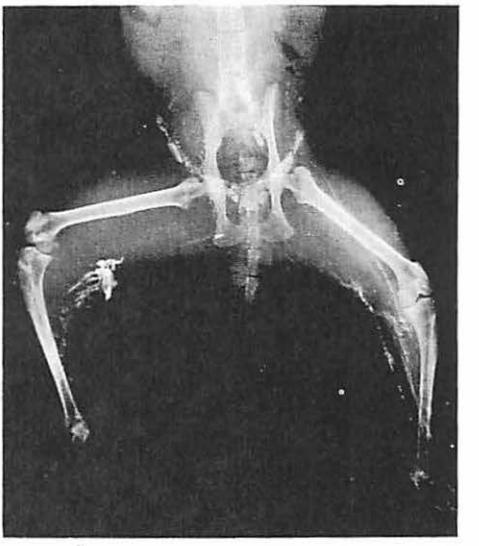


Fig. 16. Stasis of contrast medium 24 hours post-lymphography in a long standing *B. patei* infection.

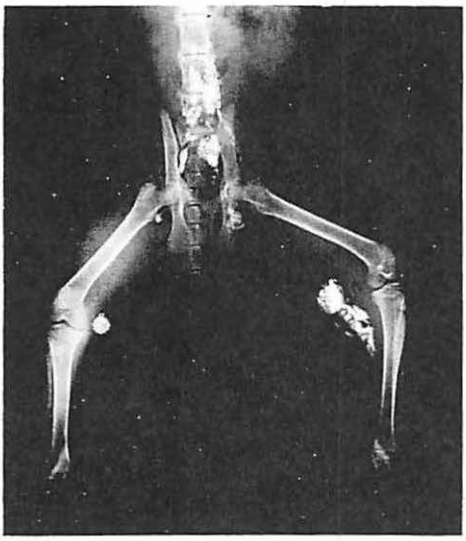


Fig. 17. Stasis of contrast medium in an affected popliteal afferent lymphatic. 82 days old infection. See Fig. 7.

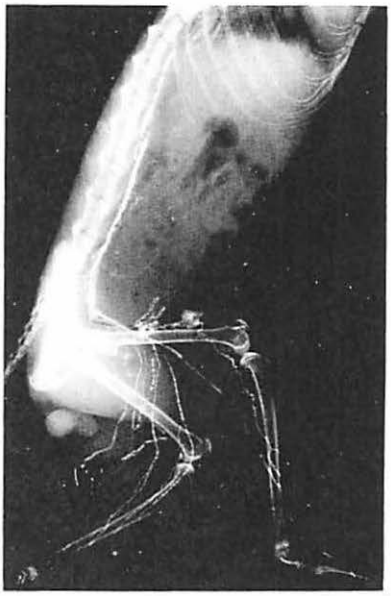


Fig. 18. A long standing *B. patei* infection showing collateral formation and absence of popliteal nodes. Note lymphangiomatous transformation of inguinal nodes.

In the old *B. patei* infected cats popliteal nodes were absent in 50% of the limbs investigated by lymphography but inguinal nodes were frequently outlined.

Only rarely were worms found in section in the node itself and when they did occur, concomitant giant cell formation was seen, with the worms usually found dead inside the nodal substance. Worms were however found frequently in the subcapsular region of the node, which seemed to be a preferential site for the developing and adult worms of *Brugia pahangi*.

Tortuosity of the lymphatics with simultaneous dilatation (Figs. 13 and 14)

The beaded appearance characteristic of the lymphatic vessels in normal cats was poorly outlined when the dilated lymphatics were tortuous as well.

In 3 cats the tortuous vessels were also dilated and showed leakage of contrast medium indicating either a damaged endothelial lining or a change in the permeability of the affected lymphatic vessels. The characteristic beaded appearance was absent and the suggestion that impaired valvular function together with a change in the lining wall of the lymphatic resulted due to the presence of worms was thus strong.

