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Plant Phenolics Behave as Radical Scavengers in the Context of Insect (*Manduca sexta*) Hemolymph and Midgut Fluid

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To evaluate the prooxidant versus antioxidant properties of plant phenolics toward leaf-feeding caterpillars, quenching of the stable ABTS radical by five phenolics was measured in two physiological contexts: hemolymph and midgut fluid. Addition of tannic acid, chlorogenic acid, quercetin, or catechin to *Manduca sexta* (L.) gut fluid increased its total antioxidant capacity by 12−45%, with tannic acid and quercetin being the most powerful in this regard. The antioxidant contribution of the phenolics increased with longer (30−60 min) incubation time in gut fluid. Chlorogenic acid and caffeic acid exhibited the weakest antioxidant activity in gut fluid. The total antioxidant capacity of hemolymph is considerably less than that of gut fluid, and in hemolymph chlorogenic and caffeic acids sometimes acted as mild prooxidants, particularly after longer incubation periods (30−60 min), although this trend was not statistically significant. Tannic acid, catechin, and quercetin behaved as antioxidants in hemolymph. These results suggest that many phenolics have radical scavenging activity in the digestive tract, but some may have more detrimental effects after absorption into the hemolymph compartment.

KEYWORDS: Herbivore; ABTS radical; total antioxidant capacity; phenolic; tannin

INTRODUCTION

Phenolics are widely distributed in the leaves of vascular plants, where they play important roles in protecting foliage from ultraviolet radiation, herbivores, and pathogens (*1*−*3*). Although many phenolics are recognized as having antioxidant properties, they can also produce reactive oxygen byproducts such as semiquinones, peroxyl radicals, hydroperoxides, and other reactive oxygen species as they undergo oxidation (*4*, *5*). These reactive oxygen intermediates can exert prooxidant effects on animal tissues in vitro and in vivo (*4*, *6*−*8*). Thus, foliar phenolics have the capacity to act either as beneficial antioxidants or dangerous prooxidants to herbivores ingesting green leaves, depending on the biochemical context (*9*).

Insect herbivores are thought to be particularly susceptible to oxidative stress generated from ingested phenolics, especially taxa that maintain alkaline conditions in their digestive tracts. For example, larval *Lepidoptera* (caterpillars), some *Orthoptera* (walkingsticks), and some *Hymenoptera* (sawflies) have midgut pH values ranging from 9.0 to 11.5 (*10*, *11*). Because the pK_a of the phenolic hydroxyl is ∼8.5, autoxidation of most phenolics would be expected to proceed rapidly in the digestive tracts of these herbivores (*11*), with potentially detrimental effects.

The view that many plant phenolics are prooxidant toward insect herbivores has been supported by experiments with artificial diet. For example, white-marked tussock moth caterpillars (*Orygia leucostigma*) have higher quantities of hydroperoxides, a marker of oxidative damage, in the midgut, reduced growth, pupal deformities, and higher mortality when fed artificial diet containing tannic acid (*7*). When chlorogenic acid is mixed with polyphenol oxidase (PPO) in artificial diet, the subsequent oxidation reaction lowers the nutritional quality of dietary proteins and produces biochemical markers of oxidative damage in the midgut tissue of caterpillars fed the treated diet (*6*, *7*, *12*, *13*). The negative effects of chlorogenic acid on growth are alleviated by dietary ascorbate, implicating oxidative stress as a primary toxicological effect under these conditions (*6*).

Despite the evidence from artificial diet experiments, many caterpillars feed successfully on host plants that are rich in tannins and phenolics and show no signs of oxidative stress (*14*−*18*). Indeed, concentrations of chlorogenic acid that produce oxidative stress in artificial diet experiments (*6*) do not induce oxidative stress in caterpillars when consumed in the context of fresh leaf tissue, for example, in transgenic tobacco that overexpresses chlorogenic acid at a 10-fold higher concentration (*16*, *17*).

