Dietary protein and the growth of rats infected with the tapeworm *Hymenolepis diminuta*

BY ANDREW HALL*

Department of Nutrition, Queen Elizabeth College, University of London, Campden Hill Road, London W8 7AH

(*Received 29 March 1982 – Accepted 28 June 1982*)

1. Weanling rats fed on a relatively low protein diet were infected with several intestinal tapeworms. The weight gain and daily food intake of the rats were measured for 3 weeks before the animals were killed and the adult tapeworms recovered. The tissues of the rats and worms were then analysed for protein. Controls were provided by uninfected rats either pair fed or fed *ad lib.* For comparison, a similar experiment was conducted on rats fed on a relatively high protein diet.

2. The effect of the infection on the protein-malnourished rats and of the low level of protein on the worms were apparently not significant. The amount of protein contained in the worm burdens was less than 1·5% of the average total intake of the protein-malnourished rats.

Nutrition may be considered a fundamental basis of the relationship between a parasite and its host (Crompton & Hall, 1981). A parasite is often dependent on a host for its nutritional needs and, perhaps, by appropriating nutrients, disrupting digestion and absorption, impairing general function or causing a needless diversion of resources, may thereby impair the nutritional status of the host or exacerbate current malnutrition.

Parasitic organisms are responsible for some of the most widespread and common infections of man, many of which are highly prevalent among malnourished people in developing countries. For example, it is estimated by the World Health Organization that 65 million people are infected with adult tapeworms of the genus *Taenia* (Markell & Voge, 1976). Another member of the same order of tapeworms, *Hymenolepis diminuta*, is a parasite of rats. During its life-cycle the egg develops to the cysticercoid stage in the body cavity of an insect which, when consumed by a rat, grows during the next 18–21 d to an adult tapeworm of approximately 450 mm in length. Tapeworms have no alimentary tract; the body surface, which is increased in area by microscopical projections and is covered by a thin, living tegument, acts as an absorptive surface (Rothman, 1963). The biology and nutritional requirements of tapeworms, and of *H. diminuta* in particular, have been quite intensively studied. However, little attention appears to have been paid to the nutritional consequences to the host of a developing tapeworm burden.

The aims of the experiments to be described here were as follows. First, to investigate the effect of a developing tapeworm burden on the food intake and weight gain of growing rats fed on a relatively low protein diet. Second, to study the effects of the tapeworm infection on the carcass composition of their protein malnourished hosts. Third, to compare the experiments conducted using rats fed on the relatively low protein diet with similarly treated animals fed on a relatively high protein diet. Finally, to investigate the effect of the level of protein in the diet on the establishment and protein content of the tapeworm burdens.

MATERIALS AND METHODS

Isoenergetic diets containing relatively high (HP) or relatively low (LP) levels of protein were provided by substituting starch for casein on a weight-for-weight basis. The LP and

* Present address: 87 Linden Gardens, London W2 4EX.

Table 1. *Experimental design and details of groups of rats*

Expt no.	Dietary protein (g/kg)	Treatment	Group
1	54	Infected, fed *ad lib.*	A
		Uninfected, fed *ad lib.*	B
		Uninfected, pair-fed	C
2	108	Infected, fed *ad lib.*	D
		Uninfected, fed *ad lib.*	E
		Uninfected, pair-fed	F

HP diets provided protein as casein at a concentration of 60 and 120 g/kg respectively of the major energy producing nutrients. Both diets were supplemented with methionine at a concentration of 1% of the protein content. Fat was provided as maize oil at 150 g/kg and the diet was made up to 1 kg with starch. To each kg of this mixture was added 40 g/kg mineral mix and 20 g/kg vitamin mix as described by Greenfield *et al.* (1969) plus 50 g/kg of cellulose powder. Thus the LP and HP diets provided protein at concentrations of 54 g/kg and 108 g/kg respectively. Rats given a diet providing 54 g/kg protein supplemented with methionine should theoretically receive enough protein for maintenance and a limited amount of growth (Miller & Payne, 1961) and should be sensitive to any change in digestibility due to the demands or effects of a tapeworm burden.

Twenty-one Sprague-Dawley, weanling male rats approximately 5 weeks old and weighing from 75 to 113 g were used in each experiment. Each animal was placed separately in a cage and allowed to consume *ad lib.* one of the two diets for a week before their allocation to experimental groups. Then the twenty-one animals were ranked according to weight and divided into groups of three. Each of the three rats was randomly assigned to one of three groups as follows: one group of seven animals was fed the diet *ad lib.* and infected with *H. diminuta*, the second group acted as uninfected controls and were also fed *ad lib.* while a third group, also uninfected controls, were pair-fed to infected partners after infection had taken place. The arrangement of each group of animals, their experimental treatments and the letter used to identify each group in both experiments are shown in Table 1.

