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Objektyp: **Article**

Zeitschrift: **Acta Tropica**

Band (Jahr): **38 (1981)**

Heft 3

PDF erstellt am: **02.05.2024**

Persistenter Link: <https://doi.org/10.5169/seals-312829>

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Filarial infections of *Mastomys natalensis* and their relevance for experimental chemotherapy³

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Summary

Experimental filarial infections of *Mastomys natalensis*, strain GRA Giessen, with *Litomosoides carinii*, *Dipetalonema viteae*, *Brugia malayi* (subperiodic), and *Brugia pahangi* were compared. Mean prepatent periods of 52, 57, 107, and 73 days p.i. were observed after subcutaneous inoculation of 40, 50, 85, and 70 infective larvae of *L. carinii*, *D. viteae*, *B. malayi*, and *B. pahangi*, respectively, in the neck region. All of the *L. carinii*, *D. viteae*, and *B. pahangi* infected *Mastomys* showed a regularly detectable microfilaraemia. In *B. malayi* infections 95.5% of the animals developed parasitaemias, when the larvae had been inoculated in the neck region, whereas after groin infections only in 66.7% of the animals became patent. For both *Brugia* species, infections in the groin resulted in considerably lower microfilarial levels.

Maximum microfilariae densities could be detected at day 120 (*L. carinii*) and at day 190 (*D. viteae*) p.i. In the case of *Brugia* neck infections, the microfilarial levels increased usually until the end of the observation period, 300–350 days p.i. Worm recovery rates were 63% (*L. carinii*), 20.6% (*D. viteae*), 21.1% (*B. malayi*), and 31.4% (*B. pahangi*) of the inoculated larvae. When third stage larvae of *Brugia* species were inoculated in the neck region, adults of *B. malayi* and *B. pahangi* were isolated predominantly from the heart and lungs (84.4 and 78.5%, respectively). Only 12.3% of *B. pahangi* parasites were found in the testes; 3.4% and 18.1% were localized in the lymphatics. After inoculation of infective larvae in the groin more worms could be recovered in the testes and lymphatics, i.e. 23.4% and 14.9% (*B. malayi*) or 19.1% and 45.2% (*B. pahangi*), respectively.

³ This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

The results are discussed under the aspect of chemotherapeutic investigations for the evaluation of microfilaricidal, macrofilaricidal or chemoprophylactic compounds. It is concluded, that *Mastomys natalensis*, an animal with a broad spectrum of susceptibility for filarial infections, can be used as an alternative experimental model system, similar to that of the jird.

Key words: *Litomosoides carinii*; *Dipetalonema viteae*; *Brugia malayi* (subperiodic); *Brugia pahangi*; *Mastomys natalensis*; microfilariae; adult worms.

Introduction

There has been an increased international effort, largely coordinated through WHO, to find animal model systems for screening of potentially filaricidal agents. Of main interest are small animal models, especially for filarial species, which are pathogenic to man. In this connection, the successful introduction of the multimammate rat, *Mastomys natalensis*, as a laboratory animal and host for various trematodes, cestodes, and nematodes (Lämmler et al., 1968b), led to further investigations on the susceptibility of the *Mastomys* also for experimental filarial infections. At first, successful infections of *M. natalensis* with the cavity-dwelling filaria *Litomosoides carinii* were reported by Lämmler et al. (1968a), Pringle and King (1968), and Petrànyi and Mieth (1972). Later on, the tissue-dwelling filaria *Dipetalonema viteae* could be established in *Mastomys* (Holdstock, 1974), followed by further detailed studies of Sànger and Lämmler (1979), and Müller (1980). Furthermore, the susceptibility of the multimammate rat also for human pathogenic, lymphatic-dwelling filariae was described for *Brugia malayi* (subperiodic) by Petrànyi et al. (1975), and for *B. pahangi* by Ahmed (1966) and Benjamin and Soulsby (1976).

This paper summarizes observations on *L. carinii*, *D. viteae*, *B. malayi* (subperiodic), and *B. pahangi* infections in *M. natalensis* (strain GRA Giessen, Zahner et al., 1980) over a period of 300 days p.i., under the special aspect of the suitability of this animal for chemotherapeutic trials of filaricides.

