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Effects of Parasitism by the Braconid Wasp *Cotesia congregata* on Metabolic Rate in Host Larvae of the Tobacco Hornworm, *Manduca sexta*

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We examined growth rates, gas exchange patterns and energy metabolism of tobacco hornworm *(Manduca sexta)* larvae parasitized by the braconid wasp *Cotesia congragata.* Larvae parasitized at the beginning of the fourth-instar had reduced growth compared to unparasitized larvae of the same age and short-term differences in metabolism (measured as rates of CO_2 production, ($\dot{V}$ CO_2) were apparent almost immediately after wasp oviposition. However, over the growth period between parasitization and the last part of the fifth-instar, there was no significant difference between parasitized and unparasitized hosts as seen in the relationship between mass and ($\dot{V}$ CO_2. One day prior to parasitoid emergence, host larvae stopped eating, ceased spontaneous locomotor activity and showed a dramatic decline in metabolism. The 60% decline of ($\dot{V}$ CO_2 at this time is consistent with lack of specific dynamic action because the animals were not feeding. Gas exchange became highly cyclical on the day of parasitoid emergence, but the cause and significance of this phenomenon, which disappeared by the third day following emergence, are not clear. This pattern of cycling was not induced by starving nonparasitized larvae for 6 days, nor by immobilizing nonparasitized larvae with tetrodotoxin. Ecdysteroid levels in the host's hemolymph significantly increased on the day when parasitoids completed their L2–L3 molt and began emerging, but not during the wasps' L1–L2 molt which occurred a few days earlier. Contrary to our initial expectation that hemolymph ecdysteroid titers might be linked to alterations in the host's metabolic rate, we observed no such correlation.

Parasitism Parasitoid Ventilation Gas exchange Ecdysteroid titers Host regulation Feeding inhibition Starvation Tetrodotoxin Developmental arrest Host–parasite relationships

INTRODUCTION

The functional and evolutionary relationships between insect parasitoids and their hosts are intricate (Waage and Greathead, 1986; Godfray, 1994; Van Driesche and Bellows, 1996) and in this context several aspects of the energy metabolism of the host–parasite complex are of considerable interest. In a host–parasite system in which the host continues to feed and grow while parasitized and continues to accumulate energy and nutrients, the parasites must maintain a balance between two conflicting factors. On the one hand, the parasites need to keep their host sufficiently viable so that it can effectively feed, avoid predation and perform other critical survival functions while the parasites mature. Presumably this situation requires parasites to minimize or limit their impact on host physiology, behavior and energy budgets. Yet effects on host physiology and metabolism are clearly evident during parasitism.

The host–parasite relationship is even more complex in systems where hosts are parasitized by gregarious or polyembryonic species, or are super- or multi-parasitized.

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Here, both intra- and interspecific competition for nutrients (parasites vs hosts and parasites vs each other) are likely to occur. The host–parasitoid interactions seen during parasitism of *Manduca sexta* larvae by the braconid wasp *Cotesia congregata* serve as an excellent example of this complexity. The wasp larvae develop in the host hemocoel, absorbing nutrients from hemolymph while leaving other tissues intact, although the fat body is diminished in mass relative to that seen in nonparasitized larvae of the same weight class. During the first-instar and the early second-instar of *C. congregata* development, parasitized *M. sexta* larvae exhibit relatively normal behaviors, although their feeding and growth rates are reduced (Beckage and Riddiford, 1978, 1982, 1983; Alleyne and Beckage, 1996). Interestingly, the dry weight of the host at emergence is directly proportional to the number of parasitoids developing within it, suggesting the parasitoids manipulate the growth of their host for their own benefit (Alleyne and Beckage, 1996). Immediately prior to the time parasitoids molt to the third-instar and emerge to spin cocoons and pupate, the host stops feeding and most other spontaneous activity ceases, although the larvae continue to exhibit normal reflex behaviors (Adamo *et al*., 1996; Beckage and Templeton, 1986). Subsequently the host lingers in a state of arrested development for several weeks and development of the testes is disrupted (Reed and Beckage, 1996). Developmental arrest arises due to induction of multiple neuroendocrine and hormonal disruptions which interfere with molting and pupation (Beckage, 1996; Beckage and Riddiford, 1982; Gelman and Beckage, 1995; Zitnan *et al*., 1995a, b).

Here we describe the effects of parasitism by *C. congregata* on the whole-animal metabolic rate of *M. sexta* larvae, using respirometry to measure gas exchange and energy metabolism (Krogh, 1941). Our major goals were to determine the approximate impact of these parasitoids on metabolism of parasitized caterpillars and to estimate the metabolic rates of the parasitoids themselves following emergence from the host. Examination of the metabolic impact of parasitism at different developmental stages proved physiologically informative.

Because ecdysteroids and other hormones have been shown to influence behavior and metabolism of several insects (Raabe, 1982; Nijhout, 1994) we hypothesized that ecdysteroids released by the parasitoids during molting while the wasps are associated with the host (Grossniklaus-Buergin *et al*., 1989; Lawrence and Lanzrein, 1993) could also impact host metabolism. For this reason, we quantified ecdysteroid titers in host hemolymph during the endoparasitic phase of the wasps' development and assessed the relationship of those with host metabolism. Previously, ecdysteroid titers on hosts parasitized in the first-instar have been determined (Beckage and Riddiford, 1982), but not for larvae parasitized later during the fourth-instar.

Host–parasitoid endocrine interactions and effects of parasitism on host hemolymph proteins and peptides have now been well-defined for many species (Lawrence and Lanzrein, 1993; Beckage, 1993a, b, 1996; Balgopal *et al*., 1995; Pennacchio *et al*., 1995; Hayakawa, 1995). However, we are only now beginning to appreciate how parasitism affects other aspects of the host insect's physiology including its metabolism. For example, while the dynamics of gas exchange in insects has attracted recent attention (Slama, 1988, 1994; Lighton, 1994, 1996; Lighton and Duncan, 1995) the effects of parasites or parasitoids on respiratory processes remain largely unknown and only a few recent studies have described effects of parasitism on host respiratory metabolism (e.g. Rivers and Denlinger, 1994). Hence the data described here provide important insights into how parasites affect the metabolic physiology of a host insect.