Cysticercoids were recovered from the body cavity of grain beetles (*Tribolium confusum*) and fifteen were administered by stomach tube to each rat in groups A and D in approximately 0·5 ml of saline (9 g sodium chloride/l). All the other animals were sham-treated but were given only 0·5 ml saline.

All animals were fed *ad lib.* for the first day after the process of infection; thereafter rats in groups C and F were pair-fed. Food consumption was recorded daily. Body-weight was recorded regularly at the same time each day and just before death.

All infected animals were killed by cervical dislocation, 21 d after infection, between 10.00 and 12.00 hours. The small intestine was removed, measured, then opened longitudinally. The tapeworms were found to be spread along almost the whole length of the intestine of animals in both experiments and appeared to occupy a substantial part of the lumen. The worms were carefully removed, washed in warm saline and then blotted dry before being weighed. The worms from each animal were dried on foil trays in an oven set at 105° for 24 h in order to estimate their water content. The infected rats in groups A and D were partly eviscerated by removing the remainder of the gut from the cardiac sphincter to the anus, the liver, spleen and kidneys. The uninfected rats in each experiment were killed on the same day as the infected animals, between 14·00 and 16.00 hours and each was

Table 2. *The food consumption, weight gain and efficiency of food utilization by rats fed for 21 d on either a 54 (groups A, B and C) or a 108 (groups D, E and F) g protein/kg diet*

(Mean values with their standard errors for seven rats/group. Animals in groups A and D were infected with *Hymenolepis diminuta* and fed *ad lib.*, those in groups B and E were uninfected and fed *ad lib.*, while rats in groups C and F were uninfected and pair-fed to infected partners)

Dietary protein (g/kg)...	54						108					
Group...	A		B		C		D		E		F	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total food consumed (g)	253·6	17·35	234·3	21·32	230·6	15·42	467·9	13·53	449·3	13·04	426·9	17·39
Initial body-wt (g)	83·6	3·14	84·6	3·52	84·3	3·17	123·6	2·46	125·2	2·83	123·6	2·99
Final body-wt (g)	128·9	5·93	126·0	7·48	127·6	6·20	298·3	5·93	289·8	7·29	279·1	10·47
Wt gain (g)	45·3	5·14	41·4	5·78	43·3	4·34	174·7	7·46	164·7	7·26	155·6	11·68
Protein consumed (g)	13·7	0·94	12·6	1·13	12·4	0·83	51·0	1·47	49·0	1·44	46·5	1·78
Protein efficiency ratio*	3·2	0·20	3·2	0·26	3·4	0·17	3·4	0·07	3·4	0·07	3·3	0·13

* $\frac{\text{Wt gained (g)}}{\text{Protein consumed (g)}}$.

Table 3. *Results of the eviscerated carcass analysis performed on groups of rats fed on 54 (groups A, B and C) or 108 (groups D, E and F) g protein/kg diets*

(Mean values with their standard errors for seven rats/group. Animals in groups A and D were infected with *Hymenolepis diminuta* and fed *ad lib.*, those in groups B and E were uninfected and fed *ad lib.*, while those in groups C and F were uninfected and pair-fed to infected partners)

Dietary protein (g/kg)...	54						108					
Group...	A		B		C		D		E		F	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Carcass wet wt (g)	104·7	4·95	102·3	7·18	106·7	5·82	252·0	4·95	252·6	5·97	239·7	8·20
Carcass dry wt (g)	39·6	2·64	39·3	3·86	40·3	2·95	112·9	8·62	105·4	3·48	96·3	3·93
Dry wt (g/100 g)	36·7	1·13	38·0	1·15	37·6	0·83	44·6	2·65	41·8	1·59	40·1	0·53
Carcass total fat (g)	16·0	1·74	13·3	1·29	16·3	1·97	48·4	5·57	44·6	1·63	42·6	3·06
Carcass total fat (g/100 g carcass weight)	15·1	1·21	12·9	0·79	15·0	1·1	17·4	1·4	17·8	0·94	17·7	0·94
Carcass total protein (g)*	17·2	0·79	17·7	1·10	18·2	0·64	44·1	0·64	44·0	1·36	41·6	1·21
Carcass total protein (g/100 g carcass wt)*	16·0	0·45	17·0	0·45	17·2	0·49	17·5	0·23	17·4	0·23	17·4	0·23

* Nitrogen × 6·25.