Tortuosity was also seen in old *B. patei* infections and this occurred among the clusters of anastomatic lymphatics in the inguinal region.

Leakage of contrast medium (Figs. 13 and 15)

Leakage was most noticeable in cats showing marked tortuosity of the lymphatic vessels.

This was sometimes accompanied by a leakage of contrast medium from the popliteal node of the affected side, while the popliteal node of the opposite uninfected limb effectively dealt with the contrast medium without any leakage occurring.

Leakage of contrast medium was also seen in the right popliteal node from an old *B. patei* infection and the histological picture suggested a poorly functioning node with a lack of lymphoid tissue.

Stasis of Contrast medium (Figs. 16 and 17)

In practically all the infected cats with marked dilatation of the lymphatic vessels there was stasis of contrast medium which was visible on follow-up X-ray.

Stasis of contrast medium was also visible in the early *B. patei* infections with tortuous lymphatics, and it was a feature in all the long-standing *B. patei* infections which showed numerous collaterals and anastomoses.

The thoracic duct in all 5 old *B. patei* infections showed stasis of contrast medium for varying periods of time.

Collateral formation and lymphatic anastomoses (Fig. 17)

In the long standing *B. patei* infections ranging from 3 years to 10 months to 5 years and 9 months, the most characteristic and diagnostic feature was the presence of numerous afferent collateral vessels in the hind limbs and the formation of anastomotic channels. In the inguinal region the anastomoses appeared as tumour like masses forming clusters with some of the lymphatics showing dilatation. Blindly ending lymphatics were outlined in these old infections which invariably showed stasis of contrast medium on follow-up X-Ray.

The collateral afferent channels numbered up to 11 in one cat but were usually 5-7 in number and were only occasionally found to be dilated. The majority of the outlined collateral lymphatics showed stasis of contrast medium on follow-up X-ray.

Collateral formation was not seen in any of the *B. pahangi* infected cats or in early *B. patei* infections.

In all 5 old *B. patei* infections the abdominal part of the thoracic duct was significantly dilated and numerous anastomotic channels were outlined. In one cat, in addition, signs of early obstruction to the thoracic duct were found with leakage of contrast medium occurring. Stasis was visible on follow-up X-ray.

Discussion

Since *Hendy* (11) nearly 200 years ago cited tropical elephantiasis as a disease of the lymphatic system, and *Demarquay* (1893) and *Wucherer* (1869) discovered the aetiological agent responsible for this disease, there has been controversy as to the pathological mechanisms responsible for chronic lymphoedema, elephantiasis and urogenital lesions in patients infected with *Wuchereria bancrofti* or *Brugia malayi.*

Until an experimental model was available with filarial parasites living in the lymphatics as is the case in human filariasis, it has not been possible to test the hypotheses put forward to explain the mechanism of lymphoedema and elephantiasis.

Dilatation of the Lymphatics

In a preliminary communication, *Gooneratne* et al. (12) reported lymphographic findings including dilatation of diseased lymphatic vessels in cats infected with *Brugia* spp., and *Schacher* et al. (13) working with *B. pahangi* infected dogs, reported dilatation as the most commonly noted change in the afferent lymphatic vessels. *Ewert* et al. (14) found dilated lymphatics in some *B. malayi* infected cats, and *Cohen* et al. (15), *Da Rocha* (16), *Carayon* et al. (17), *Kanetkar* et al. (18) and *Cahill and Kaiser* (19) have found dilated lymphatics in human beings in endemic filarial areas but the age of the infection could not be determined and accurate interpretation was therefore not possible. It is of significance that dilatation and cystic enlargement of the lymphatic vessels occurred in all 24 infected cats investigated from the 15th day after inoculation of larvae and the knowledge that the lymphatics can undergo changes detectable by lymphography as early as the 15th day of infection could provide a new understanding in the diagnosis of human filariasis in endemic areas.

