sperms are not found in ARG, this could suggest that the precipitation arcs formed with Te and Sp immunogens could have been of sperm origin. Anti-Te serum, however, detected an additional arc which could be attributed solely to testicular contents. Anti-Sp and anti-ARG sera gave stronger and broader precipitation arcs with Sp and ARG and the type of precipitation lines were similar irrespective of the antiserum used, suggesting the presence of a sizeable number of common components in the two extracts. It is likely, therefore, that the material which goes into making the spermatophore originates in the ARG, as the ultrastructural studies of these two tissues showed common structural elements[2]. It is also not surprising that materials obtained from Sp and ARG show similar biochemical and immunological patterns. Recent studies on *Tenebrio molitor* L. extracts from Sp and ARG[6] have shown that they are immunologically similar, an observation which would further support the findings discussed in these investigations.

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Sublethal virus infection depresses cytochrome P-450 in an insect

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Summary. Insect-specific cytoplasmic polyhedrosis virus infections, endemic in many species of insects, cause the gut tissue to assume on opaque, milky-white appearance through virus multiplication and formation of polyhedral protein inclusion bodies. Electron microscopy shows that the endoplasmic reticulum membrane is severely reduced and fragmented in infected midgut cells. Metabolism of foreign, lipophilic compounds, catalyzed by the membrane-bound cytochrome P-450, is significantly depressed, and resistance to insecticides disappears. In the absence of toxicants, most insects in this condition survive with somewhat impaired fitness.
Key words. Insect cytoplasmic polyhedrosis virus; insecticide resistance, cytochrome P-450; *Heliothis virescens; Manduca sexta.*

Insecticide toxicity testing and biochemical toxicology studies depend on a supply of insects that are healthy, of uniform instar and age within the instar, and have constant and known exposure to temperature, relative humidity, crowding, and nutrients. It is also important to know the natural feeding habits of the species and the prehistory of insecticide exposure of the population. Insecticide toxicity is often directly related to the activities of several foreign compound-metabolizing enzymes including cytochrome P-450, glutathione transferases, and carboxylesterases, all of which are sensitive to the physiological condition of the insects and external factors to which they are exposed[2–4].

A laboratory colony of the tobacco budworm, *Heliothis virescens* (Fabr.) was established from individuals collected in 1980 from an insecticide-resistant population in Texas. The colony was reared at constant temperature, 26 °C, relative humidity, 40–60%, and photoperiod, 16/8 h of light/darkness; the larvae were fed an agar-wheatgerm-soy flour diet, containing chloramphenicol, sorbic acid and methyl paraben as preservatives[5]. During the course of a study undertaken to characterize the stable resistance mechanism(s) in the colony, abnormal, milky-looking guts were observed with increasing frequency. This observation coincided with a marked decrease in specific cytochrome P-450 content in the larval midguts (table 1). The sudden decrease in cytochrome P-450 content between October 3 and October 18 could not be attributed to experimental error, any changes in the nutritional or physical rearing conditions of the colony, or with its obvious developmental or reproductive behavior.

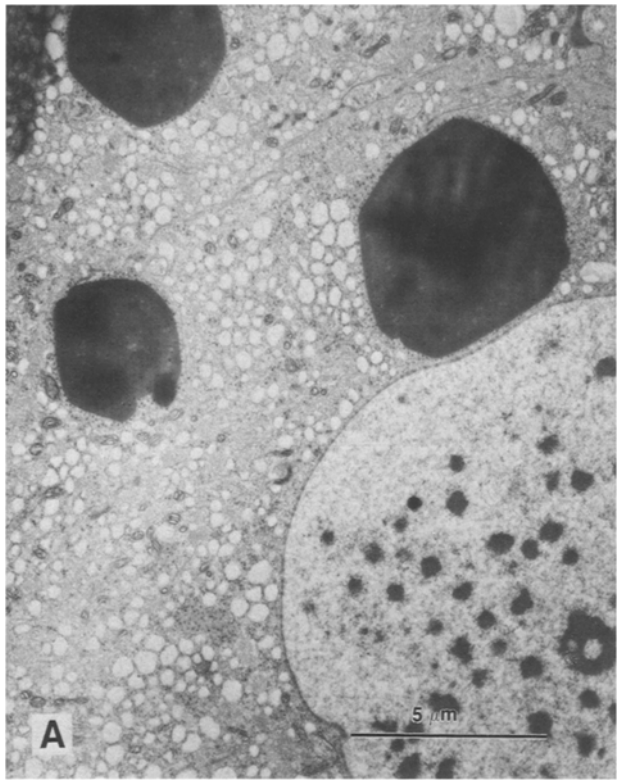
Cytochrome P-450 was quantified as the reduced carbon monoxide difference spectrum[6]. NADPH cytochrome c (P-450) reductase activity was measured with cytochrome c as substrate[7]. N-Demethylation of p-chloro N-methylaniline and epoxidation of aldrin were measured as previously described[8,9]. Glutathione transferase activity was measured with 1-chloro-2,4-dinitrobenzene as substrate[10]. Microsomal and soluble esterase activities were measured with l-naphthyl acetate as substrate[11]. Microsomes containing the cytochrome P-450 and associated activities and membrane-bound esterases and post-microsomal supernatants containing glutathione transferase and soluble esterase activities were prepared from midgut tissues as described earlier[12].

