Parasitol Res (1987) 73:171–177

Parasitology Research
Founded as Zeitschrift für Parasitenkunde

Effects on the development of *Dipylidium caninum* and on the host reaction to this parasite in the adult flea *(Ctenocephalides felis felis)*

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Abstract. Temperature was found to be a major factor affecting the development of *Dipylidium caninum* and the presence of a host reaction of adult *Ctenocephalides felis felis* to *D. caninum*. Adult fleas reared at 30–32° C contained fully developed metacestodes when they emerged from their cocoons. However at lower temperatures, *D. caninum* could not complete development until the flea hosts had spent some time on their mammalian hosts. It was the surface temperature of the mammals (31–36° C) and not the fleas' blood meals which resulted in the metacestodes completing their development. This development of *D. caninum* was therefore independent of the flea development. At 20° C, a larger and more prolonged host reaction was mounted than at higher temperatures. The larval flea diet had a small effect on the subsequent cestode development and the adult flea's reaction to it.

Ctenocephalides felis was shown to be an intermediate host of *Dipylidium caninum* by Chen (1934) who found that the parasite developed very slowly until the flea became an adult feeding on a mammal. He reported that this intermediate host encapsulated many developing parasites. However subsequent studies of *D. caninum* in dog and/or cat fleas by Zimmerman (1937), Venard (1938), Marshall (1967) Yasuda et al. (1968) and Joseph (1974) reported different rates of parasite development and did not record any host reaction. These experiments were performed under different environment conditions and in fact Chen (1934), Zimmerman (1937) and Venard (1938) did not specify the physical conditions under which their experiments were performed. Pugh and Moorhouse (1985) studied the infection of *D. caninum* in *C. f. felis* up to the 12th day of infection and found the parasites grew very little at 20° C. However, as the temperature was raised above 20° C, development rate increased. They also reported a significant host reaction in fleas reared at 30° C/3 mm Hg saturation deficit (s.d.) after 6 days of infection but this was reduced significantly after 9 days. However the proportion of parasites with a host reaction in fleas reared at 25° C/3 mm Hg s.d. and 20° C/3 mm Hg remained high even after 12 days of infection. The parasite development and the host reaction was unaffected by the host development (Pugh and Moorhouse 1985).

The term metacestode is used to encompass all growth forms following metamorphosis of *D. caninum* oncospheres and before development of proglottids as defined by Freeman (1970). *D. caninum* metacestodes cannot be considered cysticercoids as their primary lacunae develop but then disappear. Thus they do not have a double-walled bladder enclosing an invaginated holdfast with a bladder-like tail, as defined for cysticercoids by Wardle and McLeod (1952). The general terms of cestode, tapeworm or parasite are used in this paper to include the oncosphere and metacestode forms of *D. caninum*.

Materials and methods

Fleas

Twenty to thirty larval fleas were placed in 6 cm wide petri dishes and reared on a range of diet combinations in a range of temperatures and saturation deficits (s.d.). Saturation deficits were controlled using sulphuric acid solutions according to So-

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Table 1. Flea larval diets

Proportion of food in diet	Diet					
	1	3	4	5	7	9
Fibrin	10	1	10	10	1	10
Starch	1	10	10	1	10	1
Yeast	0.5	0.5	0.5	10	0.5	0

lomon (1951) and tested using a hydrometer (Buxton and Mellanby 1934) and cobalt papers (Solomon 1957). The composition of the larval diets is given in Table 1. Each petri dish contained 0.25 g food and 1 g of vermiculite (an inert substance 3 mm × 3 mm). Environmental conditions used were 20° C/3 mm Hg s.d., 25° C/3 mm Hg; 30° C/8 mm Hg, 30° C/3 mm Hg and 32° C/3 mm Hg. When the larval fleas were 3 days old, they were starved for 48 h, and then offered gravid proglottids (3–4 proglottids in each dish). These proglottids were placed on absorbent paper and allowed to dry for 2–3 h before being placed in the dishes; larvae became stuck to wet proglottids, could not escape or feed and eventually died. After 48 h, the fleas were placed in 5 cm long, 2.5 cm diameter bottles containing 0.25 g larval food and 1 g finely ground vermiculite and allowed to pupate and mature to adulthood. Twenty control fleas were reared at 20° C/3 mm Hg and 30° C/3 mm Hg in the same manner as the above test fleas; but were not offered cestode proglottids. Starvation resulted in the death of some larvae but other (unpublished) studies by the author showed this was not a significant factor affecting flea development and survival or the infection, but ensured test larval fleas fed quickly on the proglottids.