Material and methods

Animals: Male and female multimammate rats *Mastomys natalensis*, strain GRA Giessen, conventionally bred at our institute were kept in groups in polycarbonate cages (PAG-Presswerk AG, Essen) and were fed on a diet developed for Syrian hamsters (Schuster et al., 1973). Drinking water was available ad libitum.

Infections

***Litomosoides carinii*:** Male and female animals were infected subcutaneously at an age of 6 weeks with single doses of 25–200 4-day-old third stage larvae (groups 1–4: Tab. 1). The larvae were collected 4 days p.i. from the pleural cavities of naturally, by *Bdellonyssus bacoti* infected jirds (*Meriones unguiculatus*) (Lämmler et al., 1968a; McCall, pers. com.).

Table 1. Results of necropsy of *Mastomys natalensis*, various times after infections with *Litomosoides carinii*, *Dipetalonema viteae*, *Brugia malayi*, and *Brugia pahangi*

Parasite	Group no.	Infection (no. of infective larvae)	No. of animals	Sex of the host	Necropsy (days after infection)	Average number of living adult worms per animal		Mean recovery rate (%)		
						Σ				
						♀♀	♂♂		\bar{x}	\pm S.D.
<i>L. carinii</i>	1	25	9	♂♂	319	5.4	8.0	13.4	4.7	53.6
	2	40	62	♀♀/♂♂	120-319*	13.3	11.9	25.2	7.2	63.0
	3	100	7	♂♂	319	31.4	28.9	60.3	17.7	60.3
	4	200	10	♂♂	184	76.7	66.3	143.0	22.2	71.5
<i>D. viteae</i>	5	50	21	♂♂	175-189	4.2	6.1	10.3	6.4	20.6
	6	50	11	♂♂	248-261	0.5	5.2	5.7	3.7	11.4
<i>B. malayi</i>	7	85	40	♂♂	153-197	9.6	8.3	17.9	6.2	21.1
	8	85	18	♂♂	335-442	5.9	6.9	12.8	4.7	15.1
	9	**	4	♂♂	224-277	24.5	9.0	33.5	4.7	**
<i>B. pahangi</i>	10	50	10	♂♂	170	7.7	3.1	10.8	5.6	21.6
	11	70	47	♀♀/♂♂***	150	12.4	9.6	22.0	9.6	31.4
	12	70	11	♂♂	300	7.0	5.3	12.3	6.7	17.6
	13	100	15	♂♂	150	12.2	11.8	24.0	11.2	24.0
	14	150	10	♂♂	170	18.8	12.3	31.1	9.7	20.7

* No significant differences could be observed between 5 groups dissected after 120, 150, 184, 255 and 319 days p.i. respectively, neither concerning the different date of dissection nor the different sex of the host.

** Infection by infected mosquitoes by natural way.

*** No significant differences could be observed between male and female animals for the recovery rate.
S. D. = standard deviation

Table 2. Differences in the localization of adult worms in *Brugia malayi* and *Brugia pahangi* infected male *Mastomys natalensis* after inoculation of the infective stage at different sites (i. e. neck or groin)

Parasite	Group no.	Infection (no. of infective larvae)	Site of inoculation	No. of animals	Necropsy (days after infection)	Average number of living adult worms per animal		Mean recovery rate (%)	Localization of adult worms		
						\bar{x}	\pm S.D.		%		
									Heart and lungs	Testes	Lymphatics
<i>B. malayi</i>	7	85	neck	40	153-197	17.9	6.2	21.1	84.4	12.3	3.3
	15	85	groin	12	170-176	9.4	5.4	11.1	61.7	23.4	14.9
<i>B. pahangi</i>	16	70	neck	33	150	23.2	10.4	33.1	78.5	3.4	18.1
	17	70	groin	10	150	15.7	5.2	22.4	35.7	19.1	45.2

S. D. = standard deviation

Dipetalonema viteae: Male *M. natalensis*, 4 to 6 weeks old, were infected subcutaneously with 50 third stage larvae (groups 5 and 6: Tab. 1), isolated from *Ornithodoros moubata* 30 days p.i. (Sänger and Lämmler, 1979).

