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Behavioral and Anatomical Responses of the Convergent Lady Beetle to Parasitism by *Perilitus coccinellae* (Schrank) (Hymenoptera: Braconidae)

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The development of larval *Perilitus coccinellae* in the hemocoel of male *Hippodamia convergens* is described. Especial emphasis is placed upon the development of the trophic cells which originate from the dissociation of the parasite's embryonic membrane.

Some aspects of the response of male beetles to parasitism by *P. coccinellae* are considered. The influence of parasitism on the feeding and respiration rates of the beetles is described. There was no effect on the mating behavior or testicular morphology in parasitized beetles. Two types of lesions are described from the fat body of host beetles: (1) those ascribed to the activity of the *P. coccinellae* larvae, and (2) those ascribed to the activity of the parasitic cells.

Microsporidian infections were found in 10–20% of the *P. coccinellae* and in about 50% of the *H. convergens*. The lesions caused by the microsporidian infections in beetles are described including neoplasms in the midgut epithelium and in Malpighian tubules. The influence of the microsporidioses of both *H. convergens* and *P. coccinellae* on the development of the wasp are discussed.

INTRODUCTION

The braconid *Perilitus coccinellae* is a parasite of several species of coccinellid beetles. The species of beetles which are natural hosts of *P. coccinellae* have been listed by Iperti (1964).

The original description of *Perilitus coccinellae* was given by Schrank in 1803 under the generic name of *Ichneumon* (Muesebeck et al., 1959). The synonomy and taxonomy were clarified by Cushman (1922) and by Muesebeck (1936).

This report is based on the results of laboratory investigations of *P. coccinellae* parasitizing males of the coccinellid *Hippodamia convergens* emphasizing the development of the parasite, the pathology to the host, and the host–parasite relationships.

The biology of *P. coccinellae* has been summarized by Clausen (1940).

When the egg of *P. coccinellae*, and that of some of the other parasitic Hymenoptera, hatch, the cells which constituted one of the embryonic membranes disassociate and are freed into the hemolymph of the host. These cells have been termed "trophamnion cells" in the literature. However, in most cases it has not been established that the cells originate from the amnionic membrane. Since the precise origin of most of the "trophamnion cells" is unknown, it is suggested that these cells be referred to simply as "trophic" cells until their origins are clarified.

Groups of serosal cells were observed by Spencer (1922) in several species of Hymenoptera aphid parasites. Following the

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hatch of the parasite egg, these cells form into groups of varying sizes, become round, and later their nuclei fuse and degenerate into a chromatin mass. The cytoplasm of these cells becomes progressively more vacuolated as the parasite develops. As the parasite matures, the cells degenerate in the hemocoel of the aphid.

Trophic cells, apparently very similar to those which occur in *P. coccinellae*, were described by Jackson (1928) from *Perilitus rutilis* (Nees) parasitizing *Sitona lineata*. Prior to hatching, the embryonic membrane which will break up into trophic cells, consists of cells with oval nuclei, and with refractile particles and vesicles present in the cytoplasm. When the parasite embryo hatches from its egg these membrane cells disassociate into individual "trophic cells." The trophic cells increase in size as the parasite larva grows. The developing larva feeds on the trophic cells.

A similar development of trophic cells was observed to be associated with a species in the closely related euphorine genus *Microtonus* (Smith, 1952).

Both Jackson (1928) and Smith (1952) reported that trophic cells continue to increase in size and effectively parasitize the host insect following the death of the associated parasite larvae. Hence, the pathology caused by parasites which have well-developed trophic cells is at least in part caused by the trophic cells and perhaps, in part, caused by the parasite larva itself.

Spencer studied the pathology on insects (aphids) parasitized by hymenopterous parasites which possess well-developed trophic cells. In these aphids only the fat body and reproductive tissues were morphologically affected by the parasitism. The cell boundaries of the fat body became more distinct, increased vacuolization occurred in the cytoplasm of the cells, and the nuclei became irregular. The fat body appeared similar to that in aphids which were starved. Embryo development was arrested and overwintering ova were reduced in the reproductive tissue of the aphids.

The majority of parasitic Hymenoptera feed directly upon the host tissues. However, there are several references to indirect pathologies to the host insects (Doutt, 1963).

Materials and Methods

Adult *H. convergens* were collected from overwintering aggregation sites in the Sierra Nevada foothills at the 2000-ft level near Nevada City, California. Some of the investigations were carried out using these field-collected beetles while for other experiments newly emerged laboratory-reared beetles were used. The initial stock of *Perilitus coccinellae* was obtained by rearing them from field-collected beetles. Thereafter, a laboratory culture of *P. coccinellae* was maintained by exposing 15 to 20 adult beetles to one or two parasites for 2 days in a small ($4 \times 4 \times 2$ inch) plastic box. These exposed beetles were then reared individually until the parasites emerged from them.

The male beetles used for the various investigations were exposed to the parasites as outlined above and then handled in different ways depending on the type of food to be given them. Those beetles which were fed pea aphids (*Acyrthosiphon pisum*) were individually placed into 8-dr vials which were then plugged with cotton. A fresh supply of pea aphids was added daily to each vial. Since it was difficult to supply a sufficient number of pea aphids for all studies, in some cases beetles were fed an artificial diet. This diet, suggested by K. S. Hagen and based on unpublished results of nutritional studies with *H. convergens*, consisted of casein hydrolysate (4 g), vitamin mixtures [2] (1 g), fructose (6 g), distilled water (9 ml), and 1 ml

[2] Vitamin Diet Fortification Mixture, Nutritional Biochemical Corp., Cleveland, Ohio, No. 7227.

of a mixture [3] containing cholesterol plus oleic acid. Beetles which were fed the artificial diets were placed individually in a compartment of a plastic box originally designed to store fishing flies. A hole was cut in the lid over each compartment into which droplets of fresh diet were introduced on small pieces of wax paper. Each compartment was also equipped with a small wad of cotton which was moistened daily with distilled water. The holes in the lid of the box were plugged with cotton.

In order to determine the effect that parasitism by *P. coccinellae* had on the quantity of aphids eaten, the amount of feeding by ten parasitized beetles was compared to that of 10 nonparasitized beetles. Each day the beetles were transferred to clean vials containing 20 large pea aphids each. The vials from the previous day's feeding were examined and the aphids were counted in each of the following categories: (1) live, (2) less than ½ eaten, (3) more than ½ eaten, and (4) dead. The numbers tallied in each category were added and the sum subtracted from 20 to give the number of aphids totally destroyed. By assigning numerical values of 1, 2, and 3 for less than ½ eaten, more than ½ eaten, and totally destroyed, respectively, it was possible to rate numerically the amount of feeding by summing these values.

To test the effect on the respiration of male *H. convergens* parasitized with *P. coccinellae*, the CO_2 absorption was measured in 10 parasitized and 10 unparasitized beetles with the aid of a Warburg apparatus. The beetles were fed pea aphids except during the periods when the respiration rates were being measured. For the measurements each beetle was weighed individually and placed in a Number 0 gelatin capsule which had been perforated with a hot needle. Four measurements were taken between the time the test beetles were exposed to *P. coccinellae* and the time when the parasite larvae emerged.

For the determination of the general development of *P. coccinellae*, parasitized male beetles were dissected daily under a dissecting microscope.

