On the basis of available chemical data, it would appear that all known diamondiferous peridotites have come from depleted sections of the upper mantle. As yet unanswered are the questions of whether the diamonds grew in the presence of melt during that depletion process and what relation such diamonds bear to the great majority of diamonds in kimberlite.

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A Novel Means for Dealing with L-Canavanine, a Toxic Metabolite

Abstract. *L-Canavanine is a highly toxic L-arginine analog found in some leguminous seeds. Larvae of the bruchid beetle* Caryedes brasiliensis, *collected in Costa Rica, subsist solely on tissues of the mature seed of* Dioclea megacarpa, *which contains more than 8 percent L-canavanine by dry weight. The arginyl-tRNA synthetase of the bruchid beetle larvae discriminates between L-arginine and L-canavanine, and canavanyl proteins are not synthesized. In this way, bruchid beetle larvae avoid an adverse biochemical effect of L-canavanine.*

Host plants of some mono- and oligophagous insects contain high concentrations of toxic compounds that must be either detoxified metabolically or otherwise avoided by these feeding specialists. While our understanding of insect detoxification of certain synthetic insecticides is often quite detailed, a comparable appreciation for naturally occurring defensive compounds is lacking. This deficiency will become increasingly important as humans continue to intensify their management of agricultural systems.

Many plants synthesize and accumulate large quantities of amino acid analogs (*1*, *2*) that probably function as chemical barriers to the feeding activity of phytophagous insects, rodents, and other herbivores (*2*–*6*). An example of such a compound is L-canavanine, $H_2N-C(=NH)-NH-O-CH_2-CH_2-CH(NH_2)CO_2H$, the naturally occurring guanidinooxy structural analog of L-arginine. This amino acid is found in high concentrations in several leguminous seeds, including tropical species of *Canavalia* and *Dioclea*, where it can represent 5 to 10 percent of the dry weight (*5*, *7*). In some species, arginyl-tRNA synthetase can esterify L-canavanine to arginine transfer RNA ($tRNA^{Arg}$), and this amino acid analog is readily incorporated into the nascent polypeptide chain (*8*, *9*). These canavanine-containing proteins, structurally altered, severely disrupt numerous reactions of DNA and RNA metabolism as well as protein synthesis (*9*, *10*). In this report, we describe how a specialist insect deals with high concentrations of a naturally occurring toxic compound in its food.

Canavanine feeding studies with the larvae of the tobacco hornworm, *Manduca sexta* (L.) (*11*); the southern cowpea weevil, *Callosobruchus maculatus* (Fabricius) (*6*); the southern armyworm, *Prodenia eridania* (Cramer) (*4*); and the boll weevil, *Anthonomus grandis* Boheman (*12*) have demonstrated the potent insecticidal qualities of this amino acid analog. In contrast, larvae of the bru-

Table 1. Fate of [^{14}C]guanidinooxy-L-canavanine injected into *Manduca sexta* and *Caryedes brasiliensis* larvae; *N* is the number of injected larvae. Comparable data were obtained in two replications of this experiment. See text for additional details.

Organism	*N*	Radioactivity (count/min)				Radioactivity recovered as $^{14}CO_2$ (%)
		Injected	TCA precipitate	Column: NH_4OH effluent	Evolved CO_2	
Manduca sexta	5	4,442,500	166,020	158,950	156,570	3.5
Caryedes brasiliensis	33	948,750	3,510	2,475	Trace	None

chid beetle *Caryedes brasiliensis* Thunberg collected in Costa Rica subsist solely on the cotyledons of the mature seeds of *Dioclea megacarpa* Rolfe (*5*, *6*), which contain more than 8 percent L-canavanine by dry weight (*5*, *13*). Resistance to L-canavanine could be due to decomposition of canavanine or the occurrence of an arginyl-tRNA synthetase that discriminates between arginine and canavanine. Either model predicts an inability of the bruchid beetle larvae to incorporate canavanine into protein. This prediction was evaluated in the following experiment.