Experimental infections

The adult fleas were dissected within a day or two of emerging from their cocoons. Fleas reared under the same conditions, although of the same age, did not emerge from their cocoons at the same time. Hence their parasites and any host reaction to them could be observed over a number of days. Adult fleas were dissected in Insect Ringer's solution (Taylor 1935) and observed by light and phase microscopy with and without vital staining using 0.01% Janus green, 0.1% toluidine blue or 0.1% neutral red. Parasite volume was estimated to be width squared times length, according to Soltice et al. (1971) who worked with *Hymenolopis diminuta*. Parasites were measured individually while alive and the number of measurements depended on their activity. When this was high, many measurements were made to get a reasonable estimate.

Thirty-seven and thirteen adult fleas reared at 20° C/3 mm Hg and 25° C/3 mm Hg respectively were placed on rats kept in boxes 30 cms × 16 cms × 13 cms in rooms at 20° C/3 mm Hg and 25° C/3 mmHg respectively. Neither the fleas nor the rats were restrained from moving freely in the boxes but the boxes were covered with wire tops and muslin. Up to 7 days later, the fleas were retrieved from the rats, dissected and their parasites observed. The surface temperature of the rats was measured with a thermistor. Other adult fleas were kept in their bottles at 20° C/3 mm Hg and 25° C/3 mm Hg but fed 3–4 times a day for 3 days through the muslin top on a human arm, i.e. intermittent feeding. Another 500 fleas were prepared for transmission electron microscopy (TEM) and flea haemocyte counts. For TEM, fleas were fixed in cold (4° C) 4% glutaraldehyde and 4% paraformaldehyde (1:4) in cacodylate buffer pH 7.2 for 4–6 h, washed in the buffer, post fixed in osmium tetroxide for 2 h, dehydrated and embedded in araldite. Ultrathin sections were cut with glass knives on the LKB Nova, stained with uranyl acetate (25 min) and lead citrate (4 min) and studied in a A.E.I. Corinth electron microscope. Flea haemocyte counts were done by dissecting the adult flea in 10 µl of a 0.2% acetic acid solution in Insect Ringer's with 2% versene and tinted with gentian violet. *Z*-tests for proportions and *t*-tests were performed and any result was considered to be significant if $P<0.05$.

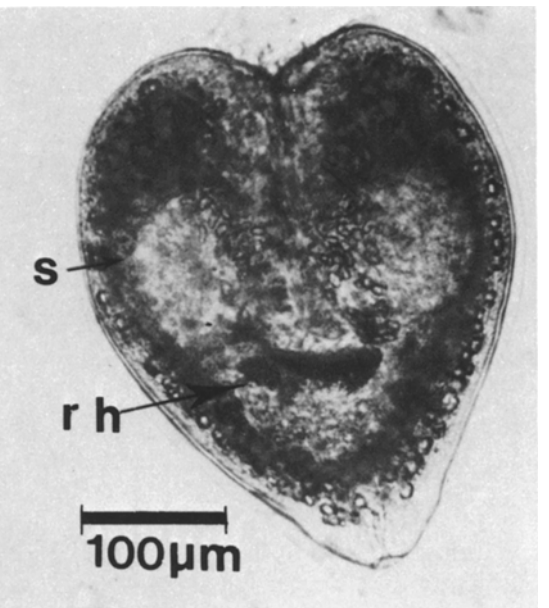


Fig. 1. Fully developed *Dipylidium caninum* metacestode

Results

A total of 4,400 adult fleas were dissected and a total 8,592 *D. caninum* were observed in 654 infected adult cat fleas.