The morphopathological changes in the beetles and the details of the development of the parasite were studied by means of paraffin sections. Whole beetles were sectioned. The fixatives used included Gilson's, Bouin's, Weaver-Thomas', and Carnoy's (Gray, 1952). For general purposes, the Weaver-Thomas fixative was found to be most satisfactory. The specimens were dehydrated in ethyl alcohol, passed into methyl benzoate and benzine and embedded in Parawax (56–58° C). Serial sections 5–8 μ thick, were cut on a Spencer (820) microtome. For general purposes the sections were stained with Harris hematoxylineosine or with iron hematoxylin (Gray, 1952). Canada balsam was the mounting media employed.

The fine structure of trophic cells associated with the first instar *P. coccinellae* was studied with the aid of an electron microscope. The trophic cells were removed from the beetles and immediately placed in 1% $OhOsO_4Oh$ buffered with veronal acetate at *p*H 7.2 and fixed for 30–40 min at 0–5° C. The cells were then dehydrated by ethyl alcohol, stained in absolute alcohol with 2% phosphotungstic acid and embedded in maraglass. Ultrathin sections were cut with a glass knife on a Porter-Blum microtome, placed on Formvar-coated copper grids and examined in an RCA EMU-F3 electron microscope.

Results

Ovipositional Behavior of P. coccinellae

P. coccinellae oviposits in both adults and pupal *H. convergens*. Motionless pupae can be detected by the parasite from a distance of about ¾ inch, while adult beetles can

[3] One ml of a mixture of 1 ml oleic acid, 500 mg cholesterol, 0.1 ml Tween 80, and 100 ml distilled water.

be detected from a distance of up to 4 inches depending on the rapidity of their movement. Occasionally adult beetles would fall from the lid to the floor of the plastic box. These beetles would move violently in an effort to right themselves and in so doing would attract a parasite up to 4 inches away.

Upon sensing a host, the parasite faces in that direction and waves its antennae a few seconds before moving closer to the beetle. When about ½ inch distant from the beetle, the parasite folds its abdomen under the thorax such that the ovipositor extends slightly forward of its head. Contact with the beetle is first made by the antennae; a period of 1 to 3 min of antennal palpating and light probing with the ovipositor follows. In the case of a walking adult beetle, this palpating and probing may be accomplished as the parasite follows alongside of or behind the beetle.

The attempted deposition of the parasite egg is made by a powerful forward thrust of the ovipositor, followed by a slight backing up of the parasite and unfolding of the abdomen. About one-half of the attempts result in actual egg deposition. Beetle pupae are nearly always stung in the pleura; adults are stung in the coxae and abdomen in a ratio of about 1:2, respectively. Pupae which have just been stung show considerable irritation by continuing to snap up and down for about 5 min. Adults also show some irritation by moving rapidly for 30 sec to 1 min following the sting.

Five to 20 min of rest are required for the female *P. coccinellae* following three oviposition attempts. During this period, the parasite is occasionally attracted to a beetle. She will then go through the palpating and probing procedure but will withdraw without attempting to oviposit.

There was considerable variation in the speed with which individual parasites attempted to deposit eggs. The length of larval and pupal developmental period was directly related to the speed with which the parent parasite deposited eggs. It was later discovered that many of the parasites were infected by microsporidia. However, it was not determined if the speed of "working" beetles and the extent of microsporidiosis were related to one another.

General Development of P. coccinellae *in Male* H. convergens *Adults*

Ripe ovarian eggs of *P. coccinellae* average 255 μ by 30 μ. Two days following deposition in a beetle the eggs had expanded to an average size of 570 μ by 345 μ. Maximum egg expansion was reached by the 3rd day at room temperatures of 70° ± 5° F; at this time the eggs average 1010 μ by 570 μ.

The egg hatches in about 5 days, releasing the first-instar *P. coccinellae* larva and clumps of unseparated trophic cells each consisting of 30 to 100 cells. By the 6th day trophic cell mass separates into individual, spherical, opaque cells averaging 46.4 μ in diameter. By the next day, the trophic cells have increased in size to an average of 76.8 μ in diameter and have begun to assume a slightly yellow tincture. The *Perilitus* larva by this time has transformed to the second instar. Two days later the larva has transformed to the third instar and the trophic cells are a deep yellow to orange color averaging 196.5 μ by 204 μ. Meanwhile, the cells have lost their spherical form and have assumed various irregular shapes. These trophic cells continue to increase in size as the larva passes into the fourth instar on about the 12th day. By this time, the cells measured 370.5 μ by 441.8 μ. On the 13th and 14th days, the mature *P. coccinellae* larvae emerged from their host beetles by boring through the membrane between the 6th and 7th abdominal tergites.

Following the emergence of the parasite, the beetle is greatly weakened and usually remains clinging to the parasite cocoon. Death of the beetles follows in 3 or 4

days after the parasite emerges. No trophic cells were found in beetles from which a parasite had emerged. This was also the case when an abnormally large number of trophic cells should have been present due to superparasitism.

Superparasitism was common, with as many as five live first-instar larvae being found in one beetle. However, only one live second-instar larva was found per beetle. In some of these cases of superparasitism an abnormally large number of trophic cells were found, but curiously, not in all cases.

In a diapausing beetle, the trophic cells were found to average between 45 and 50 μ in diameter and the *P. coccinellae* larvae were in the first instar. While the trophic cells were similar in size to those found associated with first-instar larvae in nondiapausing beetles, their color was yellow-orange as contrasted to the faintly yellow color of the cells in the nondiapausing beetles.

The Morphology and Development of Trophic Cells Associated with Perilitus coccinellae

Prior to hatching of the *P. coccinellae* egg, the membrane which will disassociate into the trophic cells can be seen between the egg chorion and developing embryo (Fig. 1). The cells of the embryonic membrane begin to enlarge before hatching. Figure 2 shows a cross section through a *Perilitus* egg which is in the process of hatching. The posterior end of the parasite has freed itself of the chorion (Fig. 2, A) but the chorion can be seen in the anterior portion of the cross section (Fig. 2, B). The cells of the posterior portion of the embryonic membrane in the anterior portion of the egg are less enlarged and remain intact. Much of the enlargement of these membrane cells is due to the increased size of their nuclei.