Table 4. *The number, weight and protein content of tapeworms* (Hymenolepis diminuta) *recovered from animals given diets containing 54 (group A) or 108 (group D) g protein/kg and infected 21 d previously with fifteen cysticercoids of* H. diminuta

(Mean values with their standard errors for seven rats/group)

Group...	A		D	
	Mean	SE	Mean	SE
No. of worms recovered	12·4	0·64	10·0	0·87
Wet wt of worms (g)	2·32	0·11	2·19	0·34
Dry wt of worms (g)	0·55	0·02	0·53	0·08
Dry wt (g/100 g)	23·9	0·3	24·9	0·54
Total protein of worms (mg)*	175	2·65	174	25·7
Total protein (g/100 g wet tissue)*	7·53	0·17	8·21	0·33

* Nitrogen × 6·25.

eviscerated to the same extent. All carcasses were weighed, then dried on foil trays to constant weight in an oven set at 105°.

The dried worms from each rat were pooled and their nitrogen content was estimated by the Kjeldahl method (Bradstreet, 1965). The dried, partly eviscerated carcasses were dissolved in warm laboratory grade, concentrated hydrochloric acid (360 g/kg) and samples were taken for analysis for their N content.

RESULTS

There was no evidence of any significant change in the daily food consumption of infected rats during either experiment when compared with their uninfected controls fed *ad lib.* During the course of both experiments the infected rats ate more in total than those in either control (Table 2) but within each experiment the differences were not statistically significant. There were no significant differences either between groups of rats in each experiment in terms of their final body-weight, weight gain during the experiments or protein efficiency ratio (PER; Table 2). If comparisons were made of the PER of groups of rats fed on different diets but receiving the same treatment, for example groups B and E, then there were no apparent differences.

In each experiment there were no significant differences in the average composition of the eviscerated carcasses of each group in terms of dry weight, total fat/kg wet weight or total protein/kg wet weight (Table 3).

In both experiments, over 65% of the administered cysticercoids developed into tapeworms (Table 4). There were no apparent differences between worms recovered from rats fed on the low or high protein diets; in a relaxed state worms were up to 400 mm long in intestines with a mean length of 960 mm (group A) or 1140 mm (group D) and the worms generally occupied the lumen from 100 mm below the pylorus to the caecum. A Mann–Whitney test indicated that there were no statistically significant differences between the number of worms recovered from rats fed on diets containing either 54 g (group A, ten to fourteen worms) or 108 g (group B, eight to thirteen worms) protein/kg. The average wet weight of worm tissue recovered from rats in groups A and D, their dry weight, total protein content and total protein/kg wet tissue are shown in Table 4; there were no significant differences indicated by an unrelated samples *t* test.

DISCUSSION

The experiments described here indicate that a developing burden of several tapeworms seems to have no effect on the nutritional status of growing, protein malnourished rats; the infection does not apparently affect food consumption, weight gain or the carcass fat and protein content. The establishment, growth and protein content of *H. diminuta* is also apparently unaffected by the relatively low level of protein in the diet.

The absence of any apparent effect of the tapeworm burdens administered in this experiment is evidence first of no significant impairment of digestive or absorptive function and second, of the negligible protein requirements of the worms. During the 21 d of Expt 1 the infected rats fed on a 54 g protein/kg diet consumed approximately 13·7 g protein as casein (Table 2). The tissue analysis of the worms recovered from these animals indicated that they contained on average only 175 mg protein, 1·28% of the total intake (Table 4). If rat tissue is taken to be approximately 160 g protein/kg, then this amount of protein is equivalent to approximately 1·0 g of the body-weight. Given the variation in rat weight between infected animals (Table 2) it would thus be hard to detect any difference in weight from an uninfected control group. In the other experiment using a 108 g protein/kg diet, the worms contained even less of the protein consumed over the 21 d period, only 0·34%. However, the analysis of the worm burden for its protein content only indicates the end of a process, it provides no information about protein turnover in the worms or its efficiency of utilization during growth.