MATERIALS AND METHODS

Insect cultures

Manduca sexta larvae were reared on an artificial diet (Bell and Joachim, 1976) under a 17L:7D photoperiod in a chamber maintained at 26°C. The fourth and fifth-instar larvae used in this study were maintained individually in plastic cups and fed *ad libitum.* Developmentally synchronous pharate fourth-instar larvae with air-filled head capsules and brown mandibles were selected for metabolic studies; these larvae usually ecdysed within 2–3 h of selection. The ensuing light–dark cycle was designated as day 0 of the fifth-instar (Beckage *et al*., 1994). To induce pupal diapause, some larvae were reared under a short-day photophase (12L:12D) at the same temperature. Pupae exposed to these conditions failed to initiate adult development and were judged to be in diapause.

Cotesia congregata were reared using first-instar *M. sexta* for parasitization. Parasite cocoons were stripped from terminal fifth-instar hosts about 24 h after emergence (Beckage and Riddiford, 1983). Adult wasps were maintained at 25°C in a plastic cage and had continuous access to clover honey and distilled water.

Parasitization protocol

Day 0 fourth-instar *M. sexta* larvae were exposed to *C. congregata* adults approximately 7 h after photophase onset. Individual hosts received 1 to 6 ovipositions within 30 min, insuring a wide range of parasitoid loads (multiple ovipositions into a single host are common in the natural environment; Fulton, 1940; Postley and Thurston, 1974). We observed a signficant positive correlation between the number of parasitoids present in the host and the final weight attained by the host–parasite complex which was virtually identical to that described by Alleyne and Beckage (1996) (results not shown).

Closed-System respirometry

We used both closed- and open-system respirometry to determine rates of gas exchange and energy metabolism. For closed-system measurements on *M. sexta* lar-

vae we used glass jars (volume 245 or 985 ml) fitted with airtight lids equipped with stopcock valves. Measurements were made on larvae housed individually. During all measurements of gas exchange, larvae were kept in an environmental cabinet maintained at constant temperature (27±0.5°C) without food.

Animals were weighed on an electronic balance (±0.01 g) and put into jars, which were then flushed with dry, CO_2-free air, sealed and placed into the constant-temperature cabinet. At the conclusion of measurement periods (2–6 h), a 60 ml syringe equipped with a stopcock valve was used to withdraw samples of air from the metabolism chamber. Each sample was rapidly injected into a narrow tube connected to the gas analyzers. The tube contained a stream of dry, CO_2-free reference air. The bolus of sample gas passed through a desiccant column (magnesium perchlorate) into a CO_2 analyzer (Anarad AR-50 or Beckman LB-2). The sample was then scrubbed of CO_2 (Ascarite), redried and passed through an oxygen analyzer (Applied Electrochemistry S-3A). Deflections in CO_2 and O_2 concentrations from reference values were recorded on a Macintosh computer that was connected to the analyzers with an analog to digital converter (Remote Measurement Systems ADC-1). The CO_2 analyzers were calibrated against certified reference gases (0.251, 0.505, or 5.10% CO_2 in air). We calculated rates of carbon dioxide production ($\dot{V}\,CO_2$: ml/min) as:

$$\dot{V}\,CO_2 = F_eCO_2 \cdot V/T$$

where V is the chamber volume (corrected to dry air at standard temperature and pressure; STPD), T is elapsed time in minutes and F_eCO_2 is the fractional concentration of CO_2 in the sample gas. We calculated rates of oxygen consumption ($\dot{V}\,O_2$; ml/min) according to Withers (1977) as:

$$\dot{V}\,O_2 = (V/T) \cdot (F_iO_2 - F_eO_2)/(1 - F_iO_2)$$

where F_iO_2 and F_eO_2 are the fractional concentrations of O_2 in reference and sample air, respectively. The duration of measurement periods was adjusted in accordance with chamber volume and animal mass to keep changes in gas concentration within the range of 0.3% to 2.0%.

We used a similar system to determine the metabolic rates of emerged *C. congregata* pupae, except that 60 ml syringes equipped with stopcock valves were substituted for the sealed jars. Twenty-four hours after emergence, all the wasp pupae collected from a single host larva were stripped from the host and weighed *en masse.* It was necessary to wait until 1 day post-emergence to avoid injury to the emerging larvae, which fail to complete cocoon spinning or pupation if disturbed. Much like lepidopteran larvae (e.g. *Ephestia kuhniella)* which fail to spin cocoons and show a delay in metamphosis (Giebultowicz *et al*., 1980, 1984), the wasps appear to require the proprioreceptive feedback provided by the cocoon to complete their metamorphosis. The group of pupae was placed into a syringe, which was then flushed with reference air, sealed and left for 3.25 h at 27°C. At the end of this interval the air in the syringe was injected directly into the reference air stream leading to the gas analyzers. Since these wasps pupate the day following emergence, their metamorphosis was in progress when measurements were performed. The metabolic rate per pupa was determined by dividing the group ($\dot{V}\,O_2$) and ($\dot{V}\,CO_2$) by the number of cocoons in the pooled sample.

Open-System respirometry

A continuous flow open system was used to obtain minimal resting metabolic rates of *M. sexta* larvae and to examine short-term fluctuations in gas exchange. Metabolism chambers were airtight plastic jars (100–200 ml) or modified 30 ml plastic syringes, equipped with input and output ports. Flow rates of dry, CO_2-free air (110 ml/min) were maintained (±1%) by Applied Materials mass flow controllers. Approximately 40 ml/min of the excurrent air stream was dried (magnesium perchlorate) and pulled through a LiCor 6251 CO_2 analyzer, then scrubbed of CO_2, redried and passed through the S-3A O_2 analyzer. Outputs from the analyzers were recorded every 2 s by a Macintosh computer. We calculated ($\dot{V}\,CO_2$) as:

$$\dot{V}\,CO_2 = \dot{V}\,F_eCO_2$$

where $O\dot{V}$ is the flow rate in ml/min (corrected to STPD). We calculated $\dot{V}\,O_2$ according to Withers (1977) as:

$$\dot{V}\,O_2 = \dot{V}(F_iO_2 - F_eO_2)\,/(1 - F_iO_2)$$

Changes in gas concentrations during open-system measurements were usually too small to be accurately determined with the oxygen analyzer (its best resolution was about 25 parts per million). Accordingly, most of these measurements were made using a LiCor 6251, which had a resolution of better than 1 part per million and was considerably more stable than the S-3A instrument. Data on respiratory quotients (see Results section), together with simultaneous open-system measurements of $\dot{V}\,O_2$ and $\dot{V}\,CO_2$ on several large *M. sexta* larvae (Fig. 1) indicated that $\dot{V}\,O_2$ and $\dot{V}\,CO_2$ fluctuated in close synchrony. Accordingly, it was possible to use $\dot{V}\,CO_2$ to estimate $\dot{V}\,O_2$ and calculate energy metabolism with acceptable accuracy.