*Brugia malayi*⁴ (subperiodic): Male animals were infected at an age of 6 to 8 weeks with 85 third stage larvae subcutaneously either in the neck (groups 7 and 8: Tab. 1) or in the groin (group 15: Tab. 2). The larvae were collected from *Aedes togoi* at day 11 after ingestion of infective blood by crushing the ether stunned mosquitoes and liberating the larvae in Tyrode solution. The larvae were concentrated with the Baermann funnel (Ash and Riley, 1970). For single natural infections (group 9: Tab. 1) the animals were anaesthetized and placed for 2 h in a cage with infected mosquitoes (100 ♀♀/animal). These mosquitoes had been fed on *B. malayi* infected *Mastomys* 11 days before.

*Brugia pahangi*⁵: Male and female *M. natalensis*, 6 to 8 weeks old, were infected with 50–150 third stage larvae (groups 10–14, 16 and 17: Tab. 1 and 2), isolated from *Aedes aegypti* 11 days after an infective blood meal. The procedures of collection and inoculation of the larvae were the same as for *B. malayi*.

Counting of microfilariae: Blood was taken from the retroorbital venous plexus between 8 and 10 a.m. at various times after infection. The density of microfilariae per mm³ blood was determined in *L. carinii* and *D. viteae* infected animals using the chamber of Fuchs-Rosenthal and Jessen, respectively (Raether and Meyerhofer, 1967). The microfilariae of *Brugia* were counted in the Nageotte chamber and evaluated per 20 mm³ blood. The geometric means (x_G) of the data were calculated for all infection groups.

Dissection of the animals: The animals were stunned and decapitated. Adult worms were recovered from the pleural cavities (*L. carinii*) or from the connective tissues (*D. viteae*). In case of *Brugia*, heart, lungs and testes as well as lymph nodes and superficial lymphatic vessels (= lymphatics) were removed and placed in saline for 24 h. Afterwards, the emigrated worms were isolated, and heart, lungs and testes were examined for further living worms by direct observation with a stereoscopic microscope during dissection in saline. Squash preparation of the lymphatics were made between glass slides, and examined at higher magnification to detect remained parasites.

The results for adult worms are expressed as the arithmetical mean of the individual data for each group.

Results

Litomosoides carinii infection

Microfilarial levels: The prepatent period of 88 *M. natalensis* (45 males, 43 females), infected with 25–200 larvae, lasted from 48 to 56 days. The course of microfilaraemia in 43 females is demonstrated in Fig. 1: The low level of 2.27 microfilariae/mm³ at day 53 p.i. increased considerably to a maximum of 6.80 microfilariae/mm³ after 120 days of infection. Thereafter, microfilarial counts decreased gradually to 3.30 microfilariae/mm³ at the end of the observation period 312 days p.i. The infection of 28 males with 25 or 40 larvae revealed maximum microfilarial counts of 6.20 and 6.35 microfilariae/mm³ after 95 days p.i., whereas in males inoculated with 100 or 200 larvae a maximum of 6.74 and 6.84 microfilariae/mm³ have occurred 120 days p.i., respectively.

⁴ The supply of the *B. malayi* strain in 1976 by Dr. G. Petrányi, Sandoz Forschungsinstitut, Vienna, is grateful acknowledged.

⁵ The supply of the *B. pahangi* strain by Dr. P. Kimmig, Institute for Parasitology, University Bonn, is gratefully acknowledged.

