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With 2 plates and 6 figures in the text

Brugia malayi microfilaraemia in mice: a model for the study of the host response to microfilariae

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SUMMARY

Microfilariae of *Brugia malayi* were obtained from the peritoneal cavities of infected gerbils and were then injected intravenously into mice. A sub-periodic, nocturnal microfilaraemia was produced. The level of microfilaraemia was proportional to the number of parasites injected, with approximately 1–3% of microfilariae being found in the peripheral circulation. The duration of microfilaraemia was proportional to the number of parasites injected; it subsided by 30 days after injection of 10^4 microfilariae but was still present at a low level 120 days after injection of 2×10^5 microfilariae. A transient splenomegaly developed after injection of microfilariae. Histopathological examination revealed large numbers of microfilariae free in the lumens of pulmonary small blood vessels and without any accompanying inflammatory reaction. Lesser numbers of microfilariae were seen in the cardiac blood and hepatic and renal blood vessels for the first few days after injection. There was cellular proliferation in the splenic white pulp and vascular congestion of the red pulp. Microfilariae labelled with ^{51}Cr were injected intravenously; 57% of radioactivity was found in the lungs, 8·5% in the liver and 2·9% in the spleen. Mice developed immediate hypersensitivity reactions to *B. malayi* antigen by 4 weeks after injection, but Arthus and delayed hypersensitivity reactions were not seen at any time. When mice which had been injected 5 months previously were challenged with a 2nd injection of microfilariae, there was an accelerated clearance of parasites over 2 weeks and a marked peripheral blood eosinophilia developed. In contrast with natural infections, in which the continuous production of microfilariae complicates assessment, this model provides a system in which factors controlling the circulation of microfilariae in the bloodstream can be studied independently.

INTRODUCTION

Microfilariae may be found for many years in the peripheral blood of patients with bancroftian and malayan filariasis, but the mechanisms by which they evade the host's immune responses are ill-understood. Microfilariae are generally

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non-pathogenic but their ability to persist in the bloodstream over long periods is essential for the maintenance of the life-cycle of the parasite. If a vaccine against microfilariae could be developed, it may be possible to interrupt transmission by effectively isolating the parasite reservoir from the mosquito vectors.

An understanding of the factors influencing the circulation of microfilariae is essential for the development of such a vaccine. The continuous production of microfilariae by adult worms in natural infections makes such an assessment difficult. We have attempted to circumvent this problem by developing a model in which *Brugia malayi* microfilariae obtained from infected gerbils were injected intravenously into mice. Under these circumstances parasites will circulate for up to 4 months after injection.

MATERIALS AND METHODS

Isolation of microfilariae

Gerbils (*Meriones unguiculatus*) infected with a sub-periodic strain of *Brugia malayi* were obtained from Dr J. McCall of the University of Georgia, Athens (provided by the US–Japan Medical Science Program–NIAID). Four months or more after inoculation with 300–400 infective larvae, they were anaesthetized with sodium pentobarbital, then their abdomens were shaved and sterile 18 gauge angiocaths (Deseret Co., Sandy, Utah) were inserted into the midline. The peritoneal cavities were washed with 20 ml of 50 % (v/v) sterile phosphate-buffered saline – 50 % (v/v) Dulbecco's modified Eagles medium (K.C. Biological Inc., Lenexa, Kansas) (PBS/medium). A yield of up to 1×10^6 microfilariae could be obtained from the peritoneal cavity of a single animal. Any specimens that contained only small numbers of microfilariae or were contaminated by large numbers of peritoneal exudate cells were discarded. In the remaining specimens, there were only minimal numbers of peritoneal cells; microfilariae constituted more than 98 % of particulate matter. Since the contamination with peritoneal exudate cells was insignificant, and attempts to remove the remainder may damage the parasites, microfilariae were simply washed 3 times by centrifugation at 600 *g* with phosphate-buffered saline and then resuspended in PBS/medium at the appropriate concentration.

Assessment of microfilaraemia

Outbred Swiss albino mice, 20 g in weight (CF_1, Carworth Farms, New City, New York), were injected with 0·5 ml of microfilarial suspension via the tail vein. Blood samples were taken from the retro-orbital venous plexus of lightly anaesthetized mice with heparinized microhaematocrit capillary tubes (Fisher Scientific Co., Pittsburg, Penn.). In long-term experiments, samples were always taken between 10.00 and 14.00 h. Microfilariae were counted on Sedgewick–Rafter chambers and the results expressed as the number of microfilariae/100 μl blood.