These discrepancies illustrate the importance of considering dietary and physiological context when evaluating the pro- or antioxidant behavior of phenolics. Phenolics can behave either as prooxidants or antioxidants, depending on their concentration, ambient pH, and the oxidizing potential of other reactants in the mixture (*19*). Many phenolic acids and flavonoids quench free radicals (*20*, *21*), chelate redox active metal ions, or form more stable intermediates that inhibit chain propagation reactions (*22*, *23*) in foodstuffs and body fluids. Tannins (epicatechin, catechin) and tannin−protein complexes can be even more (15−30 times) effective at scavenging the 2,2′-azinobis(3-ethylben-

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zythiazoline-6-sulfonic acid) (ABTS) radical than monomeric phenolics (*24*).

When caterpillars feed on green plant tissue, nutrients and allelochemicals are released from macerated leaf fragments into the fluid-filled lumen of the digestive tract. Although the alkaline pH of the fluid would be expected to promote rapid oxidation of ingested phenolics, many caterpillars have low midgut oxygen tensions (*25*) and strongly reducing concentrations of ascorbate and/or thiols in gut fluid (*7*, *26*) that can limit oxidation reactions. Moreover, green plant foliage is rich in low molecular weight chemical antioxidants, including ascorbate, thiols, tocopherols, carotenoids, and phenolics themselves (*26*, *27*) that can further enhance the total antioxidant capacity (TAC) of fluid in the midgut. TAC is defined as the capacity of a mixture to quench a known quantity of radical and thus provides a functional indicator of the nature, quantity, and degree of oxidation/reduction of antioxidants. The TAC of physiological fluids is commonly used to assess oxidative stress and existing protection against reactive oxygen species in animal tissues (*28*, *29*).

In the present study, the net effect of selected phenolics (chlorogenic acid, caffeic acid, catechin, quercetin, and tannic acid) on the TAC of freshly collected alkaline *Manduca sexta* midgut fluid and hemolymph was measured. These compounds are among the most widespread phenolics in plant foliage and are thus commonly encountered by phytophagous insects. We used the decolorization of a preformed stable ABTS radical because this assay is appropriate for measuring both water- and lipid-soluble antioxidants and has been used to characterize the antioxidant properties of carotenoids, flavonoids, simple phenolics, and tannins in simple buffered solutions and phenolic-rich foods and beverages (*30*, *31*). It can simultaneously measure prooxidant activity, because additional ABTS radical is generated in the presence of phenoxyl radicals (*32*).

Previous studies have shown that the TAC of caterpillar midgut fluid is both substantial and variable across host plants (*16*, *26*). However, the specific contribution of phenolics relative to other dietary antioxidants such as α-tocopherol, carotenoids, ascorbate, or glutathione to this capacity has not been assessed. Low molecular weight phenolics also cross the gut wall; for example, up to 14% of dietary chlorogenic acid can be found in the hemolymph, mostly in the free (unbound) form, 24 h after ingestion (*12*, *33*). We hypothesize that if conditions in the caterpillar midgut are sufficiently reducing, unoxidized phenolics may retain their antioxidant properties before and after absorption across the gut epithelium and, thereby, make beneficial contributions to the TAC of major body fluid compartments (*16*).

MATERIALS AND METHODS

Chemicals and Reagents. Tannic acid, quercetin, catechin, caffeic acid, chlorogenic acid, and 2,2′azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were obtained from Sigma Aldrich Chemicals (St. Louis, MO) and used without additional purification.

Insects. *Manduca sexta* caterpillars (actively feeding penultimate instar) were from a colony maintained at Ohio University. They were reared on commercial *M. sexta* artificial diet (Bioserve, Frenchtown, NY).

Collection of Physiological Fluids. Hemolymph from chilled, 2−3-day-old penultimate instar larvae was collected into a test tube by snipping a rear proleg with dissecting scissors. The hemolymph was chilled on ice and used within 5 min. To collect midgut fluid, the entire gut was removed from a chilled larva and rinsed with saline. A small incision was made in the gut wall to allow the peritrophic envelope and its contents to slide out into a preweighed test tube. The gut contents were weighed, then diluted with an equal mass (volume) of 0.5 mM potassium phosphate buffer (prepared as pH 8.6; the final pH of diluted gut fluid ranged from 8.8 to 9.2). The peritrophic envelope was broken up with a spatula, and each sample was centrifuged at 10000*g* for 3 min. The supernatant was used immediately in the ABTS radical assays. Early trials showed that gut fluid quenched the ABTS radical much more rapidly than hemolymph or the phenolics at the concentrations tested (complete quenching in <1 min) so the gut fluid supernatant was further diluted (1:1) before use in assays.