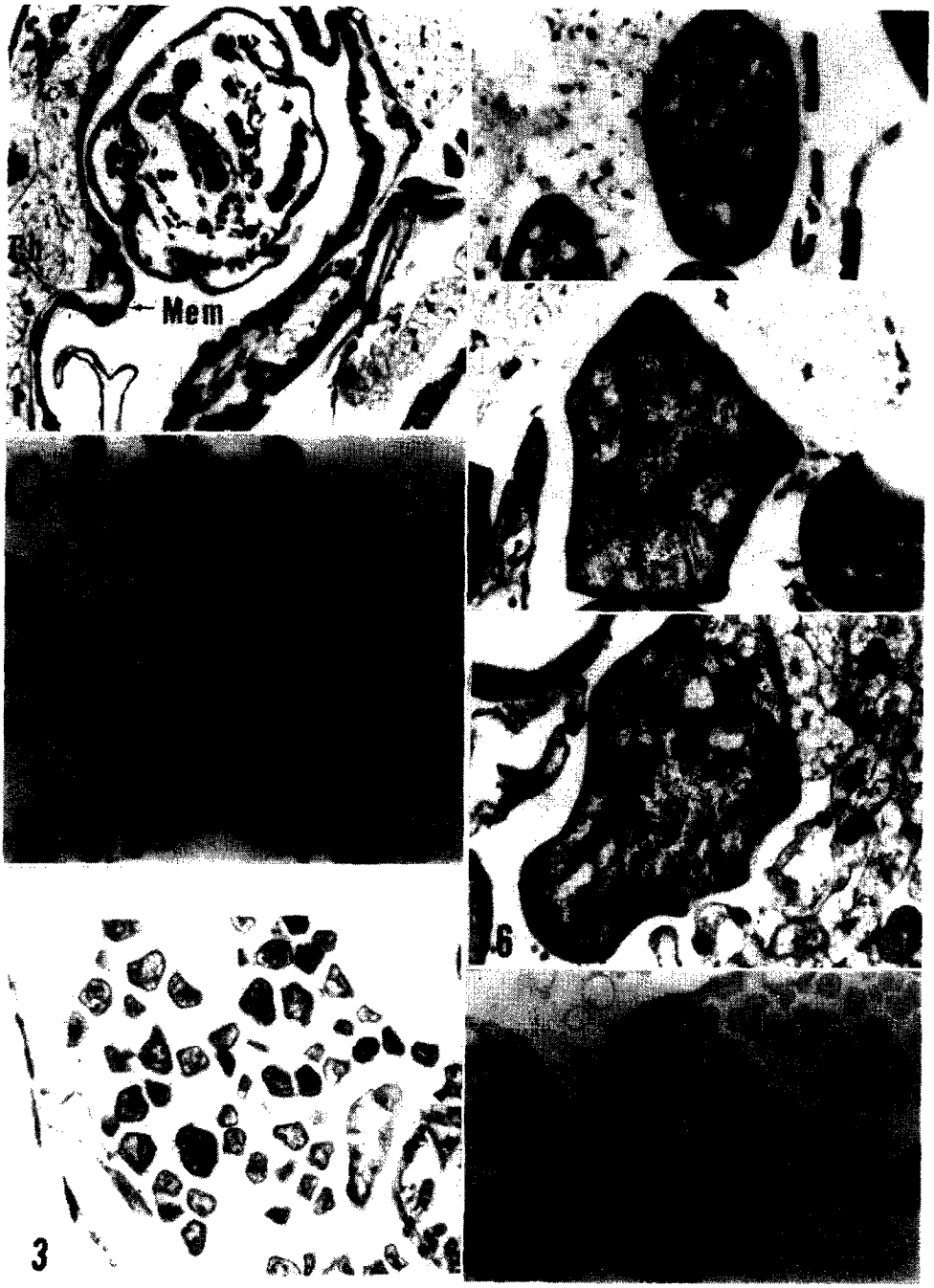
During the next few days the trophic cells which are associated with the first-instar larva become completely separated and continue to increase in size and the chromatin material becomes scattered, giving a vacuolated appearance to the nuclei. The cytoplasm appears to consist of small granules which have a strong affinity for hematoxylin stain (Fig. 3).

Ultrathin sections of trophic cells of similar age and size to those depicted by Fig. 3, were prepared for electron microscope examination. Two of the electron micrographs, prepared from these sections, are shown by Figs. 8 and 9. These figures show the surface of the trophic cells to be covered by many microvillae. The profuse mitochondria and cytoplasmic membranes suggest that these trophic cells are metabolically very active. The large, dark inclusions seen in the cytoplasm may be lipoid inclusions.

These trophic cells increase in size and are eaten by the parasite larvae. Identifiable trophic cell material was seen in numerous cross sections of the larval gut of the parasite.

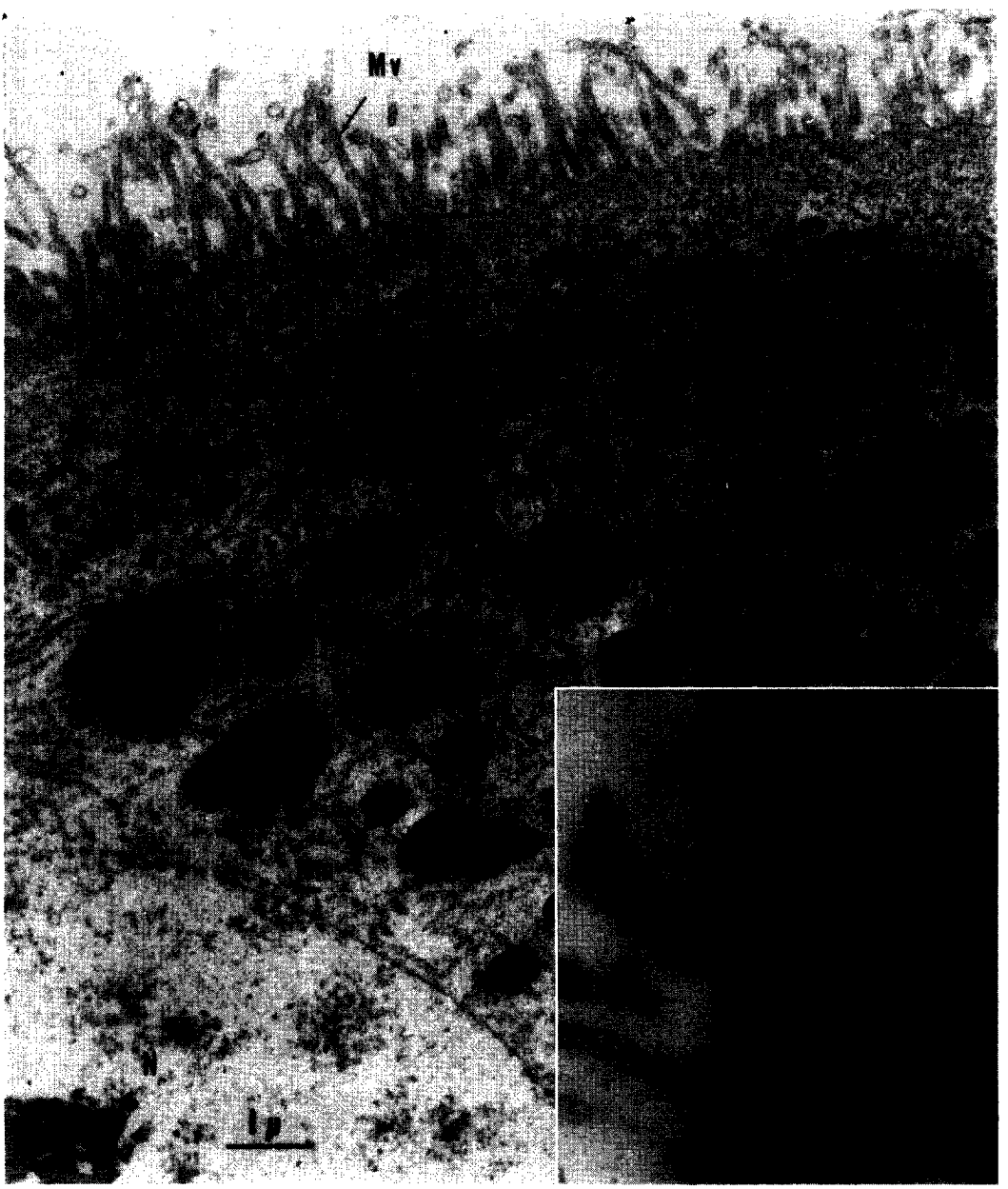
Figures 4, 5, and 6 show successively older trophic cells which are associated with the second-, third-, and fourth-instar parasite larvae. In addition to increasing in size, by the time the larva is in its second instar the nuclei of the trophic cells have degenerated into strands of chromatin. The cytoplasm consists of small granular inclusion which stain with hematoxylin and larger eosinophilic inclusions (Fig. 4). As the trophic cells age, the proportion of the larger inclusions to the smaller ones becomes greater. At the same time the chromatin strands become more scattered and larger nonstained vacuoles appear in the cells.

