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The effect of p-aminobenzoic acid and folic acid on the development of infective larvae of *Brugia malayi* in *Aedes aegypti*

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Summary

Adult *Aedes aegypti* mosquitoes, infected with the subperiodic *Brugia malayi*, were found to enhance the development of the filarial parasites to the infective stage when they were exposed to a cotton pad soaked in 10% sucrose solution containing p-aminobenzoic acid (PABA) in 0.001, 0.005, 0.01, 0.05 and 0.1% concentrations. Similarly, larval development increased when the mosquitoes were fed with folic acid at 0.001, 0.01 and 0.1% concentrations. This stimulation was more when PABA or folic acid was given prior to the infected blood meal through the developmental period of the larvae. The data thus suggest that PABA and folic acid are nutrients for the development of *B. malayi*-microfilariae to the infective stage in *A. aegypti*.

Key words: *Brugia malayi; Aedes aegypti;* susceptibility; nutrition; p-aminobenzoic acid (PABA); folic acid; *Mastomys natalensis;* microfilariae; infective larvae (L_3).

Introduction

The elegant studies of Macdonald (1975) demonstrated the genetic basis of susceptibility of *Aedes aegypti* to filarial infection. However, knowledge as to the nutritional factors required for development of microfilariae to the infective stage in the mosquito is fragmentary. Studies reported in the present communication reveal that p-aminobenzoic acid (PABA) is a nutrient for the development of *Brugia malayi* microfilariae to the infective stage in *Aedes aegypti*. PABA may act as a component of folic acid since the latter was equally stimulatory in the development of the larvae in the mosquito.

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Materials and Methods

Animals. – *Mastomys natalensis* 'GRA Giessen' strain, is bred and reared at the vivarium of the Research Centre under standard environmental conditions. 30–45-day-old animals were infected by subcutaneous injection of 80–100 third stage larvae (L_3) per animal of diurnally subperiodic strain of *B. malayi*. Methods for maintenance and monitoring of the infection were as described by Sänger et al. (1981).

Mosquitoes, infection and feeding schedules

The Liverpool black eye strain of *A. aegypti* used in these studies was maintained at 25° C and 70% relative humidity. The mosquitoes were sexed at the pupal stage and groups of 60 female pupae were placed in plastic dishes and allowed to emerge in nylon-net cages. Six-day-old female mosquitoes were used for feeding experiments. The mosquitoes were starved for 24 h prior to receiving a blood meal. The rat with the filarial infection was anaesthetized by an i.p. injection of pentothal, 60 mg/kg and kept in the cage for mosquito feeding.

The microfilarial counts of the tail blood before and after feeding were taken. After the blood meal, the unfed mosquitoes, if any, were removed from the cages.

The mosquitoes were maintained on a cotton pad soaked in 10% sucrose containing PABA or folic acid concentrations as specified below. Controls were maintained on pads soaked in 10% sucrose alone. Fourteen days after the blood meal, the mosquitoes were dissected in tyrode solution and the infective larvae released were counted with the aid of a dissecting microscope.

PABA in concentrations of 0.001, 0.005, 0.01, 0.05, 0.1% and folic acid in concentration of 0.001, 0.01, 0.1% were used in the feeding experiments. 60 mosquitoes were taken in each experiment which was done in duplicate. PABA and folic acid were fed to the mosquitoes daily by one of the three regimens. In the first regimen, the compounds were given from emergence of adult mosquitoes until the 14th day after the infected blood meal. In the second regimen, the compounds were given only from emergence till 24 h before the blood meal. In the third regimen, the mosquitoes were fed with the compounds soon after their infected blood meal till the 14th day. At the end of the experiment, the number of L_3 developed in 50 mosquitoes of each study was determined and the data were analyzed by Students' t test for significance.

Results

I. The effect of PABA feeding on the development of B. malayi-L_3 in Aedes aegypti

a) Effect of continuous feeding from emergence till 14th day after infected meal: The results on the development of L_3 in *A. aegypti* under continuous feeding with PABA from emergence to the end of the experiment are given in Table 1. An increase in the infective larval counts was observed in both the groups when the insects were fed with PABA concentrations from 0.001 to 0.1%. It was, however, statistically significant in groups receiving 0.05 and 0.1% concentrations with almost 2-fold increase in the number of the larvae. The increase in one of the groups fed with 0.005 and 0.01% PABA was also significant compared to controls.

b) Effect of feeding PABA from emergence to before infection: The second phase of the experiment was to evaluate the influence of PABA when present in the diet of the mosquitoes from emergence to their taking an infected blood meal. From the data presented in Table 2, it is seen that although there was a marginal increase in the L_3 as compared to the controls, the results were not statistically significant.

