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Effects of a diet deficient in the B complex vitamins on infectivity, growth and distribution of *Echinostoma caproni* in ICR mice

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Abstract

The effects of a diet deficient in the B vitamins on infectivity, growth, and distribution of *Echinostoma caproni* in ICR mice were studied. The vitamin-deficient diet (experimental) was isocaloric to the control diet but lacked the B vitamins. Thirty-six female, 6- to 8-week-old ICR mice were each infected with 25 metacercarial cysts. From the day of infection to the day of necropsy, 18 mice were fed the experimental diet and the remaining mice received the control diet. Equal numbers of experimental and control mice were necropsied at 2, 3 and 4 weeks postinfection (p.i.). Mice on the experimental diet showed a significant loss in body weight between 2 and 4 weeks p.i. There was no significant difference in worm recovery at 2 to 4 weeks p.i. from mice on either diet. Worms from hosts on the experimental diet were more dispersed and located more posteriad in the small intestine than those from mice on the control diet. Worm dry weight was significantly less in hosts on the experimental diet at all weeks p.i. compared with that of hosts on the control diet. The body area of worms on the experimental diet was significantly less at 2 and 3 weeks p.i. than that of worms on the control diet. An isocaloric diet deficient in the B vitamins had a detrimental effect on the growth of *E. caproni* in ICR mice.

Introduction

Relatively little research has been done on the effects of vitamin B-deficient host diets on helminths, and no study has examined this effect on gastrointestinal trematodes. Beg *et al.* (1995) demonstrated that fewer and smaller *Litomosoides carinii* (Nematoda) were recovered from vitamin B_6 (pyroxidine) deficient cotton rats. Experiments with vitamin-deficient diets on the cestode *Hymenolepis diminuta* grown in rats showed variable results. Platzer & Roberts (1970) showed that *H. diminuta* in vitamin B_2 (riboflavin) deficient rats had a greater length than worms recovered from hosts on isocaloric diets with this vitamin; they also demonstrated that the absence of choline and pantothenate had no effect on the growth of *H. diminuta*. In contrast, Platzer & Roberts (1969) showed that few *H. diminuta* established in vitamin B_6 (pyroxidine) deficient rats when host coprophagy was prevented (coprophagy may supplement the host diet with B vitamins), and worms that established were stunted and contained fewer and less differentiated proglottids than cestodes grown in hosts on normal diets. Some of these effects were also noted in the presence of coprophagy (Plazter & Roberts, 1969). Because of the absence of studies on gastrointestinal trematodes grown in hosts deficient in the B vitamins, this study was done on *Echinostoma caproni* in ICR mice. This model has been used for numerous studies on host–parasite relationships of a gastrointestinal trematode in a vertebrate host (see review in Fried & Huffman, 1996). The purpose of this study was to report our observations on infectivity, growth, development and distribution of *E. caproni* in ICR mice deprived of the B complex vitamins.

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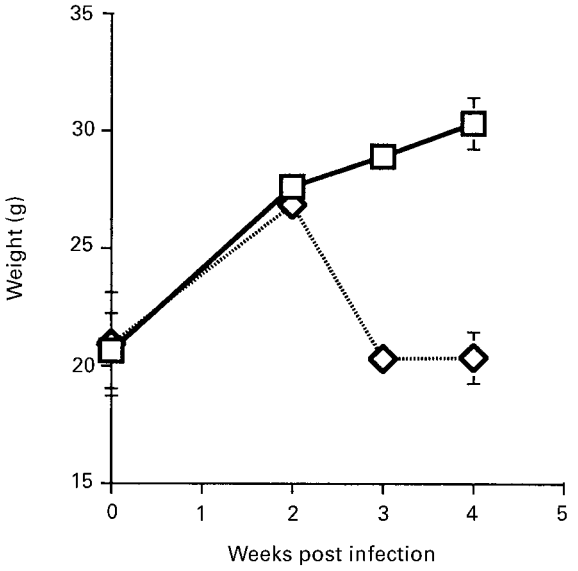

Fig. 1. Mean weights of mice ± SE on B vitamin free diet (◇) versus control diet (□) at 0–4 weeks postinfection. Where SE bars are not shown, they are smaller than the symbol that denotes the mean.