ABTS Radical Cation Decolorization Assay. Phenolics were tested for their ability to quench the ABTS radical in three experimental conditions: (1) phenolic alone (dissolved in dH_2O); (2) phenolic in combination with alkaline gut fluid; and (3) phenolic in combination with hemolymph. We chose to mix phenolics directly with aqueous gut fluid and hemolymph to simulate their in vivo solubility and redox properties as closely as possible. The ABTS radical (7.0 mM) was prepared as described in ref *30* in 5 mM potassium phosphate buffer (adjusted to pH 7.4 for hemolymph assays or to pH 8.6 for gut fluid assays) and reacted in a dark bottle overnight with potassium persulfate to generate the radical cation. This solution was diluted with buffer to obtain the desired working solution. Although slightly more ABTS radical formed overnight at the more alkaline pH, the solution performed comparably to the pH 7.4 solution in assays. ABTS solutions were used within 48 h.

Experiment 1. Radical Quenching of Phenolics in Insect Hemolymph (pH 7.4) and Gut Fluid (pH 8.6−9.0). Caffeic acid, chlorogenic acid, catechin, and quercetin were tested at a final concentration of 4 μM. Foliar concentrations of individual phenolics are quite variable, but chlorogenic acid has been reported to occur at 6.0 μmol/g of fresh weight (fw) in tomato (*34*) and at 0.59−6.21 μmol/g of fw in tobacco (*15*), both natural host plants of *M. sexta*. Plasma concentrations of catechin and quercetin in mammals can range from 4 to 50 μM after ingestion of phenolic-rich meals (*35*). Because the tannic acid we used was impure (precluding accurate calculation of molarity), it was tested at 0.24 μg/mL. If we assume a molecular weight of 908 g [the median of the 789−1027 g range reported for commercial tannic acid (*36*)], this molar concentration (0.26 μM) is considerably lower than that of the other phenolics, but it was selected because it quenched a similar amount (50%) of the ABTS radical. For the assays, phenolic solutions were combined with an equal volume (10 μL) of distilled water, midgut fluid, or hemolymph. Two minutes was allowed for mixing and reaction, and then 980 μL of the prepared ABTS radical solution was added. The change in absorbance at 734 nm, which indicated the degree of radical quenching (decolorization) or production (increased absorbance), was monitored for 6 min using a Shimadzu UV spectrophotometer. Assays were replicated with fluid from five to six insects, each in duplicate cuvettes. The radical quenching of midgut fluid, hemolymph, or phenolic in buffer was expressed as the percent of decolorization relative to dH_2O blanks, which generally did not change over the 6 min assay period. The radical quenching abilities of phenolics in combination with each body fluid were expressed relative to blanks containing the body fluid plus 10 μL of dH_2O, which progressively quenched the radical over the 6 min period.

Experiment 2. Concentration-Dependent Antioxidant Properties of Phenolics in Hemolymph and Gut Fluid. Caffeic acid, chlorogenic acid, catechin, and quercetin were tested at five concentrations (final concentrations of 2, 4, 6, 8, and 10 μM). Tannic acid was tested at 0.08, 0.16, 0.24, and 0.40 μg/mL (estimated to be 0.09, 0.18, 0.26, 0.35, and 0.44 μM). As before, radical quenching of each phenolic was measured in three contexts: (1) phenolic alone (combined with an equal volume of dH_2O as described previously); (2) phenolic in combination with hemolymph; and (3) phenolic in combination with gut fluid. Blanks consisted of dH_2O, hemolymph, or gut fluid with no phenolic. Assays were run in duplicate and repeated three times with body fluids collected from different caterpillars.