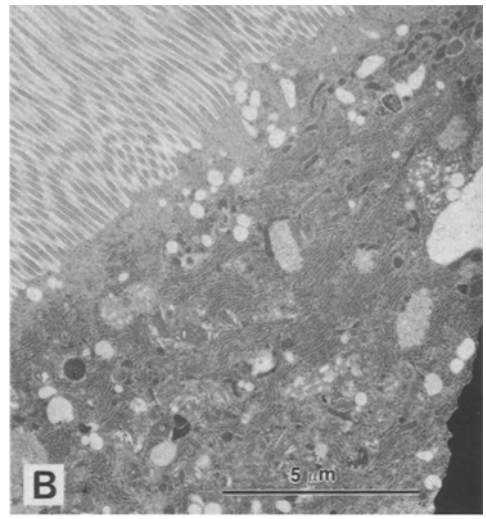
There was also an increase in toxicity of the carbamate insecticide methomyl; spraying third instar larvae, placed in petri dishes, with an acetone/water solution (75/25% v/v) containing known concentrations of the insecticide yielded the following data: in May, 1983 the 48-h LD_{50} was 412 ppm and in March of 1984 it was 119 ppm.

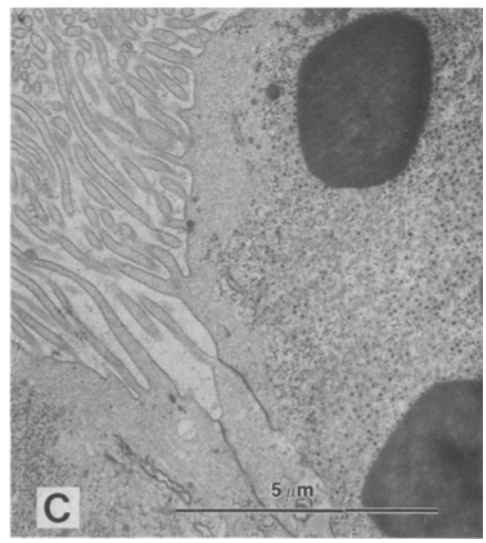
Table 1. Specific cytochrome P-450 content in midgut microsomes from 3-day-old fifth instar tobacco budworm larvae

Date of experiment	Cytochrome P-450 (nmole/mg protein)
August 12	0.401
August 15	0.531
September 15	0.470
October 3	0.643
October 18	0.148
October 24	0.154
October 25	0.208
October 27[1]	0.236
October 28	0.159
November 15	0.165
November 17	0.127
December 28[2]	0.060

The data are averages of duplicate determinations. The experiments were done in 1983. [1] 35% of the guts were milky; [2] 95% of the guts were milky.







Transmission electron micrographs of tobacco budworm larval midgut cells. *A* shows a columnar cell from a milky gut, with polyhedral inclusion bodies in the cytoplasm but not in the nucleus; *B* shows the apical portion with microvilli of a columnar cell from the midgut of a healthy larva, containing many clusters of endoplasmic reticulum membranes; *C* shows the corresponding cell portion in a milky gut, containing only fragments of the endoplasmic reticulum membrane. The midgut tissues were fixed in glutaraldehyde and osmium tetroxide, dehydrated with ethanol, and embedded in epon. The sections, 60–80-nm-thick, were collected on 400 open mesh copper grids and stained with uranyl acetate and lead citrate. Five or more grids form each gut were inspected and photographed with a Nitashi H-600 instrument.