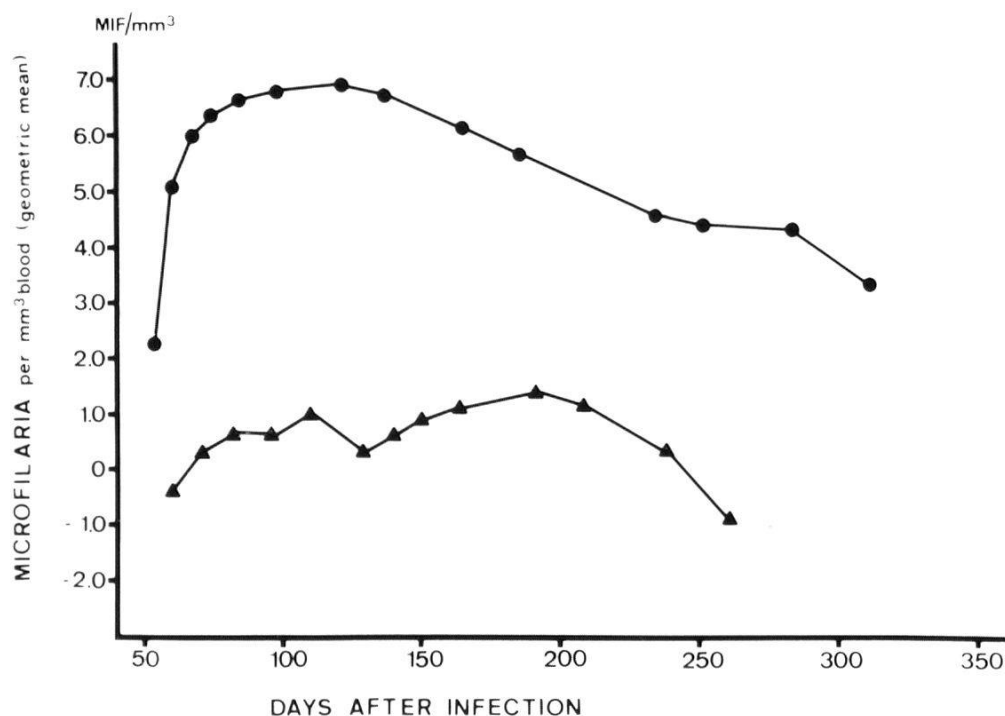


Fig. 1. Course of microfilaraemia in *Litomosoides carinii* and *Dipetalonema viteae* infections of *Mastomys natalensis* (geometric mean/mm³ blood). ● — ● = *L. carinii* (n = 43 ♀♀) 40 infective larvae s.c.; ▲ — ▲ = *D. viteae* (n = 32 ♂♂) 50 infective larvae s.c.

Adult worms: The recovery rates of living adult worms, after different infection doses, ranged from 53.6 to 71.5% of the inoculated larvae (groups 1–4: Tab. 1). No significant differences could be observed with regard to the different dates of dissection, the number of inoculated larvae, and the sex of the hosts.

Dipetalonema viteae infection

Microfilarial levels: 32 male animals, infected with 50 third stage larvae, developed only moderate microfilarial levels (Fig. 1) after a mean prepatent period of 57 days p.i. At day 60 p.i. – 0.39 microfilariae/mm³ were determined and during the patency the microfilarial counts did not exceed to more than 1.4 microfilariae/mm³. 2 of 6 animals, observed for 261 days p.i., still showed microfilariae in the peripheral blood.

Adult worms: In 21 animals, dissected 175–189 days after infection, the recovery rate of adult worms was 20.6% of the inoculated larvae (group 5: Tab. 1). In 11 *Mastomys* (group 6: Tab. 1) followed for 248–261 days p.i. worm recovery was 11.4%, thereof only 1% living females. Finally, later than 300 days p.i. no more living female worms could be found.

Brugia malayi (subperiodic) infection

Microfilarial levels: 33 of 346 male *Mastomys*, infected with 85 larvae in the neck failed to develop microfilaraemia. In 95.5% of the remaining 313 animals a constantly detectable microfilaraemia could be observed after a mean pre-

