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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

THE INFLUENCE OF HOST HIBERNATION ON *HYMENOLEPIS CITELLI* (McLEOD, 1933)

A DISSERTATION

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THE INFLUENCE OF HOST HIBERNATION ON

HYMENOLEPIS CITELLI (McLEOD, 1933)

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THE INFLUENCE OF HOST HIBERNATION ON *HYMENOLEPIS CITELLI* (McLEOD, 1933)

INTRODUCTION

Hibernation, which is beneficial for a host species, appears to be a barrier for many of the parasites of such hosts. Kayser (1961) theorized that hibernation increases the resistance of hosts to various parasitic organisms. Such resistance is probably due to mechanical and physiological interferences rather than to antibody-antigen interactions since several reports show a decrease in the general immune response of animals during hibernation or hypothermia (Bisset, 1948; Cahill *et al.*, 1967; Jarslow and Smith, 1961; Schmidt, 1963). Therefore, for the successful continuance as a species in this type of association, the parasite must adapt, in some phase of its life cycle, for survival when the definitive host's core temperature may fall to as low as 2 C. Inevitably, adaptations of a parasitic species to a hibernating host species must occur in the intermediate stages if the adult form does not survive, particularly if the duration of the adult infection is short. Babero (1960) suggested that the extensive larval

migration of Ascaris laevis Leidy, 1856 in Alaskan marmots may be an adaptation to hibernation.

The nature of such a host-parasite association is poorly defined but is obviously complex when the host can survive for several months with a relatively low body temperature, while the parasite succumbs to the cooled environment even though as a species it may have evolved with the host species. The inability to survive wide temperature fluctuations is most evident in those parasites which do not normally occur in hibernating hosts. For example, in Trichinella spiralis (Owen, 1835) development is slowed and viability reduced in hibernating hamsters, dormice, and bats, particularly if hibernation occurs within 72 hours postinfection (Chute, 1960a, 1961, 1964; Chute and Covalt, 1960). Of related interest is the report by Munchberg and Kniewaller (1960) that T. spiralis larvae will survive freezing in pork at -8 C for 45 days. However, a 10 minute exposure at -10 C is reported by Shaver (1952) to have killed T. spiralis larvae. Some parasitic animals or their intermediate stages can be kept viable in a refrigerated water medium with no apparent consequence, while survival time of the same organisms in a hibernating host is reduced (Chute, 1964; Ford and Lang, in press). A preliminary investigation indicated that Fasciola hepatica (Linnaeus) in hibernating Citellus tridecemlineatus tridecemlineatus (Mitchill) is eliminated if the fluke reaches the common

bile duct before the induction of hibernation (Ford and Lang, in press). On the other hand, its metacercariae may survive refrigeration at 7-10 C for a year or more. This information suggests that the concomitant change in host physiology during hibernation may be a greater factor in the survival of a parasite than is the low temperature alone.

Several investigators have attempted to demonstrate the detrimental or beneficial effects of host hibernation on parasites. Blanchard (1903a, b) and Blanchard and Blatin (1907) presented experimental evidence that hibernation in marmots provides protection against helminths in general and particularly against lethal infections of Trypanosoma brucei Plimmer and Bradford. Survival of parasites naturally occurring in hibernators seems to be dependent upon both the species concerned and the duration of continuous hibernation. Simitch and Petrovitch (1954) reported that several intestinal protozoa of Citellus citellus Linnaeus remain in the host during hibernation while others are eliminated, depending on the depth and duration of hibernation. Various helminths are also eliminated within 10 to 25 days after the onset of hibernation. Examination of 13 woodchucks, Marmota monax (Linnaeus), at the end of hibernation by Chute (1960b) revealed several nematodes which probably overwintered in these animals. Dery (1960) demonstrated that growth and development of Schistosomatium douthitti Cort, 1914 are retarded but that reproduction can occur in hibernating ground

squirrels in which periodic arousals occur. Conversely, Nippostrongylus brasiliensis (Travassos, 1914) is found not to survive in hibernating Citellus tridecemlineatus (Cahill et al., 1967). Schmidt et al. (1960) recovered coliform bacilli and fecal streptococci from hibernating ground squirrels and noted that psychrophilic organisms actually increased in some of these animals. Schmidt (1963) found that Coxsacki B-3 virus can survive in hibernating arctic ground squirrels whereas the virus is eliminated from active animals within 5 days after inoculation.

The foregoing reports suggest that survival of parasitic organisms in hibernating hosts depends in part on the temperature tolerance of the parasite species involved plus the depth and duration of the hibernating period. Even though there are several reports on host-parasite relationships among hibernating hosts, Dery (1960) is the only investigator who has attempted to demonstrate analytically the influence of hibernation on a parasite. He critically evaluated the growth and development of S. douthitti in the 13-lined ground squirrel. His work has the undesirable feature of the parasite being abnormal to the 13-lined ground squirrel. This host has been reported by some workers to be refractory to S. douthitti. Most other workers also utilized unnatural parasites in the hibernating host.

My investigation was designed to analyze and define the influence of the hibernating phenomenon in a host

species C. tridecemlineatus on the developmental physiology and survival of its natural parasite, H. citelli. Beginning with the fourth and extending through the 13th day post-infection, the growth rate, development, maturation, and reproduction were observed and recorded under non-hibernating conditions. In addition, certain changes in the major biochemical constituents were analyzed in the worms. Using this as a base with which to make comparisons, the influence of hibernation on the above variables was studied.

MATERIALS AND METHODS

The 13-lined ground squirrel, Citellus tridecemlineatus tridecemlineatus (Mitchill), and Hymenolepis citelli (McLeod, 1933), which parasitizes it, were selected as the model for this investigation. This selection is significant in that H. citelli is a natural parasite in the 13-lined ground squirrel, a hibernating mammal. The model has the advantage of having a parasite which being normal for its host may be assumed to be adapted to surviving the rigors placed upon it by host hibernation.

C. t. tridecemlineatus was obtained by capturing gravid females in late April and early May and allowing them to parturate in the laboratory. All hosts came from the University of Oklahoma golf course and from Lincoln Park in Oklahoma City. Since the hosts were from similar ecological habitats, strain differences were assumed to be minor. Females were housed in separate wire cages provided with nesting material throughout the lactating period. Rations for the lactating females consisted of Purina Laboratory Guinea Pig Chow plus a daily supplement of fresh clover and grass. Young ground squirrels were weaned by 2 months of age and maintained in separate cages during experiments. All

experimental animals were fed Purina Guinea Pig Chow and were 3 months of age by the time experiments were initiated.

H. citelli was isolated from the University of Oklahoma golf course host population, and has been maintained in the laboratory for the past 3 years in hamsters and ground squirrels. Tribolium confusum (Duval) was utilized as the intermediate host in this investigation. The beetles were starved for approximately 1 week and then fed on crushed gravid proglottids for 24 hours. The proglottids were refrigerated at 4 C for 24 hours prior to being fed to the beetles. Cysticercoids were removed from beetles into 0.85% saline 17 days after the beetles were exposed and fed to ground squirrels by gavage.

Four major experiments were conducted. Experiment I was designed to determine the daily growth and developmental pattern of H. citelli during the late summer period, which approximates the maximum feeding period of young squirrels. These hosts also served as summer controls and established a basis for later comparisons. Experiment II was designed to determine the influence of host hibernation on the growth and development of H. citelli. The third experiment was designed to determine the effects of host hibernation on adult worms well established in the host before the induction of hibernation. Experiment IV determined whether H. citelli could survive the winter in a hibernating host.

In the first experiment each host was given 15

cysticercoids. Four animals were necropsied per day from the fourth through the 13th days postinfection. All ground squirrels for a given experiment were necropsied approximately the same time each day. After killing each squirrel with a sharp blow to the head, the small intestine was immediately removed and the worms flushed out with 0.85% NaCl. Worms from a given host were divided into 3 lots of 3 to 5 worms each, using a random numbers table. However, due to the small size of day 4 worms, individual lots were too small for biochemical analysis. It was necessary to pool these lots from all hosts for assaying.

In characterizing the growth and development of H. citelli during the summer months, the initial growth periods were not observed since large numbers of hosts and massive infections would have been necessary to obtain maximal recovery of small worms. Therefore, observations began on the fourth day postinfection and continued through the 13th day.

From each host 2 lots were washed 5 times in 0.85% saline, weighed, and quick-frozen at -20 C. The third lot was relaxed and killed in water heated to 70 C. They were then fixed in alcohol-formalin-acetic acid, stained in Meyer's paracarmine and mounted. These specimens were used for noting physical measurements, proglottid counts, organogeny, and sperm and ova production.