Experiment 3. Phenolic Radical Quenching in Caterpillar Body Fluids after 5, 30, and 60 min. The radical quenching properties of phenolics were investigated after longer incubation times to more closely approximate the expected residence time of compounds in the insect digestive tract and hemolymph. Samples (40 μL) of hemolymph or gut fluid were mixed with equal volumes of aqueous phenolic

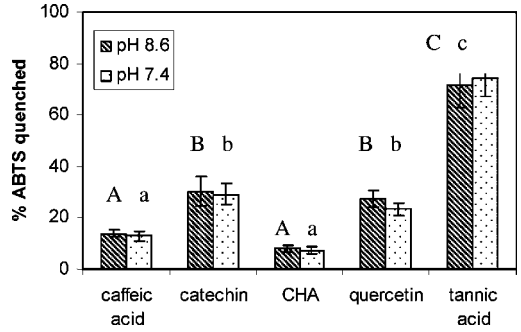

Figure 1. ABTS radical quenching by phenolics in alkaline (pH 8.6, $N = 5$) and neutral (pH 7.4, $N = 6$) buffer. Monomeric phenolics were tested at 4 μM, and tannic acid was tested at 0.24 μg/mL (~0.26 μM). The percent decolorization (radical quenched) is expressed relative to buffer-only blanks. Significant differences among phenolics within a body fluid are noted with different letters, Student–Newman–Keuls test ($P > 0.005$).

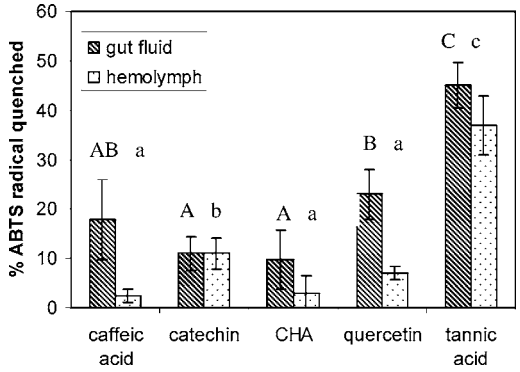

Figure 2. Increase in ABTS radical quenching by caterpillar gut fluid (1:1 dilution, $N = 5$) and hemolymph 5 min after addition of phenolics. The percent decolorization (quenching) is expressed relative to a blank containing the respective body fluid without phenolic. Significant differences among phenolics within a body fluid are noted with different letters, Student–Newman–Keuls test ($P > 0.005$).

solutions (4 μM, or 0.26 μM for tannic acid), sealed in 0.5 mL microcentrifuge tubes, and left at room temperature for 5, 30, or 60 min. After these intervals, tubes were uncapped and the mixtures tested for their ability to quench the ABTS radical as described above. Each time, *X* body fluid combination was assayed in duplicate and replicated using gut fluid and hemolymph collected from $N = 3$ and $N = 4$ caterpillars, respectively. Blanks consisted of dH_2O, hemolymph, or gut fluid only. The hemolymph and gut fluid data were analyzed separately with two-way ANOVA, with phenolics and time as main effects.

RESULTS

Experiment 1. Antioxidant Capacity of Phenolics under Neutral and Alkaline pH, in Caterpillar Hemolymph and Gut Fluid. In neutral (pH 7.4) and alkaline buffer (pH 8.6), all five phenolics exhibited weak to moderate radical scavenging behavior (**Figure 1**). Tannic acid exerted significantly more quenching power than the other phenolics in both buffers, quercetin and catechin were intermediate, and chlorogenic acid and caffeic acid were the least antioxidant (Student−Newman−Keuls test, $P > 0.005$).

Both hemolymph and diluted midgut fluid exhibited strong radical quenching properties in the absence of phenolics. The TAC of gut fluid was more than double that of hemolymph, because even after 1:4 dilution with buffer, it quenched a comparable amount of ABTS radical (70−80% decolorization). Addition of phenolics to gut fluid and hemolymph increased their TAC from 3 to 45% (**Figure 2**), and in no case did it decrease the TAC. The five phenolics ranked in a manner similar to that observed in the two buffer systems, with tannic acid acting as the strongest antioxidant in both hemolymph and gut fluid and caffeic and chlorogenic acids being the weakest. However, the differences among phenolics were not statistically significant at $P < 0.05$ in either body fluid (one-way ANOVA for gut fluid; Kruskal−Wallis ANOVA on ranks for hemolymph), due to substantial variation among the individual caterpillars from which body fluids were collected. In fact, in some hemolymph replicates, caffeic and chlorogenic acids generated radicals instead of scavenging them (see Discussion).