Table 1. Effect of PABA on the development of infective larvae (L_3) of *B. malayi* in *Aedes aegypti:* continuous feeding from emergence till 14th day after infection

PABA concentration (%)	Group number	Larvae mean ±S.E./ mosquito	P
0.001	I	4.00 ± 0.55	not significant
	II	2.92 ± 0.39	not significant
0.005	I	4.80 ± 0.60	<0.05
	II	3.24 ± 0.26	not significant
0.01	I	5.80 ± 0.57	<0.001
	II	3.75 ± 0.45	not significant
0.05	I	6.20 ± 1.00	<0.001
	II	5.04 ± 0.57	<0.01
0.1	I	6.50 ± 0.93	<0.001
	II	4.86 ± 0.47	<0.01
Control	I	2.72 ± 0.34	
	II	3.07 ± 0.27	

The animals used for transmission of infection had 60–65 microfilariae per 10 μl of peripheral blood.

Table 2. Effect of PABA on the development of infective larvae (L_3) of *B. malayi* in *Aedes aegypti:* feeding before infection

PABA concentration (%)	Group number	Larvae mean ±S.E./ mosquito	P
0.001	I	3.00 ± 0.33	not significant
	II	3.48 ± 0.25	not significant
0.005	I	2.95 ± 0.35	not significant
	II	3.91 ± 0.38	not significant
0.01	I	3.38 ± 0.26	not significant
	II	5.41 ± 0.61	not significant
0.05	I	3.64 ± 0.37	not significant
	II	5.61 ± 0.52	not significant
0.1	I	3.00 ± 0.32	not significant
	II	5.17 ± 0.42	not significant
Control	I	2.72 ± 0.34	
	II	4.23 ± 0.43	

The animals used for transmission of infection to group I had 45–50 and group II had 60–65 microfilariae per 10 μl of peripheral blood.

Table 3. Effect of PABA on the development of infective larvae (L_3) of *B. malayi* in *Aedes aegypti:* feeding after infection

PABA concentration (%)	Group number	Larvae mean ± S.E./ mosquito	P
0.001	I	7.78 ± 0.76	<0.001
	II	5.64 ± 0.58	<0.001
0.005	I	6.14 ± 0.65	<0.001
	II	4.30 ± 0.32	not significant
0.01	I	4.44 ± 0.41	not significant
	II	4.14 ± 0.26	not significant
0.05	I	3.56 ± 0.33	not significant
	II	4.00 ± 0.40	not significant
0.1	I	3.51 ± 0.33	not significant
	II	4.02 ± 0.36	not significant
Control	I	3.70 ± 0.39	
	II	3.31 ± 0.36	

The animals used for transmission of infection had 65–70 microfilariae per 10 μl of peripheral blood.

c) Effect of feeding PABA soon after infection till the 14th day: Six-day-old female mosquitoes were infected at the same time in a single cage and later they were separated into different groups and were given various concentrations of PABA. Under these conditions, PABA at low concentrations (0.001% and one group of 0.005%) produced nearly two-fold increase in the mean number of L_3 and as the concentration of PABA increased there was no change in the yield of L_3 compared to controls (Table 3).

II. The effect of folic acid on the susceptibility of A. aegypti to B. malayi infection

a) Effect of continuous feeding of folic acid from emergence of the mosquitoes to complete development of the larvae: Folic acid administered in the diet in 0.001, 0.01 and 0.1% concentrations were found to enhance the infection rate similar to that seen with PABA (Table 4). Folic acid at 0.001 and 0.01% concentration had the most significant effect ($P<0.001$) on the L_3 development.

b) Effect of feeding folic acid from emergence till infection: The results of this experiment are shown in Table 5. Although there was an increase in L_3 in the experimental group the data was not statistically significant.

c) Effect of feeding folic acid after infection till the development of larvae: The data presented in Table 5 suggest that under these conditions, increase in L_3 counts was registered in groups receiving folic acid at 0.001 and 0.01% concentrations. Thus, folic acid enhances L_3 development in the mosquitoes when fed at low concentrations.