Total white cell and eosinophil counts

Further blood samples were taken for measurement of total white cell counts and eosinophil counts in Neubauer's counting chambers using Carpentier's fluid.

Histopathology

The heart and lungs, liver, spleen, kidney and mesenteric lymph nodes were removed after exsanguination of anaesthetized mice. The liver and spleen were dried lightly with filter paper and weighed, and then all specimens were fixed in 10% (v/v) formalin. After routine processing, biopsies were stained with haematoxylin and eosin.

Labelling of microfilariae with ^{51}chromium

Microfilariae were labelled with radioactive chromium. One million microfilariae, prepared as described before, were incubated with 1 mCi of ^{51}Cr (New England Nuclear, Boston, Mass.) in 0·5 ml of PBS/medium for 3–4 h. They were then washed 5 times by centrifugation at 600 *g* with phosphate-buffered saline and finally resuspended in PBS/medium. At varying intervals after intravenous injection of ^{51}Cr-labelled microfilariae, blood and urine samples were taken and then the heart, lungs, liver, spleen, kidney, mesenteric lymph nodes and brain were removed, weighed and the total radioactivity of each organ was measured.

Footpad reactions

B. malayi microfilarial antigen was prepared as described previously (Grove, Cabrera, Valeza, Guinto, Ash & Warren, 1977). Protein (10 μg) in 0·03 ml of phosphate-buffered saline was injected into one hind footpad of each mouse and 0·03 ml of phosphate-buffered saline was injected into the other footpad. The size of the reactions at 15 min, 6, 24 and 48 h was measured with a micrometer.

RESULTS

Diurnal variation

Twenty-five mice were injected intravenously with 5×10^4 microfilariae and kept in the light by day and in darkness by night. Samples were taken every 6 h for the next 48 h. A mild nocturnal periodicity was observed with the numbers of microfilariae in the peripheral blood at night being approximately 3 times that seen during the day (Fig. 1).

Intensity of microfilaraemia

Groups of 6 mice were injected intravenously with numbers of microfilariae ranging from 10^4 to 2×10^5. Peripheral blood microfilarial counts were determined on days 1, 2, 3, 4, 8, 12, 16 and 20. There was no significant variation between these days so the counts were pooled. There was a direct relationship between the mean number of microfilariae in the blood and the number of microfilariae injected (Fig. 2); the correlation coefficient $r = 0{\cdot}988$ ($P < 0{\cdot}001$). If a mouse is estimated to have a circulating blood volume of 2·0 ml, then the proportions of microfilariae circulating in the blood during the day and night were approximately 1·0% and 3·0% respectively.

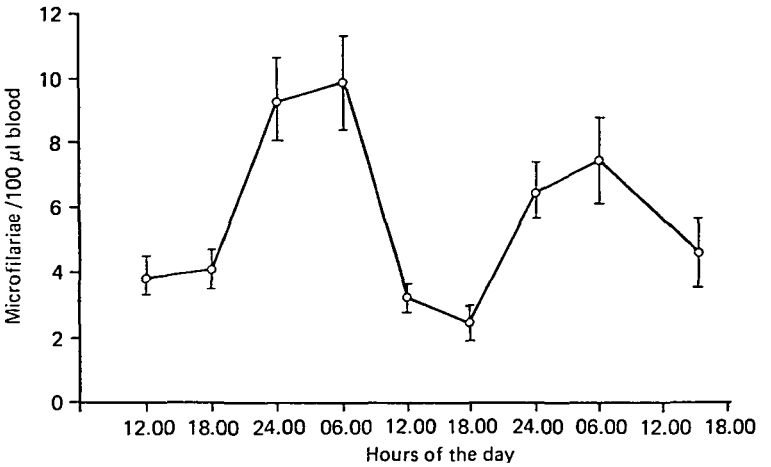

Fig. 1. Nocturnal sub-periodicity of *Brugia malayi* microfilaraemia. Twenty-five mice were given 5×10^4 microfilariae each by intravenous injection.