Experiment 2. Concentration-Dependent Radical Quenching by Phenolics in Hemolymph and Gut Fluid. All of the phenolics except caffeic acid quenched the ABTS radical in a linear and concentration-dependent manner (**Figure 3a−e**). Caffeic acid was unusual in that under the conditions of experiment 2, it generated 10−20% more radical instead of quenching it, but not in a concentration-dependent fashion (**Figure 3e**). In general, the contribution of each phenolic to the TAC was similar when measured in each of the three different contexts (buffer, hemolymph, and gut fluid), as evidenced by the degree of overlap in the three concentration−response curves in each figure.

Experiment 3. Phenolic Radical Quenching in Caterpillar Body Fluids after 5, 30, and 60 min. Differences between the radical quenching properties of phenolics in hemolymph and gut fluid became more apparent when compounds were incubated for >6−10 min. In gut fluid, the contribution of phenolics to TAC tended to increase after they were incubated for longer in the fluid (**Figure 4a**), although the effect was not statistically significant (two-way ANOVA, time, $F = 2.16$, df = 2, $P = 0.126$). Differences among phenolics reached statistical significance in this experiment (phenolic effect, $F = 6.25$, df = 4, $P = 0.001$), with tannic acid acting as a stronger antioxidant than the other four compounds at the concentrations tested (Tukey test). In hemolymph, different trends were observed. Over time, the contribution of caffeic and chlorogenic acids to the TAC decreased over the 60 min incubation period, as they showed a trend toward prooxidant properties, although again the effect was not statistically significant effect (two-way ANOVA, time, $F = 0.71$, df = 2, $P = 0.497$; **Figure 4b**). Tannic acid was a stronger antioxidant than the other four phenolics (phenolic effect, $F = 11.77$, df = 4, $P = 0.001$, followed by Tukey test). In the case of both fluids, the changes that occurred over time (1 h) summed to a <10% change in the percent radical quenched.

DISCUSSION

The possibility that phenolics might provide beneficial antioxidant benefits for phytophagous insects has been noted by numerous workers, but not tested directly (*7*, *14*, *15*, *26*, *37*−*39*). In a previous study, we found that caterpillars fed phenolic-rich tobacco leaves had a higher hemolymph antioxidant capacity than those fed low-phenolic foliage, but we could not eliminate the possibility that other dietary antioxidants such as ascorbate, glutathione, or carotenoids also differed between leaf treatments (*16*). Barbehenn et al. (*26*) reported the antioxidant capacity of midgut fluid in two tree-feeding caterpillars to be relatively constant across host plant and season and suggested that foliar phenolics made a greater contribution to the TAC of this fluid compartment than leaf ascorbate and glutathione, which varied substantially during the growing season. The present study demonstrates that individual phenolics

