

Development of Enteric Parasites in Parenterally Fed Rats¹ (38176)

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Life cycles of intestinal parasites are complex, often involving several stages within a single host. Successful maturation depends on an orderly development of successive stages, a process that is linked inseparably to the acquisition of food from habitats within the host. To survive, parasites must have specific nutrients available in their environment as well as specific chemical and physical stimuli that control feeding behavior. These strict nutritional, chemical, and physical requirements, which may differ greatly from one stage to the next, are maintained by the host's homeostatic mechanisms.

Knowledge of how and what parasites eat may be obtained from consideration of anatomical mechanisms that have been adapted for acquisition of foodstuffs; from studies of the chemical composition and intermediary metabolism of the parasites; from *in vitro* cultivation experiments and from examination of the types of tissues the parasites feed upon. Intestinal parasites may compete with the host for orally ingested food, acquire nutrients from the parenteral or exocrino-enteric circulations, or receive nourishment from the solid tissues of the host. The degree to which each source is utilized has been difficult to assess experimentally because of the problems of studying one in the absence of the others.

The objective of this investigation was to determine if the helminths, *Trichinella spiralis* and *Hymenolepis diminuta*, could develop normally in rats which did not ingest food orally, i.e., animals in which

all nutrients in the intestine were derived from the parenteral or exocrino-enteric circulations.

Methods and Materials. *T. spiralis* is a nematode that dwells in the small bowel with its head embedded in the mucosa and the remainder of its body exposed to the lumen. It is bisexual and matures within 72 hr after infection. *H. diminuta* is a monoecious, lumen-dwelling tapeworm that reaches maturity within 14-16 days following infection. Long-term intravenous hyperalimentation (IVH) was used to nourish host animals for 3 wk in the absence of enteral feeding.

Twelve Sprague-Dawley rats weighing 180-201 g were sustained by intravenous hyperalimentation as previously described (1). Thirty to 40 ml of the nutrient solution were administered daily to each rat. After the rats received IVH for 72 hr, 6 were infected with 5×10^3 *T. spiralis* larvae and 6 were infected with 10 cysticercoids of *H. diminuta*. Two groups of 6 control animals weighing 194-210 g were also infected with *T. spiralis* and *H. diminuta*, respectively. Three rats in each of the 2 control groups were fitted with harness assemblies similar to those fastened to the backs of the IVH animals, and three were left unharnessed. All controls were caged separately and maintained on an oral *ad libitum* diet of Purina rat chow and water.

The strain of *T. spiralis* used was obtained originally from Carolina Biological Supply Company (Burlington, NC) and has been maintained for seven years in CF-1 mice. Larvae were recovered from infected mouse skeletal muscle 40-60 days postinfection as previously described (2). *H.*

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diminuta cysticeroid larvae of the Rice Strain (3) were reared in *Tenebrio molitor*. Cysticeroids were recovered from the hemocoels of dissected beetles.

Rats were infected with either nematode or tapeworm larvae by oral intubation. Parenterally fed and orally fed control rats infected with *T. spiralis* were killed seven days postinfection, and worms were recovered alive according to published methods (4). Worms from each rat were fixed in 10% formalin, counted, differentiated by sex and measured microscopically with the aid of an ocular micrometer. Tapeworm-infected animals were killed 16 days postinfection, and mature worms were recovered by perfusing the small intestine with Krebs-Ringer bicarbonate buffer (pH 7.4). Worms from each animal were counted, washed in buffer and weighed immediately.

Results. Mature, adult *T. spiralis* were recovered from both harnessed and unharnessed controls as well as from IVH animals. There was no significant difference between the mean worm burden of harnessed and unharnessed controls. However, the mean worm burdens of both control groups were significantly greater than that of hyperalimented animals. Although there were significant differences in population sizes, the sex ratios and individual worm size were similar in control rats and rats fed parenterally (Table I). All female worms recovered were gravid.

Tapeworm-infected animals on the IVH regimen harbored no worms when killed 16 days postinfection; whereas, both harnessed and unharnessed orally-fed controls were infected. The 2 control groups were similar with respect to both mean worm number and worm mass present in the small intestine (Table II).