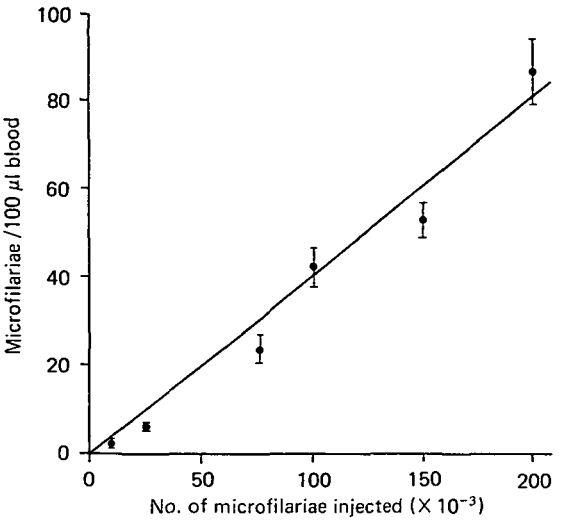

Fig. 2. The relationship between the level of microfilaraemia and the number of microfilariae injected intravenously. Six mice were in each group.

Duration of microfilaraemia

Groups of 6 mice were injected intravenously with numbers of microfilariae ranging from 10^4 to 2×10^5. Peripheral blood samples were taken at intervals for the next 120 days. The duration of microfilaraemia was proportional to the dose of microfilariae injected (Fig. 3). Circulation of microfilariae in the peripheral blood ceased by 30 days after injection of 10^4 microfilariae. A small persistent microfilaraemia was still seen, however, 120 days after injection of 2×10^5 microfilariae.

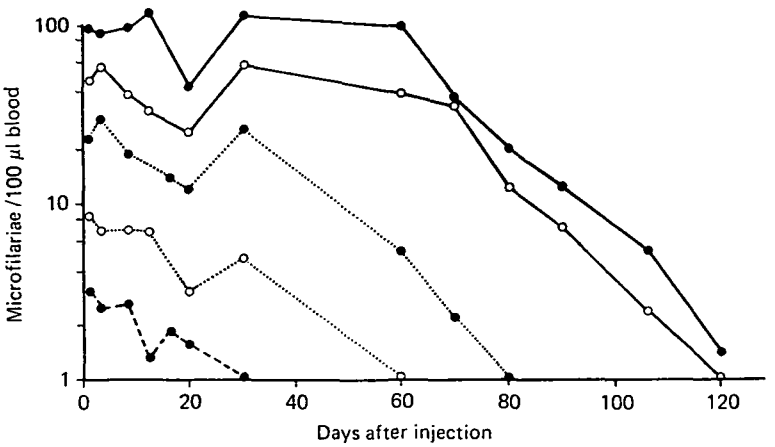

Fig. 3. The duration of microfilaraemia after intravenous injection of between 1×10^4 and 2×10^5 microfilariae. Six mice were in each group. ● – –●, 1×10^4; ○ · · · · ○, $2{\cdot}5 \times 10^4$; ● · · · · ●, $7{\cdot}5 \times 10^4$; ○——○, 1×10^5; ●——●, 2×10^5.

Table 1. *Liver weights expressed as a percentage of body weight after intravenous injection of* 10^5 *microfilariae*

(Six mice were in each group.)

Time after injection	Liver weight (Mean ± S.E.)
0	4·8 ± 0·2
1 h	4·8 ± 0·2
4 h	4·8 ± 0·2
1 day	5·9 ± 0·2
2 days	5·7 ± 0·2
4 days	6·1 ± 0·2
8 days	5·9 ± 0·4
16 days	5·6 ± 0·2
32 days	5·6 ± 0·2
64 days	4·7 ± 0·2
128 days	4·7 ± 0·1

White cell counts

Ten mice were injected with 5×10^4 microfilariae. Total white cell and absolute eosinophil counts were measured at intervals for the next 4 weeks. No significant variations were seen.

Liver and spleen weights

Mice were injected intravenously with 10^5 microfilariae. The livers and spleens were removed from groups of 6 mice at intervals for the next 128 days and expressed as percentages of the body weights. A slight increase in liver weight of approximately 20% was seen by 1 day after injection. This mild hepatomegaly persisted for 16 days and then slowly declined (Table 1). There was considerable splenic enlargement. The maximal increase of 2·5 times the original weight was seen 4 days after injection; the splenomegaly then declined slowly (Fig. 4).