Most of the trophic cells are ingested by the parasite larva during its development. However, an occasional trophic cell can be found in beetles from which a parasite has emerged. These remaining trophic cells appear to be disintegrating (Fig. 7) and none



LEGEND FOR FIG. 1–29. Caps, capsule; Cf, central fat body; Ch, chorion; Gr, eosinophilic granulation; Hg, hind gut; Lip, lipid inclusion; M, muscle; Mel, melanin; Mem, membrane; Mit, mitochondria; Mg, midgut; Mp, Malpighian tubule; Mv, microvilli; N, nucleus; Nch, nerve chord; Nm, nuclear membrane; P, parasite; Pe, parasite egg; Pemb, parasite embryo; Pf, peripheral fat body; Sp, sporelike body; T, trophic cell; Te, testes; Tr, tracheole.

FIGS. 1–7. Origin and development of the trophic cells associated with *P. coccinellae*. 1. 4-day-old *P. coccinellae* showing membrane which will disassociate into trophic cells. 200 ×. 2. Parasite egg in process of hatching: A, posterior part of egg free of chorion; B, anterior, unhatched portion of egg. 200 ×. 3. Trophic cells associated with first-instar parasite larva. 200 ×. 4. 13-day-old trophic cells. 200 ×. 5. 15-day-old trophic cells. 200 ×. 6. Trophic cell associated with fully grown parasite larva. 200 ×. 7. Disintegrating trophic cell remaining in beetle following emergence of a *P. coccinellae* larva.



FIGS. 8 and 9. Electron micrographs of sectioned trophic cells associated with first-instar parasite larva.

could be found later than the 1st day following parasite emergence.

In superparasitized hosts, the fate of the trophic cells appears to depend on their proximity to the dead supernumerary larvae. Those trophic cells which lie close to the dead larvae increase very little in size and retain the characteristics of cells which are normally associated with first-instar larvae. However, those trophic cells which are more remote from the dead larvae develop normally (Fig. 24). Figure 24 was prepared

from a section of a newly emerged, laboratory-reared beetle which had been exposed to a parasite 11 days prior to fixation. Therefore, all of the trophic cells shown are within 24 hr of each other in age.

The Effect of Parasitism by P. coccinellae *on the Quantity of Aphids Eaten by Male* H. convergens

Twenty male *H. convergens*, collected from overwintering aggregations, were used in the feeding experiment. Ten of these beetles were exposed to a parasite adult and 10 of the beetles were not exposed. All of the test insects were fed pea aphids and the amount of feeding was recorded as stated in the material and methods section. The beetles were held in a cabinet set for a 16-hr photoperiod and 78-80° F (26-27° C).

One parasite larva emerged from its host in 13 days, two in 14 days and the remaining five in 15 days. One beetle from the nonexposed, control sample was naturally parasitized and was, therefore, placed into the test sample. Four of the beetles died in from 1 to 8 days after feeding had begun; these beetles are not included in the results of the experiment.

The results of the feeding experiment are summarized by Tables 1 and 2.

The average feeding values for parasitized beetles were significantly (0.05 level) less than that for the nonparasitized ones, according to the results of the distribution significance test for unpaired means.

The feeding data were also analyzed by comparing the average food values for each day for the parasitized beetles with that for the nonparasitized beetles. The amount of feeding was significantly (0.05 level) less in the parasitized beetles than it was in the nonparasitized ones on the 5th, 13th, and 14th day following exposure to the parasite adult. The parasite larvae were emerging from the beetles on the 13th, 14th, and 15th days and the beetle activity was

TABLE 1

FEEDING ACTIVITY FOR MALE *H. convergens* PARASITIZED BY *P. coccinellae*

	Beetles [a]									
Days	1	2	3	4	5	6	7	8	Total	Average
1	18	15	14	15	18	22	13	12	127	15.86
3	28	27	13	10	15	27	17	29	166	20.75
4	22	17	15	14	23	23	15	18	147	18.38
5	12	8	6	7	8	12	18	23	94	11.74
7	19	26	14	29	27	36	31	34	216	27.00
8	25	30	17	23	34	39	34	33	235	29.38
9	30	28	12	17	31	32	31	24	205	25.63
10	17	18	16	16	25	16	23	24	155	19.38
11	9	23	12	18	18	8	2	7	97	12.12
12	23	21	9	18	7	12	0	7	97	12.12
13	16	16	13	23	4	12	—	17	101	14.43
14	9	6	16	34	4	6	—	7	82	11.70
Total	228	235	183	224	214	247	184	235		
Average	19.0	19.6	15.2	18.7	17.8	20.6	18.4	19.6		

[a] Formula for computation of feeding activity: aphids less than ½ eaten = 1; aphids more than ½ eaten = 2; aphids missing from the vials = 3.

TABLE 2

FEEDING ACTIVITY FOR MALE *H. convergens* NOT PARASITIZED

	Beetles								
Days	1	2	3	4	5	6	7	Total	Average
1	15	15	25	21	25	15	12	128	18.28
3	24	13	26	18	17	22	23	143	20.43
4	35	22	34	23	20	25	19	178	25.43
5	23	31	24	13	34	17	34	176	25.14
7	29	16	24	27	16	29	35	176	25.14
8	29	24	40	31	27	36	31	218	31.14
9	39	26	38	21	32	23	18	197	28.14
10	24	9	25	7	13	21	13	112	16.00
11	22	10	21	12	11	8	10	94	13.43
12	24	12	20	14	22	13	20	125	17.86
13	24	22	31	21	19	29	19	165	23.57
14	28	39	25	26	39	26	30	213	30.43
Total	316	239	333	234	275	264	264		
Average	26.3	19.9	27.8	19.5	22.9	22.0	22.0		

greatly reduced. Hence the feeding values were expected to be reduced on the 13th and 14th days following oviposition by the parasite. On the 5th day most of the parasite eggs were hatching. This was indicated by the results of daily dissection of a group of parasitized beetles which were held under similar conditions as the test beetles.

The Effect of Parasitism by P. coccinellae *on the Respiration Rate of Male* H. convergens

In order to determine the effect of parasitism on the respiration rate of the beetle hosts, the same individual beetles used in the feeding experiments were subjected to measurements of the oxygen utilized per 2-hr interval in a Warburg apparatus. These rate measurements were determined on the 2nd, 5th, 7th, and 13th days following exposure to an adult parasite. Similar measurements were obtained from nonparasitized beetles.

Tables 3 and 4 summarize the results of the respiration measurements in terms of total microliters of O_2 consumed during a 2-hr measurement for each beetle.

Analyses of these data showed no significant difference between the average O_2 consumed by parasitized beetles and that of nonparasitized beetles. However, when the daily averages of the two groups of beetles were compared, there was a significant difference on the 7th day and nearly a significant difference on the 13th day. On the 7th day following oviposition by *P. coccinellae* the parasitized beetles utilized less O_2 than did the nonparasitized beetles.

