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GUT REDOX CONDITIONS IN HERBIVOROUS LEPIDOPTERAN LARVAE

HEIDI M. APPEL[1,2] and MICHAEL M. MARTIN

Department of Biology
University of Michigan
Ann Arbor, Michigan 48109-1048

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Abstract—Large interspecific differences in redox potential exist among herbivorous lepidopteran larvae. Reducing conditions occur in the midguts of *Manduca sexta* (Sphingidae) and *Polia latex* (Noctuidae), whereas oxidizing conditions prevail in the midguts of *Lymantria dispar* (Lymantriidae), *Danaus plexippus* (Danaidae), and *Papilio glaucus* (Papilionidae). The epithelium of the posterior midgut of *M. sexta* fed a diet containing bismuth subnitrate accumulates bismuth sulfide, suggesting that sulfide might be one of the reducing agents responsible for the maintenance of reducing conditions in this species. We propose that the effects of plant allelochemicals in insect herbivores will be strongly affected by gut redox conditions and that the regulation of gut redox conditions is an important adaptation of insect herbivores to the chemical defenses of plants. The redox state of the gut is yet another insect trait that must be included in the analysis of plant–insect interactions.

Key Words—Plant–insect interactions, redox, reduction potential, digestion, Lepidoptera, *Danaus plexippus*, *Lymantria dispar*, *Manduca sexta*, *Papilio glaucus*, *Polia latex*, Danaidae, Lymantriidae, Sphingidae, Papilionidae, Noctuidae.

INTRODUCTION

The redox conditions that prevail in an insect's gut can have a major impact on the digestion, metabolism, and assimilation of ingested nutrients. Highly reducing conditions (negative redox potentials) in the midguts of tineid moths (Water-

[1] To whom correspondence should be addressed.
[2] Present address: Pesticide Research Laboratory, Pennsylvania State University, University Park, Pennsylvania 16802.

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house, 1952b), dermestid beetles (Waterhouse, 1952c), and bird lice (Waterhouse, 1953) facilitate the digestion of keratin, the major protein of fur and feathers, which is highly resistant to digestion by most animals. Under reducing conditions the disulfide bonds that stabilize keratin are cleved, resulting in the liberation of polypeptide subunits that are readily degraded by insect digestive proteases. Reducing conditions are also commonly encountered in portions of the gut that house large populations of metabolically active microorganisms. Negative redox potentials have been reported in the hindguts of termites (Veivers et al., 1980; Bignell, 1984), roaches (Day and Waterhouse, 1953; Bignell, 1984), and scarab beetles (Bayon, 1980), where symbiont-mediated fermentative digestion of cellulose occurs.

Gut redox conditions are also likely to mediate the impact of many classes of ingested allelochemicals on herbivorous insects. For example, the redox state of the alimentary tract is likely to determine whether ingested phenols remain in the reduced state or are oxidized to quinones, which are generally more reactive and more toxic substances than phenols. Thus, gut redox conditions are a potential adaptive mechanism by which an insect herbivore might minimize the adverse effects of ingesting the defensive chemicals present in its food plant. Prior to this study, there had been only one measurement of gut redox potential in an herbivorous insect. Bignell (1984) reported oxidizing conditions throughout the gut of *Locusta migratoria*. In the present study we have assessed the redox conditions in the alimentary tracts of larvae of five species of herbivorous Lepidoptera: the tobacco hornworm, *Manduca sexta* (Sphingidae); the cutworm *Polia latex* (Noctuidae); the gypsy moth, *Lymantria dispar* (Lymantriidae); the common monarch butterfly, *Danaus plexippus* (Danaidae); and the black swallowtail, *Papilio glaucus* (Papilionidae). In reporting and discussing our results, we have borrowed heavily from approaches developed by investigators studying the redox properties of soils.

METHODS AND MATERIALS

Oxidation–Reduction Potentials. A redox reaction is one in which there is a transfer of electrons, with or without the accompanying transfer of protons (reaction 1):

$$\text{oxidized form} + n\text{H}^+ + ne^- = \text{reduced form} \qquad (1)$$

Consequently, whether a redox couple exists primarily in its oxidized or reduced form depends upon the availability of both protons and electrons in the system. Proton availability in a system is a function of its acid–base status. Electron availability is a function of the tendencies of the various chemical species pres-

ent to lose electrons (and act as reducing agents) or gain electrons (and act as oxidizing agents). In a system in which proton activity and electron availability are high, reduction is favored; if proton activity and electron availability are low, oxidation is favored. If electron availability is held constant, oxidation is more favored under alkaline conditions than under acidic conditions.