Table 2 shows the time *D. caninum* took to reach the developed stage (stage 6) and the fully developed stage (stage 7) (Fig. 1) in adult fleas reared under different conditions. The parasites at stage 6 had developed suckers and armed rostella and had lost their cercomers containing the oncospheral hooks. Further development to stage 7 resulted in metacestodes with volumes of 10–20,000 × 10^3 µm^3, withdrawn suckers and rostella, surrounded by clear outer layers. Below 30° C, the parasites could not reach these final stages of development until their adult flea hosts had fed on rats for 5–7 days (Table 2). The rat surface temperature was found to be 31° C–36° C depending on the amount of hair and environmental temperature. Some fleas were only fed briefly and intermittently and were kept at 20° C or 25° C between meals so that the mammal's body temperature did not have time to affect the tapeworms

Table 2. Development times* of *Dipylidium caninum* metacestodes in *Ctenocephalides felis felis* reared at a range of temperatures/saturation deficits and flea larval diets

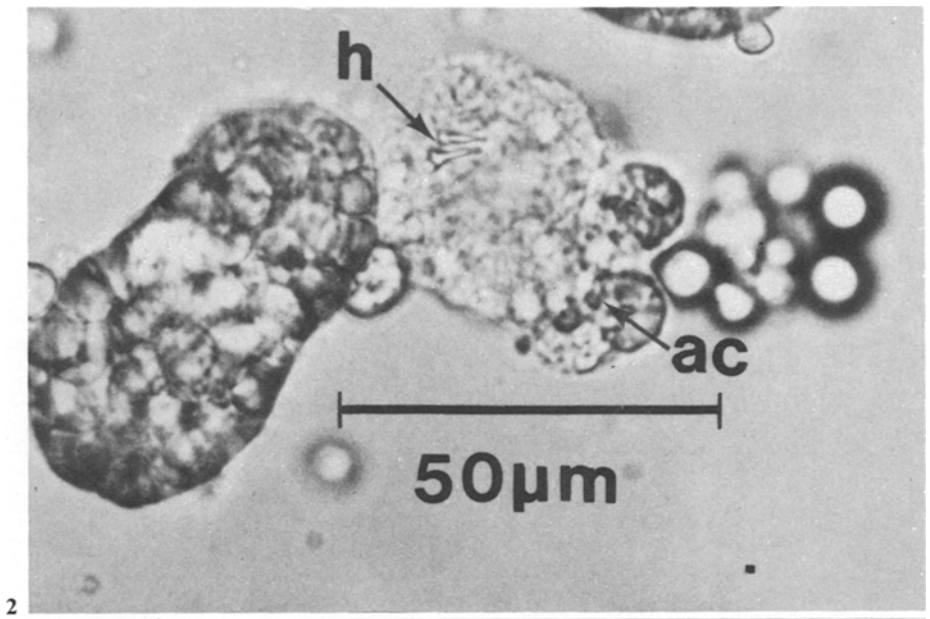
Flea larval diet	Developmental stage of *D. caninum*	Temperature/saturation deficit (°C/mm Hg)				
		20/3	25/3	30/8	30/3	32/3
1	6	–	–	15 (F); 17 (M)	16 (F and M)	9 (F and M)
	7	7 (R)	–	– (>17)	18 (M)	10 (F); 12 (M)
3	6	–	–	18 (M)	15 (F); 18 (M)	12 (F and M)
	7	–	5 (R)	–	15 (F); – (M) (>19)	12 (F and M)
4	6	–	–	– (>21)	– (>19)	12 (F and M)
	7	–	–	–	–	12 (F and M)
5	6	–	–	12 (F); 16 (M)	11 (F); 13 (M)	9 (F and M)
	7	7 (R)	6 (R)	15 (F)	13 (F); 16 (M)	9 (F); 12 (M)
7	6	–	–	17 (M)	16 (M)	12 (F and M)
	7	–	–	– (>19)	– (>19)	15 (F)
9	6	–	–	19 (M)	18 (M)	10 (F)
	7	–	– >6 (R)	–	–	14 (F)
	6	–	–	19 (M)	13 (F)	
Adult flea dejecta	7	–	–	–	15 (M)	