Hyperalimented animals infected with *T. spiralis* lost an average of 1.28% of their starting body weight per day. Mean daily weight loss in harnessed and unharnessed controls was 0.6% and 1.21% of initial body weight, respectively. Hyperalimented animals given cysticeroid larvae lost 0.33% of their initial body weight per day, whereas, tapeworm infected harnessed and unharnessed controls had daily gains

TABLE I. Recovery of *Trichinella spiralis* Adults from Infected Rats.^a

Group	Number of worms	Female worms			Male worms		
		Percent of total population	Length (μ)	Width (μ)	% of total population	Length (μ)	Width (μ)
Unharnessed controls ($n = 3$)	1,652 \pm 755	71.01 \pm 2.5	1,051 \pm 77	47.4 \pm 2.3	29.0 \pm 2.0	593 \pm 38	47.7 \pm 2.6
Harnessed controls ($n = 3$)	1,234 \pm 364	54.0 \pm 3.0	1,045 \pm 66	55.5 \pm 3.4	46.0 \pm 3.0	675 \pm 44	40.1 \pm 2.1
Hyperalimented ($n = 6$)	258 \pm 102	61.0 \pm 1.0	898 \pm 58	44.3 \pm 1.5	39.0 \pm 0.5	698 \pm 31	39.1 \pm 1.13

^a Values are means \pm S.E. Sex ratios were obtained by selecting two worm populations from each group and determining the sex of 100 randomly selected worms. Mean length and width were determined by measuring 10 worms of each sex randomly selected from each group.

TABLE II. Recovery of *Hymenolepis Diminuta* from Infected Rats.^a

Group	Number of worms	Worm mass (mg)
Unharnessed control (<i>n</i> = 3)	5.0 ± 1.5	412 ± 60
Harnessed control (<i>n</i> = 3)	4.7 ± 1.9	434 ± 42
Hyperalimented (<i>n</i> = 6)	0	0

^a Values are means ± S.E.

that averaged 0.75% and 1.56% of their starting weight.

Discussion. Metabolic differences between muscle larvae and intestinal stages of *T. spiralis* (2, 5, 6) and stringent physical and chemical requirements for successful *in vitro* culture (7, 8) suggest that factors necessary for growth and development of this nematode are varied and complex. The presence of a population of *T. spiralis* characterized by normal numbers of adult males, gravid females, and individual worms of normal size indicates that the environment of the lamina propria and the nutrients derived from the parenteral and/or exocrino-enteric circulations were sufficient to support maturation in hyperalimented rats. However, the failure of hyperalimented animals to harbor as large a worm population as either control group (Table I) is a finding that merits attention. A reduction in adult worm burden satisfies one of the criteria used to measure acquired resistance to *T. spiralis*. According to current opinion, immunity to this nematode is related to physical and chemical changes in the intestinal mucosa induced by inflammatory reactions (9, 10).

There is evidence that normal cestode development is regulated to a large degree by the composition of the host's diet. Low carbohydrate diets drastically retard growth and reproductive capacity of *H. diminuta*, whereas, the parasite is insensitive to protein deficiencies. Deletion of carbohydrate from the diet of the host does not, however, completely suppress growth (11). Thus, the failure to recover tapeworms

from parenterally fed rats 16 days post-infection cannot be attributed simply to the absence of carbohydrates in the intestine. More complex causes must be involved. Nondietary factors known to be of critical importance in the maturation of cestodes within the definitive host are temperature, pH, pCO₂, pO₂, Eh, bile composition, morphology of the mucosa and diet (12). Also, the role of gastrointestinal secretions is probably important in normal development, since tapeworms can assimilate materials from the exocrino-enteric (13) circulation. *H. diminuta* obtains pyridoxine, thiamine, nitrogenous compounds (14) and fatty acids (15) via this route, and most of these substances influence growth and maturation of the parasite (14).

Results of this study do not afford definitive answers but do provide clues regarding the relative importance of food in the gut in regulating the development of tissue and lumen-dwelling parasites. It is concluded that differences in the normal population size of *T. spiralis* and the failure of *H. diminuta* to establish in the gut of hyperalimented rats is indicative of adverse physical or chemical alterations in the parasites microenvironments caused by the cessation of enteral feeding. Additional experiments involving direct measurements of chemical and physical parameters are indicated to clarify this point.

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