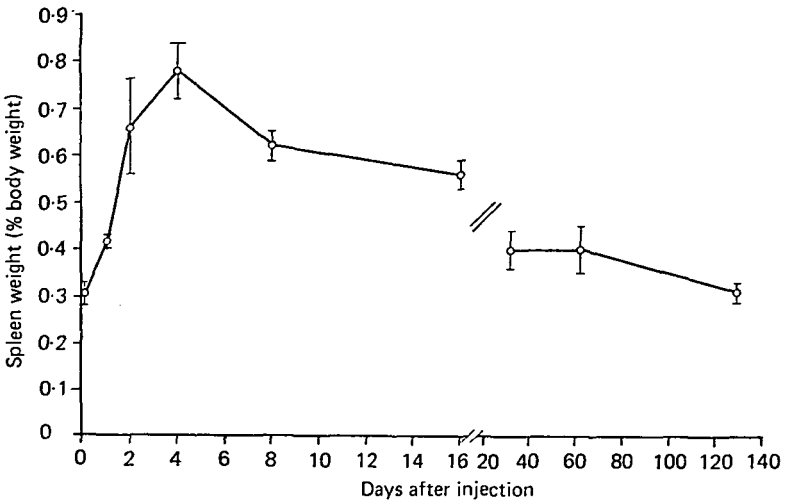

Fig. 4. Spleen weights at varying times after intravenous injection of 10^5 microfilariae. Six mice were in each group.

Histopathology

Sixty mice were injected intravenously with 10^5 microfilariae. Tissue samples were taken from groups of 6 mice at 1 and 4 h and 1, 2, 4, 8, 16, 32, 64 and 128 days after injection.

Lungs

Large numbers of microfilariae were seen in the pulmonary capillaries and small vessels from 1 h to 16 days after injection. The microfilariae were not marginated on the walls of the vessels and were not associated with an inflammatory reaction (Pl. 1). Similar appearances were seen 32 days after injection but the numbers of microfilariae were beginning to decline. Only small numbers of microfilariae were found at 64 days and none were seen after 128 days. There was no inflammatory reaction at any time.

Heart

Moderate numbers of microfilariae were seen in the cavity blood for the first 16 days, after which the numbers declined. An occasional microfilaria was seen in the coronary vessels or in a myocardial capillary.

Liver

Moderate numbers of microfilariae were seen free in the lumens of vessels in the portal areas for the first 16 days; microfilarial numbers subsequently declined. There was no significant inflammatory reaction.

Spleen

By 1–2 days after injection there was moderate enlargement of the follicles in the white pulp with the appearance of large cells with a pale-staining nucleus and

Table 2. *Radioactivity of tissues at varying times after intravenous injection of* 10^5 *microfilariae labelled with* ^{51}Cr

(Results are expressed as the mean number of counts/min with the standard error in parentheses. Four animals were in each group.)

	Time after injection				
	2 h	6 h	18 h	24 h	42 h
Lungs	179000 (7000)	150000 (16000)	124000 (22000)	156000 (8000)	133000 (9000)
Heart	4200 (550)	3600 (280)	3300 (290)	3700 (310)	2500 (300)
Liver	16000 (1700)	24000 (2500)	21000 (1700)	29000 (550)	32000 (1400)
Spleen	6200 (1200)	6100 (1200)	8100 (1400)	10000 (360)	7600 (550)
Lymph nodes	N.D.	160 (20)	190 (45)	210 (28)	170 (18)
Brain	1700 (90)	1100 (75)	980 (170)	880 (40)	460 (33)
Kidney	4200 (160)	3700 (240)	3600 (350)	4500 (150)	4800 (300)
Urine*	1400 (200)	1100 (210)	1800 (270)	1400 (220)	N.D.
Blood†	130 (8)	100 (5)	100 (15)	170 (8)	70 (6)
10^5 microfilariae	290000	275000	243000	255000	N.D.

* Sample vol. = 100 μl.
† Sample vol. = 80 μl.
N.D., Not done.

cytoplasm (Pl. 2). There was some vascular congestion of the red pulp. These changes were subsiding by 32 days after injection. Microfilariae were not seen.

Mesenteric lymph nodes

No specific changes were noted. Microfilariae were not seen.