Open-system measurements lasted 1–2 h and included inactive periods as well as bouts of activity. Minimal resting metabolic rates were obtained by using custom software to scan data files and find the lowest running average during periods of 3 min; these corresponded to times when the animals were not locomoting.

Weights and resting metabolic rates were monitored beginning on day 1 of the hosts' fourth-instar. Unparasitized individuals were followed daily until the wandering stage (fifth-instar) and parasitized individuals were moni-

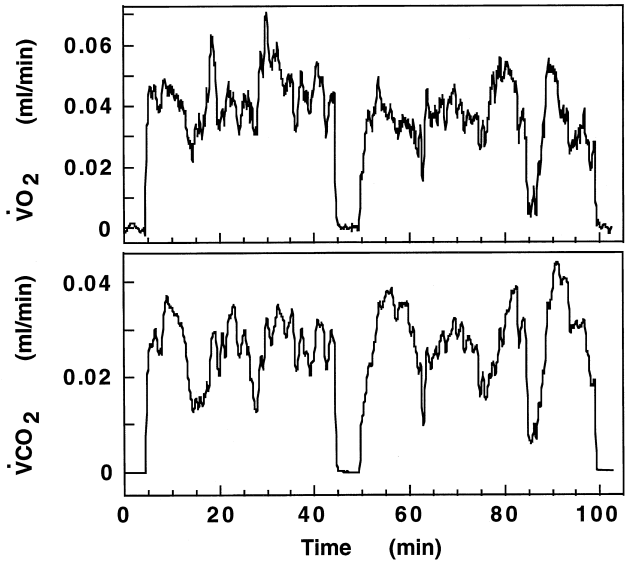

FIGURE 1. Graph depicts the simultaneous measurements of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) from an unparasitized *Manduca sexta* larva. The low, stable readings at the beginning and end of the record and at an elapsed time of 45–50 min. are readings of reference air. Note the close synchronization between fluctuations of $\dot{V}O_2$ and $\dot{V}CO_2$.

tored daily until wasps emerged. To study host metabolic rate during the period of post-emergence developmental arrest, another group of parasitized larvae was monitored 1 day prior to parasitoid emergence, on the day of parasitoid emergence and at one and three days post-emergence. The parasitoid cocoons were gently stripped from hosts within 6 h of emergence to eliminate the effects of the emerging wasps' metabolism on measurements of the host's metabolism.

To examine the interactions between short-term ventilation patterns and activity during developmental arrest, additional comparisons were made between post-emergence hosts, fasted unparasitized *M. sexta* larvae and unparasitized larvae immobilized with a paralytic agent, the puffer fish toxin tetrodotoxin (TTX; Sigma) (Gammon, 1978). Post-emergence hosts fail to reinitiate feeding and hence might be physiologically comparable to starved unparasitized larvae deprived of food for several days. The purpose of TTX paralysis was to induce a state of basal mimimal metabolism similar to that exhibited by hosts which have ceased spontaneous locomoter behavior following emergence of the wasps. To immobilize larvae, we administered 4 μg/g of TTX to several nonparasitized larvae weighing approximately 5 g; this dose is sufficient to induce flaccid paralysis within 1–2 h (Nijhout and Williams, 1974; Beckage and Riddiford, 1982). Metabolic measurements began 4–5 h after TTX was administered.

Effects of fasting were studied in a group of 2.5–3 g larvae which had been denied food for 5–6 days. We used this mass range because larger starved larvae frequently undergo dorsal vessel exposure and molting to larval-pupal intermediates (N.E. Beckage, unpublished data). Larval mass after the period of starvation ranged from 1.5–2 g.

Ecdysteroid titers

To examine the possible influence of parasitoid ecdysis on host hormones or metabolism, we measured ecdysteroid titers daily in parasitized fifth-instar *M. sexta* larva, beginning with the onset of the molt to instar 5. The wasps' L1–L2 ecdysis normally occurs between day 1 and 2 of the host's fifth-instar and a distinct pulse of ecdysteroid appears in host hemolymph just before the wasps ecdyse to L3 and emerge (Beckage and Riddiford, 1982, 1983; D. Gelman, D. Reed and N. Beckage, in prep.). Parasitoid secretion of ecdysteroids has been reported in several other species (Lawrence and Lanzrein, 1993), suggesting the possibility of an ecdysteroid peak might plausibly be synchronized with the wasps' first larval molt, so we examined ecdysteroid titers in the host. Moreover, there was a decline in host metabolism on days 6 and 7 coincident with the parasitoids' molt to the second-instar. Approximately 6 h after the onset of photophase, larvae were anesthetized with 95% CO_2 / 5% O_2 and hemolymph from a cut proleg was collected into an iced microcentrifuge tube. Aliquots (10 μl) were transferred (in duplicate) to two microcentrifuge tubes with 300 μl of absolute methanol and the mixture was vortexed and centrifuged at 16,000 g for 4 min at 4°C to remove hemocytes and teratocytes. Two 100 μl aliquots were transferred to 6×50 mm glass tubes. After the methanol was removed under nitrogen, the ecdysteroid content of the residue was measured by radioimmunoassay (RIA) as described in Gelman and Woods (1983). The antibody we used has a high affinity for ecdysone, 20-hydroxyecdysone, 26-hydroxyecdysone, 20,26-dihydroxyecdysone and makisterone A. The radioactively-labeled antigen was tritiated ecdysone (63.5 Ci/mmole; New England Nuclear). Results are expressed as pg 20-hydroxyecdysone equivalents/μl of hemolymph.