* Days after infection to reach the developed stage (6) and the fully developed state (7); (*F*) in female flea; (*M*) in male flea; indicates parasites did not develop to this stage in unfed adult fleas reared under these conditions up until time of death of fleas; (*R*) days after fleas placed on rats

they contained. These tapeworms did not develop any more than those in unfed adult fleas kept at these low temperatures for the same length of time. Fastest cestode development occurred in adult fleas at all temperatures if as larvae they had fed on a diet with a high yeast content (diet 5). Slowest development occurred on diets with high concentrations of starch and low concentrations of yeast and/or fibrin (diets 3, 4, 7). Rates of development of cestodes in fleas reared as larvae on adult flea dejecta or on diet 5 at 30° C/3 mm Hg were similar. At 30° C parasites in adult fleas reared as larvae on the high protein diets reached the fully developed stage but those in adult fleas reared on larval diets with high starch content still had their cercomers. They did not develop further unless the lives of their adult flea hosts were prolonged by being fed. Faster development occurred at 30° C at the lower saturation deficit of 3 mm Hg. At 20° C even after 50 days of infection *D. caninum* in unfed adult fleas or intermittently fed adult fleas remained at the oncospheral stage with a volume of $1{,}500 \times 10^3$ μm^3 and with a primary lacuna. Small cercomers had begun to develop on some. At 25° C after 25 days of infection when all fleas had emerged from their cocoons, the metacestodes in unfed adults had reached volumes of $4{,}000 \times 10^3$ μm^3. They had also lost their primary lacunae but developed rostella and cercomers of 200 μm.

The host reaction was characterised by adhering flea haemocytes which could not be dislodged (Fig. 2) or by full encapsulation (Fig. 3) or pigment substances attached to the parasites. No additional cells adhered to the parasites after they were dissected from the fleas.

Z-tests showed that a significant proportion of parasites with a host reaction were observed at 20° C/3 mm Hg (0.34 ± 0.38, $n = 1{,}505$) and 25° C/3 mm Hg (0.11 ± 0.22, $n = 1{,}943$) and very little or none were seen at 30° C and 32° C in adult fleas. At 20° C/3 mm Hg, significantly fewer parasites (0.13 ± 0.12, $n = 174$) had an observable host reaction in adult fleas reared as larvae on a diet lacking yeast but with a higher concentration of fibrin (9) than those on the other diets. Adult fleas reared as larvae on a diet high in fibrin and containing yeast (1) had significantly fewer parasites with a host reaction (0.23 ± 0.31, $n = 370$) than those on a high yeast diet (5) (0.33 ± 0.38, $n = 417$). They also had significantly fewer than those on the high starch diets 4, 3 or 7 (0.57 ± 0.49, $n = 88$; 0.48 ± 0.39, $n = 243$; 0.44 ± 0.46, $n = 213$ respectively). No such differences were found in adult fleas reared on different diets at 25° C/3 mm Hg. A significant rise in the proportion of parasites with a host reaction in adult fleas reared at 20° C/3 mm Hg occurred from the period 20–30 days (0.20 ± 0.25; $n = 367$) to the period 41–50 days (0.64 ± 0.38, $n = 220$) following infection. A significant fall in the proportion of parasites with a host reaction occurred in adult fleas reared at 25° C/3 mm Hg as the time following infection proceeded from 10–15 days (0.22 ± 0.27) to 21–25 days