Edeson and Buckley (20) in their study of the migration and rate of growth of *Brugia malayi* in experimentally infected cats, found that on inoculation of infective larvae into the groin, the inguinal, popliteal and sacral nodes were most commonly infected but what struck them as interesting was the "downstream" migration of infective larvae to the popliteal region. They went on to suggest a correlation between the possible preference of *B. malayi* for the popliteal region in cats with the fact that in human beings elephantiasis due to *B. malayi* almost always occurs in the legs and rarely above the knee. In the present study the lymphatics of the limbs in the cats receiving the infective larvae were demonstrated by lymphography to be diseased, as evidenced by their X-ray appearances of dilatation and cystic enlargement. Confirmation of this was obtained by finding worms in histological sections of the lymphatics of the infected limb in all but two of the autopsied cats. No worms were found other than in those lymphatics shown to be diseased on lymphography.

Cohen et al. (15) investigating filarial patients in East Africa found dilatation of the lymphatic trunks with filling of collecting radial channels and dermal backflow. *Kanetkar* et al. (18) graded their findings of lymphographic patterns obtained in a series of 40 patients from endemic filarial areas into 4 distinct clinical grades and dilatation was clinical staging 2 of the lymphatic changes observed by them.

Why dilatation occurs is not definitely known but what is evident from the findings obtained in the present study is that the presence of the worm, especially the live worms, is the governing factor that triggers off the widening of the calibre of the lymphatic vessel. The localized and segmental dilatation almost always observed in the affected lymphatics and perilymphatic cellular response strongly suggests that the physical presence of the worms with a local hypersensitivity reaction, results in dilatation and this mechanism operates not only as a local irritant effect but also an immunological response to the presence of the worms. The histological sections of tissue removed during the prepatent period of the infection showed a mild allergic type of immune response manifested by the presence of numerous eosinophils, fewer plasma cells, and macrophages.

It is interesting to record that *Napier* (21) had made similar observations in human material from patients infected with *W. bancrofti* where he described papillomatous growths projecting into the lumen of the lymphatic and forming a vascular granulation mass resulting, he said, from the entrance of more and more adult worms into the affected lymphatics constantly irritating the endothelial cells of the vessel wall which hypertrophy.

Hartz (22) indicated that these changes were isolated granulomatous masses connected with the wall of the lymphatic are covered with endothelium, and typical of human filariasis. *Michael* (24) and *Cooray* (23) also described the proliferation of filarial granulation tissue as pathognomonic of the disease.

The histological findings in a 317 days old *B. pahangi* infection where the right afferent popliteal lymphatics were practically occluded by the striking obliterative endolymphangitis seen surrounding dead and disintegrating worms in the lumen (Figs. 11 and 12) strongly suggest that the blockage of the lymphatics occurs as an individual response of the host to the presence of the parasite in its lymphatic system.

In the longstanding *B. patei* infections in contradistinction to the early infections of *B. patei* dilatation was rarely observed but instead collateral channels and anastomoses were the characteristic features. This suggests that lymphatic dilatation is an early manifestation of the disease process of filariasis.

Lymph node enlargement

Nodal enlargement is one of the clinical signs of filarial infection and has been detected on lymphography in human patients by *Cahill and Kaiser* (19), *Da Rocha* (16) and *Kanetkar* et al. (18) In most of the longstanding cases of filariasis with lymphoedema and elephantiasis the nodes are hardly palpable or visible on lymphography and if outlined, they are small (*Kanetkar* et al. (18).

Nodal changes along with lymphatic vessel dilatation were visible as early as the 15th day after larval inoculation, and at this stage the histopathological findings were of a non-specific nature – reactive hyperplasia and cellular proliferation displaying numerous mitotic figures. This seemed to be part of the nodal response to the presence of the worm further down the limb, while the local lymphatic vessel containing the worm manifested a perilymphatic and endolymphatic reaction only from the 54th day onwards when the adult worms had appeared.