Thirty-three bruchid larvae (*14*), having a total fresh weight of 2.85 g, were removed live from *D. megacarpa* seeds. Each larva was injected with 2 μl of 200 m*M* L-canavanine containing 0.04 μc of [^{14}C]guanidinooxy-labeled L-canavanine per micromole. Five tobacco hornworm larvae (each having a fresh weight of 2.4 to 3.0 g) were injected with 66 μl of the labeled canavanine solution per 2.85 g fresh weight. After 24 hours at 25°C, the bruchid larvae were frozen in liquid N_2 and stored at –70°C. The alimentary canal and associated flora of the more massive tobacco hornworm larvae were removed before storage.

The treated larvae were ground mechanically with 50 ml of 100 m*M* glycylglycine buffer (*p*H 9.5). The slurry was centrifuged at 10,000*g* for 15 minutes and the pellet reextracted. Floating debris was removed from the combined supernatant solutions by filtration before incubation at 37°C for 30 minutes to discharge canavanyl-tRNA^{Arg} (*15*).

Soluble larval protein was precipitated with ammonium sulfate (60 g per 100 ml) and then dialyzed against 200 volumes of 20 m*M* tris(hydroxymethyl)aminomethane (tris) HCl buffer (*p*H 7.2) for 2 days at 4°C with four buffer changes. Dialyzed proteins were precipitated with 15 percent (weight to volume) trichloroacetic acid (TCA) and centrifuged at 15,000*g* for 10 minutes. The TCA-precipitated material was washed repeatedly with cold 10 percent TCA and recentrifuged until the supernatant solution was free of detectable radioactivity. After the TCA-precipitated material was thoroughly extracted with a mixture of absolute ethanol and ether (1:1 by volume), it was dried in vacuo at 80°C over P_2O_5.

To determine whether radioactive canavanine was incorporated into larval proteins, the TCA-precipitated material was hydrolyzed with 6*N* HCl in vacuo for 72 hours at 110°C. After removal of HCl by evaporation in vacuo, the protein hydrolyzate was neutralized and placed on a Dowex 50-X8 (NH_4^+) column and the resin was washed with a liter of H_2O. Canavanine, but not arginine, was eluted from the resin with 250 ml of 0.1*N* NH_4OH. Evaporation in vacuo was employed to remove NH_4OH before dissolving the protein hydrolyzate in 50 m*M* *N*-tris(hydroxymethyl) methylglycine (tricine) buffer (*p*H 7.2).

Table 2. Charging capacity of insect arginyl-tRNA synthetase. Bruchid beetle larvae, 100 individuals with a total fresh weight of 5.8 g, were ground with 20 ml of 50 m*M* tricine buffer, *p*H 7.8, as described in the text. The crude insect extract was centrifuged at 13,000*g* for 15 minutes and then at 100,000*g* for 90 minutes. Three fifth-instar tobacco hornworm larvae were similarly processed. The larval extracts were incubated with 0.1 m*M* [^{14}C]-guanidinooxy-L-canavanine (10 μc/μmole), 50 m*M* tricine buffer at *p*H 7.8, 1 m*M* adenosine triphosphate, 5 m*M* $MgCl_2$, and partially purified tRNA (*17*) in a final volume of 0.75 ml at 37°C. Samples (0.1 ml) were removed at 3-minute intervals for 15 minutes and analyzed by the paper disk method of Mans and Novelli (*18*). Radioactivity was measured by liquid scintillation spectroscopy (*16*). Boiled larval extract served as the control.

Organism	Charging capacity (nmole min^{-1} mg^{-1})	
	L-Arginine	L-Canavanine
Manduca sexta	68.2	5.3
Caryedes brasiliensis	42.6	Trace

The incorporation of [^{14}C]guanidinooxy-L-canavanine into larval protein was further tested by treating the buffered protein hydrolyzate with arginase and urease (*16*). These enzymes mediate the sequential hydrolysis of the guanidinooxy-labeled canavanine to L-canaline and [^{14}C]urea with the subsequent formation of $^{14}CO_2$. Arginase and urease-dependent stoichiometric production of $^{14}CO_2$ from protein hydrolyzate, free of labeled arginine, unambiguously establishes the presence of [^{14}C]guanidinooxy-L-canavanine.