Kidneys

Moderate numbers of microfilariae were seen in the renal blood vessels and occasionally in the glomerular capillaries 1 h after injection, but only sparse organisms were found thereafter. There was no significant inflammatory reaction.

Distribution of ^{51}Cr*-labelled microfilariae*

Twenty mice were injected intravenously with 10^5 microfilariae labelled with ^{51}Cr. Groups of 4 mice were studied at varying intervals after injection. The levels of radioactivity are shown in Table 2. Similar results were found at 2, 6, 18, 24 and 42 h after injection of microfilariae. By far the greatest radioactivity was found in the lungs, which accounted for 57 % of all radioactivity injected. The organs with

Table 3. *Radioactivity/mg tissue at 2, 6, 18, 24 and 42 h after intravenous injection of 10^5 microfilariae labelled with ^{51}Cr*

(There were 4 animals in each group.)

	Counts/min/mg tissue (mean ± S.E.)
Lungs	860 ± 95
Heart	23 ± 4·6
Liver	23 ± 2·9
Spleen	42 ± 7·5
Lymph nodes	2·4 ± 0·2
Brain	2·6 ± 0·6
Kidney	36 ± 3·4

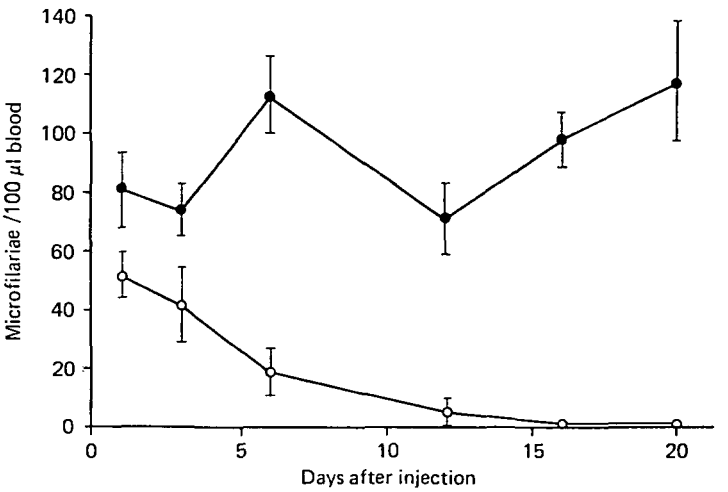

Fig. 5. The level of microfilaraemia at varying times after intravenous injection of $1{\cdot}5 \times 10^5$ microfilariae. ●——●, Previously unexposed control animals; ○——○, animals which had been sensitized with 10^5 microfilariae 5 months previously. Ten animals were in each group.

the next greatest levels of radioactivity were the liver (8·5%) and spleen (2·9%). The preferential localization of microfilariae in the lungs was confirmed when the results were expressed as counts/mg tissue (Table 3); the level of radioactivity in the lungs was 200–300 times that found in the liver, spleen and kidneys. Only small amounts of radioactivity were found in the blood and urine.

Footpad reactions

Thirty mice were injected intravenously with 10^5 microfilariae. Footpad reactions in groups of 6 mice were measured 6, 14, 30, 62 and 126 days later (Table 4). A small immediate hypersensitivity reaction, which was first seen at day 14, was marked by day 30 ($P < 0{\cdot}001$, Student's t-test) when compared with day 6. No further increase was noted at day 62 but the reaction at day 126 was significantly larger than at day 30 ($P < 0{\cdot}025$, Student's t-test). No significant 6, 24 or 48 h reactions were seen at any time.