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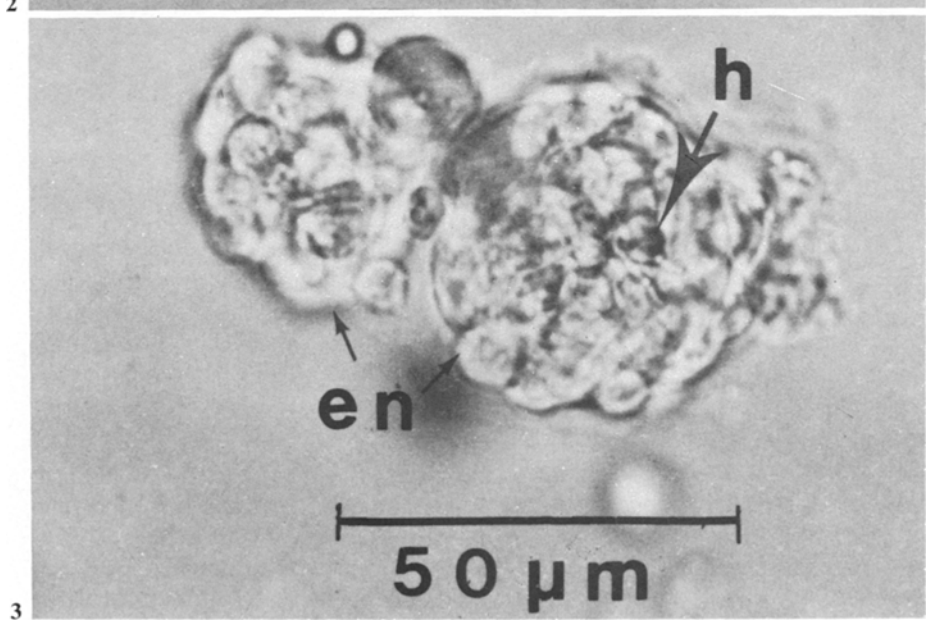

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Fig. 2. Oncosphere with adhering haemocytes. *ac* adhering haemocytes, *h* hooks

Fig. 3. Encapsulated oncospheres. *en* encapsulation, *h* hooks

(0.05 ± 0.15). Figure 4 shows the effect of temperature on the host reaction in adult fleas reared on the range of larval diets as time progressed. The parasite load did not significantly affect the parasite development or the host reaction.

TEM studies revealed flea haemocytes lay close to the basal folding or microvilli of the parasites (Fig. 5). Some parasites with these adhering cells showed signs of degeneration and some had broken tegments. Secretory droplets were observed near the attachment site. Table 3 shows that the haemocyte count was significantly raised in infected adult fleas reared at 20° C/3 mm Hg.

Discussion

Temperature had the most effect on the rate of development of *D. caninum* in adult *C. f. felis*. At temperatures below 30° C the metacestodes could not complete their development until their adult flea hosts were placed for some days on mammals. It was the effect of the mammalian surface temperature and not the blood meals which enabled the parasites to develop fully. It was only in fleas allowed to stay on mammals that tapeworms could complete their development at these lower temperatures. Intermittent blood meals, while allowing

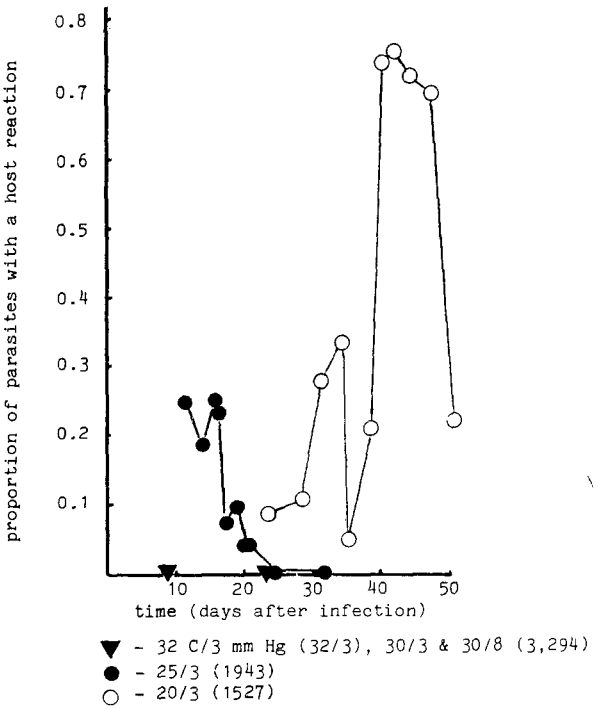

Table 3. Haemocyte counts of adult *Ctenocephalides felis felis*

	20° C/3 mm Hg	30° C/3 mm Hg
Control fleas	728± 289 $n=20$	477±255 $n=20$
Test fleas	3784±2370 $n=14$	712±474 $n=15$
	(9.7±12 parasites/flea)	(3.1±2.5 parasites/flea)