The first of the 2 lots of frozen worms was used for total protein, glycogen, and alcohol soluble

carbohydrate analysis, while the second sample was used for total lipid analysis. The first sample was dried in tared foil pans at 90 C for 24 hours and then dry weight determined. The sample was proportionately diluted with distilled water and homogenized in a glass tissue grinder for 5 minutes or longer. The second lot was homogenized in a 2:1 chloroform-methanol mixture for lipid extraction.

For glycogen determination the method of Kemp and van Heijningen (1954) was used and a phenol-sulfuric acid colorimetric test was employed for alcohol soluble sugars determination (Dubois et al., 1956). Protein was determined by the method of Lowry et al. (1951). A gravimetric method modified from the extracting and washing procedures described by Folch et al. (1957) was used for total lipid determination.

Experiment II was conducted during the winter months when hibernation normally occurs in the ground squirrels. A pilot experiment indicated that growth, development, and recovery of H. citelli are greatly suppressed during host hibernation. Further, it had been found impossible to predict whether young ground squirrels will enter hibernation within a short period of time after exposure to cold. These variables necessitated large numbers of hosts for any given experiment. For these reasons, 3 days (fifth, ninth, and 13th days postinfection) were arbitrarily selected for the experimental phase. The fifth day was selected as the

earliest age when worms would be most easily processed, while the time just before patency of worms in non-hibernating hosts was selected for the ninth day. The 13th day was selected because it coincides with the oldest age of the worms in the summer controls. Fifteen ground squirrels were used for each day selected and all animals were exposed to 15 cysticercoids each. Control hosts were selected as those which did not hibernate for the duration of any one experimental period. Those animals that did hibernate were used as experimentals. Cestodes recovered from each host were divided into 3 lots and analyzed as described in Experiment I. Where samples were insufficient for individual analysis, they were pooled with those from other hosts.

Hibernation was induced by placing the squirrels in a walk-in cooler at 6 ± 1 C. All animals were caged individually with nesting material and placed in the cold room 24 hours postinfection. The animals were checked twice daily, and to determine whether a squirrel had been awake during the interval between checks, wood shavings were placed over its body after it had entered hibernation. Dislodged shavings were taken to indicate that hibernation had been interrupted.

The third experiment was designed to determine the effects of host hibernation on adult worms well established in the host. Twenty-five ground squirrels were exposed to 10 cysticercoids each. At 20 days postinfection all

squirrels had patent cestodes, determined by the presence of ova in the feces. At this time 8 ground squirrels were selected as controls and kept at room temperature (ca. 25-28 C). The remaining squirrels were placed in the cooler at 6 C and observed daily. All animals did not enter hibernation at the same time and the periods of uninterrupted hibernation were not equal. Consequently, animals were selected for observation which had approximately equal hibernating periods. They were divided into 2 groups with 4 experimentals and 4 controls in each group. The first group was necropsied after 10 days of refrigeration. Upon necropsy worms were divided into 2 lots of 3 to 5 worms per lot and analyzed for change in biochemical composition as described earlier. No worms from this experiment were stained and mounted.

In the fourth experiment, 18 squirrels were infected in early August, 1966 and placed in the cold room 20 days later. These animals were caged separately and observed daily as described above. They served to determine whether H. citelli could survive experimentally several months in a hibernating host. Fecal checks were made periodically for 4 weeks to determine whether the worms were aborted. They were removed from the cold room on March 1, 1967.

All of the experimental groups had food and water ad libitum. Where applicable, Students's t-test was used to test the significance between the means of analytical

results. In other cases, one and two-way analyses of variance were applied to the data. Where the data were expressed as percentages, significance was tested by converting the percent values to the arcsine before applying the analysis of variance test (Steele and Torrie, 1960). Probability values of .05 or less were considered significant.

RESULTS

Experiment I

Physical Measurements

Results of this experiment provided worms which served as a basis for comparisons with both winter controls and hibernating experimentals. Mature proglottids were present by the fourth day. Genital organs were well developed, including the seminal vesicles and seminal receptacles, in the most posterior proglottids. By the fifth day immature ova were present in the uterus, and sperms were in the seminal receptacles of the last 2 or 3 proglottids. Completely shelled ova were present in 8 day old worms. The 11th was the earliest day when egg liberation occurred *en mass* and is considered herein as the day of patency.

Table I shows the number of hosts infected and worm recovery rates per day in each experimental group. Summer control animals showed a 67% to 97% recovery rate for any given day during the experimental period. In no case was there 100% recovery from all hosts on any given day. However, all squirrels became infected. The low recoveries of 12 and 13 day old worms cannot presently be explained.

TABLE I. Number of hosts infected with H. citelli and recovery rate per day in each group. Hosts received 15 cysticercoids each.

Days Post-infection	Summer Controls			Winter Controls			Experimentals		
	No. of Hosts	No. of Worms Recovered	% Recovery Per Day	No. of Hosts	No. of Worms Recovered	% Recovery Per Day	No. of Hosts	No. of Worms Recovered	% Recovery Per Day
4	4	53	88.5	.	..	. .	..	..	. .
5	4	58	96.8	7	80	76.2	7	1	1.0
6	4	51	85.0	.	..	. .	..	..	. .
7	4	49	82.7	.	..	. .	..	..	. .
8	4	58	96.8	.	..	. .	..	..	. .
9	4	57	95.0	4	35	58.3	11	59	35.8
10	4	54	90.0	.	..	. .	..	..	. .
11	4	54	90.0	.	..	. .	..	..	. .
12	4	40	66.6	.	..	. .	..	..	. .
13	4	40	66.6	2	21	70.0	10	57	38.9

TABLE II. Physical measurements of H. citelli from non-hibernating summer squirrels, (n) = number of worms in sample.

Days Post-infection	Fresh Weight[a]	Dry Weight[a]	D/W[b]	(n)	Total Length[c]
4	0.41	0.131	0.317	(40)	16.6 ± .81
5	2.14	0.422	0.200	(20)	24.8 ± .93
6	5.64	1.206	0.207	(24)	42.9 ± 2.00
7	10.8	2.45	0.224	(21)	58.6 ± 2.16
8	16.3	3.80	0.229	(20)	65.5 ± 6.88
9	15.1	3.28	0.215	(20)	68.4 ± 6.59
10	24.2	5.40	0.218	(20)	74.1 ± 5.08
11	29.8	6.67	0.221	(18)	89.9 ± 9.51
12	32.8	7.73	0.236	(17)	103.7 ± 10.52
13	35.5	7.51	0.211	(19)	120.0 ± 11.60

[a]Expressed as the average weight in mg per worm.

[b]D/W means the dry weight-fresh weight ratio.

[c]Expressed as the mean length of worms in mm ± standard error of the mean.

[d]Expressed as the mean number of proglottids ± standard error of the mean.

TABLE II. Continued

Immature Proglottids[d]	Mature Proglottids[d]	Gravid Proglottids[d]	Total Proglottids[d]	(n)
131 ± 6.3	45 ± 4.6	0	173 ± 10.0	(13)
133 ± 4.2	124 ± 3.7	0	256 ± 6.4	(18)
158 ± 4.6	237 ± 7.8	0	388 ± 16.9	(12)
214 ± 12.6	299 ± 18.7	0	514 ± 24.2	(13)
290 ± 22.6	278 ± 27.0	5 ± 2.0	574 ± 37.1	(18)
211 ± 12.1	229 ± 20.6	22 ± 3.7	484 ± 47.8	(17)
256 ± 12.4	278 ± 21.5	28 ± 7.5	556 ± 21.1	(16)
275 ± 20.9	307 ± 26.4	48 ± 14.5	631 ± 38.2	(18)
247 ± 3.5	325 ± 18.2	110 ± 14.0	671 ± 28.9	(10)
316 ± 20.9	325 ± 29.7	68 ± 16.4	731 ± 14.3	(12)

As the worms matured in non-hibernating hosts under summer conditions, there was an increase in all values of physical measurements (Table II). One area of interest is the dry weight-fresh weight ratio for 4 day old worms. This high value is the average of all worms from 3 separately pooled lots, the ratios of which ranged from 0.302 to 0.338. High dry weight percentages are of interest since Roberts (1961) obtained varying ratios in H. diminuta with values of 0.197 to 0.288 depending on the worm population per host.