The assessment of the redox conditions in an insect's gut requires a measurement of both acid–base status and electron availability. The acid–base status of the gut can, of course, be determined by a simple pH measurement. However, the measurement of electron availability in a complex aqueous mixture is more problematic. The standard approach has been to determine the redox potential by measuring the emf of a platinum electrode inserted into the gut and to add to the observed potential the potential difference between the reference electrode and the standard hydrogen electrode. Although redox potentials measured in this way routinely are called "standard reduction potentials" and designated by the symbol "E_h," which implies that they are equilibrium redox potentials, they are actually mixed potentials that are the resultants of all of the redox couples present in the system, most of which are not at equilibrium. The contribution of each redox couple present in the mixture is an unknown function of its concentration and its reversibility. Such mixed potentials are, therefore, only rough measures of electron availability.

In describing the redox conditions in insect guts, we have used terms developed by soil scientists to characterize the redox conditions of soils (Lindsay, 1979; Bohn et al., 1985; Bartlett, 1986). Electron availability, or "pe," is derived from the Nernst equation, and at 25°C, is defined as:

$$\text{pe} = E_h/59.2$$

where E_h is the observed standard reduction potential in millivolts. Just as pH expresses the negative log of proton activity, pe expresses the negative log of electron activity. On either the pH or pe scales, zero is the standard state, and a unit of pH or pe is equivalent to 2.3 RT joules of free energy per mole of protons or electrons, respectively. A low pe system has a surplus of electrons and, therefore, a high tendency to lose electrons and become more oxidized. A high pe system has a deficiency of electrons and a high tendency to gain electrons and become more reduced.

To characterize the redox status of a complex aqueous system, Lindsay (1979) has suggested using the sum of pH and pe as a convenient single term expression that accords equal weight to acid–base status and electron availability. He has defined pe + pH as the "redox parameter." By taking the logarithm of the expression for the equilibrium constant of a generalized redox half-reaction, such as reaction 1, in which the activities of the oxidized and reduced

species are equal, and substituting pH and pe for their defined equivalents, it follows that

$$\text{pe} + \text{pH} = (1/n) \log K$$

Thus, for any given system at equilibrium, which is probably not actually the case for either soil samples or insect gut contents, pe and pH should change in a compensatory manner such that pe + pH remains constant. In an aqueous system, in which the maximum oxidizing and reducing capacities are set by the water–oxygen and hydrogen–proton couples, respectively, pe + pH can range from a high value of 20.78 (highly oxidizing conditions) to a low value of 0 (highly reducing conditions).

Insects. Fifth-instar *Manduca sexta* were reared from eggs (Carolina Biological Supply) on an artificial diet (Bio-Serv #9783) in a growth chamber at 25°C with a 16:8 light–dark photoperiod. Sixth-instar *Lymantria dispar* were reared from sterilized egg masses of the New Jersey-OTIS strain supplied by the USDA-ARS (APHIS, Otis AFB, Massachusetts) on artificial diet prepared according to ODell and Rollinson (1966), but lacking casein, in a growth chamber at 25°C with a 16:8 light–dark photoperiod. Fifth-instar *Papilio glaucus* were obtained from a lab culture (J.M. Scriber, Michigan State University) reared on excised foliage of tulip tree (*Liriodendron tulipifera*). Final instars of *Polia latex* and *Danaus plexippus* were collected from black walnut (*Juglans nigra*) and common milkweed (*Asclepias syrica*) foliage, respectively, in Centre County, Pennsylvania, and were fed excised foliage of their host until measurements of gut pH and E_h were conducted.

Gut pH, E_h, and pe. Three to five larvae of each species were immobilized on ice or by exposure to diethyl ether, and the gut was exposed by a ventral longitudinal incision. The integument was folded back and secured away from the gut using insect pins, and hemolymph was blotted from the integument and gut surfaces. The preparation of a larval gut required less than a minute. If dissection resulted in puncture of the gut wall, or if the gut was not full, larvae were discarded. In *M. sexta*, the shortness of the foregut precluded the accurate measurement of foregut pH and E_h in all but one larva.

