action is probably related to the varying abilities of the drug to penetrate the cuticle of the worms[3] and to reach the receptive sites or to differences in the susceptibility of receptive sites.

As reported previously regarding the sensitivity of various neuropharmacological agents[8], it may be likely that blood nematodes are more susceptible to various drugs than intestinal nematodes. The difference may arise from the fact that the habitat of blood nematodes is highly homeostatic compared to that of intestinal nematodes. Since an intact worm or an anterior piece of *A. suum* was little influenced by neuropharmacological agents[8-10], this worm was used in the form of muscle strips with a longitudinal cut along the lateral line or as eviscerated preparations. It was reported that in vitro, pyrantel immediately caused contracture in muscle strips of *A. suum* at lower doses ($10^{-9}$–$8\times10^{-9}$ g/ml), compared with rather slow spastic paralysis in the whole worm preparation of this worm at higher doses ($2.5\times10^{-5}$ g/ml)[5].

*T. vulpis* was not sensitive to pyrantel at concentrations of $10^{-5}$ M or less, and this result agrees well with those in chemotherapy of trichuriasis in animals and man[1,2,4]. Since *T. vulpis* was remarkably less sensitive to various drugs examined in our laboratory, it may be that the worm is less permeable and/or less susceptible to drugs. Pyrantel at the concentrations of $10^{-5}$ M or less had little effect against cestodes such as *Dipylidium caninum, Diplogonoporus grandis* and plerocercoids of *Diphyllobothrium erinacei,* and trematodes such as *Metagonimus yokogawai, Paragonimus westermani, P. miyazakii, Fasciola hepatica* and *Schistosoma japonicum.*

Aubry et al.[5] suggested that pyrantel acts by stimulating nicotinic receptors in *A. suum,* and recently we have reported that this anthelmintic may act similarly in another nematode, *A. cantonensis*[11]. The cholinergic mechanism in nematodes such as *A. suum*[12,13] and *A. cantonensis*[8] is suggested to be nicotinic from results on both cholinergic agonists and antagonists. In cestodes such as *D. caninum*[14] and trematodes such as *Schistosoma mansoni*[15] and *F. hepatica*[15], however, the cholinergic mechanism was suggested to be neither nicotinic nor muscarinic. Thus, differences between the nature of the cholinoceptors of nemathelminths and plathelminths may give an explanation for the lower susceptibility of plathelminths to drugs such as pyrantel which affects nematodes through its nicotinic actions.

Isolated host tissues such as the mouse ileum and the frog rectus were also sensitive to pyrantel at the concentrations of $10^{-6}$ M or more (fig., A). The effects of pyrantel against both skeletal and smooth muscles have been reported in experiments in vivo as well as in vitro[5,16]. It was reported that these effects on the host tissues are also caused by stimulating the nicotinic receptors in autonomic ganglia and skeletal neuromuscular junctions[5,16]. However, no particular clinical syndrome of toxicity has been reported for pyrantel[16,17]. This discrepancy may be due to the fact that pyrantel is always given orally and is poorly absorbed from intestine[16].

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## Inhibition of host phenoloxidase activity by parasitoid hymenoptera

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*Summary.* Partial or complete inhibition of hemolymph phenoloxidase activity occurred in host species attacked by some parasitoid wasps. In one system, inhibition of enzyme activity could be achieved by injection of a virus purified from parasitoid ovaries.

The most common response of insect larvae to the presence of large foreign objects is the formation of a multicellular capsule of hemocytes[1]. Such encapsulation reactions are often (but not necessarily) associated with the deposition of melanin[1-4], and several workers have accordingly suggested that melanization per se might play an important role in insect immunity[1,5]. In support of this view, recent studies have shown that phenoloxidases responsible for the formation of melanin can be activated by cell wall components of parasitic fungi[6] and bacteria[7]. Furthermore, it has been suggested that phenoloxidases might act as opsonins[8], and indeed it seems clear that active enzyme is deposited on some foreign surfaces[6]. Thus it might seem reasonable to suppose that some parasitoids might have evolved mechanisms to inhibit the activation and/or activity of phenoloxidase (PO). Earlier studies have in this regard provided equivocal results[9]. We now report that at least in some systems, PO activity in the hemolymph of parasitized insect larvae is suppressed.