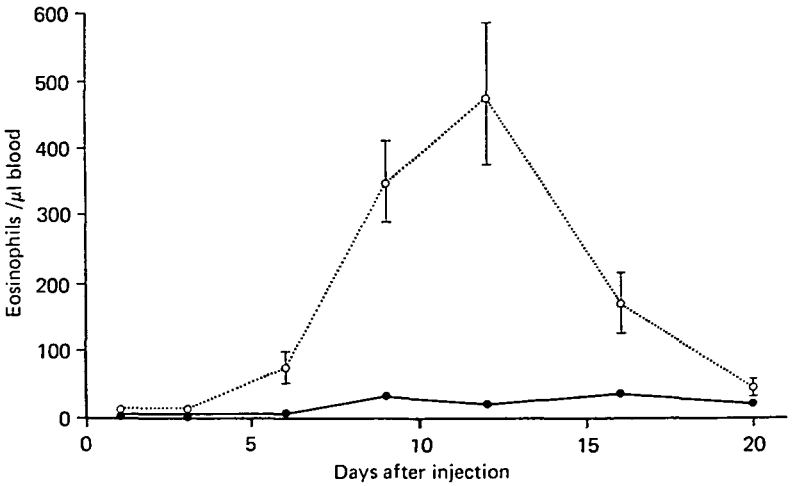

Fig. 6. Blood eosinophil levels at varying times after intravenous injection of $1{\cdot}5 \times 10^5$ microfilariae. ●——●, Previously unexposed control animals; ○ · · · · ○, animals which had been sensitized with 10^5 microfilariae 5 months previously. Ten animals were in each group.

Table 4. *Footpad reactions of mice* 15 *min and* 6, 24 *and* 48 *h after injection of microfilarial antigen at varying intervals after intravenous injection of* 10^5 *microfilariae*

(Results are expressed as the mean ± S.E. Six mice were in each group.)

Days after injection of microfilariae	Footpad thickness (mm)			
	15 min	6 h	24 h	48 h
6	−0·05 ± 0·04	0·02 ± 0·11	−0·03 ± 0·12	0·01 ± 0·05
14	0·14 ± 0·14	0·05 ± 0·09	0·06 ± 0·07	−0·05 ± 0·09
30	0·49 ± 0·05	0·05 ± 0·08	0·09 ± 0·10	0·06 ± 0·06
62	0·35 ± 0·07	0·12 ± 0·07	N.D.	0·15 ± 0·06
126	0·91 ± 0·13	0·09 ± 0·08	0·08 ± 0·07	0·06 ± 0·07

N.D., Not done.

Re-infection

Ten mice were given 10^5 microfilariae by intravenous injection. Five months later, when the microfilaraemia had subsided, these mice together with a control group of previously unexposed animals were injected intravenously with $1{\cdot}5 \times 10^5$ microfilariae. Microfilarial levels and eosinophil counts were determined at intervals for the next 20 days. A persistent microfilaraemia developed in control animals but microfilariae were eliminated from the circulation in those mice which had previously been exposed (Fig. 5); the difference was statistically significant by 6 days ($P < 0{\cdot}001$, Student's t-test) and microfilariae were not found by 2 weeks after challenge. Animals which had not been previously exposed failed to develop an eosinophilia. Mice which were sensitized, however, developed a marked eosinophilia which reached a maximum 12 days after injection ($P < 0{\cdot}001$,

Student's *t*-test) (Fig. 6). The experiment was repeated and similar results were obtained.

DISCUSSION

This study has shown that the mouse provides a model in which the factors controlling the circulation of *B. malayi* microfilariae in the bloodstream can be investigated. Microfilariae of a number of different species have been transferred from one host to another in both animals and man. These include *Dirofilaria repens* (Gruby & Delafond, 1852; Fulleborn, 1912; Hawking, 1953) and *D. immitis* (Hinman, Faust & De Bakey, 1934; Augustine & Drinker, 1935; Underwood & Harwood, 1939; Wong, 1964*a*; Pachecho, 1974) in dogs, *B. pahangi* in cats (Ponnudurai, Denham & Rogers, 1975), *Setaria labiatopapillosa* into rabbits, guinea pigs and rats (Nelson, 1962), *D. corynodes* (Pachecho & Orihel, 1968) and *Edesonfilaria malayensis* (Hawking, Worms & Walker, 1965) in monkeys, and *Loa loa* (Gonnert, 1942), *Mansonella ozzardi* (Mazzotti & Palomo, 1957) and *Wuchereria bancrofti* (Knott, 1935) in man. In most of these instances, however, large numbers of microfilariae were required as the recipients were large animals. Since the mouse is a small animal, we have been able to observe the fate of microfilariae after the injection of a relatively small number of parasites.