The growth curves based on length and fresh weight for H. diminuta and H. citelli are in close agreement (Figs. 1, 2; Table II). The curves were fitted to the mean points. The greatest relative growth rate for the period plotted is the fourth through the sixth days. Thereafter growth in length and fresh weight declined as patency was approached. On the curves for H. citelli in Figures 1 and 2, the length and fresh weights of 9 day old worms are less than for 8 day old worms. All worms except those from 1 host were small for this day, indicating influences from unknown factors. H. diminuta data are taken from Roberts (1961) for 15 to 20 worms per host. This cestode showed a similar pattern of growth. Initially it is smaller than H. citelli but surpasses the latter in length by the seventh day and in weight by the eighth day postinfection. It is apparent that after 8 days relative growth decelerates and approaches a maximum by 13 days postinfection in H. citelli.

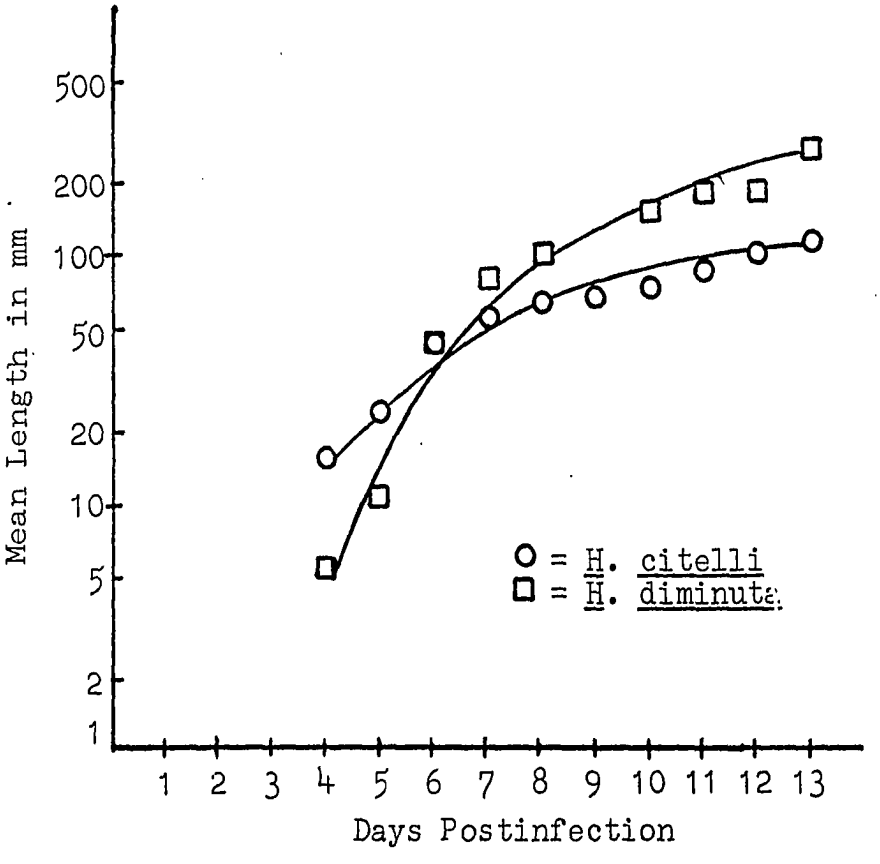

Figure 1. Growth curves in length for H. citelli and H. diminuta from 4-13 days postinfection in non-hibernating hosts. H. diminuta plotted from data by Roberts, 1961.

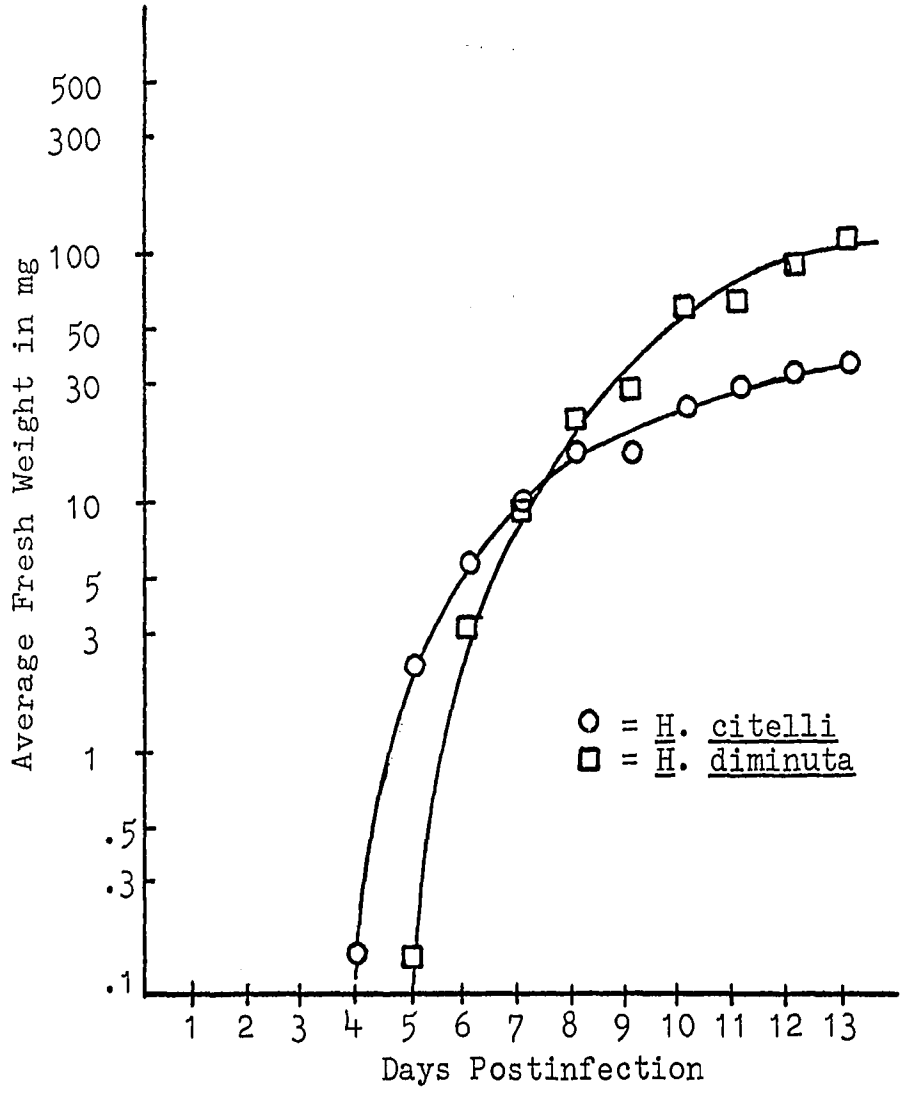

Figure 2. Curves of growth in fresh weight for H. citelli and H. diminuta. H. diminuta data from Roberts, 1961.

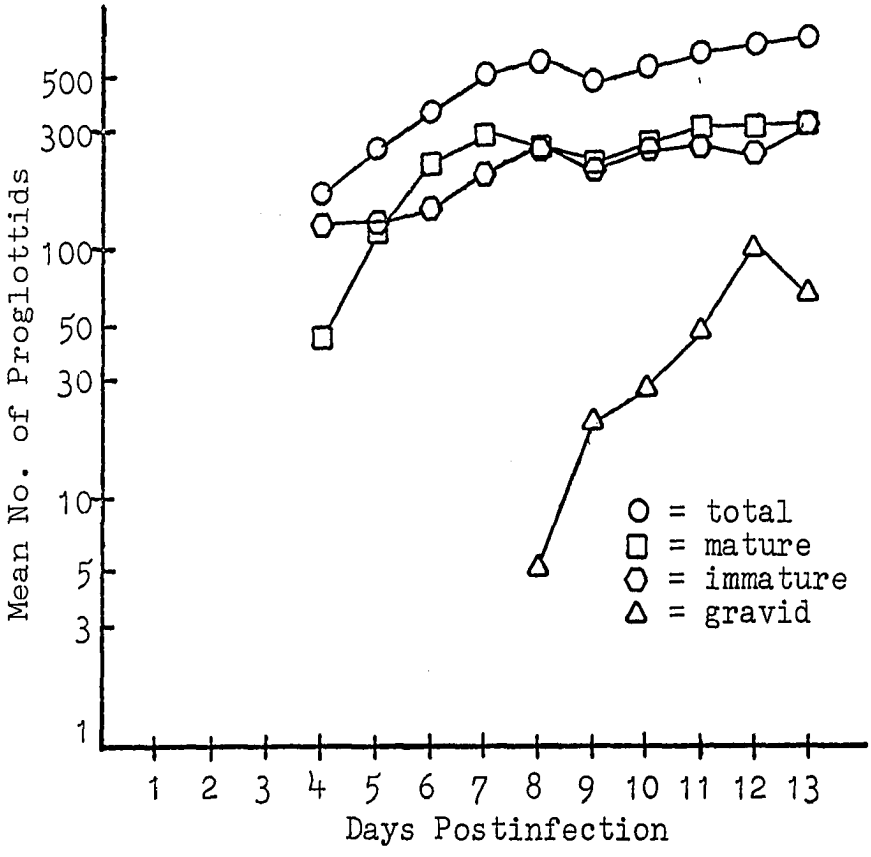

Figure 3. Increase in the number of proglottid types in worms from non-hibernating squirrels during summer months.