The most obvious histopathological changes were seen around adult worms in the *B. pahangi* infections manifesting a plasma cell and eosinophil response. No reactions were seen around prematurely liberated ova as suggested by *Manson* in 1882 in *W. bancrofti* infections in man, and more recently by *Schacher and Sahyoun* (25) in their studies on cats infected with *B. pahangi.* It is possible that they were observing ova that were dispersed in the tissues due to mechanical damage to the gravid females when the material was being dissected from the infected patient or animal and prepared for histology. The new technique used in the present study of intra-lymphatic fixation of the adult worms *in situ in vivo* (*Gooneratne* [5]) would probably prevent rupture of the gravid females.

The absence of popliteal lymph nodes in the long standing *B. patei* infections could have been due to non-functioning diseased nodes but it may have been a peculiarity of the laboratory strain of inbred cats that were used for maintaining *B. patei* infections in the department. The lymphangiomatous change seen in these cats had been similarly observed by *Servelle* et al. (26) in congenital lymphoedematous patients.

Large filling defects were seen in the left popliteal node in two *B. pahangi* infected cats suggesting the presence of adult worms. Filling defects were seen in the inguinal nodes in human patients investigated by *Cahill and Kaiser* (19).

Tortuosity of the lymphatics

The tortuous lymphatic vessels were found to be dilated as well and showed leakage of contrast medium indicating a change in the permeability of the afferent vessels and/or damage to the endothelial lining. The characteristic beaded appearance was absent and the suggestion that valvular function was being impaired, causing in addition a change in the wall of the lymphatic due to the presence of the worms in it, was thus strong.

Cohen et al. (15) investigating lymphoedematous patients with a typical history of filarial fever in endemic areas in East Africa found dilatation and tortuosity of the lymphatic trunks with filling of the collecting radials and dermal backflow. They also reported autopsy findings in cats and dogs in Kenya infected with *B. patei* where tortuous and varicose lymphatic vessels were seen, and careful examination revealed adult worms "thrashing to and fro" in dilated vessels.

Tortuous afferent lymphatics were also reported by *Cahill and Kaiser* (19) in filarial lymphoedematous patients, while *Kanetkar* et al. (18) mentioned tortuosity as the 2nd stage of the clinical staging of the filarial process in *W. bancrofti* infections in endemic areas in India. *Da Rocha* (16) investigating adenolymphocoeles found obstruction at the iliac level causing lymph flow to deviate through the pre-sacral anastomoses and reflux into the inguinal nodes. This, he said, was the cause of the dilatation and tortuosity of the afferent lymphatics. He argued that the increased permeability of the tortuous lymphatics he observed in filarial patients with lymphangitis was the result of the inflammation.

Carayon et al. (17) made similar observations in their study of bancroftian filariasis patients presenting with lymphangitis.

Leakage of contrast medium was found only in cats with early infections manifesting lymphatic dilatation and tortuosity resulting from local reactions and the degree of obstruction further up the lymphatic system. It seems likely therefore that lymphangitis, together with valvular impairment and resultant stagnation of lymph, are responsible for the leakage that results. This effect was probably exaggerated by the external pressure needed to inject the contrast medium thus causing a greater leakage than would have occurred without lymphography being performed. The association of pharmacologically active substances such as histamine present in allergic manifestations of most helminthiases could possibly explain the increased permeability of the vessel wall and is a pointer to what probably happens in the initial stages of lymphoedema where seepage of protein-rich lymph occurs into the surrounding tissues, causing stagnation of lymph, together with an increased permeability of the vessel wall.

Stasis of contrast medium

Stasis of contrast medium has been attributed to valvular incompetence and to obstruction to lymph flow both in the node and within the lymphatic itself. The very presence of the active adult filarial worms in the lumen must act as a partial obstruction to lymph flow but it is surprising that in spite of gross changes visible on lymphography, contrast flow occurs in these diseased, though patent, lymphatics. Valvular thickening with cellular infiltration into the swollen valvular substance was demonstrated clearly by histology (Fig. 10). The occurrence of such a thickened and obviously poorly functioning valve must play an important role in the resulting stagnation of lymph within the lymphatic vessel.