In contrast to the preceding studies, we transferred human filarial parasites to unnatural hosts. This was first attempted by Hawking (1940) who separated *W. bancrofti* microfilariae from human blood, concentrated them in hydrocele fluid and Locke's solution and injected them intravenously into mice. In his experiments, severe prostration and sometimes death were produced and microfilariae failed to circulate for more than 2 days. We did not have such problems, however, for the mice remained well and microfilariae were able to continue to circulate in a fashion similar to that seen after transfer to the normal host species. These differences probably represent an improved capacity to purify microfilariae. It appears, therefore, that the major difficulty with the establishment of filarial parasites in an abnormal host lies not in the inability of microfilariae to circulate, but in the failure of maturation of infective larvae into adult worms (Ahmed, 1967; Ash & Schacher, 1971).

When microfilariae were injected into mice, they continued to show a mild periodicity with the number found at midday, being approximately 30% of that seen during the night. This persistence of periodicity in the transfused host has also been observed with other microfilarial species (Hinman *et al.* 1934; Knott, 1935; Hawking, 1953; Hawking *et al.* 1965). In order to obtain comparable results, therefore, microfilaraemia levels were always measured between 10.00 and 14.00 h.

Only a small proportion of the microfilariae injected intravenously circulated in the peripheral bloodstream. The maximal proportion of 3% at night does not differ greatly from the 7% found circulating after infusion of *D. immitis* in dogs (Pachecho, 1974). Those microfilariae which did circulate, continued to do so for from 1 to 4 months. The reported survival of transfused microfilariae has varied widely; microfilariae of *Litomosoides carinii* persisted for a few days to 5 months

(Kershaw, 1949), *D. immitis* for several weeks to 2 years (Hinman *et al.* 1934; Underwood & Harwood, 1939; Wong 1964*a*), *D. repens* for 2 months to 3 years (Gruby & Delafond, 1852; Fulleborn, 1912; Hawking, 1953), *B. pahangi* for 2 to 136 days (Ponnudurai *et al* 1975), *D. perstans* for 3 years (Gonnert, 1942) and those of *M. ozzardi* for up to 2 years (Mazzotti & Palomo, 1957). In the present study, the duration of microfilaraemia was proportional to the size of the original inoculum of parasites. Such a relationship has been suggested previously by Mazzotti & Palomo (1957) who found in a small study, in which *M. ozzardi* microfilariae were injected into humans, that the microfilaraemia persisted longer in those given more microfilariae.

The microfilariae which did not circulate were not destroyed but were sequestered in the capillaries and small vessels of the viscera. When examined by histological section, the majority of microfilariae were found in the pulmonary vasculature, while moderate numbers were seen in the hepatic and renal vessels. These results are consistent with the findings of Hawking & Thurston (1951) who demonstrated that microfilariae accumulated in the small vessels of the lungs during the daytime in natural infections of monkeys and dogs with various *Dirofilaria* species. These observations were confirmed when the distribution of microfilariae was assessed by labelling organisms with radioactive chromium. When expressed on a weight basis, the radioactivity in the lungs was 200–300 times that found in the liver, spleen or kidneys. This may reflect the much larger contribution which the vasculature makes to the total pulmonary mass as compared with the other organs. Hawking (1975) has suggested that the absolute number of microfilariae in the lungs during the day is probably several times greater than that seen in the peripheral blood, and that there is a passive phase at night when microfilariae are distributed evenly throughout the blood and, therefore, are more numerous in the peripheral circulation. On the other hand, Pachecho & Orihel (1968) and Pachecho (1974) using periodic *D. corynodes* and non-periodic *D. immitis*, respectively, concluded that there are many more microfilariae in the viscera than in the peripheral circulation, and that the phenomenon of periodicity is not the result of a shift in the entire population of microfilariae from the central to the peripheral circulation. In the present model, there are at least 50 times as many microfilariae in the lungs as in the peripheral blood during the day. Nevertheless, it seems likely that the more intense microfilaraemia seen at night with subperiodic *B. malayi* is due to at least a partial transfer of parasites from the pulmonary vasculature to the peripheral vessels.

There was remarkably little host reaction to microfilariae in all the tissues studied with the exception of the spleen. Microfilariae were generally observed to be free in the small vessels and capillaries with no surrounding cellular infiltrate. Similarly, Augustine & Drinker (1935) found no evidence of a tissue cellular response after transfusion of *D. immitis* in dogs. Considerable splenic enlargement occurred in our mice; the maximal increase in weight of 2·5 times was seen 4 days after injection and was followed by a gradual subsidence over the next few weeks. Splenomegaly has also been observed in jirds infected with *B. malayi*, *B. pahangi* and *B. patei* when the infections became patent (Vincent & Ash, 1978). The

histological changes in the mouse spleen of expansion of both white and red pulp with follicular hyperplasia and vascular congestion are similar to the findings in *B. malayi*-infected gerbils (Vincent & Ash, 1978). We did not find granulomata associated with degenerating microfilariae, nor did Vincent & Ash (1978) in *B. malayi*-infected gerbils, although those authors did observe such foci in gerbils infected with *B. pahangi* and *B. patei*. It seems likely that the splenic reaction represents a response to chronic antigenic stimulation by excretory and secretory products of microfilariae.