*Materials and methods.* The parasitoid used for most ex-

periments described here was *Hyposoter exiguae* (Hymenoptera: Ichneumonidae); the host insect was *Trichoplusia ni* (Lepidoptera: Noctuidae). *Heliothis virescens* (Noctuidae) and *Campoletis sonorensis* (Ichneumonidae) were provided, as required, by Peter Krell, University of Guelph. Both *T. ni* and *H. virescens* are successfully parasitized by the 2 ichneumonid species employed in this study; *Anticarsia gemmatalis*, on the other hand, is a non-permissive host. Noctuid larvae were reared on artificial diets purchased from BioServ, Inc. (Frenchtown, N.J.).

Larvae to be parasitized were exposed to female wasps and removed after 2 attacks had been observed. Confirmation of successful oviposition was determined by subsequent dissection. For assay of enzyme activity, samples of hemolymph were diluted 1:12 (v/v) with distilled water and incubated for 2 h at 4 °C in order to 'activate' phenoloxidase; no visible signs of melanization could be observed in such samples for a minimum of 4–5 h. Enzyme activity was determined spectrophotometrically by measuring the formation of dopachrome, read at 480 nm, from 3,4,-D,L-dihydroxyphenylalanine (500 µl of $2\times10^{-2}$ M DOPA in 0.1 M $KPO_4$, pH 6.57, was mixed with 30 µl of activated hemolymph and incubated at room temperature for 5 min). *H. exiguae* virus was purified on continuous sucrose gradients as described[10]. Virus bands were diluted, pelleted (30,000×g/30 min), and resuspended in sterile Dulbecco phosphate buffered saline (PBS, containing 0.7 mM $Ca^{2+}$ and 0.5 mM $Mg^{2+}$, pH 7.3). Prior to injection, protein concentration was determined by the Lowry method, and adjusted to 0.3 mg/ml. Larvae were asphyxsiated by immersion in water and injected with 1-2 µl of PBS or virus; antibiotics were not used.

*Results and discussion.* Our studies were prompted initially by the observation that cabbage looper *(T. ni)* larvae parasitized by *H. exiguae* seemed to bleed somewhat more readily than control larvae; significantly, the hemolymph failed to melanize (fig. 1). The effect of parasitism on PO activity was subsequently examined by spectrophotometric assay, the results of which are shown in figure 2 and table 1. Following oviposition by *H. exiguae*, PO activity in host blood rapidly declined to non-detectable levels. On the other hand, parasitization of *T. ni* larvae by a different ichneumonid species, *Campoletis sonorensis*, led to only an initial decrease of less than 30%, at which level enzyme activity stabilized. PO activity was reduced, but not eliminated, in *H. virescens* larvae parasitized by either ichneumonid species. No change in enzyme activity was observed in hemolymph from *A. gemmatalis* larvae parasitized by *H. exiguae*. More recently, we have observed complete inhibition of PO activity in *Manduca sexta* larvae parasitized either by *H. exiguae* or *Apanteles congregatus* (unpublished data). PO activity in control *T. ni* larvae was occa-

Table 1. Effect of parasitization on phenoloxidase activity in hemolymph of host larvae

| Host | Parasitoid(s) used | Phenoloxidase activity (±SD) |
|---|---|---|
| *T. ni* | None | 20.4 (20) ± 16 |
| *T. ni* | *H. exiguae* | 0 (15) |
| *T. ni* | *C. sonorensis* | 13.5 (9) ± 7 |
| *T. ni* | Both | 0 (10) |
| *H. virescens* | None | 53.0 (19) ± 26 |
| *H. virescens* | *H. exiguae* | 17.4 (6) ± 14 |
| *H. virescens* | *C. sonorensis* | 19.6 (16) ± 11.8 |
| *A. gemmatalis* | None | 15.1 (5) ± 7.6 |
| *A. gemmatalis* | *H. exiguae* | 10.0 (18) ± 5.5 |

Figures given are average values; the number of larvae examined is given in parentheses. All spectrophotometric assays were carried out 24 h after oviposition (ie. prior to hatching of parasitoid eggs). Activity is expressed in terms of photometric units (1 unit = 0.001 $OD_{480}$/min/2.5 µl of hemolymph).