Differential proglottisation (Fig. 3; Table II) occurs early in H. citelli. Immature and mature proglottids are present by the fourth day. Immature proglottids increase gradually between the fourth and eighth days of observation but thereafter approximate the number of mature proglottids. There is a rapid increase from day 4 through day 6 in mature proglottids. Thereafter a near maximum is reached and a decline in the number of mature proglottids occurs in the seventh and eighth days as the most posterior proglottids become gravid. For a similar worm load (15-20 per host) Roberts (1961) observed that mature proglottids appear on the sixth day in H. diminuta and gravid proglottids are present by day 13. There is a very rapid increase in the number of gravid proglottids of H. citelli through day 12 but the number declines on day 13 as the most posterior proglottids are shed. The total number of proglottids increases exponentially from the fourth through the seventh days, but reaches a plateau after 8 days. The decline observed in all proglottid types between the eighth and tenth days is influenced by several factors and is discussed later.

Chemistry

With the exception of alcohol soluble sugars, which varies considerably, all biochemical components gradually increase in absolute amounts as worm size increases. Glycogen is initially low (7-10% of dry weight) and increases

to more than 36% on the later growth days (Table III; Fig. 4). Alcohol soluble carbohydrates are initially near 10% but decrease to less than 2%. The alcohol soluble sugars increased dramatically on the seventh day. Since all samples were treated similarly on any given day and were collected at approximately the same time, no definite explanation can be given for the increase. Total carbohydrates range from about 17% in 4 day old worms to about 40% in gravid worms. This is in close agreement with data from other workers (Read and Rothman, 1957; Roberts, 1961). Figure 4 illustrates the relative changes in the carbohydrates. The highest relative value observed for protein is 60.6% in 4 day old worms. This declines to 37% in 13 day old cestodes. Lipids are also initially higher on the fifth and sixth days (29-31%) but decrease to less than 19% as age increases.

Experiment II

Since young ground squirrels were used in this investigation, hibernation was not uniform for all animals used in any given experiment. Many of the animals appeared to undergo "conditioning" responses to hibernation as the body temperature decreased gradually over several days with frequent arousals. Strumwasser (1960) observed the same phenomenon in California ground squirrels. For this reason it is difficult to assess the actual initiation of hibernation for the animals in this experiment. If an animal was

TABLE III. Biochemical analysis of H. citelli from summer control squirrels, expressed as per cent of dry weight. (n) = number of worms in samples.

Days Post-infection	Glycogen	Alcohol Soluble Sugar	Total Carbohydrates	Protein	(n)	Total Lipid (Based on the Calculated Dry Weight)	(n)
4	10.18	6.89	17.07	60.65	(40)	. .	. .
5	7.31	9.60	16.91	50.48	(20)	29.42	(20)
6	9.79	5.56	15.35	54.03	(24)	31.13	(15)
7	12.45	17.46	29.91	42.62	(21)	21.46	(15)
8	22.32	9.72	32.04	42.99	(20)	20.16	(20)
9	27.29	1.92	29.21	51.41	(20)	17.68	(20)
10	35.48	3.16	38.64	40.00	(20)	16.46	(18)
11	40.19	1.16	41.35	37.59	(18)	18.74	(18)
12	27.41	1.80	29.21	39.01	(17)	17.66	(17)
13	36.07	2.68	38.75	37.35	(19)	19.28	(19)

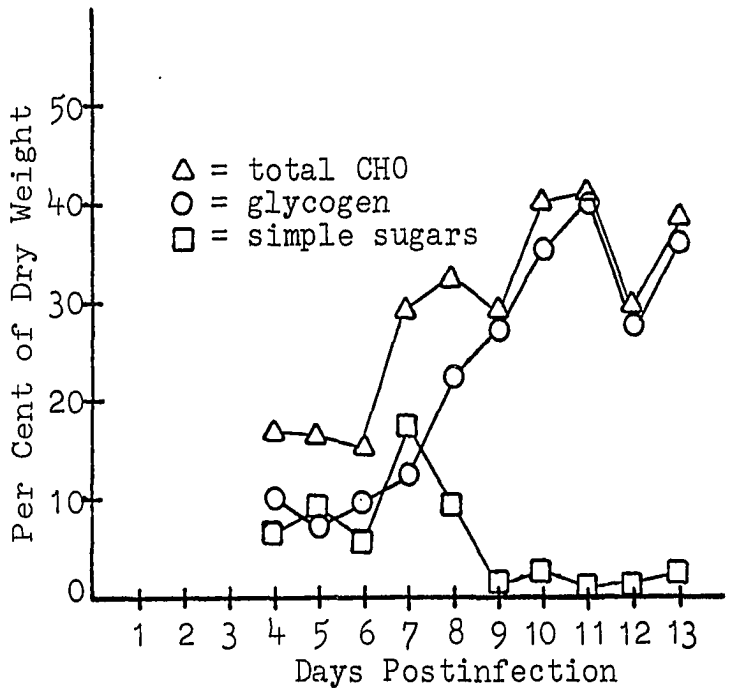

Figure 4. Relative changes in carbohydrate composition of H. citelli from 4-13 days postinfection during non-hibernating summer conditions.

observed to have aroused and moved about, it was not recorded as having hibernated on that day. Ground squirrels used in the 5 day period hibernated an average of 2.5 days per animal, those for the 9 day period averaged 4.4 days, and those in the 13 day period averaged 7.4 days. In the latter 2 groups, the longest period of uninterrupted hibernation for a single animal was 7 days.

Since it takes several hours for H. citelli to establish itself in the host's gut, 24 hours were allowed to elapse between exposure of the squirrels to it and the time when the squirrels were placed in the cold room. Its ability to remain established in both hibernating and non-hibernating hosts is indicated in the recovery rates shown in Table I. There is no significant difference between the recovery rates of summer and winter controls but the latter rates are slightly lower because of several possible influences reviewed in the Discussion. However, there is a significantly greater recovery rate of the 2 control groups over that of the experimental group ranging from 96.8% to 66.6% in the summer controls and 76.2% to 58.3% in the winter controls as compared to 1.0% to 38.9% for the experimentals. A correlation is evident between recovery rates and the time the host enters hibernation after infection (Table IV). If hibernation occurs within 72 hours postinfection, survival of H. citelli is greatly reduced. Controls were infected at the same time the experimentals and the 83% recovery

TABLE IV. Relationship between recovery rate of *H. citelli* and time postinfection that hosts entered hibernation.

Time in Hours Hosts Entered Hibernation Postinfection	Number of Infected Hosts	Number of Non-infected Hosts	Number of Worms Recovered	% of Recovery
24 - 48	1	6	1	1.0
49 - 72	1	6	6	5.7
73 - 96	2	1	24	53.3
97 - 144	2	0	11	36.7
145 - 192	4	0	41	68.3
193 - 322	3	2	23	30.6
Controls	12	1	151	83.3

for controls reflects the importance of the non-hibernating physiology of the host to worm survival during this period.

On day 5, all the squirrels which hibernated had done so within 48 hours postinfection. Only 1 worm was recovered from 7 hibernating hosts on that day. Because of this no data were collected for experimentals on day 5 since the 1 worm did not provide enough tissue to give reliable results for any one measurement in this experiment. However, this demonstrates that worms must reach a given stage of maturity before hibernation or they will not survive.