Collateral formation and lymphatic anastomoses

Cohen et al. (15) investigating human lymphoedematous patients from endemic filarial areas in East Africa, found that more lymphatic trunks are apparent than in a normal lymphogram and that some of the abnormal trunks ended blindly. They confirmed this by visual lymphography where the diseased lymphatic trunks were dissected proximally and some were found to end blindly in fibrous tissue. Numerous anastomotic channels were discovered by them, and these transported the lymph upwards. Similar appearances were seen in the present study in the old infected *B. patei* cats. *Da Rocha* (16) examining patients with filarial lymphoedema and elephantiasis found that collateral channels were due to lymphatic obstruction further up and that this resulted in shunting the lymph flow to the superficial network of lymphatics. He confirmed these findings with radio-active serum albumin (RISA) injections. More recently *Kanetkar* et al. (18) in their study of patients in filarial endemic areas in India suggested that progressive obliteration of the lymphatics results in collateral formation and abnormal channels opening up in the leg. This stage, they found, progressed to gradual obliteration of the lumina of the lymphatic vessels with development of extensive backflow. The findings in longstanding *B. patei* infections in cats suggest that a similar process has been occurring and the appearance of numerous lymphatic channels, some ending blindly, is a sign that interference and obstruction to lymph flow has occurred further up with back pressure causing the opening of new, though existing channels, as a compensatory mechanism.

Cellular response to filariasis in the lymphatic system

The most striking cellular reaction visible in histological sections of the lymphatics and nodes containing worms, was the presence of eosinophils, plasma cells and macrophages.

In the early prepatent infections there were much fewer eosinophils seen in histological section.

Basten et al. (27) has suggested that eosinophilia is an immune reaction of the cell-mediated type as in delayed hypersensitivity.

The predominance of plasma cells in the patent infections of *B. pahangi* infected cats were most striking. Their association as antibody producing cells is now established (*Holbrow* [28]).

Coombs (29) suggested that macrophages in some way process antigen which interacts with antigen-sensitive lymphocytes containing "recognition units", and stimulates cell division to produce more receptive or primed lymphocytes of specificity, setting in motion "the synthesis of immunoglobulin according to the cells innate genetic code".

Value of lymphography *in elucidating the nature of the lymphoedema in patients from endemic filarial areas*

The significance of the present lymphographic observations to the problem of filariasis is that it may now be possible to demonstrate lesions at a very early stage of the disease at a time when lymphoedema and elephantiasis can be prevented. But perhaps of more importance is that the observations in infected cats may provide several new criteria distinguishing filarial from non-filarial lymphatic disease. In areas where filariasis is endemic it is all too often assumed that enlargement of the lymph nodes and limbs are due to filariasis. *Poynton and Hodgkin* (2) considered that symptomless enlargement

of lymph nodes in areas of endemic filariasis were due to filariasis when no other obvious cause was found. When this occurred in a high proportion of children they considered it one of the most important indications of active and intensive transmission of filariasis. *Loewenthal* (30) and *Clark* (31) described a number of cases of elephantiasis from Uganda and Kenya in areas where *Wuchereria bancrofti* was absent. Their patients had swelling below the knees with characteristic verrucose barnacle-like growths on the affected areas which led *Loewenthal* to name this disease of non-filarial aetiology "Lymphostatic verrucosis". The technique of lymphography was not available at the time to help elucidate the nature of the disease syndrome. *Cohen* (32) and *Cohen* et al. (15) found non-filarial lymphoedema and elephantiasis prevalent in Ethiopia and Kenya, and lymphography of value is establishing the correct diagnosis. The cause of this non-filarial elephantiasis is still unknown (*Oomen* [33]).