Fig. 4. Proportion of *Dipylidium caninum* with a host reaction from adult *Ctenocephalides felis felis* reared at different temperatures/saturation deficits

Note: this figure is constructed from the observations of 6,764 parasites. Approximately 20–50 parasites were observed for the presence of adhering haemocytes and crystals or for complete encapsulation, at intervals of 1, 2 or 3 days. The total number of parasites at each temperature/saturation deficit is given in parenthesis

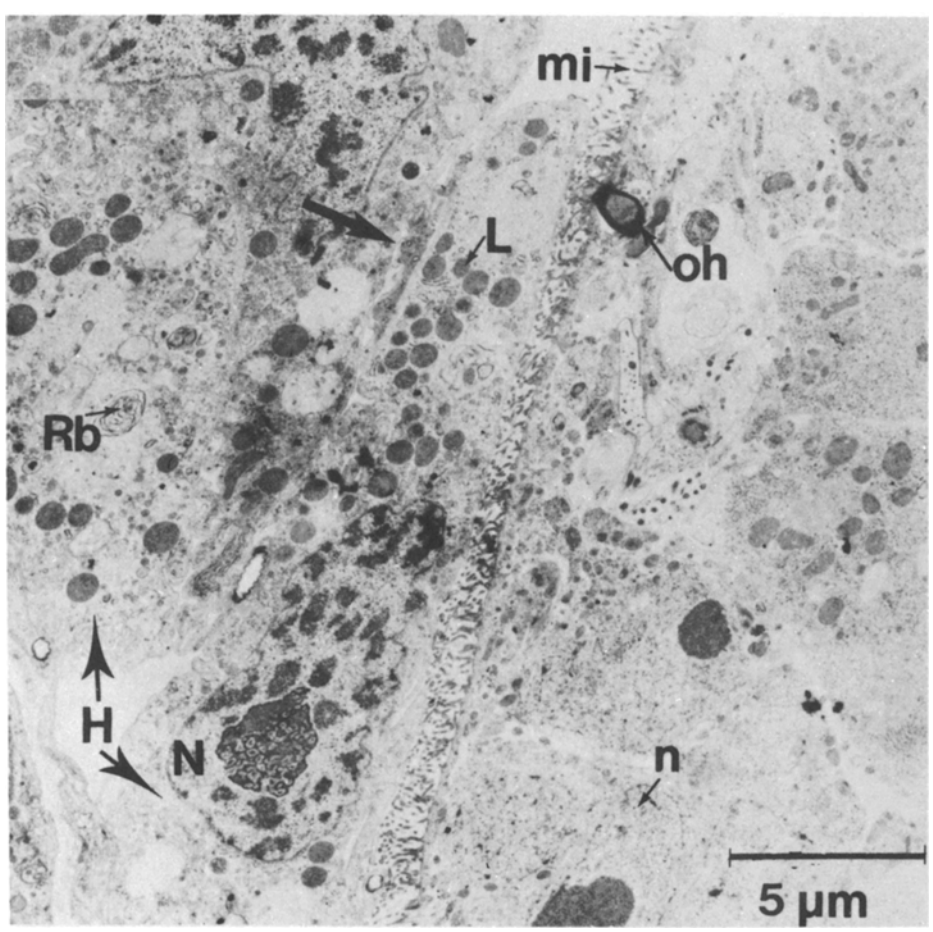

Fig. 5. Transmission electron micrograph of haemocytes adhering to a cestode in a flea reared at 20° C/3 mm Hg, infected for 38 days. *H* haemocyte, *L* presumptive lysosome, *mi* microvilli, *N* nucleus of haemocyte, *n* nucleus of parasite cells, *oh* oncospheral hooks, *Rb* residual body, *arrow* indicates close contact between haemocytes

the flea to live, had no effect on the cestode, Voge and Turner (1956) and Schiller (1959) found *Hymenolepis* spp developed faster in their intermediate hosts if the temperature was raised and Soltice et al. (1971) suggested this may have been mediated through the effect on the parasites' enzyme systems. This probably also occurred with *D. caninum* in *C. f. felis*. This temperature effect explains why Chen (1934) and Venard (1938) observed that most *D. caninum* growth occurred in the adult flea, whereas Marshall (1967) and Joseph (1974) found parasites grew more in pupae since they all used different temperatures. Hence parasite development was not linked to the host development as suggested by the others.