Physical Measurements

If hibernation has a marked effect on cestode metabolism, the influence should be reflected clearly on its growth and development. A comparison of worms from summer and winter control squirrels or of both controls with hibernating experimentals indicates this to be true (Table V). Nearly all physical measurements analyzed are significantly different with probability values between .0005 and .05, whether the comparison is between summer and winter controls or between winter controls and experimentals. The average length of H. citelli from experimental hosts is significantly less than that of those from all controls. What may not be expected is the highly significant differences between the lengths of winter and summer control groups. A plausible explanation is given in the Discussion. The

TABLE V. Physical measurements of *H. citelli* from non-hibernating and hibernating squirrels, ± standard error of the mean. (n) = number of worms in samples.[a]

	Average No. Days Host Hibernated	Total Length in mm	Number of Immature Proglottids	Number of Mature Proglottids	Gravid Proglottids	Total Proglottids	(n)
5 Days Postinfection							
Summer Controls	0	24.8 ± .93	133 ± 4.2	124 ± 3.7	..	256 ± 6.4	(18)
Winter Controls	0	10.7 ± 1.06	72 ± 8.0	30 ± 5.6	..	94 ± 11.8	(24)
9 Days Postinfection							
Summer Controls	0	68.4 ± 6.59	211 ± 12.1	229 ± 20.6	22 ± 3.7	484 ± 47.8	(17)
Winter Controls	0	42.6 ± 4.30	155 ± 15.4	197 ± 28.4*	..	373 ± 7.6*	(12)
Experimentals	2	21.5 ± 2.70	81 ± 9.4	88 ± 19.0	..	189 ± 27.8	(18)
13 Days Postinfection							
Summer Controls	0	120.0 ± 11.60	316 ± 20.9	325 ± 29.7	68 ± 16.4	731 ± 14.3	(12)
Winter Controls	0	52.3 ± 5.82	200 ± 10.7	183 ± 16.3	38 ± 8.9*	421 ± 35.3	(6)
Experimentals	8	17.3 ± 2.38	110 ± 11.6	59 ± 12.4	..	169 ± 23.5	(12)

[a]Except those marked (*), all means are significantly different from summer controls between .0005 and .05 Probabilities.

average lengths of the summer controls are over 1 1/2 to nearly 2 1/2 times those of the winter controls and are approximately 3 to 7 times longer than the experimental groups. It is interesting that winter controls fall midway between the summer control and experimental groups in average length.

There is a significant difference in weights between summer controls and the experimentals ($P < .05$) of 9 day old worms, but not between the summer and winter controls or between the winter controls and the experimentals (Table VI). In 13 day old worms, all 3 groups differ significantly in weight ($P < .05$). The average fresh weights of summer control worms are approximately 9 to 70 times greater than that of the experimentals.

Proglottisation is also affected. While there is a gradual increase in the numbers of immature proglottids from the fifth through 13th days, the number in the experimentals is significantly less than in the controls (Table V). Mature proglottids appeared in all groups. Sperm and ova production had occurred in less than 33% of the 5 day old worms used as winter controls as compared to 100% in the summer controls. In 9 day old winter control worms, ova and sperm production had occurred in all specimens and the seminal receptacles were distended with sperm. In contrast, worms from hibernating hosts produce no sperm or ova if hibernation occurs in the first 4 or 5 days of worm growth.

TABLE VI. Average fresh and dry weights in mg of H. citelli from hibernating and non-hibernating hosts.

Days Post-infection	Summer Control Group				Winter Control Group				Hibernating Group			
	Fresh Weight	Dry Weight	D/W[a]	(n)	Fresh Weight	Dry Weight	D/W[a]	(n)	Fresh Weight	Dry Weight	D/W[a]	(n)
5	2.14	0.422	0.200	(20)	0.25	0.081	0.327	(57)	. .	. .	. .	..
9	15.1	3.28	0.215	(20)	7.10	1.551	0.223	(15)	1.58	0.336	0.202	(27)
13	35.5	7.51	0.211	(19)	13.7	3.63	0.234	(8)	0.51	0.142	0.276	(24)

[a]D/W means the dry weight-fresh weight ratio.

In worms from squirrels beginning hibernation on the seventh or eighth days after infection, small immature ova were present as were sperm in the seminal vesicles. Sperm and ova production occurred in 13 day old winter control worms, as indicated by well formed oncospheres being present in the ova. The number of total proglottids in 9 and 13 day summer control worms is approximately 3 to 4 times that of the respective experimental worms (Table V). Even though the mean number of the other proglottid types differs significantly between the 2 control groups, there is no significant difference between the number of gravid proglottids in the 2 control groups of 13 day old worms. In the experimentals of this same age group, the number of mature proglottids is significantly less than in the controls. Also sperm and ova production is greatly reduced and comparable to that in 5 day old summer controls. No gravid proglottids were seen in any hibernating group for the duration of the experiment.

As shown in Tables V and VI, 9 day old experimentals are larger than 13 day old experimental worms. This is related to the fact that those squirrels harboring the 13 day old worms hibernated an average of 8 days with an average of 5 non-hibernating days per host, whereas the squirrels harboring 9 day old worms hibernated on the average of 2 days with an average of 7 non-hibernating days per squirrel.

While the dry weight-wet weight ratios do not differ significantly, the tendency is toward higher ratios in

cestodes from both the hibernating and non-hibernating squirrels. This suggests that the worms are slightly dehydrated in winter control hosts and hibernating hosts. Such seems plausible since the gut contents of the hosts in both groups are mostly enterohepatic circulation, succus entericus, and mucus which may be hypertonic to the worm tissue.

Chemistry

The general biochemistry of the cestodes was followed to determine whether host hibernation produces changes in their over-all metabolism. If metabolism continues during hibernation at relatively high rates, a reduction in the total carbohydrate and protein content would be expected during that time since host food intake is greatly reduced during the dormant periods. However, such was not the case.

In Table VII a general increase in the absolute amounts of glycogen and low molecular weight carbohydrates with increased worm size is evident. Thirteen day old worms from hibernating squirrels contained 0.046 mg of glycogen per worm as compared to 2.717 mg per worm in summer controls or 1.651 mg per worm in winter controls. Based on the per cent of dry worm weight, glycogen and alcohol soluble sugars (Table VIII) are similar for the control groups. Glycogen concentration in the experimental group (18.29% for 9 day olds and 32.64% for 13 day old worms) is only slightly lower

TABLE VII. Average carbohydrate composition in mg per worm from hibernating and non-hibernating squirrels.

Days Post-infection	From Non-hibernating Summer Hosts			From Non-hibernating Winter Hosts			From Hibernating Hosts		
	Glycogen	Alcohol Soluble Sugars	(n)	Glycogen	Alcohol Soluble Sugars	(n)	Glycogen	Alcohol Soluble Sugars	(n)
5	0.034	0.040	(20)	0.009	0.010	(57)	. .	. .	..
9	1.125	0.054	(20)	0.423	0.042	(15)	0.076	0.012	(23)
13	2.717	0.199	(19)	1.651	0.114	(8)	0.046	0.017	(24)

TABLE VIII. Carbohydrate composition of H. citelli from hibernating and non-hibernating squirrels, expressed as per cent of dry weight.

Days Post-infection	From Non-hibernating Summer Hosts		From Non-hibernating Winter Hosts		From Hibernating Hosts	
	Glycogen	Alcohol Soluble Sugars	Glycogen	Alcohol Soluble Sugars	Glycogen	Alcohol Soluble Sugars
5	7.31	9.60	10.83	9.99	. .	. .
9	27.29	1.92	21.87	3.73	18.29	5.98
13	36.07	2.68	45.52	3.15	32.64	11.76

as compared to the 2 control groups which contained 21-27% in 9 day olds and 36-46% in 13 day old worms. The per cent of alcohol soluble sugars is higher in the experimental worms than in the control groups, but is significantly higher only in the 13 day old experimental worms as compared to the controls ($P < .05$).

Figure 5 shows the relative differences in composition of the total carbohydrates as glycogen and alcohol soluble sugars between summer controls and worms from hibernating hosts. There is no significant difference between the carbohydrate content of summer and winter controls (Table IX) so the latter was not included in Figure 5. Alcohol soluble sugars constitute the major portion of total carbohydrates in 5 day old worms for both summer and winter controls (Table IX). Glycogen is the major carbohydrate component in all 3 groups in 9 and 13 day old worms, but the alcohol soluble portion constitutes a significantly larger percentage in worms from hibernating animals than it does in the summer control group ($P < .05$).