Materials and methods

Metacercarial cysts of *Echinostoma caproni* were removed from the kidney/pericardium region of experimentally infected *Biomphalaria glabrata* snails and fed by stomach tube (25 cysts per mouse) to 36 female, 6- to 8-week-old ICR mice obtained from ACE Mouse, Inc. (Boyerstown, Pennsylvania). After weighing in groups of six, half of the mice were maintained on an experimental diet containing 20% protein, 40% carbohydrate, 7% fat, but without the B complex vitamins (pelleted diet no. 116506, Dyets Inc., Bethlehem, Pennsylvania); the other half were fed an isocaloric diet (controls) containing 22.5% protein, 32% carbohydrate, 11% fat and the minimum daily requirements of the B complex vitamins (PROLAB Animal Diet, PMI® Feeds, Inc., St Louis, Missouri). Mice were weighed and necropsied at 2, 3 and 4 weeks postinfection (p.i.). At necropsy, the small intestine was removed from the pylorus to the ileo-caecal valve and divided into five equal sections, beginning with the pylorus. Worms were removed from the intestine and the location and number of worms in each section were recorded. Some worms were weighed in groups of 4–10 at 10 and 14 days p.i. and dried at 110°C for 24 h. Dry weights were calculated on a per worm basis. Additional worms recovered at 7, 10 and 14 days p.i. were fixed in hot (80–90°C) alcohol–formalin–acetic acid, stained in Gower's carmine, dehydrated in ethanol, cleared in xylene and mounted in Permount. Body and organ measurements in mm were made with a calibrated ocular micrometer on each of ten randomly selected worms from hosts on both diets at 7, 10 and 14 days p.i., and worm body area was calculated as described in Sudati *et al.* (1997). Student's *t*-test ($P < 0.05$ being considered significant) was used to compare

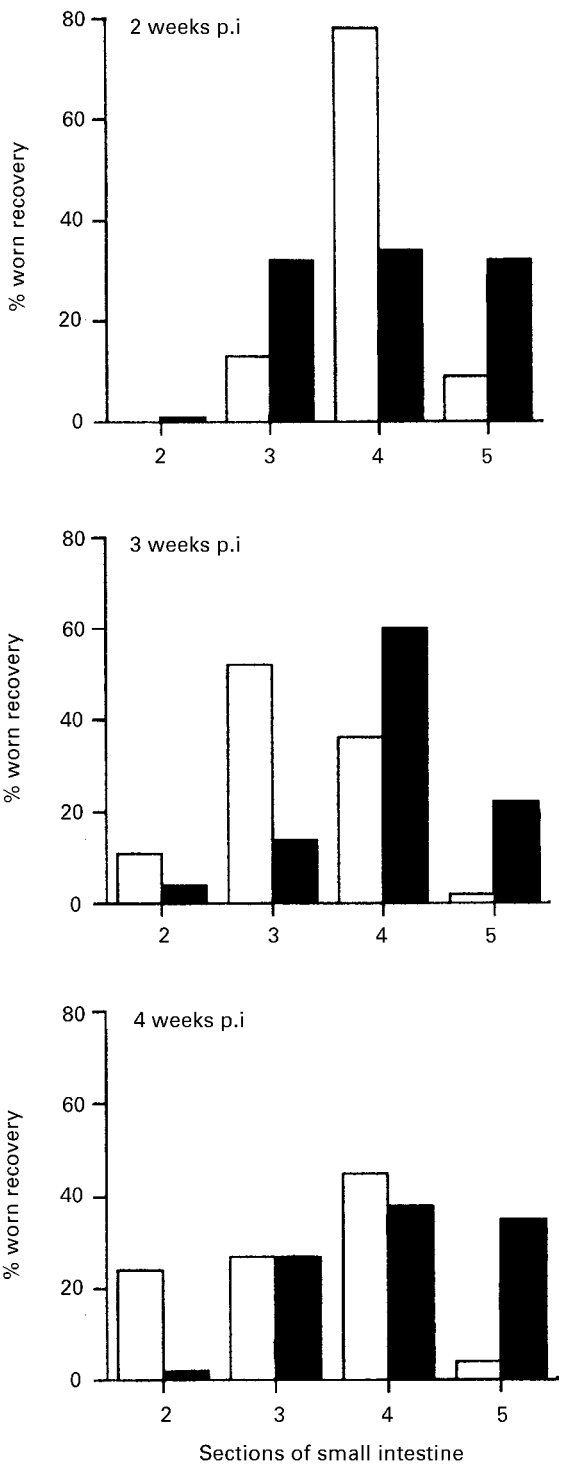

Fig. 2. Percent recovery of *Echinostoma caproni* from sections 2–5 of the small intestine of control hosts (□) and B vitamin deficient hosts (■) at 2, 3 and 4 weeks postinfection.