Ngu and Konstam (34) found tuberculous adenitis the commonest cause of chronic lymphoedema in Western Nigeria where there is a low rate of transmission of bancroftian filariasis. Out of a series of 65 patients investigated they found only one patient with a history suggestive of filariasis.

The lymphographic observations supported by histopathological findings in this series in the infected cats support *Wharton*'s contention (35) that the site of inoculation of the infective larvae by the mosquito may determine the eventual localisation of the adult worms and the ensuing lymphatic lesions. This may in part account for the predominance of elephantiasis of the lower limbs, although other factors such as vertical posture and hydrostatic pressure may play a part in the mechanism of lymphoedema and elephantiasis which are practically non-existent in nature in animals other than man (*De Magalhaes* [36], *Nelson* [37] and *Oomen* [33]).

Suggested *sequence of events in the lymphatic system following filarial infection*

Based on the findings in these experiments and on the results reported by other research workers, it appears that the following sequence of events takes place on the entry of infective filarial larvae into the host either by syringe-inoculation in experimentally infected animals or from infective mosquitoes in nature.

The infective larvae migrate to the lymphatic vessels or nodes and this migration is almost complete within 24 hours to 48 hours (*Schacher and Sahyoun* [25]). The presence of these larvae which undergo 2 moults in the vertebrate host before they become adult worms, has an irritant effect on the endothelial lining of the lymphatic vessel. On moulting, the moulting fluid and the cast-off sheaths, which are suggested to be antigenic by *Schacher and Sahyoun* (25) (was was previously found with *Ascaris* larvae by *Rogers and Somerville* (38)., may stimulate an early immunological response by the host. *Wharton* (39) had made a similar observation in the pathology produced by *Litomosoides carinii* where he observed that in the pleura the pathology was due to the liberation of substances (unnamed) by the worms. This suggestion needs further investigation with reference to filariasis, though the response seen to the worms found at this stage of the infection during the prepatent period in infected cats in this series of experiments did not manifest as a marked immunological reaction. However, the lymphographic findings of dilatation of the lymphatic vessels which occurred in *B. pahangi* infected cats by the 15th day could well be the result of the combined factors of irritation caused by the

worms together with the moulting products and exsheathing fluid, and the metabolic products of the developing worms producing the initial local immunological reaction seen. No histological evidence of cast-off sheaths and moulting fluid was visible, and this awaits further investigation. The valves at this early prepatent period were not much affected when examined histologically but significant interference to lymph flow, as evidenced by slow flow of injected contrast medium and stasis on follow-up X-ray, did occur. Valve changes were however seen in histological sections in older patent infections and some of the more marked lymphographic and histological changes were also observed during this period.

With the worms getting bigger and finally moulting to become adult worms, the irritant action and local immunological response become more marked as evidenced by the appearance of numerous plasma cells and eosinophils in the histological sections of the autopsied cats with patent infections. The clinical symptoms of early lymphangitis and allergic manifestations, especially localized reactions in human patients (*Manson-Bahr* [40] provide comparative correlating evidence for this. Histamine and histamine-like substances have been isolated in allergic disorders and the signs and symptoms of histamine induced allergy have been recorded experimentally in animals (*Archer* [41]). Recently *Cruickshank and Weir* (42) have found that lymph node permeability factor (LNPF) – one of the pharmacological agents responsible for delayed hypersensitivity (*Turk* [43]) – produces increased lymphatic permeability when given intradermally in small doses. They went on to state "there is much circumstantial and persuasive evidence that LNPF is important in reactions of the delayed type of hypersensitivity but at present direct and incontrovertible evidence is lacking".