Injection of labeled L-canavanine into *M. sexta* led to incorporation of radioactivity into the TCA-precipitable materials (Table 1). Acid and enzymatic hydrolysis of these materials yielded at least 3.5 percent of the injected L-canavanine as $^{14}CO_2$. In striking contrast, the bruchid larval proteins produced no labeled CO_2 after acid and enzymatic hydrolysis. The TCA-precipitable materials of the bruchid larvae possessed some radioactivity, but it was not uniquely associated with L-canavanine. Separate analysis of the radioactive bruchid TCA-precipitate revealed a diverse mixture of labeled amino acids. This indicates that the bruchid larvae can metabolize L-canavanine and that the evolved CO_2 is fixed into protein amino acids.

The capacity of the bruchid beetle larvae to metabolize canavanine suggests that they may avoid canavanine toxicity by converting it to an innocuous compound. This possibility was evaluated in two experiments. First, bruchid beetle larvae were injected with 1 or 3 μl of [^{14}C]guanidinooxy-L-canavanine, as previously described. After 24 hours, approximately one-half and two-thirds, respectively, of the original radioactivity was recovered as canavanine. Second, the soluble protein (24 mg) obtained from an extract of 12 bruchid larvae was incubated at 35°C with 20 m*M* [^{14}C]guanidinooxy-L-canavanine containing 12,500 count min^{-1} μmole^{-1}. The canavanine content was determined by enzymatic analysis with arginase and urease (*16*) after 3, 6, 12, and 24 hours of incubation. At least three-quarters of the substrate was not catabolized. Thus, while the bruchid beetle larvae can metabolize L-canavanine, they undoubtedly tolerate an appreciable in vivo level of the analog.

Caryedes brasiliensis larvae may also avoid the adverse effects of canavanine by mechanisms not considered in this study. Nevertheless, the bruchid beetle larvae have an amino acid activating enzyme able to discriminate between L-canavanine and L-arginine (Table 2). This capacity could be the critical factor in bruchid resistance to canavanine and likely represents an evolutionary adaptation by the bruchid to canavanine-containing food.

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Eimeria tenella (Sporozoa, Coccidia): Gametogony Following a Single Asexual Generation

Abstract. *Selection of* Eimeria tenella *for precociousness resulted in a strain with only one asexual generation prior to gametogony.*

Propagation in cell culture of a precocious strain (Wis-F) of *Eimeria tenella* (*1*) from the chicken cecum revealed that gametogony occurs after the first asexual generation and viable oocysts are produced in less than 90 hours. Until now the life cycle of this parasite was considered fixed, with a minimum of two generations of schizogony required prior to gametogony, resulting in a prepatent period of about 6 days.

After the appearance of first generation merozoites at 48 to 56 hours in cell cultures, clusters of intracellular zoites developed into immature macrogametocytes or microgametocytes by 64 to 72 hours. Macrogametocytes, recognized as early as 64 hours by the distinct karyosome prominent under phase-contrast microscopy or in stained material, were numerous by 72 hours (Fig. 1A). As the macrogametocytes matured, formation of peripheral, plastic granules and the oocyst wall was similar in appearance to that reported in the normal Wisconsin strain of *E. tenella* (*2*) (Fig. 1C).

Developing microgametocytes were recognized as multinucleate clumps of positively stained material among clusters of immature macrogametocytes (Fig. 1A). After 88 hours we found numerous mature microgametocytes, recognizable in living cultures by the sperm-like appearance of the active, flagellated microgametes within the cell (Fig. 1B). Some microgametes had escaped from the cells and were motile in the culture medium.