Based on a direct method of measurement, protein concentrations in the experimentals and winter controls are less than in summer controls (Fig. 6; Table X). Statistically, the per cent of protein in 9 day old worms from hibernating hosts is no different from the summer controls. In 13 day old worms the protein concentrations in both winter controls and experimentals are significantly below the summer

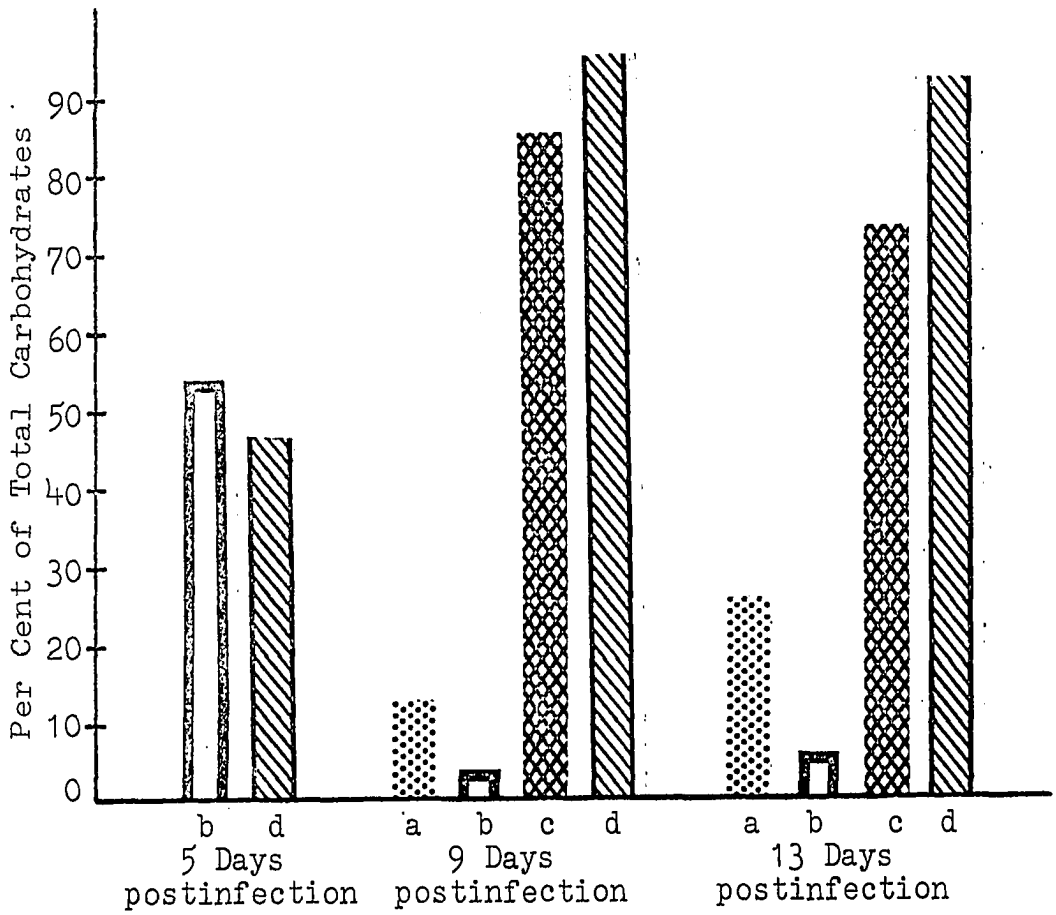

Figure 5. Relative changes in composition of glycogen and alcohol soluble sugars in worms from hibernating and non-hibernating animals. (a) = alcohol soluble sugars, hibernating hosts. (b) = alcohol soluble sugars, non-hibernating hosts. (c) = glycogen, hibernating hosts. (d) = glycogen, non-hibernating hosts.

TABLE IX. Glycogen and alcohol soluble sugars of H. citelli in hibernating and non-hibernating hosts, expressed as per cent of total carbohydrates.

Days Post-infection	Summer Control Group			Winter Control Group			Hibernating Group		
	Average Total Carbohydrates (mg) Per Worm	% Glycogen	% Alcohol Soluble Sugars	Average Total Carbohydrates (mg) Per Worm	% Glycogen	% Alcohol Soluble Sugars	Average Total Carbohydrates (mg) Per Worm	% Glycogen	% Alcohol Soluble Sugars
5	0.074	45.68	54.32	0.020	48.55	51.45	. .	. .	. .
9	1.168	96.38	3.62	0.465	90.98	9.02	0.088	86.27	13.73
13	2.916	93.32	6.68	1.765	93.53	6.47	0.063	73.52	26.48

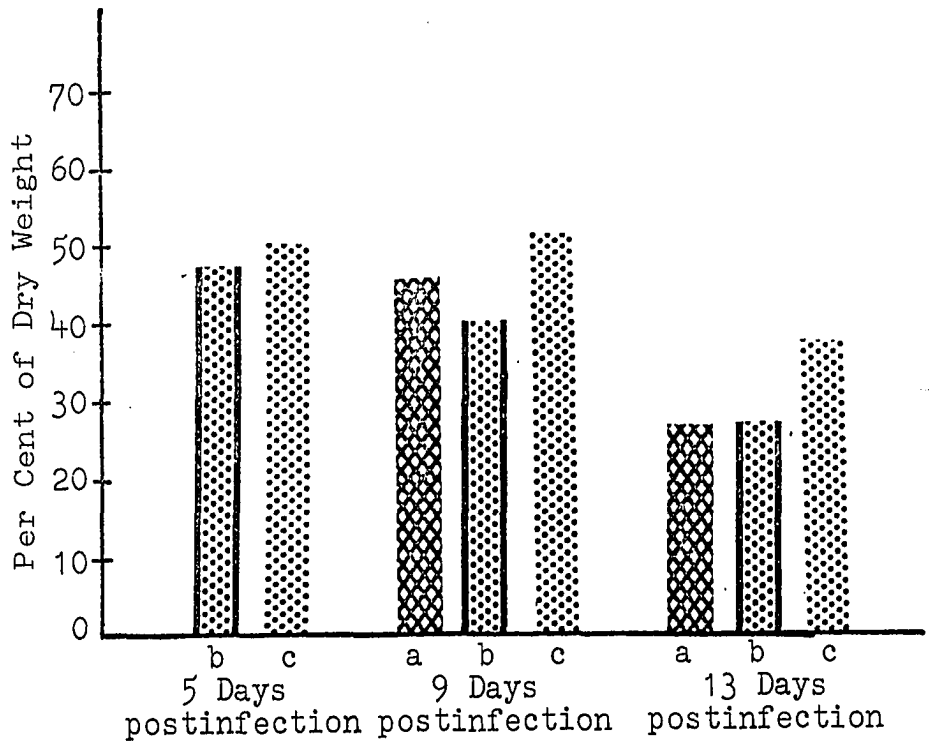

Figure 6. Relative changes in protein composition in worms from hibernating and non-hibernating hosts. (a) = worm samples, experimentals. (b) = worm samples, winter controls. (c) = worm samples, summer controls.

TABLE X. Protein in H. citelli from hibernating and non-hibernating squirrels, (n) = number of worms in samples.

Days Post-infection	From Non-hibernating Summer Group			From Non-hibernating Winter Group			From Hibernating Group		
	Mg Per Worm	(n)	% of Dry Weight	Mg Per Worm	(n)	% of Dry Weight	Mg Per Worm	(n)	% of Dry Weight
5	0.213	(20)	50.48	0.037	(57)	47.64	. .	..	. .
9	1.545	(20)	51.41	0.587	(15)	40.44	0.142	(23)	46.16
13	2.826	(19)	37.35	1.017	(8)	28.06[a]	0.040	(24)	28.19[a]

[a]Significantly different from summer controls with $P. < .005$.

control group. Based on the per cent of wet weight, there is no difference between lipid content in the control groups and the experimental groups (Table XI).

Experiment III

This experiment was conducted to determine the influence of host hibernation on the chemistry of adult worms which were gravid and well established in the ground squirrels. Fifteen ground squirrels, each harboring mature 20 day old worms, were placed in the cold room at 6 C. Six days later 4 of these animals, which hibernated an average of 4 days, and 4 control hosts were necropsied. These animals are designated as Group I. Ten days after placing the squirrels in the cold room 4 additional squirrels, which hibernated an average of 6.5 days, and 4 control animals were necropsied. These animals constitute Group II. Tables XII and XIII show the results of this experiment.

As shown in Table XII the fresh and dry weights of the experimental worms are significantly less than that of the control groups ($P < .005$). However, there is no significant difference between weights of the 2 control groups or between weights of the 2 experimental groups even though Group II is larger. Except for glycogen and total carbohydrates, there is no difference between the concentration of the other biochemical compounds in control and experimental worms in either group (Tables XII, XIII). Group I experiments contain about 6% less glycogen and total carbohydrates

TABLE XI. Total lipids per worm in hibernating and non-hibernating squirrels.

Days Post-infection	Expressed in mg					Expressed as Per Cent of Fresh Weight				
	Summer Controls	Winter Controls	(n)	Experimentals	(n)	Summer Controls	Winter Controls	(n)	Experimentals	(n)
5	0.120	. .	. .	. .	. .	6.17	. .	. .	. .	. .
9	0.499	0.674	(8)	0.130	(12)	3.80	7.08	(8)	6.50	(12)
13	1.373	0.545	(7)	0.070	(4)	4.06	4.44	(7)	5.60	(4)

TABLE XII. Composition in mg of adult worms from non-hibernating and hibernating squirrels, (n) = total number of worms in samples.