The Effect of Parasitism by P. coccinellae *on the Mating Behavior and Testes Morphology of Male* H. convergens

Five-day-old, adult, male *H. convergens* were exposed to two *P. coccinellae* adults for a 2-day period. The beetles were then fed pea aphids and on each day thereafter one of them was placed with a virgin, laboratory-reared female beetle for 24 hrs. Following exposure of the two beetles to each other, the male was fixed for sectioning and the female was fed pea aphids;

TABLE 3

MICROLITERS OF O_2 CONSUMED DURING 2-HR PERIODS OF MEASUREMENT FOR PARASITIZED *H. convergens* MALES

	Beetles									
Days	1	2	3	4	5	6	7	8	Total	Average
2	33.30	60.52	38.70	79.42	65.68	37.08	—	20.74	335.44	47.92
5	40.64	44.52	34.04	48.72	50.12	79.42	63.94	63.86	425.26	53.16
7	44.51	42.66	39.71	46.63	57.46	58.93	52.25	50.12	392.27	49.03
13	69.96	54.13	37.07	52.56	50.05	59.99	57.50	55.30	436.56	54.57
Total	188.41	201.83	149.52	227.33	233.31	235.42	—	190.02		
Average	47.10	50.45	37.38	56.83	58.33	58.86	—	47.50		

TABLE 4

MICROLITERS OF O_2 CONSUMED DURING 2-HR PERIODS OF MEASUREMENT FOR NONPARASITIZED *H. convergens* MALES

	Beetles								
Days	1	2	3	4	5	6	7	Total	Average
2	59.32	36.08	81.62	32.08	46.44	37.10	18.90	312.54	44.65
5	93.26	76.00	98.75	72.30	51.62	38.93	50.97	481.82	68.83
7	91.59	44.49	81.23	61.18	68.63	60.51	46.91	454.54	64.93
13	92.68	73.27	72.30	61.93	77.53	76.06	59.54	513.31	73.33
Total	336.85	229.84	333.90	228.49	244.22	212.60	176.32		
Average	84.21	57.46	83.47	57.12	61.05	53.15	44.08		

the subsequent eggs were observed for hatching which was taken as proof of successful insemination. The experiment was conducted at room temperature of 70° ± 5° F (21° C) during the day and 65° ± 5° F (18° C) at night. Under these conditions, the parasite larvae emerged from the beetles in 19 to 20 days.

The general activity of the male beetles was noticeably reduced following the 15th day after exposure to the parasites. However, mating occurred readily each day until a parasite emerged. The last male beetle exposed to a female was not fixed for sectioning and a parasite larva emerged from it the following day. This beetle mated almost immediately when placed with a female and the resulting eggs were viable. Following emergence of the parasite, however, this male beetle would not respond to the presence of a female. Hence, there was no effect on the mating behavior of the male beetles until after the parasite emerged, and viable sperm were transferred to the female beetles at each mating.

The fact that viable sperm was transferred does not, in itself, suggest that the parasitism caused no pathology to the testes. The males can store viable sperm for long periods of time as during periods of dormancy.

In order to determine the effect of para-

sitism on the structure of the testes, serial sections of parasitized beetles were compared to those of nonparasitized beetles. No differences between the testes of parasitized and those of nonparasitized beetles were observed. Measurements were made of the length and width of sectioned testes. Analyses of these measurements resulted in no significant differences between parasitized and nonparasitized beetles. Even the testes in beetles from which a parasite had emerged were normal in structure and size.

The Effect of Parasitism by P. coccinellae *on the Tissues of Male* H. convergens

Although the gut, muscle, nerve, testes, and other tissues were examined, only the fat body showed structural changes as a result of parasitism.

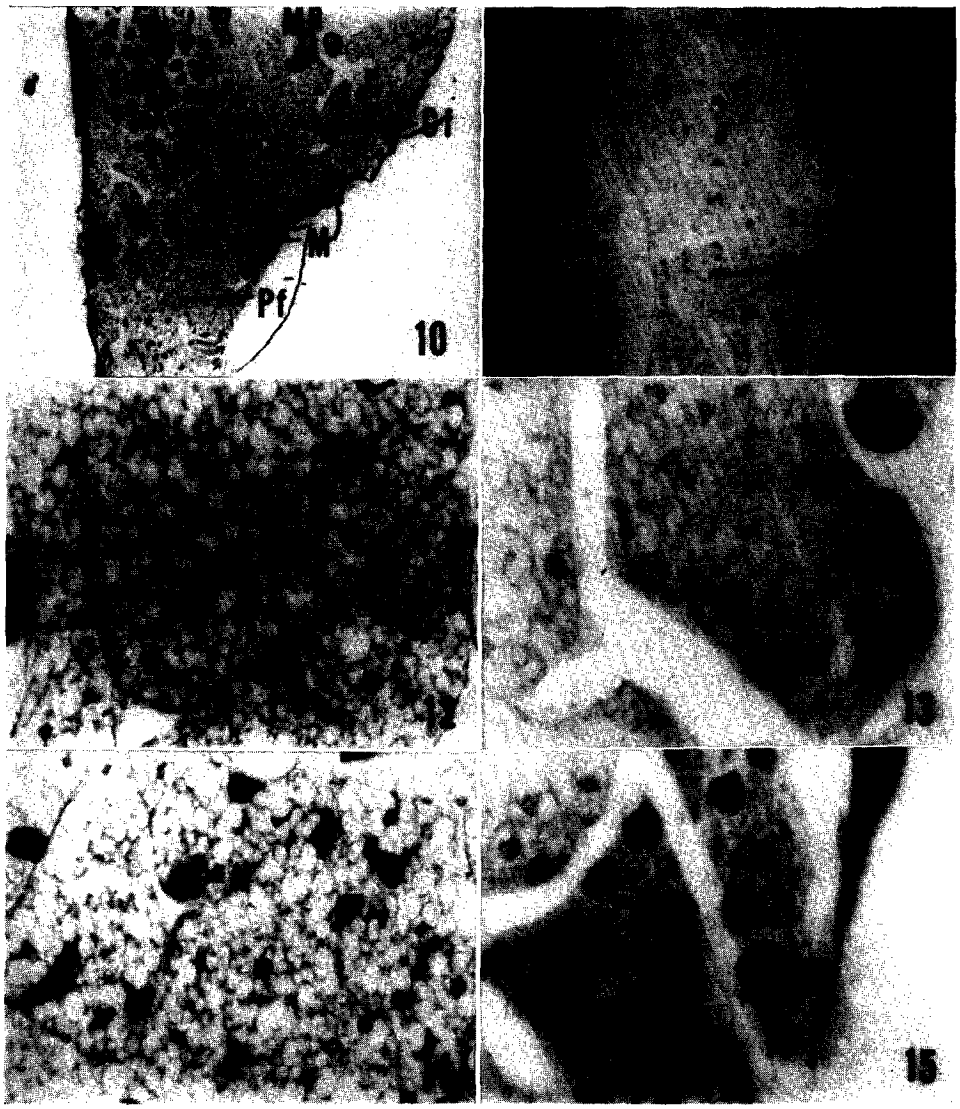
The fat body was studied in serial sections of normal and parasitized male beetles collected from overwintering aggregations, and of normal and parasitized newly emerged beetles reared in the laboratory.

The normal fat body of male *H. convergens* consists of two obvious types of tissues which are spatially separated (Figs. 10 and 11): One type is located in the central portion of the beetle and surrounds the gut, testes, and Malpighian tubules. This type of fat body consists of cells with a large number of unstained vacuoles and a few granular inclusions. Clearly distinguishable nuclei are generally not seen (Figs. 12 and 13). The cells which make up this central fat have been termed trophocytes (Snodgrass, 1935), because their main function is thought to be food absorption and storage.

The other type of fat body is made up of highly vacuolated cells which have large, obvious nuclei, many granular deposits and which occurs in the periphery of the sectioned beetles (peripheral fat body). These granular deposits appear to be of two kinds: (1) small, dense, hematoxylin-stained granules surrounding each nucleus and (2) coarse, sparse, crystallinelike granules which are lightly stained by hematoxylin and are not associated with the nuclei (Figs. 14 and 15). It is this second type of granule which may occasionally be seen in the central fat. Fat-body cells which possess granules have been termed urate and purine cells based on the nature of the granular inclusions (Snodgrass, 1935).