Separate assays of the milky-looking guts revealed that their cytochrome P-450 content was significantly lower than that of separately assayed non-milky-looking guts (table 2). NADPH cytochrome c (P-450) reductase, the enzyme upon which cytochrome P-450 depends for a supply of reducing equivalents from NADPH, was also significantly reduced in the milky-looking guts as well as the cytochrome P-450-catalyzed N-demethylation and epoxidation. The difference in microsomal esterase activity between milky and non-milky (normal) guts was much smaller than that of cytochrome P-450. There was no difference in the cytosolic glutathione transferase activity.

An opaque, milky-white appearance of the midgut is characteristic of larvae afflicted with an acute cytoplasmic polyhedrosis virus infection[13,14]. Electron microscopic examination of milky guts showed the presence of large numbers of polyhedral inclusion bodies and virus particles in the cytoplasm of the columnar cells (fig. A). There were no inclusion bodies in any of the examined nuclei. In midgut epithelia from non-milky guts, the apical portions of the columnar cells contain large masses of endoplasmic reticulum membranes (fig. B), larger than in any other portion of the columnar cells or other midgut cells. In sharp contrast, the apical portions of the columnar cells from milky guts contain only fragments of endoplasmic reticulum membranes (fig. C).

Having outlived its usefulness for screening purposes, this population was discarded. Another laboratory colony was established with individuals collected in March, 1984 from an insecticide-resistant population in Imperial Valley, Ca. It was subjected to further insecticide selection pressure in each generation with 1 µg of permethrin per larva applied topically and had very high foreign compound-metabolizing enzyme activities after one year (table 2). It became extremely resistant to carbamate, organophosphate and pyrethroid insecticides: 11 mg per gram of b.wt of azinphos-methyl caused no mortality to fifth instar larvae; likewise, 2.3 mg per gram of b.wt of methomyl caused no mortality.

Sample groups of this highly resistant colony were contaminated under controlled conditions with a suspension of polyhedral inclusion bodies isolated and purified form *Heliothis virescens*. Each fourth instar larva was contaminated orally, via the diet, with 10^8 polyhedral inclusion bodies and raised separately in a 10-ml plastic cup; their enzyme activities were measured when they were 3-day-old fifth instar, 5–6 days after treatment. The cytochrome P-450 content and activities in the infected larvae (table 2) were significantly lower than those in control larvae. There were no differences in the glutathione transferase or soluble esterase activities, and the difference in microsomal esterase activity was small. The overall effect on the enzyme activities by the virus contamination strongly resembles the differences observed between normal and milky guts in the Texas population. The toxicity of methomyl had increased more than five times, with a 48-h topical LD_{50} to newly molted fifth instar larvae of

Table 2. Cytochrome P-450, glutathione transferase, and esterase activities in midguts from 3-day-old fifth instar tobacco budworm and tobacco hornworm larvae[4]

	Tobacco budworm				Tobacco hornworm	
	Texas population		California population			
	Ordinary guts	Milky guts	Control guts	Virus-exposed guts	Control guts	Virus-exposed guts
Cytochrome P-450 (nmole/mg protein)	0.511 ± 0.102	0.019 ± 0.003*	1.340 ± 0.200	0.436 ± 0.024*	0.132 ± 0.020	0.062 ± 0.003*
Reductase (nmole/min, mg protein)	146.61 ± 29,72	35.71 ± 6.78*	255.08 ± 38.20	53.17 ± 5.62*	132.40 ± 7.00	89.48 ± 4.46*
N-demethylation (nmole/min, mg protein)	6.53 ± 1.05	1.97 ± 0.31*	9.36 ± 1.35	3.81 ± 0.15*	2.67 ± 0.23	1.56 ± 0.02*
Epoxidation (nmole/min, mg protein)	4.58 ± 0.76	0.54 ± 0.08*	5.51 ± 0.55	2.09 ± 0.23*	2.62 ± 0.10	1.09 ± 0.14*[1]
Microsomal esterase (μmole/min, mg protein)	9.38 ± 0.25	7.97 ± 0.24*	16.23 ± 1.87	12.06 ± 1.77**	15.35 ± 0.27	12.78 ± 1.36**
Soluble esterase (μmole/min, mg protein)	–	–	9.14 ± 1.57	8.46 ± 1.26	19.34 ± 0.21	19.62 ± 0.11
GSH transferase (nmole/min, mg protein)	711.03 ± 95.29	644.80 ± 83.82	1193.05 ± 233.07	1121.19 ± 57.34	1249.97 ± 156.98	1142.69 ± 250.19