Statistical analysis

Results are reported as means ± standard deviations. Differences between means with similar variance were tested with *t*-tests and we used covariance analysis (ANCOVA) to correct for the effects of mass when comparing metabolic rates among treatment groups (Zar, 1984). The significance level was 95% (α=0.05) for all comparisons. Calculations were performed using Statview (Abacus Concepts) or Statistica/Mac (StatSoft).

RESULTS

Growth and development

Unparasitized *M. sexta* larvae ecdysed to the fifth-instar 1 day prior to the parasitized larvae and attained a maximal mass of 8.43±0.72 g several days following ecdysis to the fifth-instar. Parasitized larvae grew more slowly, reaching a maximal mass of between 3 and 7 g

(mean 6.40±5.76 g) approximately 1–2 days before parasitoids emerged. Emergence occurred 11–13 days post-parasitization (days 8–10 of the host's fifth-instar). Parasitoid loads ranged from 203 to 816 wasps per host (mean 434±174). This indicates that most hosts received more than one oviposition, since a mean of about 115 wasps develops following a single oviposition in *M. sexta* larvae of this stage. There was a significant positive correlation between parasitoid load and maximal mass as shown previously, in that more heavily parasitized hosts weighed proportionately more at emergence (Alleyne and Beckage, 1996). Examination of a wide range of parasitoid loads provides useful information about the regulation of host physiology under conditions of light vs heavy parasitism (Beckage and Riddiford, 1978; Alleyne and Beckage, 1996). Moreover, in this system the host is frequently superparasitized in the field, with up to several hundred parasitoids being found within or emerging from a single host (Fulton, 1940; Postley and Thurston, 1986). We have observed a maximal number emerging (or 'host carrying capacity') of about 200 parasitoids, (Alleyne and Beckage, 1996).

Comparison of $\dot{V}O_2$ and $\dot{V}CO_2$

Typically, short-term rates of CO_2 production in unparasitized *M. sexta* larvae were irregular, largely as a result of episodic locomotory activity (Fig. 1). Similar patterns were noted in parasitized larvae that were still feeding. However, this pattern changed during and after the emergence of parasitoids as described below.

Respiratory quotients

We obtained closed-system *RQ* data for 82 *M. sexta* larvae ranging in mass from 0.78 to 8.85 g, of which 42 were parasitized by *C. congregata*. There was no effect of body mass or of parasitism (p=0.12; ANCOVA) on *RQ*, which averaged 0.877±0.081.

To obtain estimates of the parasitoids' metabolic rate, we collected emerged *C. congregata* pupae from 12 host larvae; the number of cocoons per host ranged from 20 to 172. The $\dot{V}O_2$ and $\dot{V}CO_2$ averaged 0.024± 0.003 ml/min and 0.019±0.003 ml/min per cocoon, respectively, corresponding to an *RQ* of 0.79±0.12.

Resting metabolic rates

As might be expected from its effects on host growth, parasitism by *C. congregata* was associated with significant short-term changes in host metabolism. The mass-specific resting $\dot{V}CO_2$ of hosts declined significantly on the day following parasitization (Fig. 2; $p<0.02$; ANOVA). Nevertheless, over the remainder of development prior to the onset of parasitoid emergence, the metabolic rates of both parasitized and unparasitized *M. sexta* larvae increased consistently with increasing body mass (Fig. 3). The overall allometric relationship between mass (grams) and metabolism ($\dot{V}CO_2$; ml CO_2 /min) was not significantly different in parasitized and unparasitized hosts ($\dot{V}CO_2 = 0.0277.\text{mass}^{0.774}$ and $\dot{V}CO_2 = 0.0259.\text{mass}^{0.804}$, respectively; p=.14, n=39 parasitized and 38 unparasitized).

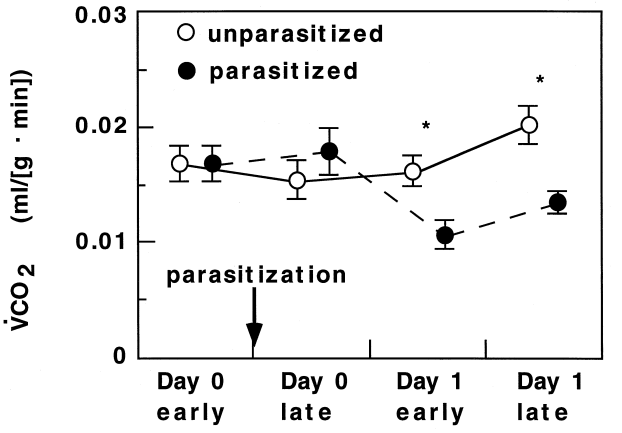

FIGURE 2. Short-term changes seen in the metabolic rate ($O\dot{V}CO_2$) of *Manduca sexta* larvae were detected almost immediately after parasitization by *Cotesia congregata.* Data are plotted as means ±SE. The * indicates the detection of significant differences between parasitized and unparasitized larvae as determined using t-test comparisons (for the day 1 early point, $p<0.03$, d.f.=13; for day 1 late, $p<0.003$, d.f.=13).

Approximately two days prior to parasitoid emergence, host larvae ceased feeding and by the day of emergence their resting metabolism had fallen to 30–40% of peak values [Figs 4 and 5(A)]. A total of 10 larvae were followed at daily intervals; the trace in Fig. 4 represents that of one such larva; pooling data from all 10 larvae would obscure the short-term changes due to the lack of synchronous ecdysis to the fifth-instar, as well as parasitoid emergence, which occurred over two days. Smaller transient declines in resting $\dot{V}CO_2$ occurred during host ecdysis to the fifth-instar and (in parasitized animals) on day 6–7 following parasitization (Fig. 4), which corresponds to the time when the parasitoids ecdyse from L1 to L2 (Beckage and Riddiford, 1983).

Temporal patterns of gas exchange

One to two days prior to emergence, host activity ceased and CO_2 production became more stable [Fig. 6(A)]. On the day of emergence, the $\dot{V}CO_2$ of most larvae became strongly cyclical, with a periodicity of 4 to 10 min [Fig. 6(A)]. The amplitude of cycling subsequently declined and the cyclic pattern usually disappeared by the third day after parasitoids had emerged [Fig. 6(A)].