	No. of Hosts	Average No. Days Hosts Hibernated	Fresh Weights	Dry Weights	Dry-to-Fresh Weight Ratio	Glycogen	Soluble Sugars	Total Carbohydrates	Protein		Total Lipid	
						Group I[a]						
										(n)		(n)
Experimentals	4	4.0	19.4	3.82	0.199	1.330	0.379	1.710	0.731	(18)	1.190	(19)
Controls	4	0	35.2	8.04	0.223	3.612	0.641	4.253	1.116	(24)	2.402	(18)
						Group II[b]						
Experimentals	4	6.5	23.1	5.50	0.232	2.552	0.266	3.358	1.078	(12)	1.157	(9)
Controls	4	0	40.5	8.92	0.219	4.976	0.304	5.285	1.723	(16)	1.621	(15)

[a]Group I represents cestodes from squirrels allowed to remain in cooler at 6 C for 6 days.

[b]Group II represents cestodes from squirrels allowed to remain in cooler at 6 C for 10 days.

TABLE XIII. Composition of adult H. citelli from non-hibernating and hibernating squirrels, expressed as per cent of dry weight.

	Glycogen	Alcohol Soluble Sugars	Total Carbohydrates	% of Total Carbohydrates as Glycogen	Protein	Total Lipids[a]
			Group I[b]			
Experimentals	34.39	10.22	44.61	77.85	18.58	5.33
Controls	40.10	9.96	51.22	84.92	17.82	6.83
			Group II[c]			
Experimentals	42.56	5.96	48.54	75.98	16.16	6.66
Controls	56.27	3.57	60.68	94.15	18.75	3.96

[a]Expressed as per cent of wet weight.

[b]Group I represents cestodes from squirrels allowed to remain in cooler at 6 C for 6 days.

[c]Group II represents cestodes from squirrels allowed to remain in cooler at 6 C for 10 days.

than the control worms. Statistically, the difference is not significant. Experimental worms in Group II are from squirrels which hibernated 2.5 days longer than the Group I squirrels. Therefore, the experimental worms have nearly 14% less glycogen ($P < .025$) and 12% less total carbohydrates ($P < .005$) than do the controls. It is interesting that glycogen in the Group I experimentals is 77.8% of the total carbohydrates while glycogen in the controls constitutes 84.9% of the total carbohydrates, a difference of 7.1%. In Group II glycogen constitutes nearly 76% of the total carbohydrates in the experimentals and 94% in the controls. This is a difference of 18% as compared to about 7% in Group I.

Experiment IV

To determine whether H. citelli survives the winter in an experimentally induced hibernating host, 18 ground squirrels were infected and placed in the cold room at 6 C 20 days postinfection. A check of the feces for worm ova prior to inducing hibernation, indicated that all squirrels were infected. They were induced to hibernate by August 20, 1966. All feces from these animals were checked for a 4 week period after cold exposure.

All animals were hibernating within 1 week after placing them in the cooler. Daily checks indicated that different hosts hibernated for continuous periods of from 3

to 14 days. Squirrels would usually arouse for only a few hours, defecate, perhaps eat, and then re-enter hibernation.

A check of fecal pellets after arousal periods indicated that worms were being destrobulated after 3 weeks. No scoleces were observed during this time when strobulae appeared in the feces. After 4 weeks no ova were found in feces of any animal.

After 60 days in the cooler, 6 animals were removed and 3 were necropsied, while the remaining 3 were maintained at room temperature. The latter were fed and watered ad libitum. No worms were found in the intestinal tract of the necropsied squirrels. A check of the fecal pellets of the 3 squirrels kept at room temperature contained ova 2 weeks after removal of the animals from the cold room, indicating the squirrels were still infected.

The 12 animals left in the cold room were removed to a warm temperature room on March 1, 1967, after 6 months of discontinuous hibernation. An immediate check of the feces showed no evidence of an infection. However, within 13 days ova of H. citelli appeared in the feces of 50% (6 squirrels) of the animals. This indicates that the scoleces were left in the host's intestine and regenerated their strobulae after conditions became favorable.

DISCUSSION

The specific growth curves of H. citelli are similar to those reported for H. diminuta by Roberts (1961). The initial larger size of H. citelli is indicative of its earlier maturation and ova liberation as compared to H. diminuta. Read (1959) reported that H. citelli is intermediate in this respect between H. nana and H. diminuta. My investigation tends to support his conclusion.

The influences of different types of hosts on growth of cestodes are obvious when H. citelli from the 13-lined ground squirrel is compared to those from the golden hamster. Read (1959) reported wet weight values of 8 day old worms as 5.7 mg for H. citelli. Wet weights of 8 day old worms from ground squirrels in my research averaged 16.3 mg while 12 day old worms from hamsters (Read, 1959) were more than twice the average wet weights of the same age worms from ground squirrels reported in this paper. Such comparisons must be viewed with caution since Read used single worm infections in hamsters on a starch diet. However, the differences between the older worms in the 2 hosts may be due to a crowding effect in the squirrels and to the lower carbohydrate diet for these animals. The Purina Guinea Pig Chow fed

ground squirrels in this research was 45% carbohydrate, but a large portion of this is probably sucrose. Read (1959) showed that H. citelli is smaller in hamsters on a sucrose diet than in the same host on a starch diet.

The low growth rate for 9 day old worms is the results of several indefinite factors (Table II). Thirteen-lined ground squirrels are not ideal laboratory animals and are easily excited, especially when caged. During the 9 through 13 day experimental period there was an unusual amount of human activity in the animal quarters. This agitated them and possibly caused a stressful situation which may be reflected in the cestodes through suppressed growth. Another cause of variation may have been the time the squirrels were infected. The experiments for 9 through 13 day old worms (Experiment I) were conducted during early September. By then laboratory reared squirrels had gained maximum weights and food consumption had declined. This resulted in less available food for the worms and reduced their growth rate since this requires a high carbohydrate supply (Read et al., 1958). Also numerous cockroaches and Norwegian rats were in the building where the squirrels were quartered. Since the 13-lined ground squirrel has insectivorous habits, some of the animals during this period acquired H. diminuta which undoubtedly occurs in roaches and rats. A pilot experiment in different animal quarters produced consistently larger worms for this same age group a

year earlier. The data from the pilot experiment are not included here because of the different experimental conditions under which it was conducted.

The high dry weight-wet weight ratio obtained for 4 day old worms may be the result of slight dehydration. The 0.85% saline used to wash these worms is hypertonic to the worm. Therefore, there was probably a loss of water during the washing phase. Roberts (1961) showed high values for this same age in H. diminuta with infection of 20 and 40 worms per host. He also used a hypertonic wash solution and calculated that his data were in error by 5.8%.

In contrast to a high growth rate of worms in actively feeding summer hosts, the foregoing results demonstrate that host hibernation markedly reduces the growth and maturation of H. citelli. The interpretation of the results is difficult since many factors are involved. In hibernating hosts temperatures are depressed, available food is low, and host physiology is radically changed. Also the effect depends in part on the duration of the hibernating period as observed in the size and maturation differences between 9 and 13 day old worms (Table VI). The 9 day old worms came from squirrels which hibernated one-fourth as long as did those which harbored 13 day old worms. The cestodes from the former group were therefore larger than the older worms.

On the basis of the morphological measurements made on worms in hibernating and non-hibernating hosts,

development and maturation were affected more than any other variable analyzed. In addition to suppressing growth in length, there were fewer than half as many mature proglottids in 13 day old experimental worms as in 5 day old summer controls. If the age of the 13 day old experimental worms is corrected by subtracting the average number of hibernating days from the actual worm age, a corrected age would correspond to 5 day old summer controls (Table VI). However, data for 4 day old summer controls (Table II) and 13 day old experimental worms correspond more closely than the latter do to 5 day old worms. This clearly indicates that development is inhibited during the hibernating period. Though a correlation is shown between the size or degree of development of H. citelli and the number of days the host hibernated, the relationship is not proportional. The size of 9 day old experimental worms and all the winter controls indicates that factors other than the decrease in body temperature are involved. Also indicative of other causative factors is the finding that the prepatent period was extended in the winter control cestodes as well as in the experimental group. While egg liberation did not occur by the 13th day in winter controls, it probably would have within another 2 days since gravid proglottids were present. This is a 2 to 4 day prolongation of the summer control prepatent period. Even if hibernation had not been interrupted, patency would probably have never occurred in worms of

hibernating squirrels. As shown from Experiment IV, the worms would likely have been destrobulated before full maturity was reached. Dery (1960) reported similar results on Schistosomatium douthitti in hibernating 13-lined ground squirrels. However, he reported that ovulation did occur in worms parasitizing hibernating squirrels that had frequent arousals.