With repeated filarial infections that occur in human patients in endemic areas, this sequence of events probably occurs so frequently that overlap of the developmental stages of the worm results. Such repeated challenges of infection must result in more damage to the lymphatic system in susceptible individuals with the production of lymph thrombi, granulation tissue, and partial or total obstruction of the lymphatics as seen in the infected cats in this series of experiments, and by *Schacher and Sahyoun* (25) in *Brugia* infected cats and dogs. What is significant is that nearly identical lesions were observed by *O'Connor* (7), *O'Connor and Hulse* (44), *Bahr* (9), *Cooray* (23), *Reddy and Ray* 45) and other workers reporting on filarial tissues from human patients. The clue as to why only some people infected with *W. bancrofti* or *B. malayi* develop lymphoedema, chyluria and elephantiasis, while others with heavier infections and prolonged exposure to reinfections and repeated challenges in endemic areas escape this, seems to lie in the individual response of the host to the presence of these worms in the body. In the 317 day-old *B. pahangi* infected cat, for instance, the right afferent popliteal lymphatics of the inoculated limb were almost totally obliterated and the striking massive reaction seen in these lymphatics on histological examination was very suggestive of a strong immunological response on the part of the host, which not only killed the adult worms in the lumina of the lymphatics but in one lymphatic produced near total occlusion of its lumen. Most of the other infected cats responded less violently and some rather mildly. In the clinical syndrome of Tropical Pulmonary Eosinophilia which occurs in areas where transmission of bancroftian filariasis is taking place, a similar immunological response by the host operates against the microfilariae (*Webb* et al. [46]). It has also been suggested that this condition is the result of sensitisation in some patients to filariae of animal origin (*Danaraj* [47], *Donohugh* [48]), but patients with Tropical Pul-

monary Eosinophilia have been described also manifesting classical clinical signs of filariasis such as lymphangitis and hydrocoele (*Friess* et al. [49], *Crosnier* et al. [50], *Galliard and Mallarme* [51]). *Buckley*'s experiments (52) however suggest that both human and animal filarial parasitisation may induce this allergic syndrome.

Gross thickening of the valves of the infected limbs occur as a later manifestation of the disease and these diseased valves not only must be unable to function efficiently, but must also add to the embarrassment to lymph flow caused by the dilated lymphatic with an altered endothelial lining and narrowed lumen due to the presence of the worm and the formation of intralymphatic thrombi. This, together with obstruction further up, would explain the slow flow of contrast medium seen in the infected cats showing marked dilatation and tortuosity with the lack of the normal characteristic beaded-appearance of the lymphatics. The erect posture of man and the resultant high hydrostatic pressure in comparison with other animals would add a further strain on the diseased valves and on the lymphatic system of the dependent parts of the body, and this is probably the reason why lymphoedema manifests in these regions, in human patients.

Rusznyak et al. (53) reported that when lymph with a high concentration of protein e.g. lymph originating from a focus of inflammation, passes through an area of comparatively low protein concentration, protein may diffuse from the lymphatics. *Yessipova* (54) investigating chronic aspecific pneumonia found that fibrosis was more commonly seen in the vicinity of important collecting lymphatics. Therefore, when congestion occurs in filariasis due to factors enumerated previously accumulation of fluid of high protein concentration in the interstitial space could be the important factor in the genesis of fibrosis seen in the terminal stages of chronic lymphoedema and elephantiasis. This could have an important bearing on the patient with filarial lymphoedema, the oedema fluid of which is protein rich. This however needs further investigation in human patients with filariasis and in filarial infected animals in whom induced lymphoedema may result from experimental infections.

The derangement in the lymphatic vessels caused by the filarial worms, is accentuated by nodal changes either directly or indirectly due to the adult worms. Several workers have suggested that nodal fibrosis occurs due to the presence of adult filarial worms and some have even attributed pathological changes leading to nodal sclerosis to microfilarial and infective larvae (*Acton and Rao* [55], *Cooray* [23] and *Reddy and Ray* [45]). Such changes were not seen in *Brugia* infected cats in the present study.