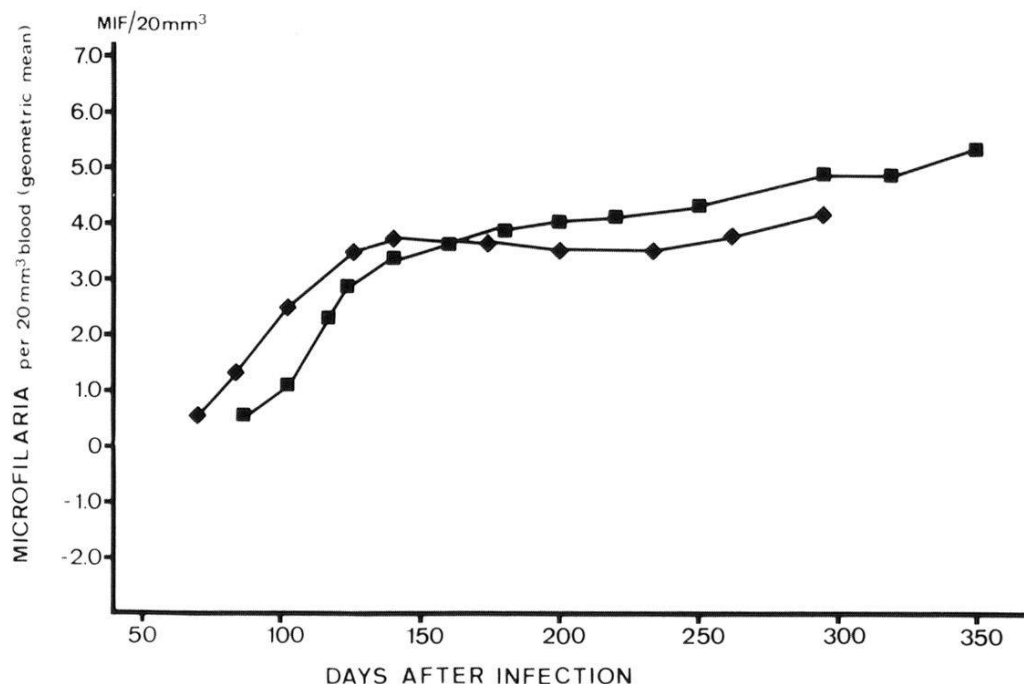


Fig. 2. Course of microfilaraemia in *Brugia malayi* and *Brugia pahangi* infections of *Mastomys natalensis* (geometric mean/20 mm³ blood). ■ — ■ = *B. malayi* (n = 58 ♂♂) 85 infective larvae s.c. neck; ▲ — ▲ = *B. pahangi* (n = 33 ♂♂) 70 infective larvae s.c. neck.

patent period of 107 days. 4.5% of the animals showed only a intermittently detectable microfilaraemia, and the prepatency ranged from 130–211 days. After the inoculation of 85 infective larvae in the groin, 8 animals showed regularly detectable microfilariae in the circulating blood after a mean prepatent period of 116 days. 4 animals developed only an irregularly microfilaraemia after 87–170 days. One animal remained negative. In the 4 naturally infected *Mastomys*, a regularly detectable microfilaraemia occurred after 111 days.

The course of microfilaraemia in neck infected animals is shown in Fig. 2: The microfilarial level increased more or less continuously until the end of the observation period, in animals with regularly detectable microfilaraemia. Finally, the parasitaemia persisted at a density of 5.07–5.22 microfilariae/20 mm³. On the contrary, the microfilaraemia was markedly reduced in the groin infected groups, and the microfilarial counts did not exceed a number of 3.62 microfilariae/20 mm³ blood.

Adult worms: In neck infected animals (groups 7 and 8: Tab. 1), 21.1% and 15.1% of the inoculated larvae were recovered as adults 153–197 and 335–442 days p.i., respectively. In 12 groin infected animals 11.1% of the larvae were found as living adult parasites (group 15: Tab. 2). In neck infected *Mastomys* (group 7: Tab. 2) the majority of the recovered worms with 84.4% were localized in heart and lungs, and only 12.3% and 3.3% in testes and lymphatics, respectively, whereas after the infection in the groin (group 15: Tab. 2) 23.4% were found in the testes and 14.9 in the lymphatics. A similar distribution of adult worms could be noticed in naturally infected animals, but surprising much higher number of female worms.

Brugia pahangi infection

Microfilarial levels: The prepatent period of 79 male *M. natalensis* lasted from 72 to 74 days after inoculation of 50–150 third stage larvae in the region of the neck. In 10 groin infected animals microfilariae could not be detected earlier than 85 days p.i. There was also a delay in the occurrence of microfilariae in the peripheral blood in 14 neck infected females. In this case, the prepatency was 90 days.

In 33 male *Mastomys* given 70 infective larvae, 0.58 microfilariae/20 mm³ were observed at day 74 p.i. (Fig. 2). The number of microfilariae increased to 3.77 microfilariae/20 mm³ 170 days p.i. Thereafter counts levelled off, but beginning from 240 days p.i. until the end of the observation period a further increase of microfilarial density in the peripheral blood was observed, up to a level of 4.26 microfilariae/20 mm³. On the contrary, and similar to the situation already described for the *B. malayi* infection, only moderate levels of microfilariae could be determined in the peripheral blood of groin infected animals. In this group maximum microfilarial densities occurred at 150 days p.i. with 3.22 microfilariae/20 mm³. Also, in male animals, given 50, or in females, given 70 infective larvae, generally the numbers of microfilariae remained low with less than 2.84 microfilariae/20 mm³ during the whole observation period. The infection with 100 or 150 third stage larvae in the neck region did not result in higher microfilarial levels than after inoculation of 70 larvae at the same region.