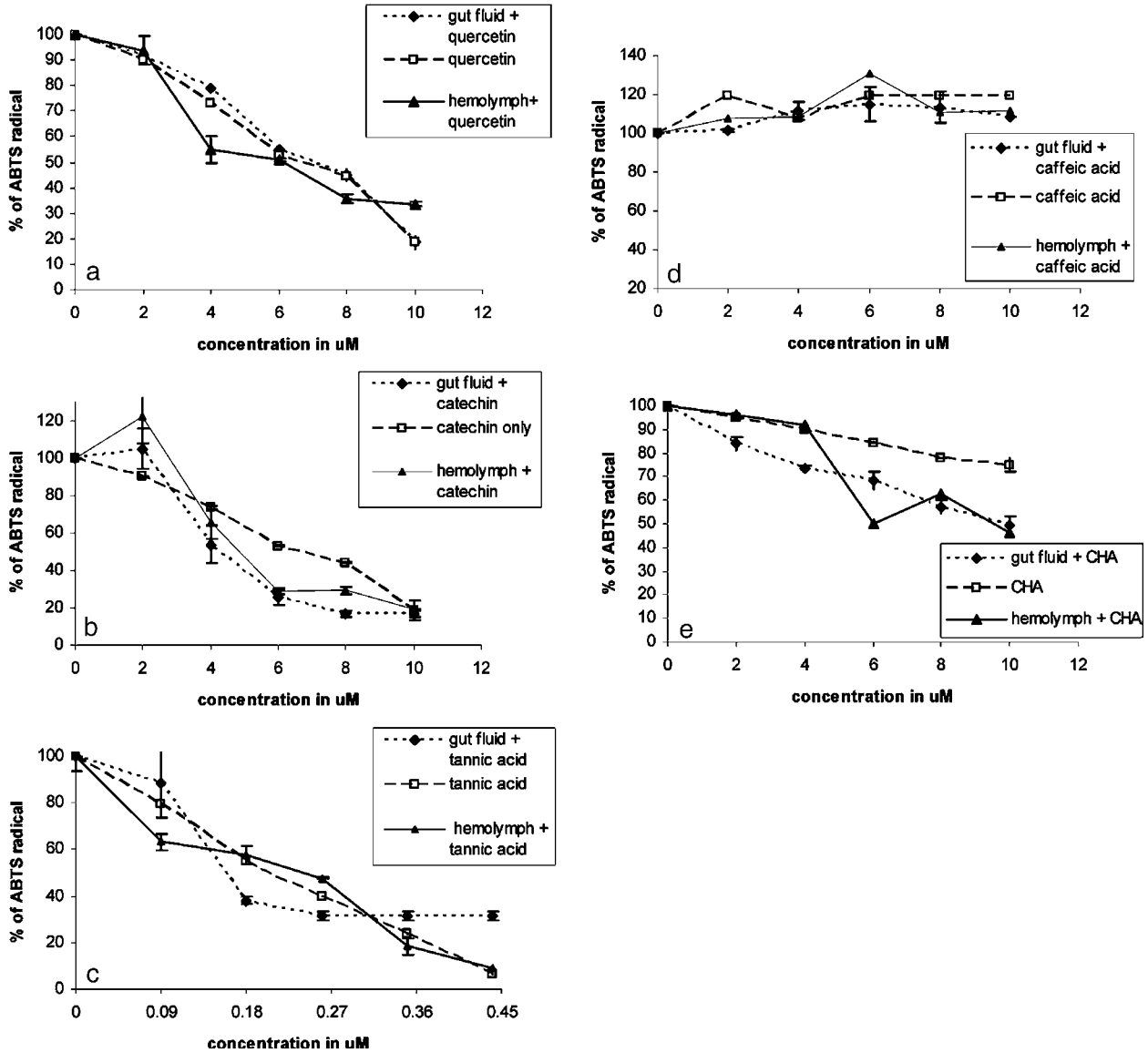

Figure 3. Concentration-dependent quenching of ABTS radical by phenolics (**a**, quercetin; **b**, catechin; **c**, tannic acid; **d**, caffeic acid; **e**, chlorogenic acid) when mixed with buffer, hemolymph, or gut fluid. The percent decolorization (percent radical quenched) is expressed relative to blanks of buffer only, hemolymph only, or gut fluid only, respectively. Bars represent standard errors.

can act as antioxidants in the physiological fluids of caterpillars and provides quantitative measures of the radical quenching activity of some commonly encountered plant phenolics.

Tannic acid behaved as the strongest antioxidant, increasing the TAC of both hemolymph and gut fluid significantly more than the other phenolics. This was particularly notable because the estimated molar concentrations used in these experiments were much lower (0.18−0.44 μM) than those of the other phenolics (all tested at 4 μM). These observations are consistent with prior reports of polymeric phenolics being 15−30 times more effective at quenching peroxyl radicals than monomeric phenolics (*24*), due to their high molecular weight, degree of polymerization, and abundance of hydroxyl groups (*24*, *40*). In caterpillar body fluids, quercetin and catechin acted as moderate antioxidants, quenching less radical than tannic acid but more than caffeic acid or chlorogenic acid. This ranking is also similar to other in vitro comparisons reported in the literature. Rice-Evans et al. (*21*) ranked the Trolox equivalent antioxidant capacities (TEAC) of these compounds as quercetin (4.7) > catechin (2.4) > caffeic acid (1.26) > chlorogenic acid (1.24). Kim et al. (*41*) also ranked quercetin > catechin > chlorogenic acid using the ABTS radical quenching method.