A longitudinal profile of gut redox potential was made using a 0.02 in. platinum electrode (Microlectrodes MI-800) and a silver–silver chloride microreference electrode (Microelectrodes MI-401) connected to a Metrohm/Brinkmann (model 103) or an Accumet (model 291) millivolt and pH meter. Potential measurements were made in the foregut, anterior midgut, medial midgut, posterior midgut, and hindgut. Redox potentials generally stabilized at each location within 10 sec, such that measurements on an individual larva were completed within a minute. The observed redox potentials (E_{obs}) were converted to standard redox potentials (E_h) by adding 200 mV, which is the poten-

tial difference between the silver–silver chloride and hydrogen reference electrodes. Calculation of pe was from E_h, using the equation pe = $E_h/59.2$ (Lindsay, 1979). Following the measurement of redox potential, a longitudinal profile of gut pH was made using a micro-needle pH electrode (Microelectrodes MI-408c) and a silver–silver chloride reference electrode (Microelectrodes MI-401). pH values generally stabilized at each location of the gut within a minute, such that all measurements on an individual larva were completed within 5 min. In *P. glaucus* and *D. plexippus*, there was some regurgitation of midgut fluid into the foregut in some animals, which compromised the accuracy of the measurements of foregut pH and E_h.

Mean values of pH, E_h, pe, and pe + pH were compared by ANOVA. Significant pairwise differences were determined by LSD analysis. When variances were not equal, treatment means were compared by the Kruskal-Wallis test, and the significance of pairwise differences was determined by the median test with alpha set at 0.01 to adjust for multiple comparisons.

Test for Sulfide in Midgut. M. sexta and *L. dispar* larvae, reared from eggs on artificial diets, were switched early in the fourth instar to a portion of the same artificial diet that had been amended by the addition of bismuth subnitrate (10% dry weight). After 24 hr, three larvae of each species were immobilized and the gut exposed as described above. The presence of sulfide ions in the gut contents or gut epithelium was indicated by a black precipitate of bismuth sulfide.

RESULTS AND DISCUSSION

Previous Reports of Redox Conditions in Insect Guts. We have calculated pe and pe + pH at various gut locations for all insect species for which we could find measurements of pH and E_h in the literature (Table 1). In most studies of gut redox conditions in insects, the results are reported as a range, not as a mean. In Table 1, we have reported the range of reported E_h values and have calculated values for pe and pe + pH based on midrange values. Midrange values for pe and pe + pH in insects range from −4.4–5.1 and 2.8–11.6, respectively. To provide a measure of the most extreme reducing conditions encountered in insect guts, we also have calculated the lowest values for pe and pe + pH observed in those species that possess gut regions with high electron availabilities.

High electron availability (pe < -3.0) is characteristic of the hindguts of termites and the midguts of the bird lice *C. columbae* and *E. stramineus*, the dermestid beetles, and the webbing clothes moth *T. bisselliella*. Of the species listed in Table 1, the conditions most conducive to reduction (pe + pH < 4.5)

TABLE 1. COMPILATION OF pH, E_h, pe, AND pe + pH FROM EARLIER STUDIES OF REDOX CONDITIONS IN INSECT GUTS[a]

Order and species	Gut location	pH	E_h (mV)	pe		pe + pH	
				Midrange	Lowest	Midrange	Lowest
Thysanura							
Ctenolepisma lineata[b]	MG	6.4–7.0	+290 to +170	3.9		10.5	
	HG	2.6–3.8	+160	2.7		5.9	
Orthoptera							
Blattella germanica[b]	MG	6.0–6.2	+30 to +10	0.3		6.4	
	HG	8.0	−90 to −120	−1.7	−2.0	6.3	6.0
Periplaneta americana[c]	FG	5.4–6.5	+347 to +252	5.1		11.0	
	MG	6.1–6.7	+157 to +37	1.6		8.0	
	HG	6.3–6.7	+167 to −173	−0.1	−2.9	6.4	3.6
Locusta migratoria[d]	FG	5.7–6.6	+327 to +77	3.4		9.5	
	MG	6.4–7.4	+267 to +67	2.8		9.7	
	HG	5.8–6.8	+287 to +97	3.2		9.5	
Isoptera							
Nine termite species[e]	MG	7.0	> +100	>1.7		>8.7	
	HG	7.0	−230 to −270	−4.2	−4.5	2.8	2.5
Cubitermes severus[f]	FG	6.7	+347 to +187	4.5		11.2	
	HG(P_1)	10.4	+27 to −183	−1.3	−3.1	9.1	7.3
	HG(P_3)	9.2	−13 to −123	−1.1	−2.1	8.1	7.1
	HG(P_{4a})	8.2	+87 to −98	−0.1	−1.7	8.1	6.5
	HG(P_{4b})	8.0	+197 to −53	1.2	−0.9	9.2	7.1
Zootermopsis nevadensis[f]	FG	6.8	+257 to +157	3.5		10.3	
	MG	7.1	+207 to +137	2.9		10.0	
	Paunch	7.1	−73 to −303	−3.2	−5.1	3.9	2.0
	Colon	7.3	+147 to −163	0.1	−2.8	7.2	4.5