The persistent circulation of microfilariae in the peripheral blood implies that these organisms are able to evade the host's immune system. Nevertheless, an immunological response does develop since immediate hypersensitivity reactions to microfilarial antigen were seen by 2 weeks after injection and persisted thereafter. The development of footpad reactions was not contemporaneous with elimination of parasites; microfilariae continued to circulate for up to 4 months. Whether the eventual disappearance of microfilariae represents the life-span of the parasite or the development of an effective immune response remains to be determined. There is some evidence from both experimental (Wong, 1964*b*; Ponnudurai, Denham, Nelson & Rogers, 1974) and clinical studies (Wong & Guest, 1969; Grove & Davis, 1978) that antibodies against microfilariae may play a role in controlling microfilaraemia in long-standing filarial infections. It is apparent that some form of effective immunological response does develop in mice given *B. malayi* microfilariae by intravenous injection. When animals were challenged with microfilariae after the primary infection subsided, there was an accelerated removal of microfilariae from the circulation. A similar situation has been seen in dogs after repeated intravenous injection of living *D. immitis* microfilariae (Wong, 1964*b*) and in hamsters after 2 intravenous injections of *D. viteae* microfilariae (Weiss, 1970). If further microfilariae were injected during the initial period of *D. immitis* microfilaraemia in dogs, however, there was no accelerated elimination of microfilariae (Pachecho, 1974). In most immunological systems, an effective primary immune response develops within 2 weeks of initial exposure to antigen. These filarial infections, therefore, are unusual; the reasons why the host should remain tolerant of microfilariae for a prolonged period then finally develop resistance are unclear and may be a fruitful field for further investigation.

It is of some interest that the rapid elimination of microfilariae in sensitized mice was associated with the development of a marked peripheral eosinophilia. This may represent a secondary response to dead and disintegrating worms. The possibility that eosinophils may be an integral part of the acquired resistance cannot be dismissed, however, for they have been shown to play a role in host resistance to *Schistosoma mansoni* (Mahmoud, Warren & Peters, 1975) and *Trichinella spiralis* (Grove, Mahmoud & Warren, 1977; Kazura & Grove, 1978) infections.

In conclusion, we have shown that when microfilariae of *B. malayi* are injected intravenously into mice, they continue to circulate in the peripheral blood for periods of up to 4 months. This infection resembles that seen in other experimental systems in which microfilariae have been transferred from one host to another, in

that innate subperiodicity is maintained, most of the parasites are located within the lungs, and there is no significant tissue reaction to the parasites. An immunological response as demonstrated by footpad reactions to microfilarial antigen develops, and when mice are challenged after subsidence of the initial microfilaraemia, resistance to re-infection is shown. This mouse model of microfilaraemia is cheap to maintain and easy to manipulate, requiring relatively small numbers of microfilariae. Furthermore, mice are well defined immunologically and genetically, thus facilitating study of the influence of these factors on the host–parasite relationship. This model provides, therefore, a useful system in which the various mechanisms controlling the circulation of microfilariae can be studied in isolation. Finally, if microfilaraemia can be abolished by the development of a vaccine and applied to human filarial infections, it may be possible to interrupt transmission and thus control filariasis.

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EXPLANATION OF PLATES

PLATE 1

Pulmonary small blood vessel 1 day after intravenous injection of 10^5 microfilariae. Note the large numbers of *Brugia malayi* microfilariae free in the lumen of the vessel and the absence of a parenchymal inflammatory reaction (Haematoxylin and eosin stain).

PLATE 2

Low-power view of the spleen

A. Normal spleen.

B. Spleen 4 days after intravenous injection of 10^5 microfilariae. Note the expansion of the white pulp by pale-staining cells (Haematoxylin and eosin stain).

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