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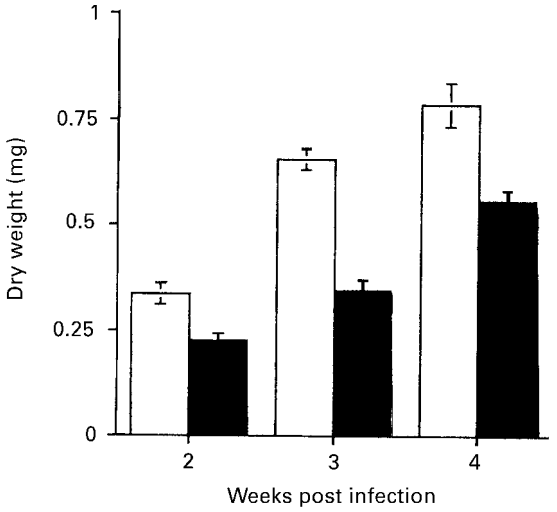

Fig. 3. Mean dry weight ± SE of *Echinostoma caproni* from ICR mice on the control diet (□) versus the B vitamin deficient diet (■) at 2, 3 and 4 weeks postinfection.

differences in mean weights and measurements of worms from hosts on the experimental versus control diets. The same test was used to compare weight differences in mice on each diet.

Results

Infectivity data showed that 35 of 36 (97%) mice on the experimental diet were infected, compared with all mice on the control diet. The percent worm recovery in experimental versus control mice was 38.2 versus 66.5 at 2 weeks p.i., respectively; 65.3 versus 52.3 at 3 weeks p.i., respectively; 34.7 versus 64.7 at 4 weeks p.i., respectively. There were no significant differences in worm recoveries at any time points. Mean weights of mice on the experimental diet were significantly less than those of mice on the control diet at 3 and 4 weeks p.i. (fig. 1). The weights of mice on the control diet increased steadily with time, but the weights of mice on the experimental diet decreased after 2 weeks p.i. Mice on the experimental diet at 3 and 4 weeks p.i. showed spastic movement when walking and a kangaroo-like posture at rest (see Kutsky, 1973). At all weeks p.i. the intestinal wall from the experimental hosts was thinner than that of the control hosts. The majority of the worms from mice on the control diet were distributed in segments 3 and 4 at all weeks p.i. (fig. 2). Worms from mice on the vitamin-deficient diet were more evenly distributed in segments 3, 4 and 5 at all weeks p.i. Worms were not found in segment 1 in either group at all weeks p.i.

Mean dry weights of worms from mice on the experimental diet were significantly less than those of worms from control mice at all weeks p.i. (fig. 3). Observations on body and organ measurements are shown in table 1. At 2 weeks p.i., all measurements of worms from control hosts except body length and oral sucker diameter were significantly greater than those from experimental hosts. All measurements except oral sucker diameter and ovary length were significantly greater in worms from control hosts than from the experimental hosts at 3 weeks p.i. At 4 weeks p.i., body width of control worms was significantly greater than that of the experimental worms. Oral sucker diameter was significantly smaller in control worms than in experimental worms at 4 weeks p.i. There were no significant differences in measurements in the other structures studied at 4 weeks p.i. At 2 and 3 weeks p.i., but not at 4 weeks p.i., body area was significantly smaller in worms from experimental hosts than in worms from control hosts. All worms from hosts on both diets were ovigerous at all weeks p.i.

Discussion

Most previous studies on helminths in rodent hosts deprived of B vitamins involved the elimination of a single vitamin. For instance, Prasad *et al.* (1980) found that greater numbers of *Litomosoides carinii* established in thiamin-deficient rats, but Beg *et al.* (1995) noted that

Table 1. Mean ± S.E. (mm) of body and organ measurements of *Echinostoma caproni* adults from ICR mice maintained on either a B complex vitamin deficient diet (experimental) or a control diet.