Data are mean ± SD of experiments; *Significant difference indicated by two-tailed student's t-test; $p < 0.001$; **$p < 0.05$; [1]pmole/min, mg protein.

400 μg per gram of larval b.wt. This effect was present already three days after infection. This residual resistance may be attributable to cytochrome P-450 activity in the fatbody tissues which were not invaded by the virus. The infected larvae also had guts with a milky appearance, and electron microscopy showed polyhedral inclusion bodies, virus particles, and fragmented remnants of endoplasmic reticulum membranes in the apical portions of the columnar cells.

The differences in enzyme activities between control and virus-infected larvae were smaller than those observed in the Texas population, probably because of the shorter time of exposure than in the naturally inoculated population. Cytoplasmic polyhedrosis viruses are normally transmitted transovarially to the next generation[13,14]. Identical effects on enzymes activities were also observed when larvae of the tobacco hornworm, *Manduca sexta* (L.) were contaminated with the polyhedral inclusion bodies (table 2). Experiments with larvae of the fall armyworm, *S.frugiperda* (J.C. Smith), indicated similar effects (data not shown); it cannot be taken for granted that all lepidopterans will respond in this way.

Although it is well established that tobacco budworm and many other lepidopterous larvae have highest cytochrome P-450 content in their midguts with lower activities in other tissues[15], it has been unclear precisely where within the midgut tissue the activity resides. Apparently, most of the cytochrome P-450 in tobacco budworm midguts is localized in the apical portions of the columnar cells, a seemingly strategic location, since here it is in closest contact with the ingested food.

Cytoplasmic polyhedrosis viruses, a group of highly similar RNA-viruses, are the most widespread of insect viruses and reported from over 100 species of lepidopterous larvae. Being relatively non-specific among insects, they also infect dipterous and hymenopterous insects[16]. Unlike nuclear polyhedrosis virus infections which are usually lethal, cytoplasmic polyhedrosis virus infections are often latent. Disease symptoms and mortality are aggravated by additional stress factors such as exposure to extreme temperatures, nutrient shortage, crowding, or exposure to certain chemicals. They often result in smaller adult insects with reduced fecundity and life span[17–19]. Sufficiently adverse conditions could occur in laboratory colonies and may, in part, explain the loss of resistance often observed after a colony has been established with field-collected, resistant individuals[20].

Attempts have been made to devise insect control strategies based on virus infections combined with synthetic chemical insecticides[21–24] but such methods have had only limited success. The effects of cytoplasmic polyhedrosis virus infections resemble those of insectide synergists, chemicals which, whilst nontoxic alone, enhance the toxicity of insecticides by inhibiting detoxifying enzyme activities, notably cytochrome P-450. Synergists are typically not effective when used against susceptible insects[25]. Instead of using virus infections as agents for large scale insect control, cytoplasmic polyhedrosis viruses may be useful tools in insecticide resistance management. A clear advantage with this approach is that it will probably be insect-specific and harmless to other organisms; the insect cytoplasmic polyhedrosis viruses, are not pathogenic to vertebrates[21–24] and to few non-insect invertebrates.