As for post-emergence parasitized larvae, the metabolic rates of fasted larvae were about 40% of those of fed larvae of similar mass (0.007 and 0.018 ml/min, respectively, for 1.5–1.8 g animals). However, cyclic CO_2 release did not occur in fasted animals [Fig. 6(B)] and their pattern of gas exchange qualitatively resembled that of fed larvae (Fig. 1), with irregular short-term fluctuations in $\dot{V}CO_2$. The average metabolic rate of larvae immobilized with tetrodotoxin [Fig. 6(B)] was a little higher than that of post–emergence parasitized larvae of similar mass [Fig. 6(A)]. However, the $\dot{V}CO_2$ of tetrodo-

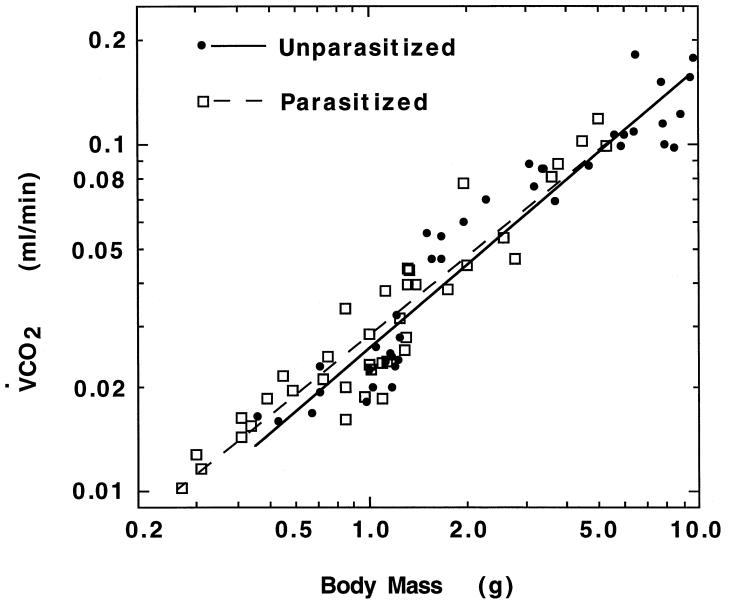

FIGURE 3. The relationship between mass and metabolism (measured as $\dot{V}\,CO_2$) of parasitized and unparasitized *Manduca sexta* larvae observed during the feeding stages of the fifth-instar. The slopes and intercepts do not differ significantly (n=39 parasitized and 38 unparasitized larvae; $\dot{V}\,CO_2 = 0.0277.\text{mass}^{0.774}$ and $\dot{V}\,CO_2 = 0.0259.\text{mass}^{0.804}$, respectively; p=0.14). Data was analyzed using ANCOVA (see text).

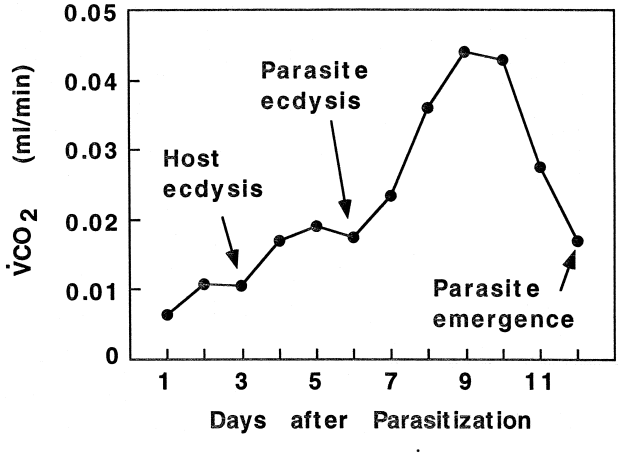

FIGURE 4. The metabolic rate (measured as $\dot{V}\,CO_2$) as measured for a single typical parasitized *Manduca sexta* larva. Feeding behavior ceases on day 9–10 after parasitization, about 24 h before the parasitoids emerge from the host. A total of 10 larvae were monitored at daily intervals; graph shows data from an individual due to interindividual temporal differences in the timing of molting to the fifth-instar and parasitoid emergence.

toxin-injected animals was highly stable rather than cyclic [Fig. 6(B)], indicating that cycling is not simply a result of the relative immobility of hosts with emerging wasps. Hence the cycling seems to have a physiological origin unique to parasitized larvae.

All of these patterns of gas exchange were qualitatively and quantitatively different from the 'classic' discontinuous ventilation seen in a diapausing female *M. sexta* pupa [Fig. 6C), which has a periodicity of many h and includes long periods without any apparent trachael gas exchange. The pattern observed in the pupae examined in the present study was similar to that reported in earlier studies of diapausing lepidopterans and other insects (Levy and Schneiderman, 1966; Burkett and Schneiderman, 1974; Slama, 1994).

Ecdysteroid titers

As expected, we found high titers of ecdysteroids in the hemolymph of pharate fourth-instar *M. sexta* larvae on the day preceding ecdysis to the fifth-instar (Fig. 6). Immediately following ecdysis (day 0 of the host fifth-instar) titers had dropped more than 10-fold and they remained low for several days. In parasitized larvae, ecdysteroid titers increased substantially on day 4 of the fifth-instar (day 9 post-parasitization), which coincides with the cessation of larval feeding and the initiation of the second molt of the wasps. However, there was no increase in ecdysteroid titers on days 1 and 2 of the host fifth-instar (Days 6 and 7 post-parasitization) (Fig. 6) coincident with the observed transient decline in $\dot{V}\,CO_2$ (Fig. 5) and the predicted time of the first larval molt of the parasitoids. The lack of a hemolymph ecdysteroid peak in the host at the time of the parasitoids' molt to the second-instar supports the view that at this time the parasitoids are not markedly influencing the host's ecdysteroid titer, though its metabolic rate is affected. In contrast, a few days later the wasps actively secrete ecdysteroids (D. Gelman, D. Reed and N. Beckage, in preparation) and generate an ecdysteroid pulse a few h prior to their emergence. Thus, different aspects of the host's physiology (gas exchange, endocrine physiology, hemolymph proteins, immune system) may be temporally affected by varying stages of parasitism, to greater or lesser degrees at different times during endoparasitism.