Mallophaga							
Columbicola columbae[g]	aMG	7.8–8.0	−170 to −215	−3.2	−3.6	4.7	4.3
	pMG	6.5	About −100	−1.7		4.8	
	HG	5.8	> +70	>1.2		>7.0	
Eomenacanthus stramineus[g]	aMG	8.0–8.4	−175 to −220	−3.3	−3.7	4.9	4.5
	pMG	6.4–7.2	About −115	−1.9		4.9	
	HG	6.4–7.2	> +30	>0.5		>7.3	
Damalina ovis[g]	MG	6.5	> +77	1.3		8.8	
	HG	5.0–6.0	> +97	1.6		7.1	
Coleoptera							
Three dermestid species[h]	MG	6.8–8.2	−190 to −230	−3.5	−3.9	4.0	3.6
	HG	4.4–4.8	+260	4.4		9.0	
Oryctes nasicornis[i]	MG	11.8	+27 ± 18	0.5		11.6	
	HG	7.8–8.5	−83 ± 22	−1.4	−1.6	6.6	6.4
Lepidoptera							
Tineola bisselliella[j]	aMG	8.9–9.0	About −200	3.4		5.6	
	mMG	9.8–10.0	−220 to −300	−4.4	−5.1	5.5	4.8
	pMG	6.2–6.5	+80 to +30	1.8		8.2	
	HG	4.5–5.8	> +250	4.2		9.6	
Hymenoptera							
Apis mellifera[k]	MG	7.5	+257 to −83	1.5		9.0	

[a] Abbreviations: FG, foregut; MG, midgut; aMG, anterior midgut; mMG, medial midgut; pMG, posterior midgut; HG, hindgut.
[b] Day and Waterhouse (1953).
[c] Redox data from Bignell (1984); pH data from Bignell (1981).
[d] Redox data from Bignell (1984); pH data from Srivastava and Srivastava (1956) and Bodine (1925).
[e] Veivers et al. (1980); pH values assumed, not measured; termite species were *Ceratokalotermes spoilator, Coptotermes lacteus, Glyptotermes brevicornis, Incistitermes barretti, Mastotermes darwiniensis, Nasutitermes exitosus, Neotermes insularis, Porotermes adamsoni,* and *Stolotermes victoriensis.*
[f] Bignell (1984); P_1, P_3, P_{4a}, P_{4b}, paunch and colon are distinct segments of the hindgut.
[g] Waterhouse (1953).
[h] Waterhouse (1952c); species were *Anthrenocerus australis, Anthrenus verbasci,* and *Attagenus piceus.*
[i] Bayon (1980).
[j] Waterhouse (1952b).
[k] Bignell and Heath (1985).

occur in the hindguts of termites and the midguts of dermestid beetles. For comparison, a flooded soil containing decomposable organic matter, with a value of pe + pH below 4.5, would be classified as a highly anaerobic soil and might be expected to accumulate methane (Rowell, 1988). Methanogenesis is a process that is known to occur in the hindguts of many termite species (Breznak, 1984). Somewhat less extreme reducing conditions (pe + pH = 4.5–7.0) occur in the hindguts of the silverfish *C. lineata*, the two roach species, the soil-feeding termite *C. severus*, the rhinocerus beetle *O. nasicornis*, and in the midguts of the German cockroach *B. germanica*, the two species of bird lice, and the webbing clothes moth. For comparison, a waterlogged organic soil with a value of pe + pH between 4.5 and 7.0 would be classified as a reducing soil and might be expected to accumulate sulfide (Rowell, 1988). Sulfide appears to be one of the reducing agents responsible for the maintenance of high electron availability in the midgut of the webbing clothes moth (Waterhouse, 1952a; Yoshimura et al., 1988).