Although the normal fat body of both newly emerged beetles and aggregated overwintering beetles consists of central and peripheral types, they differ considerably in appearance. A comparison of Fig. 10 with Fig. 11 shows that there is a greater quantity of fat body (especially central fat) in the overwintering beetle (Fig. 10) than in the newly emerged (Fig. 11) beetle. The cells of the central fat body of overwintering beetles (Fig. 12) are much more vacuolated than are those of the newly emerged beetle fat body (Fig. 13). The overwintering beetle, from which Figs. 10 and 12 were prepared, was collected in June when the amount of stored fat is at its greatest. During the remainder of the summer and throughout the winter the fat body gradually becomes depleted, so that when these dormant beetles become active again their central fat appears very similar to that in newly emerged beetles. That is, the difference between the central fat body of the newly emerged beetle and that of the overwintering beetle may be ascribed to differences in the degree of vacuolization in the body.

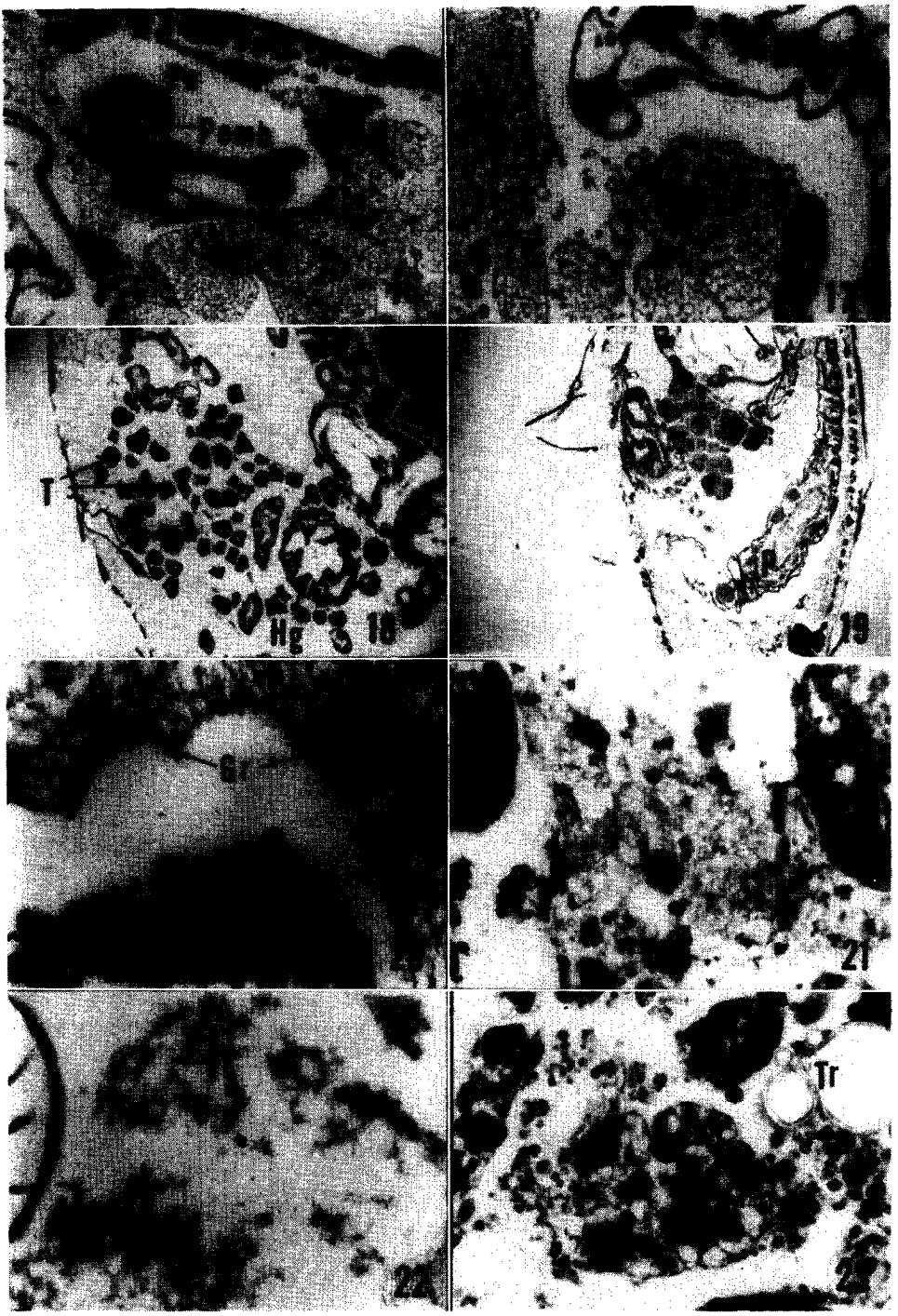
The peripheral fat body, however, shows basic structural differences in newly emerged as compared to overwintering beetles (Figs. 14 and 15). Not only are the peripheral fat tissues of the newly emerged beetle, less vacuolated than that in the overwintering beetle but the nuclei and associated granulation are markedly different than those found in overwintering beetles. The nuclei in the newly emerged beetles



FIGS. 10–15. Normal *H. convergens* fat body. 10. Cross section through abdomen of overwintering beetle. 50 ×. 11. Cross section through abdomen of newly emerged beetle. 50 ×. 12. Central fat body of overwintering beetle. 320 ×. 13. Central fat body of newly emerged beetle. 320 ×. 14. Peripheral fat body of overwintering beetle. 320 ×. 15. Peripheral fat body of newly emerged beetle.

are large, not darkly stained, and surrounded by extensive, fine, granular particles (Fig. 15). By contrast the nuclei of the overwintering beetles are small, darkly stained and the surrounding granulation is limited to a small area.

The pathological difference in the fat body of newly emerged beetles resulting from parasitism by *P. coccinellae* resemble those differences found in overwintering beetles. However, there is less change produced in newly emerged beetle peripheral fat body than in that of overwintering beetles. These results are primarily based on studies conducted with overwintering beetles; differences in the effect on the peripheral fat of newly emerged beetles are obviously only near the termination of the



FIGS. 16–23. Pathological lesions in overwintering *H. convergens* fat body caused by *P. coccinellae*. 16. 1–2 days following parasitization showing granulation of beetle fat body near the parasite egg. 130 ×. 17. Fat-body lesions in the vicinity of the parasite's head. 130 ×. 18. Fat-body loss in the vicinity of newly freed trophic cells. 65 ×. 19. 13-day-old parasite, absence of fat body in area of trophic cells and parasite larva and granulation of fat body near parasite larva. 50 ×. 20. Granulation of central fat body near parasite larva 13 days after parasitization. 320 ×. 21. Central fat body of beetle 15 days following parasitization. 320 ×. 22. Central fat body of beetle from which a parasite larva had emerged. 320 ×. 23. Peripheral fat body of beetle from which a parasite had emerged.

parasite development and these differences will be indicated in their proper sequence.

Within 1 to 2 days following deposition of the parasite egg, structural changes can be seen in the surrounding fat body (Fig. 16). Very fine, eosinophilic granulation forms in the fat body surrounding the parasite egg.

The strongly mandibulate first-instar *P. coccinellae* larva which hatches about 5 days after egg deposition, apparently feeds directly on the fat body. This is indicated in Fig. 17, which shows a longitudinal section of an early first-instar larva. The apparently affected area of the beetle fat body lies just below the parasite's head and extends for a distance equal to the length of the larval mandible. Figure 17 also shows an absence of beetle fat surrounding the parasite larva.

When the parasite larva hatches, the embryonic membrane breaks into individual trophic cells which can then be seen as a group of small, irregularly shaped cells in the hemocoel of the host beetle (Fig. 18). A day or two following larval eclusion the trophic cells completely replace the fat in their immediate area.