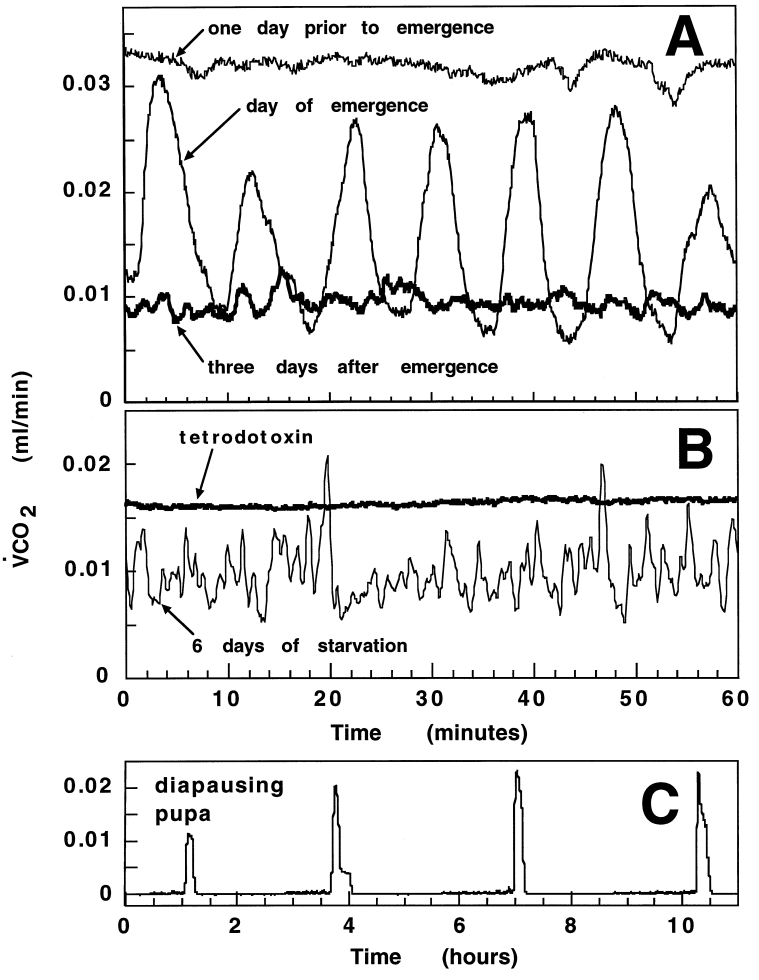

FIGURE 5. A. Cyclic pattern seen in the rate of CO_2 emission of a single *Manduca sexta* larva during the period of parasitoid emergence. B. The CO_2 emission patterns of a starved larva (held without food for 6 days) and a larva induced to exhibit flaccid paralysis by injection with tetrodotoxin. C. Discontinuous ventilation pattern seen in a diapausing female *M. sexta* pupa. Note the periodicity differs from the cyclic pattern seen in (A).

DISCUSSION

Parasitoids often induce drastic changes in the physiology and behavior of the host insect, many of which can be viewed as adaptations to ensure 'successful' parasitism and create a host environment suited to meet the needs of the parasitoids (Waage and Greathead, 1986; Strand, 1986; Horton and Moore, 1993; Godfray, 1994). In the *Manduca sexta / Cotesia congregata* system, known effects of parasitism include growth inhibition and endocrine anomalies such as suppression of metamorphosis and developmental arrest (Beckage, 1993a, 1996), behavioral changes such as the pre-emergence cessation of feeding and spontaneous motor activity (Beckage and Templeton, 1986; Adamo *et al*., 1996), alterations in neuropeptide levels in the nervous system and gut (Zitnan *et al*., 1995a, b), shifts in the metabolism of energy substrates and alterations in hemolymph proteins (Thompson 1982a, b, 1993; Thompson *et al*., 1990; Beckage, 1993b; Beckage and Kanost, 1993; Harwood *et al*., 1994), inhibition of testicular development (Reed and Beckage, 1996) and immediate immunosuppression coupled with lysis of host hemocytes (Lavine and Beckage, 1995, 1996). Some of these effects are induced by the polydnavirus *C. congregata* females inject into hosts along with their eggs; yet other factors such as teratocytes and venom may also be important (Beckage, 1993a, b; Dahlman and Vinson, 1993; Lavine and Beckage, 1995, 1996; Jones and Coudron, 1993; Wani *et al*., 1993; Beckage *et al*., 1994; Strand and Pech, 1995).

Given the extensiveness of these effects, we were not surprised to find differences in the energy metabolism of parasitized and unparasitized *M. sexta* larvae. How our findings relate to earlier studies of unparasitized larvae conducted by Siegert and Ziegler (1983) is not clear. The most obvious effect is the dramatic decline in host metabolism following parasitoid emergence (Figs 4 and 5). Prior to this, short-term changes in parasitized hosts included a significant reduction of metabolic rate on the day following wasp oviposition (Fig. 2) and a small transient decline in host metabolism on day 6 following oviposition (an example is shown in Fig. 4). The latter event was temporally consistent with the expected time of ecdysis between the first and second parasitoid-instars (Beckage and Riddiford, 1983). This pattern was consist-

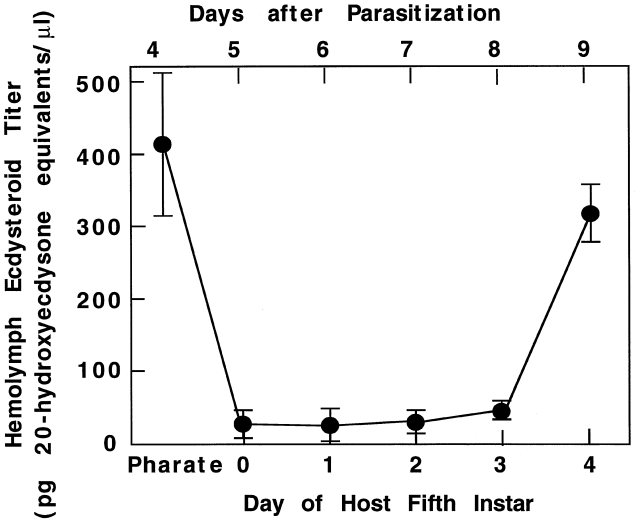

FIGURE 6. Hemolymph ecdysteroid titers in *M. sexta* larvae parasitized by *C. congregata* monitored from the time of molting to the fifth-instar (pharate fifth-instar larvae) until parasitoid emergence. Larvae were parasitized on day 0 of the fourth-instar and wasps emerged on day 4 of the fifth-instar, or 9 days post-parasitization. Note the lack of change in ecdysteroid levels at the time of the parasitoid ecdysis from the first to the second larval-instar at day 6 after parasitization. Titers are expressed as pg 20-hydroxyecdysone equivalents per microliter of hemolymph ±S.E. See Materials and Methods for details.