Redox Conditions in Gut Fluids of Herbivorous Caterpillars. Reducing conditions exist in the midguts of *M. sexta* and *P. latex* (Table 2). As in *T. bisselliella*, the reducing conditions in the midguts of *M. sexta* and *P. latex* reflect the presence of high electron availabilities (negative values of pe), which compensate for low proton availabilities (high values of pH). In all three of these lepidopteran species, the lowest proton availability (highest pH), the highest electron availability (most negative pe), and the most reducing conditions (lowest values of pe + pH) occur in the anterior and medial portions of the midgut (Table 2). Although the conditions in the midguts of *M. sexta* and *P. latex* are reducing, they are somewhat less so than those in the midguts of the webbing clothes moth and the three dermestid beetles and than those in the hindguts of nine termite species (Table 1). However, the midguts of these two herbivorous caterpillars are as reducing as the midguts of the bird lice *C. columbae* and *E. stramineus*, the hindguts of the cockroach *B. germanica*, the scarab beetle *O. nasicornis*, and the hindguts of two termite species. The redox conditions that exist in the midguts of *M. sexta* and *P. latex* are comparable to those of a waterlogged organic soil; that is, reducing but not highly anaerobic (Rowell, 1988).

Although the midguts of *M. sexta* and *P. latex* are strongly reducing, the redox conditions that exist elsewhere in the gut are weakly oxidizing or intermediate between oxidizing and reducing (Table 3). Values for E_h are uniformly positive in both the foreguts and hindguts, and values for pe and pe + pH fall in the ranges 2.1–2.9 and 8.6–8.7 in the foregut and 1.5–4.2 and 8.9–9.6 in the hindgut. For comparison, soil samples with values of pe and pe + pH comparable to those observed in the foreguts and hindguts of the larvae of these two lepidopteran species are transitional between anaerobic and aerobic conditions (Rowell, 1988).

TABLE 2. pH, E_h, pe AND pe + pH OF ANTERIOR, MEDIAL, AND POSTERIOR MIDGUTS OF FIVE SPECIES OF LEPIDOPTERAN LARVAE[a]

Species (N) and midgut location	pH	E_h	pe	pe + pH
Manduca sexta (5)				
Anterior	8.0 (0.46)[a]	−131 (66.6)[a,b]	−2.2 (1.13)[a,b]	5.8 (1.58)[a]
Medial	9.3 (1.33)[b]	−188 (26.1)[a]	−3.1 (0.44)[a]	6.1 (0.81)[a]
Posterior	8.2 (0.66)[a]	−88 (96.8)[b]	−1.5 (1.63)[b]	6.8 (1.38)[a]
LSD (0.05,12)	0.9	78	−1.3	1.5
Polia latex (4)				
Anterior	7.8 (0.68)[a]	−85 (62.1)[a,b]	−1.4 (1.05)[a,b]	6.4 (0.77)[a]
Medial	8.8 (0.15)[b]	−132 (51.1)[a]	−2.2 (0.86)[a]	6.5 (0.90)[a]
Posterior	8.3 (0.76)[a,b]	−55 (36.0)[b]	−0.9 (0.61)[b]	7.4 (0.70)[a]
LSD (0.05,9)	0.8	66	1.1	1.0
Lymantria dispar (5)				
Anterior	8.2 (0.38)[a]	+238 (45.1)[a]	4.0 (0.76)[a]	12.2 (0.70)[a]
Medial	8.2 (0.27)[a]	+214 (36.1)[a]	3.6 (0.61)[a]	11.8 (0.76)[a]
Posterior	7.9 (0.39)[a]	+218 (35.7)[a]	3.7 (0.61)[a]	11.6 (0.35)[a]
LSD (0.05,12)	0.4	44	0.7	1.1
Danaus plexippus (3)				
Anterior	8.6 (0.49)[a]	+77 (9.1)[a]	1.3 (0.15)[a]	9.9 (0.35)[a]
Medial	8.5 (0.15)[a]	+54 (4.9)[b]	0.9 (0.01)[b]	9.4 (0.11)[b]
Posterior	8.1 (0.31)[a]	+91 (8.6)[c]	1.5 (0.14)[c]	9.6 (0.40)[a,b]
LSD (0.05,6)	0.6	12	0.2	0.5
Papilio glaucus (3)				
Anterior	10.1 (0.50)[a]	+17 (58.6)[a]	0.3 (0.99)[a]	10.4 (0.98)[a]
Medial	10.1 (0.47)[a]	+48 (7.4)[a]	0.8 (0.12)[a]	11.0 (0.42)[a]
Posterior	9.8 (0.74)[a]	+57 (28.0)[a]	1.0 (0.47)[a]	10.8 (0.29)[a]
LSD (0.05,6)	0.9	179	1.0	1.0