Table 4. Effect of folic acid on the development of infective larvae (L_3) of *B. malayi* in *Aedes aegypti:* continuous feeding from emergence till 14th day after infection

Folic acid concentration (%)	Group number	Larvae mean ± S.E./ mosquito	P
0.001	I	4.72 ± 0.33	<0.001
	II	5.82 ± 0.56	<0.001
0.01	I	5.29 ± 0.53	<0.001
	II	6.65 ± 0.86	<0.001
0.1	I	3.60 ± 0.24	not significant
	II	4.28 ± 0.32	<0.01
Control	I	3.07 ± 0.30	
	II	3.00 ± 0.29	

The animals used for transmission of infection to group I had 50–55 and to group II 70–75 microfilariae per 10 μl of peripheral blood.

Table 5. Effect of folic acid on development of infective larvae (L_3) of *B. malayi* in *Aedes aegypti:* feeding before (A) and after (B) infection

Folic acid concentration (%)	Feeding before infection		Feeding after infection	
	Larvae mean ± S.E./ mosquito	P	Larvae mean ± S.E./ mosquito	P
0.001	3.24 ± 0.29	not significant	4.31 ± 0.39	<0.02
0.01	3.45 ± 0.26	not significant	4.08 ± 0.26	<0.02
0.1	4.13 ± 0.64	not significant	3.15 ± 0.24	not significant
Control	3.00 ± 0.30		3.05 ± 0.32	

The experiments were done with 50 mosquitoes at each folic acid concentration. – The animals used for transmission of infection had 50–55 microfilariae/10 μl for mosquitoes fed before infection and 65–70 microfilariae/10 μl of peripheral blood for mosquitoes fed after infection.

The L_3 collected from these experiments were infective to rodents. When these were injected subcutaneously into 3–4-week-old male *M. natalensis*, the animals developed the infection after 110–120 days as seen from the presence of microfilariae in the peripheral blood.

Discussion

The nutritional factors responsible for optimal development of filarial larvae in appropriate species of mosquitoes are largely unknown. The data of

this investigation reveal that in general, both PABA and folic acid were most effective when given during the development of the subperiodic *B. malayi* larvae in *A. aegypti* suggesting a requirement of the nutrients for the growth of microfilariae. PABA being a component of folic acid may be acting after conversion to the latter in the mosquito although direct experiments have not been done to substantiate this view.

It is seen from the data that only low amounts of PABA or folic acid had a significant stimulation in L_3 development when the mosquitoes were fed after the infection while at all levels there was an enhancing effect when the feeding was given before and after infection. While the precise reasons for this observation are not understood, the mosquito might have adapted itself for better utilisation of the nutrients for larval development and for other purposes when it received the compounds right after emergence. Terzian et al. (1952) noted that maximal effects of drugs or metabolites on the dynamic host-parasite equilibrium are seen at optimal range of concentrations of these compounds beyond which they may be depressed or eliminated.

The importance of folic acid to the filarial parasite was evident by the observation of Jaffe et al. (1977), that increased amount or turnover of dihydrofolate reductase occurs in the *A. aegypti* in response to infection with *B. pahangi.* Indeed, sulfisoxazole, and inhibitor of folate metabolism adversely affected (Jaffe et al., 1978) the development of L_3 of *B. pahangi* in the mosquito. The sulfonamide possibly inhibits the synthesis of dihydrofolates which was shown to occur from PABA, glutamic acid and a pteridine precursor in *A. aegypti* (Venters, 1972).

There are several reports to suggest that PABA is required for growth of malaria parasites. Terzian et al. (1952) discovered the relationship between PABA and susceptibility of *A. aegypti* to *P. gallinaceum.* More recently Noblet and Weathersby (1973) have shown that PABA increases the number of *P. gallinaceum* oocysts in *A. aegypti.* The influence of PABA on the transmission of *P. yoelii* and *P. berghei* by *Anopheles stephensi* was demonstrated by Peters and Ramkaran (1980). Hawking (1963) indicated that plasmodia can synthesise folic acid since PABA serves as a growth factor.

The plant parasitic nematode, *Aphelenchoides rutgersi* was reported to utilize folinic acid in vitro as a growth factor (Thirugnanam and Myers, 1974). Although the filarial parasites actively metabolize folates (Barrett, 1983), the mode of uptake of folic acid by parasites is not fully understood. Chen and Howells (1981) found no apparent utilization of either folic acid or PABA in vitro by L_3 of *B. pahangi.* However, once ingested by the insect, folic acid seems to play a vital role in the development of the filarial larva.

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