	2 weeks p.i.		3 weeks p.i.		4 weeks p.i.	
Measurements	Control	Experimental	Control	Experimental	Control	Experimental
Body length	3.92±0.08	3.85±0.07	4.8±0.1*	4.2±0.1	4.9±0.2	5.3±0.2
Body width	0.95±0.05*	0.78±0.03	1.19±0.05*	0.93±0.03	1.37±0.07*	1.13±0.03
Body area†	3.7±0.2*	2.9±0.2	5.7±0.3*	4.0±0.2	6.6±0.3	6.0±0.3
Oral sucker diameter	0.152±0.003	0.148±0.003	0.160±0.006	0.151±0.003	0.153±0.003*	0.162±0.003
Acetabulum diameter	0.490±0.008*	0.458±0.005	0.51±0.01*	0.47±0.01	0.511±0.008	0.518±0.008
Ovary length	0.156±0.005*	0.140±0.005	0.180±0.005	0.164±0.008	0.190±0.005	0.207±0.009
Ovary width	0.284±0.008*	0.233±0.009	0.316±0.008*	0.28±0.01	0.36±0.01	0.34±0.01
Anterior testis length	0.299±0.009*	0.231±0.008	0.33±0.02*	0.26±0.02	0.37±0.02	0.35±0.02
Anterior testis width	0.41±0.02*	0.30±0.01	0.42±0.02*	0.34±0.02	0.49±0.03	0.42±0.02
Posterior testis length	0.40±0.01*	0.36±0.01	0.44±0.02*	0.38±0.01	0.47±0.03	0.47±0.03
Posterior testis width	0.36±0.02*	0.28±0.01	0.37±0.01*	0.31±0.02	0.41±0.02	0.39±0.02

* Measurements were significantly greater ($P < 0.05$) in the control group than in the experimental group.
† Body area reported in mm^2.

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smaller and fewer *L. carinii* adults were recovered from pyridoxine-deficient rats. Our study was more dramatic than most of the previous ones in that all of the B vitamins were eliminated from the diet of the experimental host from the time of metacercarial cyst exposure until the day of necropsy.

The most significant finding of our study is that hosts on the B-deficient vitamin diet showed stunted growth based on worm dry weight from 2 to 4 weeks p.i. (at which time the study was terminated). Moreover, worm body area showed a significant decline in the experimental hosts at 2 and 3 weeks p.i., but not at 4 weeks p.i. The difference in worm growth observed at 2 and 3 weeks p.i., but not at 4 weeks p.i., is probably due to a slower worm growth rate in the absence of B vitamins. Body area measurements of *E. caproni* usually parallel dry weight measurements (see Hosier & Fried, 1991). We do not know why body area measurements between the two groups did not differ significantly at 4 weeks p.i.

Another significant finding is the fact that worms became more dispersed in the host small intestine on the B vitamin-deficient diet. When infected mice receive balanced diets (see Fried & Huffman, 1996), *E. caproni* is very committed to mouse intestinal segments 3 and 4 (the ileo-jejunal region). Whether the B-deficient host diet had a direct effect on worm dispersal, or an indirect effect, i.e. caused damage to the gut mucosa with subsequent secondary effects on the worm, could not be determined from the study.

Coprophagy is an important factor in the establishment of *Hymenolepis diminuta* in rats deprived of certain B vitamins and indeed worm stunting under such conditions is even greater when faecal feeding is prevented (Platzer & Roberts, 1969). We cleaned our cages daily and removed mouse faeces on a regular basis. Our incidental observations on mice on the B-deficient diet showed no evidence of mouse coprophagy. However, we did not prevent coprophagy using the rigorous design in the Platzer & Roberts (1969) study and cannot say what possible factor B vitamins in host faeces could have had on our study. The dramatic decline in mouse body weight and in worm dry weight at 2 to 4 weeks p.i. in hosts on the B-deficient diet seems to negate any significant effect coprophagy may have had on our study.

No experiments were done with uninfected animals fed the experimental diets, and therefore we do not know if such animals also undergo weight losses. Therefore, we do not know if the drop in weight observed in the infected animals fed the experimental diet is caused by the infection in the absence of B vitamins or by the absence of B vitamins itself.

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