Adult worms: The recovery rates of adult worms in 93 *Mastomys* (79 males, 14 females), dissected 150, 170 and 300 days, after infection doses of 50–150 infective larvae in the neck region are listed in Table 1. No significant differences were observed in the recovery rates for the different infection doses at day 150 or 170 after infection. 17.6% of the inoculated larvae could be detected as living adult worms in group 12, dissected 300 days p.i. Also, no dose dependent differences were observed in 79 neck infected male *Mastomys* with regard to the distribution of the worms. Most adult parasites were detected in the heart and lungs, where 68.6% of the worms were recovered in all infected groups. In testes or lymphatics only 3.5% and 27.9%, respectively, of the living adult worms occurred. There was also no change with increasing time after infection. In the 14 neck infected females 99.5% of the recovered adult worms were found in heart and lungs and 0.5% in the lymphatics of the host. However, the localization of the parasites in the host depended on the site, where the infection was performed (groups 16 and 17: Tab. 2). Opposite to group 16, in the groin infected animals 19.1% and 45.2% of the adult worms were found in the testes and lymphatics, respectively.

Discussion

Investigations on the susceptibility of the multimammate rat, *Mastomys natalensis*, have shown that *L. carinii*, *D. viteae*, *B. malayi* (subperiodic), and *B. pahangi* can be established in this host.

Concerning *L. carinii*, all animals infected with 4-day-old pleural cavity derived third stage larvae, developed a consistently detectable microfilaraemia after a mean prepatent period of 52 days. The recovery rates of living adult parasites were 53.6–71.5% of the given larvae for different infection doses.

Higher recovery rates of 75.8–96.8% were determined by Petrànyi and Mieth (1972), after intrapleural implantation of 40 third stage larvae, isolated 7 days after infections of donors, into *Mastomys*. These differences may be explained with the different age of the larvae and the different routes of administration. The time course of parasitaemia observed after infection with 4-day-old larvae accorded to natural infections described by Zahner et al. (1974). Altogether the host parasite relationship does not differ markedly from that of the cotton rat as a natural host (Pringle and King, 1968).

The results of the *D. viteae* infection of *M. natalensis*, presented in this paper, are quoted from an earlier publication (Sänger and Lämmle, 1979). In *D. viteae* infections of male *Mastomys*, only a moderate microfilaraemia developed after a mean prepatent period of 57 days. Parasitaemia lasted for 300 days, similar to observations of Holdstock (1974). Decreasing numbers of worms were observed at necropsies performed with increasing times after infection. Thus, 20.6% and 11.4% of the inoculated larvae were recovered as living adult worms 175–189 and 248–261 days p.i. In agreement with our findings, Thomas (1979) described an infection rate of 21.3% at 121 days p.i.

A similar course of infection was reported by Johnson et al. (1974) in *D. viteae* infected jirds (*Meriones unguiculatus*). In case of *Meriones libycus*, one of the natural hosts of the parasite, the prepatent period lasted from 46–59 days, similarly to the *Mastomys*. However, definitively higher levels of microfilaraemia developed, and living adult worms could be recovered until 16 months p.i. (Weiss, 1970).

With regard to *Brugia* infections, the *Mastomys* proved to be also very susceptible. The time course of microfilaraemia as well as the recovery rates depended on the site of inoculation of the infective larvae, i. e. neck or groin. In *B. malayi* prepatency lasted 107 days and 116 days in neck infected and groin infected animals, respectively. For *B. pahangi*, under same conditions, microfilariae appeared in the blood after 73 and 85 days, respectively. In general, groin infected animals showed lower levels of parasitaemia. More adult parasites were recovered in neck infected animals compared with the groin infected groups, and only a slight decrease in the worm recovery could be observed with increasing time after neck infection. Furthermore, also the distribution of *Brugia* adults depended on the site, where the larvae had been inoculated. Infections in the neck region resulted in high worm burdens in the heart and lungs, whereas after groin infection the number of worms recovered from the testes and lymphatics was increased.