By the 13th day following parasitization, both the larva and the trophic cells have increased in size (Fig. 19). Granular degeneration of the fat body surrounding the larva (Figs. 19 and 20) is similar to that which surrounded the egg prior to hatching. However, this type of fat degeneration is not associated with the loss of fat in the vicinity of the trophic cells (Figs. 18 and 19).

Much of the fat body is destroyed by the 15th day of parasitism. The central fat has lost most of its vacuoles, cell boundaries have disappeared, and the cytoplasm has assumed a rather translucent, homogeneous appearance with granules and an occasional hyperchromic nucleus (Fig. 21).

Figure 22 shows a portion of the remaining central fat body immediately following emergence of the parasite larva from the host beetle. This shows a continuation of the processes which were illustrated by Fig. 21. Nearly all of the fat body is lost and what remains has degenerated in a manner which has been termed "hyalin degeneration" (Anderson, 1953). The peripheral fat remains slightly more intact, with a few vacuoles still evident. The nuclei of the peripheral fat cells are greatly enlarged and hyperchromic (Fig. 23).

The sequence of events in newly emerged beetle fat body is similar to that in the overwintering beetles except that the changes in the peripheral fat are less evident. In these beetles there remains some peripheral fat body which appears normal even after the parasite larva has emerged.

Host–Parasite Relationships

Although a large number of parasitized beetles were dissected and serial sections were prepared from about 30 parasitized beetles, only one example of encapsulation of a *Perilitus* larva was discovered. This is shown in Fig. 25. The encapsulated larva appears to be in an early second instar. It is difficult to determine if the larva was dead or alive but some melanization can be seen in the capsule. It is interesting that the trophic cells in close proximity to the encapsulating host cells appear to be restricted in size. This beetle was superparasitized and four dead, first-instar larvae were also present; none of these dead larvae showed any indication of being encapsulated.

In spite of the low incidence of encapsulation there were many cases where *P. coccinellae* failed to complete development. This was apparently attributable to disease of (a) the parasite larvae or (b) of the beetles.

While diseased parasite larvae died in all stages of development, most succumbed in the last larval instar or in the pupal stage. Occasionally small, very weak and short-lived adults emerged from the pupa.

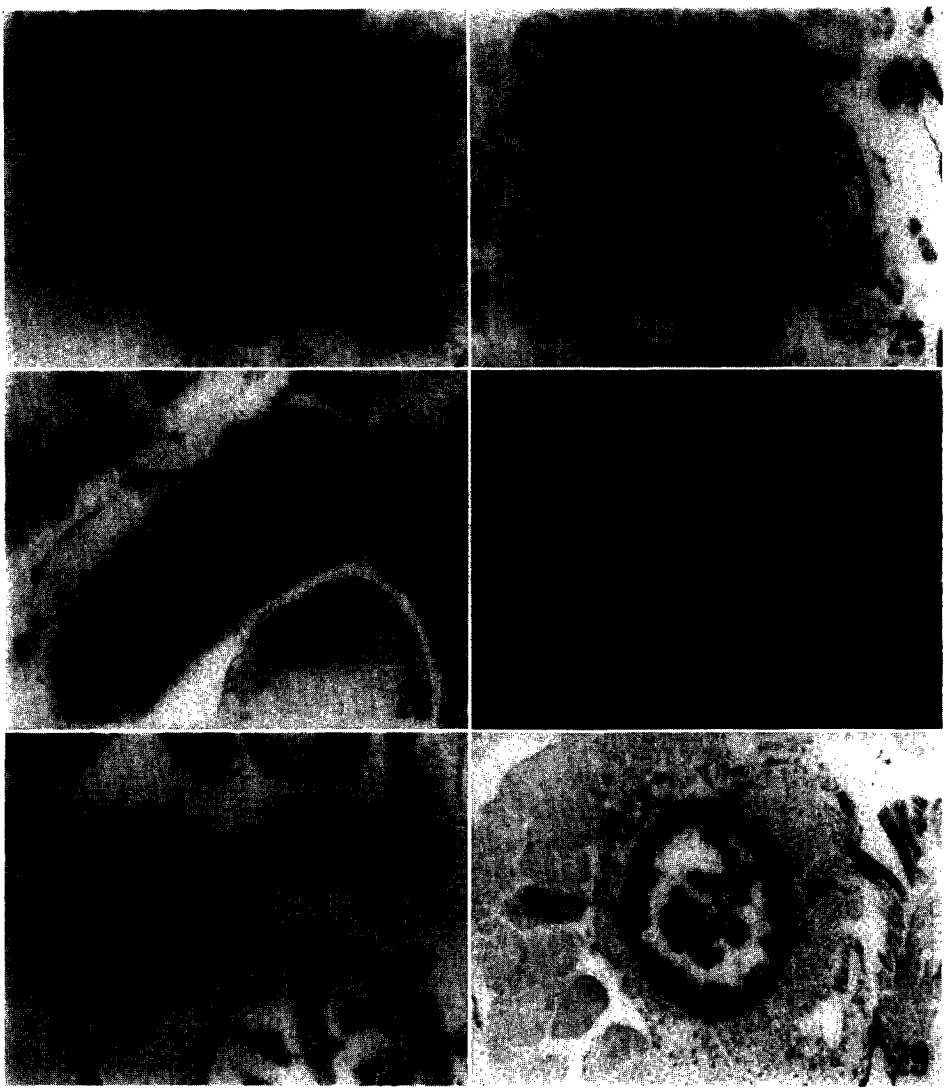


FIG. 24. Small trophic cells associated with dead parasite larva and larger trophic cells distant from the dead parasite larva.

FIG. 25. Encapsulated second-instar *P. coccinellae* larva.

FIG. 26. Abnormal central fat body of newly emerged *H. convergens* adult.

FIG. 27. Central fat and hindgut muscle with sporelike bodies.

FIG. 28. Proliferation in Malpighian tubule associated with the presence of sporelike bodies in a newly emerged beetle.

FIG. 29. Proliferation of midgut in newly emerged beetle.

Microscopic examination of dead or moribund *P. coccinellae* revealed the presence of large numbers of microsporidian spores. That the spores were microsporidian was confirmed by their possessing polar filaments. In fresh physiological saline preparations these spores averaged 7.4 μ in length and 3.1 μ in width.

Although abnormalities were observed in approximately 50% of the *H. convergens*, only in some of these were the abnormalities extensive enough to inhibit the parasite development. These lesions were more commonly encountered in the laboratory-reared beetles than they were in the field-collected beetles.

Examples of these abnormalities are shown in Figs. 26 through 29. Figure 26 shows a section through the fat body of a laboratory-reared beetle which had been stung 17 days prior to fixation by a parasite heavily infected with microsporidia. The fat body is lacking cell boundaries and vacuoles and the nuclei are greatly enlarged. In addition there were many sporelike bodies in the fat body, muscle, and gut tissue. These sporelike bodies were similar to those illustrated in Fig. 27. One dead first-instar parasite larvae was found in this beetle (Fig. 26). The trophic cells were small (25–30 μ in diameter) and occurred in groups of 30 to 60.