ently observed in several individuals and we anticipated it may be synchronized with molting of the parasitoids and peak of ecdysteroids. However, we did not observe a coincident change in ecdysteroid levels in host hemolymph (Fig. 6), which suggests that the pattern is not a direct result of the appearance of molting hormones in the hemolymph. However, samples taken at more frequent 4–6 h intervals may be necessary to detect a relatively small and transient ecdysteroid pulse.

Nor did we observe a peak in ecdysteroids synchronous with the pattern of respiratory cycling on the day of emergence, or the low level of $\dot{V}CO_2$ seen after emergence. Hence, we conclude that while emergence is preceded by or synchronous with an ecdysteroid peak, major transitions in $\dot{V}CO_2$ are not synchronized with ecdysteroid pulses appearing in the blood. The ecdysteroid titers confirm earlier data provided by Beckage and Riddiford (1982) for larvae parasitized in the first-instar. Many hormones including juvenile hormone and ecdysteroid are thought to be important in regulating insect metabolism (Raabe, 1982; Nijhout, 1994) but their role in regulating host metabolism in this system has yet to be proven.

The overall relationship between mass and resting metabolism across the cumulative period of fifth-instar growth (i.e. prior to developmental arrest), did not differ between parasitized and unparasitized hosts (Fig. 3). Unparasitized larvae grew faster than parasitized animals, but over a 10- to 15-fold mass range the resting metabolism of the host–parasitoid complex was equivalent to that of an unparasitized host of similar mass. There was no apparent effect of varying parasitoid load on this relationship, even though the maximum mass attained by parasitized *M. sexta* larvae is positively correlated with parasitoid load and heavily parasitized larvae feed slightly longer in the fifth-instar than more lightly parasitized animals (Alleyne and Beckage, 1996).

In parasitized *M. sexta* larvae, parasitioids may comprise up to 20% of the mass of the host–parasitoid complex on the day of emergence (Beckage and Riddiford, 1983) and often more when the host is heavily parasitized, although a maximum of about 200 parasitoids emerges from the host (Alleyne and Beckage, 1996). Accordingly, it seems possible that the parasitoids could contribute a substantial fraction of the total metabolism of the host–parasitoid complex. The *in vivo* metabolism of *C. congregata* larvae is poorly understood, although a few measurements have been made on other parasitoids (e.g., *Microplitis croceipes;* Edson and Vinson, 1976; see also Thorpe, 1932). Cutaneous respiration is hypothesized to occur in endoparasitoids since an open tracheal system is lacking until the onset of the last-instar when the parasitoid leaves the host (Clausen, 1940; Hagen, 1964). Nevertheless, parasitoids are presumed to have an oxygen requirement, which has been invoked to explain the physiological suppression of development of some parasitoid larvae in superparasitized or multiparasitized hosts (Fisher, 1963, 1971). Those that feed on host hemolymph components, as does *C. congregata*, appear to be highly efficient at converting host tissue to parasitoid biomass; for example, the wasp *Nemeritis canescens*, which develops in *Ephestia kuehniella*, has been reported to have an assimilation efficiency of 94% (Howell and Fisher, 1977). Parasitized *M. sexta* larvae are slightly more efficient at digesting their food compared to nonparasitized larvae (Alleyne and Beckage, 1996) suggesting the presence of the parasitoids enhances the host's metabolic efficiency.

We measured the gas exchange of emerged *C. congregata* undergoing pupation within the cocoon (0.019 ml CO_2/min per parasitoid). If the metabolic rate of this stage is similar to the metabolism of unemerged parasitoids, then the wasp larvae may account for about 20% of the resting metabolism of the host–parasitoid complex immediately prior to emergence (i.e. when host metabolism has fallen from peak values; Fig. 4). The corresponding value for the end of the feeding period (Day 9–10 after wasp oviposition) is 5–8% of total metabolism. However, these estimates are tentative because of uncertainties about the mechanism of metabolism (aerobic vs anaerobic pathways; both may be used in different stages) and the rate of gas exchange of larval wasps.

It is unclear to what extent parasitoids manipulate their host's metabolism to maximize their own benefit (Godfray, 1994). We observed a relatively minor impact on host energy metabolism during the pre-emergence feeding stage which probably reflects the need for continued host growth in the initial stages of development in koinobiont parasites like *C. congregata.* These species do not consume host tissue, relying instead on nutrients

absorbed from host hemolymph, although this strategy indirectly depletes host nutrient stores, however. Maintenance of most normal host activities and behavior during parasitoid growth allows acquisition of additional energy and materials, some of which are diverted from the host and exploited by the parasitoids (Thompson, 1982a, b, 1993; Strand, 1986; Thompson *et al*., 1990). Moreover, the teratocytes released by the serosa of *C. congregata* grow from 10 μm diameter (Beckage and de Buron, 1994) to 150–200 μm (I. de Buron and N. Beckage, in preparation) in a short period of time; furthrmore about 150 are released per embryo and their mass in the hemocoel is significant when the wasps emerge from the host. The rapid and phenomenal growth of these cells, which are packed with glycogen and lipid inclusions, likely represents another 'metabolic stressor' aside from the rapid growth phase exhibited by the second-instar parasitoids themselves (Beckage and Riddiford, 1983).