The larval flea diet affected the rate of *D. caninum* development in adult *C. f. felis*. Flanders (1937) and House and Barlow (1961) reported similar effects on other parasites in insects. Unlike Marshall (1967) and Joseph (1974) no crowding effect on the development rate was observed with *D. caninum*. No abnormal forms were seen at the higher temperatures as reported by Voge (1959) for *Hymenolepis diminuta*.

The findings by Chen (1934) on the flea reaction to *D. caninum* correlated with those in this present study only when fleas were reared at 20° C/3 mm Hg. At this temperature, the host reaction was active against more parasites, was more prolonged and increased the longer the adult fleas remained in their cocoons.

De Giusti (1949) and Walker (1959) are the only known workers who connected host reaction with temperature in invertebrate hosts, but they did not investigate or explain this connection. No difference was observed by light or electron microscopy between parasites with adhering haemocytes and those without. Degenerate bodies were seen and they may have been destroyed parasites but they lacked any distinguishing features and hence could not be identified. Lie and Heyneman (1975) stated that encapsulation occurred after the host cells had adhered to the parasites. In fleas, death of the parasite due to the host reaction would explain why the infection rates fell from 100% in larvae to 15% in adult fleas Pugh (1985). However, this fall in infection rates could also result from natural death of non-viable parasites or the unsuitable physico-chemical nature of the flea environment.

TEM observations of *D. caninum* in *C. f. felis* are similar to those observed by Ubelaker et al. (1970a, b), Collin (1970), Lackie (1976), Pesson and Leger (1978), Pesson et al. (1978) with *Hymenolepis* spp in their intermediate hosts. The secretory droplets may be a parasite response (as suggested for *Hymenolepis* spp by Ubelaker et al. 1970b and Collin 1970) or an incidental product of the digestion or excretion of the parasite (as suggested for *H. diminuta* by Lackie 1976). They seemed to be only in the vicinity where haemocytes had adhered to *D. caninum*. They therefore seem to be a response to the haemocytes, although it could not be determined if they were of parasite or host origin. Some parasites in fleas reared at the higher temperatures had degranulated haemocytes attached to them, suggesting either these parasites had fought off the host reaction or the haemocytes were spent. *D. caninum* developed more slowly at 20° C/3 mm Hg and this may have enabled the host to react to the parasite more effectively, although flea development is also slowed as temperature is lowered Pugh (1985). Temperature may affect the enzyme system of the host and the parasite and subsequently the host reaction. Marshall (1967), Yasuda et al. (1968) and Joseph (1974) performed their experiments at 28° C or above and this would explain why they did not see any host reaction in adult fleas.

Although not considered in this paper, the species/strain of host is also an important factor influencing the development of and tolerance to the parasite. The author has studied the development of *D. caninum* and the host reaction to it in other flea species and found that a colony of the dog flea, *Ctenocephalides canis* mounted an effective host reaction which prevented the parasite surviving past the oncospheral stage. However, the development in the rat flea, *Xenopsylla cheopis* and this host reaction to *D. caninum* was very similar to that observed in *C. f. felis* Pugh (1985).

The raised haemocyte counts in the test adult fleas reared at 20° C/3 mm Hg correlated with the higher proportion of parasites with a host reaction than those at 30° C/3 mm Hg. This was in agreement with the work of Nappi and Streams (1969) and Walker (1959) with *Drosophila melanogaster*. Salt (1963) and Wittig (1963) stated that normal parasites did not evoke a host reaction unless they were weak or moribund. From this study, it seems the host-parasite relationship is a dynamic one and the effect of this insect's defence mechanism can be altered by varying conditions, particularly temperature.

Acknowledgements. This work was part of a study carried out for a Ph.D. Thesis, under the supervision of Dr. D.E. Moorhouse, University of Queensland. The author wishes to acknowledge the expert help by Mrs. R. Webb, EM unit, University of Queensland in the preparation of specimens for electron microscopy.

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Accepted June 30, 1986