Figure 27 was prepared from a laboratory-reared beetle which was fixed 6 days following exposure to a parasite adult. Some of the fat body is more intact than that shown in Fig. 26, but the nuclei are enlarged. Sporelike bodies may be seen in the fat body and the muscle tissue surrounding the hindgut. These sporelike bodies ranged from 2.9 μ to 3.8 μ in length and from 1.4 μ to 1.9 μ in width. These bodies were also found in Malpighian tubules and testes. The midgut possessed enlarged nuclei, some being picnotic, and had proliferated into the gut lumen similar to that shown in Fig. 29. Figure 28 shows another section through the hindgut area of the same beetle. This figure (28) illustrates the general appearance of the fat body and the presence of sporelike bodies in the hindgut tissue. Of especial interest is the neoplasm in the cross section of a Malpighian tubule in which the sporelike bodies can be seen. The beetle from which Figs. 27 and 28 were prepared contained five unhatched *P. coccinellae* eggs and one first-instar larva accompanied by small trophic cells.

The sporelike bodies were never found in the midgut cells of beetles, however, when the other tissues were heavily infected, the midgut was neoplastic and proliferated into the lumen (Fig. 29).

Frequently beetles were encountered whose fat bodies were generally normal in appearance but with occasional scattered groups of sporelike bodies present. These bodies could also be found in muscle tissue but were lacking in the hindgut. When the hindgut lacked sporelike bodies there was no proliferation of the midgut or Malpighian tubule tissues. The disease appears to begin in the fat body and then progresses to other tissues, with the gut being the last tissue to be invaded.

Parasites are able to develop to maturity in some infected beetles, depending on the extent of the infection in the beetles. When the infection extends to the beetle gut the parasite is prevented from completing its development. But by and large successful development occurred where infection was restricted to the fat body.

Discussion

Although morphological lesions may be observed in the fat body surrounding the parasite egg within 24 hr following its deposition, the beetle shows no abnormalities of activity, feeding behavior, or respiration until the egg hatches. When the egg hatches the beetle's feeding activity is significantly decreased and 2 days later the

respiration rate is significantly lowered over that of nonparasitized beetles. However, within 24 hr the respiration was restored to a normal level where it remained until shortly before parasite emergence. This suggests the presence of a toxic material which is released when the parasite egg hatches. The beetle's respiration is also reduced just prior to parasite emergence. The respiration measurement included that of both the beetle and the parasite. Hence, as the parasite increases in size the total respiration would have been expected to increase if the beetle's respiration per unit of weight remained constant. Since there was no increase in the respiration of parasitized beetles over that of nonparasitized ones, the parasitism apparently caused a reduction in the metabolic processes of the host.

Morphological lesions associated with parasitism by *P. coccinellae* in *H. convergens* were found only in the fat body. The peripheral fat body of newly emerged beetles was less damaged than that of overwintering beetles. In newly emerged beetles, the peripheral fat appears to be a more active tissue than that in overwintering beetles. This is suggested by the nuclear differences in the two tissues and by the more extensive granulation surrounding the nuclei of peripheral fat in newly emerged beetles. Just how the more active tissue may resist the effects of parasitization is unknown.

The pathological lesions in the fat body adjacent to the parasite larva and those in fat tissues near the trophic cells appear to be of different kinds. In the vicinity of the parasite an eosinophilic granular degeneration appears in the surrounding fat-body tissue. The trophic cells appear to absorb and replace the fat-body tissue without causing a granular degeneration. The same type of granular fat degeneration associated with the parasite larva may also be found in the vicinity of the egg shortly after deposition. Apparently there is a toxic by-product which results from the metabolism of the parasite embryo and larva. This was also suggested by the shock-response of the host beetle, as indicated by the feeding and respiration experiments when the parasite egg hatches.

Following emergence of the parasite larva, the remaining fat body is degenerate, with numerous hypertrophic, chromatophilic nuclei free in loosely associated masses of hyaline degenerate cytoplasmic material.

The development and structure of the trophic cells, as well as the pathology caused to the host by these cells, suggest that their main function is the absorption and storage of nutrients from the host beetle. These trophic cells appear to have a fine structure similar to vitelline cells in the egg of *Mytilus edulis* described by Humphreys (1964). The vitelline cells of *M. edulis* absorb and store food which is later utilized by the developing sea mussel embryo.

Although many *P. coccinellae* are unsuccessful in their development, encapsulation by the hemocytes of *H. convergens* was not a significant factor. Only one example of an encapsulated parasite larva was seen, and dead supernumerary larvae were not encapsulated. There was no case where the trophic cells were encapsulated. From the data obtained no explanation of why a second-instar *P. coccinellae* larva was encapsulated can be offered.

Some of the unsuccessful parasitism was due to a microsporidian infection in *P. coccinellae.* The developmental stages of the protozoan were not observed, hence a determination of the genus is not possible.

A disease of the coccinellid beetles, when sufficiently widespread, also inhibited the development of *P. coccinellae.* The etiological agent responsible for the observed lesions in beetles was not positively identified. However, sporelike bodies were ob-

served in sections which resembled microsporidian spores. These sporelike bodies were within the size range of those of the microsporidian, *Nosema hippodamiae* (Lipa and Steinhaus, 1959). It is possible that the observed abnormalities of diseased beetles were due to parasitization by *N. hippodamiae*. In any event the probability of a *P. coccinellae* completing development is inversely proportional to the extent of the abnormalities observed in the beetle tissues. The trophic cells of the parasite apparently can not obtain the necessary nutrients for development of the *P. coccinellae* larva from severely diseased beetle fat body.

The ultimate fate of a beetle which is diseased sufficiently to prevent the development of a parasite is unknown. However, these beetles were able to live in excess of their life expectancy had the *P. coccinellae* parasite developed normally. Furthermore, some diseased beetles in which a parasite failed to develop appeared to be feeding and the testes appeared to be normal. Hence, to some extent, the disease of these beetles protected them from the effects of parasitism by *P. coccinellae*.

An interesting lesion was observed in midgut tissues of some diseased beetles. In these beetles the midgut epithelium and the intima proliferated into the lumen of the gut. This tumorous growth became sufficiently profuse in some beetles to nearly block the passage of food in the gut lumen. While sporelike bodies were never found in midgut epithelium, these tumors were seen only in beetles whose fat body and other tissues (including the hindgut epithelium) were extensively infected by the sporelike bodies. Neoplasms in the Malpighian tubules were also observed in heavily infected individuals. Sporelike bodies were present in the tumorous growths of the Malpighian tubules. *Perilitus coccinellae* were unable to develop in beetles whose gut tissues were abnormal. However this inhibition of parasite development was probably due to the extensive lesions in the fat body. The fat body was always heavily infected in beetles whose gut tissues exhibited sporelike bodies and neoplasms. Thompson (1958) reported a case where a hymenopterous parasite was prevented from completing development in spruce budworm which were infected by a microsporidian. The parasite ingested large numbers of spores along with the tissues of the host, as these spores were indigestible in the parasite they accumulated and mechanically blocked the digestive system of the wasp. In *H. convergens* the parasite appears to be unable to successfully compete with the microsporidian for the nutrients of the beetles fat body.

The disease of *P. coccinellae* and that of *H. convergens* both contribute to limiting the effectiveness of the parasite as a regulating factor of the beetle. In addition, the observation of the ovipositional behavior indicated that *P. coccinellae* has difficulty depositing eggs in the beetles. Examinations of field-collected beetles showed that *P. coccinellae* is most active in the mountain aggregations of the beetle during the late spring months. During these months beetles occur in dense aggregations, usually several layers of beetles deep. Hence, the parasite has an opportunity to oviposit only into those beetles which make up the top layer of the aggregation. Thus, only a relatively small proportion of the total *H. convergens* populations are exposed to possible parasitism and only in a portion of these is the parasite able to develop. Thus, even though *P. coccinellae* is very active in the spring and its life cycle is well synchronized with that of *H. convergens*, it is able to parasitize only a fraction of the aggregating beetles and the degree of parasitism rarely exceeds 10%.

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