[a] Values are means with standard deviations given in parentheses. In comparisons of different locations in the same species, values followed by the same letter are not significantly different at $P < 0.05$.

In contrast to *M. sexta* and *P. latex*, which have reducing midguts and mildly oxidizing foreguts and hindguts, *L. dispar*, *D. plexippus*, and *P. glaucus* larvae have guts in which oxidizing conditions prevail throughout (Tables 2 and 3). The average values of pe and pe + pH in the midguts of these species are in the ranges 0.7–3.8 and 9.5–11.9, respectively. Soils with these values for pe and pe + pH would be classified as aerobic soils (Rowell, 1988).

As the midgut is the primary site of digestion and assimilation in larval Lepidoptera, the large interspecific differences in midgut redox conditions reported here reflect significant differences in the chemical environment in which digestion and assimilation occur.

TABLE 3. pH, E_h, pe, AND pe + pH OF FOREGUTS, MIDGUTS, AND HINDGUTS OF FIVE SPECIES OF LEPIDOPTERAN LARVAE[a]

Species and gut location (N)	pH	E_h	pe	pe + pH
Manduca sexta				
Foregut (1)	5.7	+172	2.9	8.6
Midgut (5)	8.5 (0.61)^{a,1}	−136 (51.0)^{a,1}	−2.3 (0.86)^{a,1}	6.2 (0.99)^{a,1}
Hindgut (5)	7.4 (0.79)^{b,1}	+86 (48.0)^{b,1}	1.5 (0.81)^{b,1}	8.9 (0.85)^{b,1}
LSD (0.05,8)	0.8	58	1.0	1.1
Polia latex				
Foregut (4)	6.6 (1.27)^{a,1}	+123 (147.2)^{a,1}	2.1 (2.49)^{a,1}	8.7 (2.21)^{a,b,1}
Midgut (4)	8.3 (0.39)^{b,1}	−91 (43.6)^{b,1}	−1.6 (0.71)^{b,1}	6.7 (0.63)^{a,1}
Hindgut (4)	7.3 (0.92)^{a,b,1}	+99 (60.2)^{a,1,3}	1.7 (1.04)^{a,1,3}	8.9 (1.82)^{b,1,2}
LSD (0.05,9)	1.2	123	2.1	2.2
Lymantria dispar				
Foregut (5)	6.4 (0.41)^{a,1}	+236 (45.6)^{a,1}	4.0 (0.77)^{a,1}	10.5 (0.83)^{a,1}
Midgut (5)	8.1 (0.23)^{b,1}	+223 (36.4)^{a,2}	3.8 (0.61)^{a,2}	11.9 (0.56)^{b,2}
Hindgut (5)	7.1 (0.43)^{c,1}	+204 (36.6)^{a,2,3}	3.5 (0.62)^{a,2,3}	10.5 (0.51)^{a,2}
LSD (0.05,12)	0.4	45	0.8	0.8
Danaus plexippus				
Foregut (3)	7.5 (0.96)^{a,b,1,2}	+127 (73.5)^{a,1}	2.1 (1.25)^{a,1}	9.6 (0.42)^{a,1}
Midgut (3)	8.4 (0.06)^{a,1}	+74 (3.5)^{a,3}	1.1 (0.06)^{a,3}	9.5 (0.10)^{a,3}
Hindgut (3)	7.2 (0.52)^{b,1}	+114 (14.8)^{a,1}	1.8 (0.25)^{a,1}	9.0 (0.73)^{a,1,2}
LSD (0.05,6)	1.0	69	1.2	0.7
Papilio glaucus				
Foregut (3)	8.9 (1.42)^{a,b,2}	+122 (87.9)^{a,b,1}	2.1 (1.51)^{a,b,1}	11.0 (0.26)^{a,1}
Midgut (3)	10.0 (0.57)^{a,2}	+41 (26.0)^{a,3}	0.7 (0.40)^{a,3}	10.7 (0.40)^{a,2,3}
Hindgut (3)	7.6 (0.40)^{b,1}	+133 (18.9)^{b,1,3}	2.2 (0.32)^{b,1,3}	9.8 (0.10)^{b,1,2}
LSD (0.05,6)	1.4	86	1.4	0.5