Although none of the phenolics exhibited strong prooxidant behavior, caffeic acid and chlorogenic acid were mildly prooxidant under some experimental conditions in our study. For example, in some hemolymph replicates, they increased ABTS formation, while in others, they reduced it. This suggests either measurable variation in the TAC of body fluids of individual caterpillars or subtle differences in procedure during the dissection and collection of body fluids. Both caffeic acid and chlorogenic acid became increasingly prooxidant in hemolymph over time (60 min), but the effect was not statistically significant. This pattern cannot be attributed to decreased endogenous antioxidant capacity of the fluid relative to the phenolics, since there was no overall reduction in the TAC of hemolymph-only or gut fluid-only controls during the same time period. Additionally, the antioxidant contributions of the other phenolics (tannic acid, quercetin, and catechin) did not change in hemolymph over time, suggesting that the time-dependent changes to caffeic acid and chlorogenic acid are via mechanisms

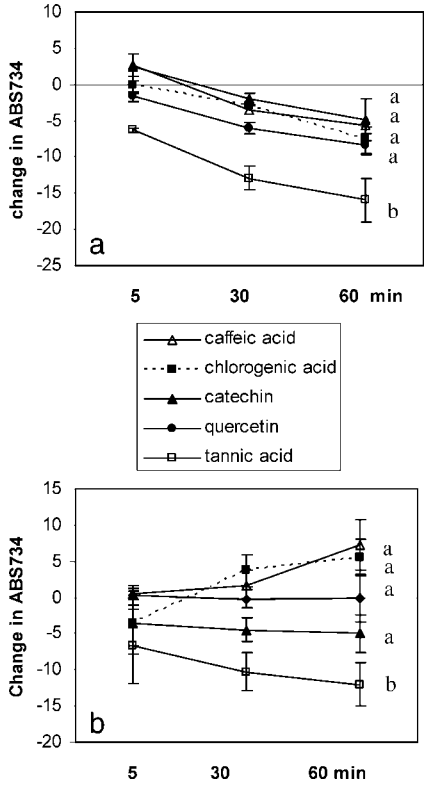

Figure 4. ABTS radical quenching by phenolics (4 μM) after 5, 30, and 60 min of incubation in (**a**) caterpillar gut fluid (1:1 dilution, $N = 3$) and (**b**) hemolymph ($N = 4$). The change in absorbance is expressed relative to blanks containing the body fluid with no added phenolic. Letters indicate significant differences among phenolics (Tukey test), and bars represent standard errors.

specific to these compounds. We can only speculate about what theses mechanisms could be. Perhaps they involve enhanced oxidation by hemocyte-associated NADPH oxidases (*42*), lipophorins (*43*), or phenol oxidases, the latter of which are capable of oxidizing phenols to quinones and producing reactive oxygen species in the plasma (*44*). Phenolics also differ in their abilities to oxidize NADH, ascorbate, or GSH in the presence of peroxidase; this is known to cause erythrocyte hemolysis to various degrees (*8*). More work is needed to understand the mechanisms by which caffeic and chlorogenic acid become more prooxidant in hemolymph over time and to determine what consequences such reactions might have in vivo.

In gut fluid, all of the phenolics, including caffeic and chlorogenic acid, tended to increase their antioxidant contribution after incubating for an hour. Typically, the residence time of food in the gut of *M. sexta* is 2−8 h, so the longer incubation periods may be more physiologically relevant than the 6−10 min assays. As was the case with hemolymph, the TAC of the gut fluid controls did not change over time. Thus, the increase in the relative contribution of phenolics appears to be due to an enhanced ability of the phenolics to scavenge the ABTS radical in this chemical context, not a relative decrease in the collective activity of other antioxidants in the fluid.