The results observed in *B. malayi* (-groin) infected animals, in principle confirmed earlier studies of Petrànyi et al. (1975) in the WSA-strain of *Masto-*

mys, but Petrànyi found more living adult worms in the lymphatics of the host. At present, it cannot be determined whether the difference depends on the host strain or other factors. With regard to *B. pahangi* infection of *Mastomys*. Ahmed (1966) recovered adult worms from heart and testes but not from the lymphatics. (Benjamin and Soulsby, 1976 did not specify the localization of the recovered worms.) The results obtained in the present investigations for *Brugia* species after infection in the groin of *M. natalensis* are largely in accordance with observations obtained in *Meriones unguiculatus* (Ash, 1973). However, it seems that higher microfilarial levels occur in the jird. In experimental infections of natural hosts, i.e. monkeys, cats and dogs, usually performed in the hind leg, adult worms were only reported in the lymphatics of the animals (Edeson and Wharton, 1958; El Bihary and Ewert, 1971; Denham et al., 1972). However, there is no indication in the cited papers whether the lungs were investigated or not (independent from difficulties to detect worms in the lungs of larger animals).

Under the aspect of screening filaricidal compounds, three main fields must be discussed: microfilaricidal and macrofilaricidal activities as well as trials on the chemoprophylactic efficacies. Screening for probably microfilaricidal drugs, *L. carinii* infected animals represent a suitable model. The high microfilarial counts in the circulating blood allowed sensitive testing. In the case of *D. viteae* two problems had to be solved: firstly, the low levels of microfilaraemia during patency are not suitable for quantitative evaluation of microfilaricidal properties and secondly, the high mortality of the animals, when a microfilaricidal treatment is performed later than 70 days p.i. (Müller, 1980). The low levels of microfilaraemia in *D. viteae* infections can be elevated to six times higher levels, 30 min after a single subcutaneous injection of 10 mg dexamethasone or 5 mg histamine/kg body weight (Sänger and Lämmle, 1979; Müller, 1980). This can be done repeatedly and without noticeable influence on the efficiency of chemotherapy. Treatment dependent incidents can be prevented by using animals earlier than 70 days p.i. For *Brugia* infections of *Mastomys* the slow increase of microfilaraemia after a long prepatent period, especially observed in groin infected animals, may be regarded as a disadvantage. However, these problems may be insignificant in comparison to the fact, that human pathogenic species are used.

Screening of macrofilaricidal drugs should be done preferably in quantitative infection. This can be easily performed in all filarial species mentioned above. The *L. carinii* infection is an optimal model system for testing microfilaricidal compounds. This filarial infection has the advantage, beside high worm recoveries and the long lifespan of the worms, that the location of adult worms is restricted to the pleural cavity of the host. *D. viteae* is disadvantaged due to the more difficult and time consuming search for adult worms in the connective tissues and skeletal muscles of the host. Also, the shorter lifespan of the female parasites may limit the screening with slow operating agents. The

most time is needed for the dissection of *Brugia* infected animals; it also requires experience and carefulness. But again, the relevance of the species must be regarded.

Concerning chemoprophylactic studies, *L. carinii* infection might represent the most suitable model system compared to the others. Worms can be easily found, even when they are retarded in size, and non effective drugs could be discovered by blood examinations for microfilariae.

Altogether, the *Mastomys natalensis*, an animal easy to breed and to handle, represents a suitable laboratory animal, with a broad spectrum of susceptibility for human and non human pathogenic filariae. The *Mastomys* can be used as an alternative experimental filarial model system to the jird (*Meriones unguiculatus*), and promises an effective screening of filaricidal drugs. This efficacy of *Mastomys* models are proven in different experimental investigations (Lämmle et al., 1971; Lämmle and Herzog, 1974; Lämmle et al., 1975; Lämmle and Wolf, 1977; Lämmle, 1977; Lämmle et al., 1978; Zahner et al., 1978; Hartmann, 1979; Sängler et al., 1980).

Acknowledgments. I am grateful for the technical assistance provided throughout this study by Mrs. Brigitte Hofmann and Mrs. Karin Schultheiss. Appreciation is due to Dr. H. Zahner for suggestions in preparation of the manuscript.

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