[a] Values are means with standard deviations given in parentheses. Midgut values are the averages of the anterior, medial, and posterior midgut values reported in Table 2. In comparisons of different gut locations within a species, values followed by the same letter are not significantly different $P < 0.05$. In comparisons of the same gut locations between different species, values followed by the same number are not significantly different at $P < 0.01$.

Absorption of Bismuth Salts by Midgut Epithelium of M. sexta. When *M. sexta* larvae consumed artificial diet that contained bismuth subnitrate, the posterior midgut epithelium became dark brown (Figure 1), presumably because of the accumulation of bismuth sulfide in the cavities of the goblet cells. A similar accumulation of insoluble metal sulfides in the goblet cells of the midgut epithelium has been reported for the larvae of *T. bisselliella* fed a diet containing soluble metal salts (Waterhouse, 1952a). The accumulation of bismuth sulfide in the midgut epithelium of *M. sexta* and *T. bisselliella* larvae fed diets amended with bismuth subnitrate suggests that in both species sulfide may be

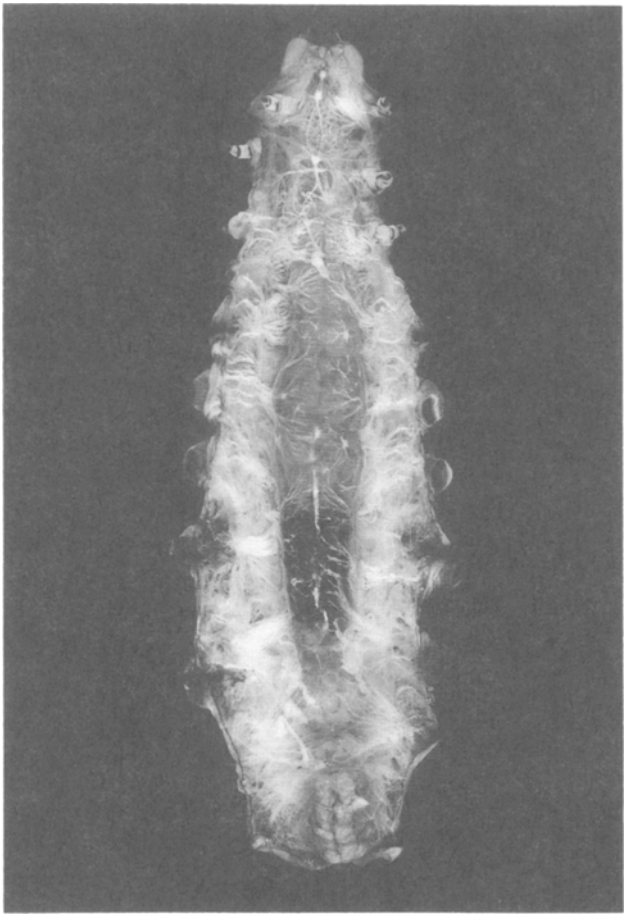

FIG. 1. Ventral view of the alimentary tract of a fourth-instar *M. sexta* larva that had been feeding on an artificial diet that included 10% bismuth subnitrate. The anterior of the larva is at the top of the photograph. The darkened region is the posterior third of the midgut. The dark substance, presumably bismuth sulfide, is in the midgut epithelium, not in the lumen contents.

one of the reducing agents responsible for the maintenance of reducing conditions. In *T. bisselliella* the sulfide is generated from cysteine by the action of L-cysteine lyase (Yoshimura et al., 1988).

No accumulation of bismuth sulfide was evident in the midgut epithelium of *L. dispar* larvae fed a diet amended with bismuth subnitrate.