Table 2. Effect of injection of purified *H. exiguae* virus on levels of phenoloxidase activity in the hemolymph of 5th instar *T. ni* larvae

| Treatment | Phenoloxidase activity |
|---|---|
| Injected with: | |
| a) phosphate buffered saline | 28.0, 16.0, 25.8, 0, 24.0, 20.8, 36.2, 27.0 (average = 22.3) |
| b) virus | 0, 1.6, 0, 0, 0, 1.0, 0, 0, 0, 0, 0, 0 (average = 0.22) |

Measurements are from individual larvae injected 6–8 h after ecdysis, and assayed for enzyme activity 40 h later.

Table 3. Effect of starvation on phenoloxidase activity in the hemolymph of early 5th-instar *T. ni* larvae[a]

| Time | Phenoloxidase activity[b] |
|---|---|
| 0 h | 33.3 (15) ± 24.8 |
| 24 h | 29.6 (15) ± 17.2 |
| 48 h | 25.1 (13) ± 21.2 |

[a] Larvae were given only water, for the time periods indicated; starvation commenced 6–8 h after ecdysis. [b] Average values ± SD; the numbers of larvae examined are in parentheses.

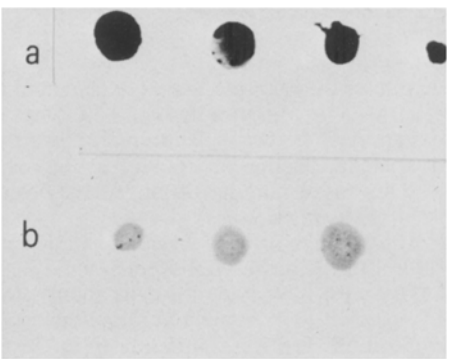


Figure 1. Appearance of *T. ni* hemolymph samples (40 µl) on glass microscope slides 24 h after bleeding. *a* Control; *b* larvae parasitized 24 h previously by *H. exiguae*.

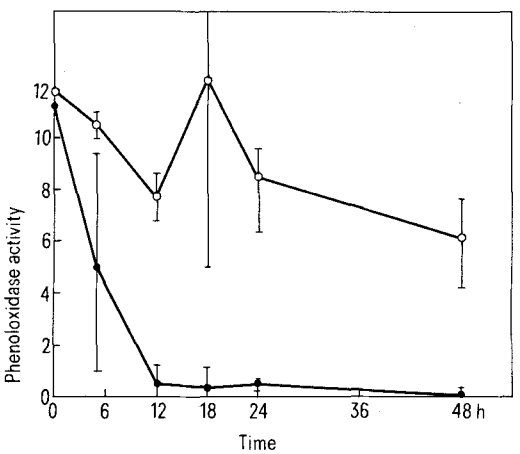


Figure 2. Effect of parasitization by *H. exiguae* on phenoloxidase activity in the hemolymph of 4th instar *T. ni* larvae. Each data point represents an average value for 4 different control (○) or parasitized (●) larvae ± SD.

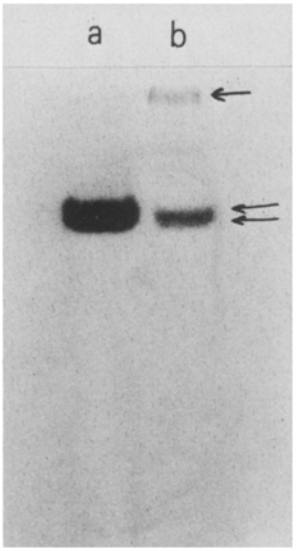


Figure 3. Zymogram showing phenoloxidase activity in polyacrylamide gel of *T. ni* hemolymph plasmas. The gel was treated with pronase and subsequently exposed to substrate (DOPA). *a* Pooled plasma from larvae 24 h after parasitization; *b* plasma from control larvae. The single arrow marks the interface between stacking and running gel, at which position endogenous activity (i.e., no pronase added) occurs in control samples; the double arrow indicates the position of a putative proenzyme.

sionally variable; for example, the peak shown in figure 2 is the result of a single abnormally high value for one of the 4 larvae examined at the 18 h interval. Nevertheless, we observed a clear decline in activity as larvae progress through the 4th instar; similar findings have been reported in the case of *Heliothis virescens* larvae[9].