The use of a conversion factor of $\times 6{\cdot}25$ to calculate the protein content of the worms from the value determined for N may lead to some error. There is evidence that tapeworm tissue may contain between 9 and 16% of the total N as non-protein-N (Smyth, 1969). The low concentration of protein, relative to that in mammalian tissue, may be partly explained, first, by the smaller dry weight and greater water content of the worm tissue and, second, by the fact that protein may in effect be diluted by the large reserves of glycogen which are known to be stored in worms (Read & Rothman, 1957).

The finding that the growth of *H. diminuta* is independent of the level of protein in the diet of its host confirms previous studies (Chandler, 1943; Mettrick & Munro, 1965). This is probably because the tapeworm obtains its nutrients from at least three sources: from the food of its host being digested in the intestine; from the gastrointestinal secretions of the host such as enzymes, bile and the exoenteric circulation which serves to maintain a relatively constant molar ratio of amino acids in the intestine (Nasset & Ju, 1961) and; finally, from the possible diffusion of nutrients across the wall of the intestine (Hopkins & Callow, 1964; Arme & Read, 1969).

Certain adaptations may also serve to ensure that *H. diminuta* is well nourished. Worms appear to undergo a circadian rhythm of movement, first down the intestine with the passage of nocturnally ingested food, eventually returning to the anterior, thereby increasing the time available for the absorption of nutrients (Read & Kilejian, 1969; Hopkins, 1970). *Hymenolepis diminuta* is also known to adsorb the host's enzymes onto its body surface (Read, 1973). This phenomenon, termed 'contact digestion', would be likely to ensure that a high concentration of digested nutrients is maintained near to the surface of the worm, thereby sustaining a concentration gradient for passive diffusion into the worms. Mediated transport and facilitated diffusion have also been shown to operate in vitro preparations of tapeworms (Pappas & Read, 1975).

The experiments described here indicated that *H. diminuta* caused no significant disruption of the digestive or absorptive function of its host. It has been reported that rats infected with *H. diminuta* and fed on a diet containing no protein showed no difference in N balance when compared with uninfected controls (Mettrick & Munro, 1965). Nearly all worm tissue

is ultimately derived from nutrients consumed by the host, but the magnitude of their requirement seems to be small even for a sizeable worm burden. The long-term consequences of a mature, egg-producing worm burden may, however, have a cumulative effect on a protein-malnourished host, not detected in this relatively short experiment: the life of the worm appears to be limited by that of its host (Read, 1967). Yet this experiment suggests that *H. diminuta* is well adapted to the rat, a quality which ensures its own existence by not endangering the survival of its undernourished host.

The author is grateful to the States of Jersey for financial assistance, Dr D. J. Naismith for advice and comments, Professor A. E. Bender for laboratory facilities, Miss S. E. Arnold for infected grain beetles, and M. G. Edwards.

REFERENCES

Arme, C. & Read, C. P. (1969). *Comp. Biochem. Physiol.* **29**, 1135.
Bradstreet, R. B. (1965). *The Kjeldahl Method for Organic Nitrogen*. New York and London: Academic Press.
Chandler, A. C. (1943). *Am. J. Hyg.* **37**, 121.
Crompton, D. W. T. & Hall, A. (1981). *Parasitology* **82**, 31.
Greenfield, H., Briggs, G. M., Watson, R. H. J. & Yudkin, J. (1969). *Proc. Nutr. Soc.* **28**, 43A.
Hopkins, C. A. (1970). *Parasitology*, **60**, 255.
Hopkins, C. A. & Callow, L. L. (1964). *Parasitology* **54**, 5P.
Markell, E. K. & Voge, M. (1976). *Medical Parasitology*, 2nd ed. Philadelphia and London: W. B. Saunders.
Mettrick, D. F. & Munro, H. N. (1965). *Parasitology* **55**, 453.
Miller, D. S. & Payne, P. R. (1961). *Br. J. Nutr.* **15**, 11.
Nasset, E. S. & Ju, J. S. (1961). *J. Nutr.* **74**, 461.
Pappas, P. W. & Read, C. P. (1975). *Expl. Parasit.* **37**, 469.
Read, C. P. (1967). *J. Parasit.* **53**, 1055.
Read, C. P. (1973). *J. Parasit.* **59**, 672.
Read, C. P. & Kilejian, A. Z. (1969). *J. Parasit.* **55**, 574.
Read, C. P. & Rothman, A. H. (1957). *Expl. Parasit.* **6**, 1.
Rothman, A. R. (1963). *Trans. Am. microsc. Soc.* **82**, 22.
Smyth, J. D. (1969). *The Physiology of Cestodes*. Edinburgh: Oliver & Boyd.

Printed in Great Britain