Significance of Midgut Redox Conditions to Lepidopteran Herbivores. The redox state of the gut is likely to mediate the effects of many plant allelochem-

icals on insect herbivores. The impact of ingested phenols is likely to be especially dependent upon the redox conditions encountered in the gut, since quinones, which are oxidation products of phenols, are generally more reactive and more toxic than their phenolic precursors. Quinones can form covalent bonds with nucleophilic groups present in a variety of nutritionally important compounds (Leatham et al., 1980; Hurrell et al., 1982). The oxidation of foliar phenols, mediated by ingested foliar phenoloxidases, has been shown to reduce the efficiency of utilization of dietary amino acids and proteins in larvae of the tomato fruitworm, *Heliothis zea* (Lepidoptera, Noctuidae) (Felton et al., 1989). In addition, the toxic effects of plant phenols may depend upon the formation of highly reactive superoxide anion radicals during phenol oxidation. These reactive intermediates have been shown to inactivate enzymes, disrupt membranes, and damage DNA in mammals (Smith, 1985). Oxygen radicals, produced during the metabolism of phenols, may be responsible for the gut lesions observed in some insect herbivores maintained on diets rich in tannins (Bernays et al., 1980; Steinly and Berenbaum, 1985). Thus, both antinutritional and toxic effects of plant phenols may be strongly influenced by the redox conditions of the gut.

On the other hand, the oxidation of ingested phenols can have beneficial consequences for insect herbivores when the adverse effects on growth and fecundity are offset by the beneficial effects of reducing susceptibility to infection by microbial pathogens. The oxidation products of ingested phenols afford protection against the infection of *H. zea* by the nuclear polyhedrosis virus, HzSNPV (Felton and Duffey, 1990). Similarly, ingested phenols have been shown to protect the gypsy moth, *L. dispar*, against infection by the nuclear polyhedrosis virus GMNPV (Keating et al., 1989), although it has not yet been demonstrated that oxidized phenols are involved. Thus, it is possible that for one herbivore it would be advantageous to prevent phenol oxidation by the maintenance of reducing conditions in the midgut, whereas in another it would be advantageous to promote phenol oxidation by the maintenance of oxidizing conditions throughout the gut.

Tree-feeding caterpillars are more likely to encounter higher concentrations of tannins and other polyphenols than forb-feeding caterpillars. We were interested in determining whether there was a relationship between the growth form of the larval food plant and the redox conditions maintained in the midgut. We observed reducing conditions in one tree-feeder (*P. latex*) and one forb-feeder (*M. sexta*) and oxidizing conditions in two tree-feeders (*L. dispar* and *P. glaucus*) and one forb-feeder (*D. plexippus*). Thus, even this limited survey reveals that factors other than host plant growth form must account for the dichotomy between those Lepidoptera with oxidizing and those with reducing midguts.

We do not know yet whether ingested phenols undergo significant oxidation in the midguts of *L. dispar*, *D. plexippus*, and *P. glaucus*. Nor do we know

whether the conditions in the midguts of *M. sexta* and *P. latex* are sufficiently reducing to prevent the oxidation of ingested foliar phenols or to reduce ingested foliar quinones. Definitive resolution of those questions can be achieved only by determining the chemical fates of phenols of known structure that have been introduced into midgut fluids. On the basis of the findings reported in this paper, however, we feel justified in drawing the following conclusions: (1) the conditions in the midguts of *M. sexta* and *P. latex* are less conducive to the oxidation of ingested foliar phenols than the conditions in the midguts of *L. dispar*, *D. plexippus*, and *P. glaucus*; (2) the extent of oxidation of some foliar constituents will be greater in the midguts of *L. dispar*, *D. plexippus*, and *P. glaucus* than in the midguts of *M. sexta* and *P. latex*; and (3) some foliar constituents that are oxidized in the midguts of *L. dispar*, *D. plexippus*, and *P. glaucus* will not be oxidized in the midguts of *M. sexta* and *P. latex*. On the basis of these conclusions, we propose that the effects of plant allelochemicals on insect herbivores will be affected by gut redox conditions and that the regulation of gut redox conditions is an important adaptation of insect herbivores to the chemical defenses of plants. The redox state of the gut is yet another insect trait that must be included in the analysis of plant–insect interactions.

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