In the *M. sexta / C. congregata* system the host continues to feed for at minimum 9 days after oviposition (after parasitization of fourth-instar larvae when the wasps emerge at 10 or 11 days post-oviposition) or for up to 17 days, as occurs following parasitization of first-instar larvae. This continued energy and nutrient intake by the host, together with the associated increase in host mass, augments the resources available to the parasitoids by at least an order of magnitude (or even several) above that contained in the host at the time of oviposition. Hosts with more parasites feed longer and the dry weight of the host is directly correlated with the parasitoid clutch size or parasitoid load (Alleyne and Beckage, 1996). In contrast, idiobiont parasites, which attack nongrowing host stages or active feeding stages that are paralyzed during oviposition, provide a fixed amount of resources (Askew and Shaw, 1986). These parasitoids usually feed directly on host tissues and often consume most, if not all, of the host's carcass (aside from the cuticle) prior to emerging, as occurs when the ichneumon *Hypososter exiguae* emerges from *M. sexta* (Beckage and Templeton, 1985).

The cause of the dramatic drop in host metabolism at the time of *C. congregata* emergence is unclear. It may partly be a result of the loss of the metabolic contribution of emerging parasitoids, but this is unlikely to account for more than a fraction of the total change. Cessation of host locomotor activity is also a factor; the striking quiescent behavior of the host (Adamo *et al*., 1996) is presumably adaptive for the wasps, which must complete cocoon spinning without disturbance. Also, the host's stoppage of active feeding ensures that the parasitoids will not be consumed by an avidly feeding host. While the neurophysiological controls regulating host feeding activity in caterpillars and other insects are now well characterized (Chapman and de Boer, 1985), we have as yet no clue as why the host stops feeding just before the wasps emerge, although hemolymph octopamine levels rise significantly on the day of emergence (Adamo *et al*., 1996), as do hemolymph dopamine levels (S. Adamo, C. Linn and N. Beckage, unpublished data). These phenomena may be temporally correlated, but not causally linked; i.e. the transitory peaks in octopamine and dopamine may be a response to the massive integumental damage occurring during emergence, rather than being physiologically linked to the observed alterations in feeding behavior or metabolism *per se* (Adamo *et al*., 1996).

Spontaneous host activity ceases well before parasitoid emergence while the host's metabolic rate continues to decline until at least 3 days post-emergence [Fig. 5(A)]. Much of the decline may result from cessation of feeding and lack of specific dynamic action (SDA; Harper, 1971). SDA is the energy cost of utilizing food and is manifested as an elevated resting metabolism during digestion and assimilation. Roughly 5–13% of the metabolizable energy of dietary carbohydrates and lipids and 30% of the metabolizable energy of dietary proteins is lost as SDA (Harper, 1971). We used these values, the composition of the diet (energy content approximately 65% protein, 25% carbohydrate and 10% lipid), metabolic rate and growth rate to estimate SDA in *M. sexta*. For a 6 g parasitized larva just prior to emergence, the metabolizable energy intake is roughly 12.4 KJ/day, of which about 20% (2.5 KJ/day) is SDA and 68% (8.4 KJ/day) is deposited in new tissue (growth). Therefore the SDA would comprise roughly 60% of the actual metabolism of 4 KJ/day (assuming relatively constant rates of metabolism, growth and feeding). Accordingly, the 60% drop in resting metabolism in post-emergence animals may simply be due to the fact that they do not eat. This hypothesis is supported by the observation that fasted, unparasitized larvae showed a reduction in resting metabolism similar to that seen during the parasitoid-induced developmental arrest.

A striking but enigmatic feature of the decline in metabolism at the time of parasitoid emergence is the strong periodicity of CO_2 release on the day of emergence [Fig. 5(A)]. Although superficially resembling the discontinuous ventilation (DV) seen in some insects [including diapausing *M. sexta* pupae; Slama, 1988; Fig. 5(C)], these cycles had a much shorter period (4–10 min) and a much lower amplitude than seen during typical DV. Moreover, CO_2 release on the day of emergence was continuous, although it varied by 3–5 fold during cycles. In adult ants, a temperature-dependent pattern of discontinuous ventilation is seen, with periodicities of $<$1–6 min being seen and the amplitudes of the ant cycles are similar to those reported here (Lighton, 1988).

Two physiological interpretations of the cycling phenomenon are possible. Cycling may be a function of periodic spiracular adjustments (with relatively constant metabolism) as occurs during discontinuous ventilation, or reflect periodicity of the whole body metabolic rate *per se*. Discontinuous ventilation has been observed in a variety of adult insects including Hymenoptera (e.g. ants; Lighton *et al*., 1993), ticks, mites and spiders (Lighton and Duncan, 1995; Lighton and Fielden, 1996) as well as in diapausing lepidopteran pupae and other diapausing

insects (Burkett and Schneiderman, 1974; Levy and Schneiderman, 1966; Slama, 1994). In many species, acidemia of the hemolymph triggers release of carbon dioxide in ventilatory bursts and the bursts have been hypothesized to be neurohormonally regulated by factors secreted by neurosecretory H-organs localized in the thorax (Slama, 1994).

The $\dot{V}CO_2$ of tetrodotoxin-injected animals was slightly lower than in control larvae probably due to flaccid paralyzed state of the TTX-injected larvae, but was constant without the cyclic pattern seen in the parasitized insects with emerging wasps. Interestingly, it was higher than that seen in starved insects but since the latter had been starved for 6 days and were near death, their metabolic rate likely was extremely low. Morever, the TTX-injected larvae likely still had food remaining within gut as well as large pools of nutrient reserves available to fuel metabolism, despite the induction of flaccid paraslysis.

The mechanistic cause and functional significance (if any) of the transient cycling seen in the hosts with emerging parasitoids are unclear. Cycling usually disappeared by 3 days after emergence [Fig. 5(A)], when the rate hovered near the nadir of the peaks seen previously on the day of emergence. Interestingly, on the day immediately preceding emergence, the rate of $\dot{V}CO_2$ slightly exceeded the peaks seen on the day of emergence, so the day of emergence may be interpretable as illustrating a transition state between the two patterns typical of pre- and post-emergence larvae. The cycling did not occur in any other natural or experimentally induced condition, including starvation or tetrodotoxin-induced immobilization [Fig. 5(B)]. We are inclined to speculate that cycling may arise from the rapidly changing physiological and metabolic milieu during the emergence of *C. congregata* larvae and is not due to the relative immobility exhibited by the host larva at this time. Although discontinuous ventilation or other cyclic ventilatory or metabolic activity have been described in a number of insect taxa (Lighton, 1994, 1996), to the best of our knowledge it has not been previously reported in caterpillars or in parasitized insects of any species.

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