With respect to worm chemistry, hibernation affected two components. First, relative amounts of soluble sugar content were greatest in worms from hibernating hosts. Soluble sugars also compose a larger portion of the total carbohydrates in worms from hibernating hosts than in those from non-hibernating hosts (Fig. 5). A plausible explanation for these relative differences may be that the rate of monosaccharide absorption from gut contents by small worms is greater than the rate of glycogenesis. This seems possible since a small amount of ingesta was present in the intestine and some digestion undoubtedly occurred in the host's gut during early periods of hibernation. This hypothesis also is supported by the fact that 4 and 5 day old worms have a higher soluble sugar concentration than do older and more mature worms. This may be explained on the basis of a smaller worm having a larger surface area in proportion to its volume than does a large worm. For this reason the rate of sugar absorption should be greater in small cestodes than in larger ones. Alternatively, the higher soluble sugar

portion may be from increased hydrolysis of glycogen with decreased catabolism of monosaccharides, allowing a higher monosaccharide concentration. The latter explanation does not seem as plausible as the first since glycogen degradation is normally influenced among other things by a low monosaccharide concentration and high inorganic phosphate concentration in many animal cells (White et al., 1964).

Second, worm size in hibernating animals is also related to protein synthesis as shown by the relatively lower protein content in both 13 day old winter controls and experimentals than in summer control worms (Table X; Fig. 6). The lower protein content is possibly the result of reduced maturation and developmental rate in these worms. It appears that synthesis of proteins is inhibited by hibernation and this is also reflected in the lack of sperm and ova production. Where metabolic rates are reduced, the normal growth and development of the worms are retarded accordingly. Lipid concentrations do not seem to be affected by hibernation (Table XI). There is a slightly higher lipid fraction in worms from hibernating squirrels than in controls.

The time at which a host enters hibernation after infection with a parasite is important for the successful establishment of the parasite. Chute (1961, 1964) observed that T. spiralis is completely eliminated if the host hibernates within 72 hours after exposure to the parasite. Blanchard and Blatin (1907) observed that the lethal effects

of 4 species of Trypanosoma are reduced in marmots if hibernation occurs within 5 days after exposure to these parasites. I have observed similar results with H. citelli. If the ground squirrel hibernates within 48 hours after infection, less than 1% of the cestodes is recovered and only 5% recovered if hibernation was induced within 72 hours. For specialized organisms such as cestodes, energy requirements during the period of rapid growth and cellular differentiation is critical. Therefore, any extreme change in temperature and consequent host physiology will be reflected in the economy of the worm parasites.

An analysis of the data from winter control worms also shows a smaller size, and retarded development and maturation as compared to summer controls. As shown in Table I there is a lower, but non-significant, recovery of winter controls than in summer controls. This indicates that the decreased body temperature alone cannot be credited with the total effects of hibernation. Ground squirrels decrease their food consumption when physiologically prepared to hibernate. The decrease is observed even though the body temperature does not decrease. Essentially this results in starving the cestode during periods of a high metabolic rate. This concept is further substantiated when the data are compared to those collected by Read et al. (1958) on H. citelli from hamsters on carbohydrate free diets. These authors recorded wet weights of 13.9 mg for 14 day old

worms. In Experiment II, 13 day old winter control worms weighed 13.7 mg (Table VI). Different hosts and worm loads do not permit accurate comparisons; however, it appears that the worms were starved for carbohydrates under both conditions.

Examination of Tables XII and XIII shows that hibernation does not completely inhibit metabolism in adult worms. Fresh and dry weights were approximately 45% less in experimental than in summer control worms. Glycogen is the principal compound which occurs in significantly smaller amounts, especially in worms from squirrels which hibernated 6.5 days (Group II) as compared to those from squirrels which hibernated 4 days (Group I). The fact that the glycogen content in Group II experimentals is nearly 14% less than the controls adds further evidence that food deprivation is an important influence of the host hibernation on the worms. When converted to a wet weight relationship, diminution in glycogen synthesis and storage in the experimentals (Groups I and II) approximates that observed by Read and Rothman (1957) in H. citelli from hamsters starved 48 hours. Although these authors used a different host and type of food in their experiments, worm load and age are comparable. Therefore, it seems reasonable to postulate that hibernation retards cestode metabolism, but does not completely inhibit it. The fact that metabolism does continue at a reduced rate may account for the destrobulisation

of adult worms after 3 weeks in a hibernating host, since Reid (1942) found that Raillietina cisticellus became destrobulated in a host starved for 24 hours.

While the maximum life span of H. citelli is not known in the 13-lined ground squirrels, I have maintained it for more than 10 months during my investigation. Therefore, the longevity of this cestode is adequate to extend through the winter of host hibernation in Oklahoma. Experiment IV indicates that the cestode may survive the winter in a hibernating squirrel. The worm is destrobulated within 3 weeks after the host enters hibernation but the scolex and probably a portion of the neck remain in the intestine. Scoleces were not recovered from any host allowed to overwinter in the cold room. However, the fact that ova did not appear until 2 or 3 weeks after the squirrels were moved to a warm room indicates that only the scolex and neck region remained. Destrobulisation appears to be a response to carbohydrate deficiency or host starvation, and in any event seems to be an adaptation for H. citelli to reduce energy requirements while its host is hibernating.

C. tridecemlineatus is a natural host for H. citelli. McCarley (1966) has made a detailed study of the 13-lined ground squirrel's behavior in North Texas. His findings are in close agreement with my knowledge of the behavior of ground squirrel populations in Central Oklahoma. Adult ground squirrels feed maximally during May and early

June. Before mid-July many of these animals go underground and most of them do not reappear above ground until mid-March of the following year. Nearly all adults are underground by mid-August. In contrast, the young of the year forage maximally the latter part of the summer and do not go underground until mid-October.

A survey of a C. tridecemlineatus population in Central Oklahoma indicated that H. citelli population fluctuations correlate very closely with the feeding habits and behavior of its host population. During the summer of 1962, 70% of the squirrel population harbored H. citelli. The average infection was 6 worms per host, but maximum number of worms recovered from a single host was 17. Adult squirrels had a peak infection during late May and early June during the maximal feeding period. Thereafter, fewer adults were captured as they remained near the burrow and seldom reappeared after being disturbed. Young squirrels showed a maximal infection rate in August. In relation to this behavior, squirrels infected 2 to 4 weeks prior to hibernation may retain at least a portion of their worm burden until the following spring. The worm is destrobulated but the scolex may remain in the host. This residual population overwintering in the hibernating squirrel is probably sufficient to maintain the species. Two advantages may exist in the cestode's behavior. First, the worm is already established when the squirrel emerges from hibernation, a time when the

insect population is still low. Therefore, the cestode is not dependent on the intermediate host alone for its continuance. Second, Read (1959) reported that the life span of H. citelli in hamsters is 70-90 days. My observations confirm Read's findings. However, this cestode lives 10 months or longer in squirrels which hibernate. It is very likely that hibernation may extend the life span of the worm from one season to the next. While it is also possible that this cestode survives the winter in an intermediate host, no attempt was made to determine the natural intermediate host or hosts.

Squirrels were allowed to eat and drink during arousal periods in this investigation but, as to what relationship this has to natural conditions is not known. However, adequate evidence is lacking to determine whether ground squirrels do or do not come above ground during warm days of the winter period. Laboratory observations indicate that they do (Pengelley and Fisher, 1961), and the 13-lined ground squirrel in Central Oklahoma has been seen above ground in early February.

SUMMARY

Host hibernation dramatically reduces the growth, maturation, and development of Hymenolepis citelli. The effects are dependent on the duration of the hibernating period and the time hibernation is induced after infection. Sperm and ova production in worms from hibernation animals is comparable to that of 5 day old summer control cestodes. In young worms glycogen content changes very little, but an increase in alcohol soluble sugars is significant. Protein content is lower in 13 day old experimentals than in 9 day old experimentals, whereas lipid content does not change significantly. In adult worms glycogen content is significantly reduced by host hibernation. Protein and lipid content in experimental adult worms approximately equal that in the controls.

H. citelli can survive the winter in a hibernating host, but is destrobulated in 3 weeks after the initiation of hibernation. The life span of this cestode may be extended at least 7 months by host hibernation, since senescence occurs within 90 days in other hosts.

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