The extensive destruction of the endoplasmic reticulum membrane in the virus-infected midgut cells could explain the depression of cytochrome P-450, an enzyme deeply embedded in and dependent on these membranes for activity[26]. This would, however, not explain the small decrease in microsomal esterase activity. Experiments with incorporation of ^{14}C-glycine into larval protein in the presence and absence of cytoplasmic polyhedrosis virus infection in the silkworm, *Bombyx mori* (L.), showed that overall protein synthesis as well as breakdown were enhanced in the presence of the virus[27,28]. If this also happens in the tobacco budworm larvae, it could explain the small or lacking reduction in glutathione transferase and esterase activities. It is possible that the effect on cytochrome P-450 is more complex. Theophylline, an anticongestant used in asthma treatments, is normally eliminated after cytochrome P-450-catalyzed conversion to a polar metabolite. During episodes of viral infections in humans, serum theophylline concentrations can reach toxic levels[29]. In addition to viral infections, several immunostimulants and interferon-inducing agents depress cytochrome P-450 in rats and mice; there is indirect evidence that interferon itself may suppress cytochrome P-450 activity in these cases[30,31] although the biochemical mechanism is not known. Interferon is not known to be part of the insect immune system, but insects do have special proteins, the cecropins, associated with their immune response[32]. The reproducible destruction of cytochrome P-450 associated with cytoplasmic polyhedrosis virus infection in the tobacco budworm larvae and other species may offer an opportunity to further explore insect immunity. This is an important area of investigation because insects constitute a major life form and can transmit microorganisms that cause crop plant diseases and even serious human diseases. Better understanding of the insect immune system at the molecular level may improve the prospects for the development of insect-specific microbial insecticides.

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Photoperiodic summation is temperature-dependent in *Pyrrhocoris apterus* (L.) (Heteroptera)

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Summary. In *Pyrrhocoris apterus*, a low temperature, 15 °C, prevented the termination of diapause by long days and, unexpectedly, also the induction of diapause by short days. Both responses were enabled at a higher temperature, 26 °C. In contrast to current concepts, it was proved that the summation of photoperiodic signals was temperature-dependent, since the morphogenetic development was prevented by starvation.
Key words. Diapause induction; diapause termination; starvation; photoperiodic response; photoperiodic counter; temperature compensation.

Temperature may considerably modify photoperiodic response in insects. With long-day species, low temperature enhances the incidence of diapause under conditions of short days, whereas high temperature and long days work together to avert diapause[1,2]. Saunders[3] interprets the relationship between temperature and photoperiod as an interaction between the photoperiodic counter and the rate of development; the numbers of days required for photoperiodic induction of diapause show a high degree of temperature compensation, but temperature affects the rate of development and thus also the number of light-dark cycles actually experienced by an individual. Hence, the higher incidence of diapause at low temperature is thought to be due to the protracted sensitive period. In contrast, the rate of photoperiodic termination of diapause is temperature-dependent[4,5]. However, the stimulatory effects of high temperature on the post-diapause morphogenesis and on the photoperiodic activation of diapause have never been discriminated.

It seems unlikely that the dependence on temperature would be so contradictory for the summation of diapause-promoting and diapause-terminating photoperiods. This study indicates that summation of both types of photoperiodic signals is restrained at low temperature in *Pyrrhocoris apterus*.

P. apterus exhibits a facultative adult diapause regulated by photoperiod. A long-day photoperiod of 18 L:6 D (LD)

Table 1. Activation of females destined for diapause

Experimental photoperiod and temperature (10 days)	n	% ovipositing females	Pre-oviposition period (days)
LD, 26 °C	20	95	14.7 ± 6.6
SD, 26 °C	20	90	29.6 ± 5.1
LD, 15 °C	30	90	24.9 ± 3.3
SD, 15 °C	30	87	27.5 ± 4.3
Control	11	100	19.0 ± 3.0

Mean ± SD. Reproductive parameters were recorded with LD, 26 °C and food supply. For experimental procedure see figure 1.

Table 2. Induction of diapause

Experimental photoperiod and temperature (10 days)	n	% ovipositing females	Pre-oviposition period (days)	Oviposition period (days)
LD, 26 °C	19	100	6.1 ± 0.9	15.1 ± 7.3
SD, 26 °C	42	24	6.3 ± 1.3	1.0 ± 2.2
LD, 15 °C	20	100	5.9 ± 0.9	10.0 ± 6.2
SD, 15 °C	32	97	5.5 ± 0.8	11.3 ± 5.8
Control	20	100	5.9 ± 0.6	7.3 ± 5.1

Mean ± SD. Reproductive parameters were recorded with SD, 26 °C and food supply. For experimental procedure see figure 1.