Despite the subtle differences in the behavior of phenolics after longer incubation times, the similarities in their contribution to the TAC of the two fluid compartments of caterpillars are more striking, given the differences in pH, ionic composition, and redox biochemistry of gut fluid and hemolymph. The lower TAC of hemolymph may increase its susceptibility to some prooxidant phenolics such as caffeic acid and chlorogenic acid. Uric acid and ascorbate are the dominant antioxidants in hemolymph (*45*, *46*), and in the blood sucking insect *Rhodnius prolixus*, TRAP assays showed that uric acid (up to 5 mM) accounts for almost all of the free radical scavenging activity (*47*). Glutathione concentrations are low in hemolymph, for example, 21−93 nmol/L in noctuid larvae (*48*), in comparison with gut fluid (in the micromolar range) (*7*, *26*). In some caterpillar midguts, the high glutathione and a luminal glutathione peroxidase work together to re-reduce and recycle ascorbate to maintain powerful reducing conditions (*7*). This is consistent with midgut fluid being a first line of defense against potentially detrimental dietary prooxidants.

Several important questions remain to be addressed. First, it is unclear whether the direct contribution of phenolics to the TAC of either compartment is large enough to be of physiological significance. Most natural phenols and polyphenols are still likely to be inferior electron donors compared to endogenous antioxidants such as ascorbate or glutathione, on the basis of their oxidation−reduction potentials. However, because the potentials of phenoxyl radicals are still significantly lower than those of most reactive oxygen species (*49*, *50*), phenolics can act as an important second line of antioxidant defense by working in coordination with ascorbate (*51*, *52*). Concentrations of total phenols in plant foliage are often higher than those of ascorbate or glutathione, sometimes reaching 10−25% dry weight (*53*, *54*); thus, their contribution to TAC in the gut lumen could be substantial. However, for plant-feeding herbivores, direct antioxidant advantages of dietary phenolics could be short-lived unless there are mechanisms to regenerate the reduced form of phenolics once they are oxidized. In plant tissues, phenolics can be reduced by ascorbate or MDA reductase (*55*−*57*), but it is not clear whether these reactions would occur (or be beneficial) in the insect gut lumen. Oxidation of some phenolics, such as caffeic acid, may, in fact, be irreversible (*58*). Nonetheless, phenolics could be important "sacrificial antioxidants", sparing other compounds such as ascorbate, glutathione, tocopherols, and carotenoids that can be more readily recycled in tissues.

A second issue that needs further study is the extent to which phenoxyl radicals versus ascorbyl radicals cause a net benefit or detriment toward target tissues. When mixtures of ascorbate and phenols undergo oxidation in vitro, a mixture of phenoxyl and ascorbyl radicals is produced, roughly in proportion to the molar ratio of the parent compounds (*59*−*61*). Both radical types are relatively stable compared to reactive oxygen species such as hydroxyl radicals, superoxide, and even H_2O_2, but both are still reactive and capable of exerting toxicological effects under a variety of conditions (*8*, *9*).

Finally, these assays were designed to measure autoxidative, not phenolase-catalyzed, oxidations of phenolics. Due caution should be used in generalizing the findings to other caterpillar taxa or larvae feeding on different host plants, because the presence of co-ingested plant oxidases could substantially alter the quantity and nature of oxidative byproducts produced in the gut lumen. For example, when PPO is the predominant oxidase in macerated leaves, catecholic phenolics retain their antioxidant properties, but when peroxidase activity dominates, they produce more free radicals (*38*, *39*).

Despite the above-mentioned limitations, these in vitro assays did not unequivocally support the prediction that phenolics would act as prooxidants in the alkaline gut fluid of *M. sexta*, even under conditions when molecular oxygen was likely more available than it would be in vivo (*25*). Instead, most phenolics improved the TAC of midgut fluid. In hemolymph, some of the same phenolics exhibited prooxidant tendencies, especially

after longer periods of time. These demonstrations of radical quenching activity of phenolics in *M. sexta* hemolymph and gut fluid under near-physiological conditions are a first step in predicting their potential benefit or detriment according to the time after ingestion and the fluid compartment they are localized in.

ABBREVIATIONS USED

ABTS, 2,2′-azoinobis(3-ethylbenzothiazoline 6-sulfonic acid); CHA, chlorogenic acid; DNA, deoxyribonucleic acid; DW, dry weight; EPR, electron paramagnetic spectrometry; GPOX, glutathione peroxidases; GSH, reduced glutathione; MDA, monodehydroascorbate; PPO, polyphenol oxidase; POD, peroxidase; TAC, total antioxidant capacity.

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