Since *H. exiguae* eggs in *T. ni* began to hatch at approximately 44 h after oviposition, it seemed clear that changes in PO activity must have been brought about either by the parasitoid egg itself or by some additional factor(s) injected during oviposition. We were particularly interested in examining the role of parasitoid viruses[11] in this context, since it was already known that these were injected during oviposition by several parasitoid species and could, moreover, invade host cells[12]. As was the case with natural parasitism, injection of gradient-purified *H. exiguae* virus virtually eliminated all PO activity in *T. ni* larvae (table 2). In preliminary studies, the kinetics of inactivation appeared similar, but we have not examined this in detail since, for example, we had no way of knowing whether the virus dose was physiological. Nor have we eliminated the possibility of synergism between virus and accessory gland secretions.

Certain parasitoid viruses, including that found in *H. exiguae*, cause severe growth inhibition when injected into host larvae. In *T. ni* larvae, this phenomenon is noticeable within 24 h, and becomes increasingly dramatic 2–3 days post injection; nevertheless, larvae eventually recover and produce normal adult moths (Stoltz, unpublished data). It was of interest to determine whether transient starvation per se would affect PO activity, and to this end we starved early 5th instar *T. ni* larvae for 1–2 days. Typical data are presented in table 3, and reveal that starvation has no effect on PO activity.

In preliminary experiments, we found that hemolymph from *T. ni* larvae parasitized by *H. exiguae* would melanize if 'activated' by pronase; this observation would seem to be in support of recent evidence to the effect that proteolysis may be required for conversion of a proenzyme to active PO[13]. It was of interest to determine whether use of a protease would allow us to detect a putative proenzyme following electrophoretic separation of hemolymph polypeptides under non-denaturing conditions. It was found, as expected, that hemolymph plasma from parasitized larvae differed from control samples in totally lacking endogenous activity. After pronase treatment, however, considerable PO activity could be detected in both control and experimental samples, with melanin deposits forming at equivalent positions in the gel (fig. 3). These observations suggest that lack of enzyme probably does not represent a limiting factor in parasitoid-induced inhibition of PO activity, at least in this system. The mechanism by which melanin biosynthesis is inhibited nevertheless remains unclear; other possibilities yet to be investigated include the absence of a necessary activating factor or, alternatively, the appearance of an inhibitor.

Our initial observations do not answer the question of whether inhibition or reduction of PO activity is in any way relevant to, or even routinely associated with, successful parasitism. The evidence from earlier studies indicates that purified ichneumonid viruses may be required for successful parasitism[14]; *H. exiguae* virus not only promotes the successful development of *H. exiguae* larvae in *T. ni* (unpublished observations) but in addition, as shown here, completely inhibits PO activity in that host. However, the ability of this virus to suppress PO activity could be a natural consequence of some more significant, but as yet unidentified, primary event. While others have searched for a relationship between PO and insect immunity, results obtained have been somewhat difficult to interpret[2,3,15]; this may be due to the fact that inhibition of PO was achieved in earlier studies by the administration of phenylthiourea, which is likely to have exerted a nonspecific toxic effect[16]. The *H. exiguae*/*T. ni* system offers a unique opportunity to reassess the causal role, if any, of PO activity in relation to invertebrate immunity under physiological conditions. Aside from contributing to an understanding of the physiology of host/parasitoid interaction, the availability of hemolymph which fails to melanize may facilitate research in a number of areas; these include: a) studies on the control of melanin biosynthesis in general; b) purification of PO; and c) the establishment of hemocyte cell lines.

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