That the infected lymph nodes and lymphatics showing gross changes are not functioning normally was demonstrated by the presence of contrast medium in the dilated and tortuous lymphatics of the diseased limbs on follow-up X-ray, and also by the presence of contrast medium densely outlining the infected nodes.

With further progress of the disease, compensatory collateral channels develop to carry the lymph to the thoracic duct and then on to the right heart. But if the majority of the lymphatics have been thus affected the number of channels will be few in the chronic long-standing infections, and these may be incapable of coping with the total volume of lymph which has to drain into the general circulatory system every day. Lymphatics commence blindly in the tissues and the lymph in the interstitial spaces has to actively enter these blindly-ending vessels. With derangement of the lymphatic vessels and nodes caused by filariasis and stagnation of lymph flow as seen in most of the infected limbs of the infected cats showing marked dilatation of tortuosity, it is possible that this

lymph could find its way out of the lymph vessels in a reversal of the physiological process by which it first entered the lymphatic. What could also happen due to a derangement caused by a partial or total lymphatic occlusion, intra-lymphatic thrombi, the presence of adult worms and thickened diseased and incompetent valves, is that the larger protein molecules in the tissues which are normally transported in the lymph remain in the interstitial compartment unable to enter the lymphatic, and be carried in the circulation as happens in the normally functioning lymphatic system. This observation was made in injuries due to cold, by *Zimmerman and Takats* (56), where they found a fibrinous exudative network obstructing the lymphatics and preventing the transportation of the protein from the tissues. They also remarked that fibroblasts wander into the lymphoedematous area so formed, leading to a thickening of the subcutaneous connective tissue, a multiplication of collagenous and elastic fibres then occurs leading finally to the classic picture of chronic lymphoedema.

Chronic filarial lymphoedema is a high-protein lymphoedema and according to *Crockett* (57) who examined 400 samples of oedema fluid from patients with diverse aetiology, this high-protein oedema is due to a failure of drainage of tissue-fluid protein and is therefore "a failure of lymphatic function resulting from obliteration of lymphatic pathways, through incompetence of their valves, or through the removal of the normal agencies of lymph propulsion".

It is hoped that a more extensive study of patients in endemic filarial areas of different ages, sexes, microfilarial counts and clinical manifestations will reveal further knowledge of the disease process of filariasis, confirming the importance and significance of the results obtained in the laboratory model chosen for this study in relation to what occurs in nature in human beings who are infected with filariasis.

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B.W.M. Gooneratne, M.D., Ph.D., Department of Diagnostic Radiology the Prince Henry Hospital, Little Bay, Sydney N.S.W. 2036 (Australia)
Address for reprints: 29 Dunmore Road, Epping, N.S.W. 2121, Australia.

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Experimental Studies on Lymphatic Drainage of the Peritoneal Cavity Using ^{198}Au-Colloid*

H. Langhammer[+], U. Büll[++], K.J. Pfeiffer[++], G. Hör[+], H.W. Pabst[+]

[+]Nuclear Medicine Clinic and Policlinic, Technical University of Munich
and [++]Clinic and Policlinic for Radiology, University of Munich, West Germany

Summary

The lymphatic drainage from the peritoneal cavity was studied scintigraphically and by determination of the specific accumulation of ^{198}Au-colloid and of the ^{198}Au-contents within the regional lymphatics, liver and spleen following intraperitoneal injection of 25 µCi in 29 rabbits. The investigation revealed the selective ^{198}Au-accumulation in the mediastinal lymph nodes and within the lymphatics of the greater omentum, whose absorptive capacity was shown to be significant. The theoretical background for visualization of the mediastinal lymph nodes scintigraphically and for the transposition of the greater omentum in the treatment of lymphedema was explained by these results. Moreover, it was previously suggested, that intraperitoneal radiotherapy affects the regional lymphatic system of the peritoneal cavity as well. The lack of radioactivity in the mesenteric lymph nodes and in the thymus indicated, that these organs do not participate in the lymphatic drainage mechanism of the peritoneal cavity.

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