Clusters of mature oocysts were numerous after 96 hours of incubation. Transition from macrogamete to oocyst was marked by wall formation and constriction of the oocyte away from the wall. At this point in fixed, stained cultures, oocysts were easily recognized because they were impervious to the stains and have a golden appearance when viewed microscopically (Fig. 1D).

To test viability of cell culture–produced oocysts we allowed them to sporulate at room temperature for 2 days before feeding to 7-day-old chickens. Oocysts and gametocytes were found in cecal smears at 120 hours after inoculation, indicating that at least some of the oocysts were viable. For this experiment, we maintained controls similar to those described by Doran (*3*) by incubating sporulated oocysts at 40°C for 96 hours. These oocysts were not viable when fed to chickens.

Primary cell cultures were derived from kidneys of 2-week-old chicks by conventional tissue culture techniques. Leighton tubes with cover slips were inoculated with 200,000 cells per milliliter of culture medium (0.16 percent lactalbumin hydrolyzate in Earle's balanced salt mixture and 5 percent fetal calf serum) and incubated at 40°C for 3 days prior to inoculation with sporozoites.

Sporulated oocysts of the Wis-F strain were washed free of fecal debris and sterilized with sodium hypochlorite solution. Sporocysts were released by grinding in a tissue homogenizer, then excystation was effected by incubation for 2 hours in a solution of trypsin and bile (0.25 percent : 5 percent). Freshly excysted sporozoites were washed in saline, then inoculated into cultures at 200,000 per milliliter (2 ml per tube).

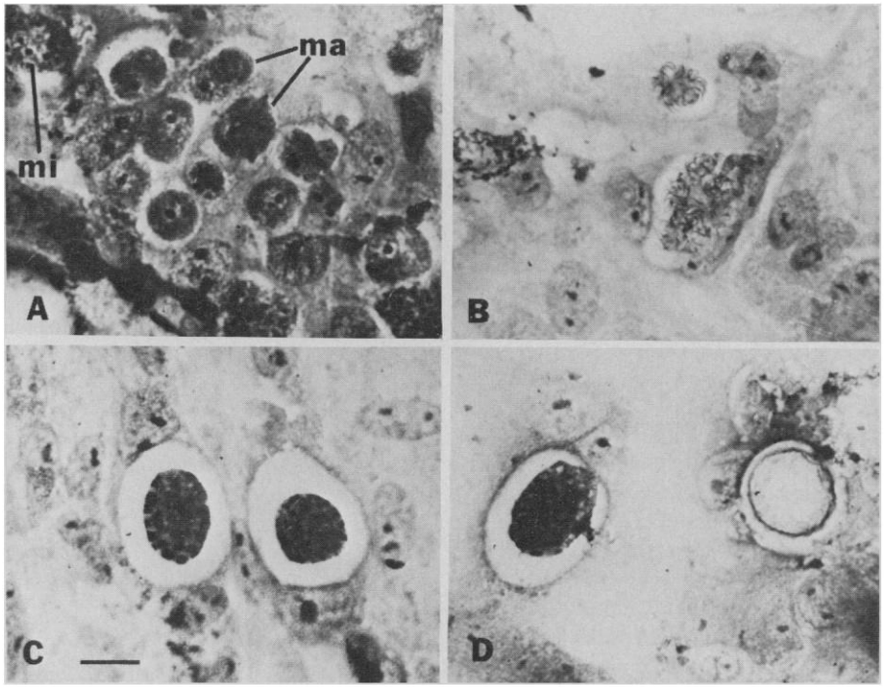

Fig. 1. Sexual development of the Wis-F strain of *Eimeria tenella* in chick kidney cell cultures. (A) Immature macrogametocytes (*ma*) and microgametocytes (*mi*) at 72 hours; (B) mature microgametes at 88 hours; (C) mature macrogametes with prominent wall-forming bodies at 88 hours; and (D) mature macrogamete and oocyst at 96 hours (scale marker, 10 μm).