teral dorsal medulla, most likely to the DCN. This finding cannot be ascribed to diffusion of injected HRP into the ventral spinocerebellar tract, which takes origin from such cells but is mainly crossed and runs along the ventrolateral margin of the medulla (*10*).

Cells in lamina IV, in which the majority of spino-DCN neurons appear to be located, are responsive to a wide range of mechanical stimuli applied to glabrous and hairy skin (*11*), and are modulated by activity in descending supraspinal pathways (*12*). Thus, besides receiving direct afferents via dorsal root fibers, the DCN are the relay of an ascending input which has undergone complex integration at segmental levels. Spinothalamic cells activated by hair movements and by low threshold mechanical deformation of the skin (*13*) and cells of origin of spinocervical tract (*14*) are also found in this region of the primate dorsal horn. Whether at least some of the fibers in the spinocervical, spinothalamic, and spino-DCN tracts have common cells of origin in the dorsal horn is a matter for further anatomical and electrophysiological investigation. However, it is legitimate to postulate, on the basis of the present observations, that these three pathways in the rhesus monkey may share some common functional properties.

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Degradation and Detoxification of Canavanine by a Specialized Seed Predator

Abstract. *Larvae of the bruchid beetle* Caryedes brasiliensis *feed exclusively on seeds of the Neotropical legume* Dioclea megacarpa, *which contains 13 percent* L-*canavanine by dry weight.* L-*Canavanine, a nonprotein amino acid analog of* L-*arginine, exhibits potent insecticidal properties. Most of the seed nitrogen is sequestered in canavanine, and bruchid beetle larvae do not simply excrete this toxic compound. Instead, these larvae possess extraordinarily high urease activity, which facilitates the conversion of canavanine to ammonia through urea. In this way, canavanine is effectively detoxified and a supply of nitrogen for fixation into organic linkage is ensured.*

Larvae of the bruchid beetle *Caryedes brasiliensis* feed exclusively on the seeds of the Neotropical legume *Dioclea megacarpa* (*1*), which contains 13 percent L-canavanine by dry weight (*2*). L-Canavanine is a toxic structural analog of L-arginine whose potent insecticidal properties have been thoroughly established in studies with various insects (*3*). We recently reported that larvae of *C. brasiliensis* possess an arginyl-tRNA synthetase that discriminates between arginine and canavanine so that canavanine-containing proteins are not synthesized (*4*). As a result, these bruchid beetle larvae avoid the most deleterious consequence of canavanine consumption (*5*). This bruchid beetle may simply excrete canavanine, sacrificing this abundant source of seed nitrogen, or it may have developed the ability to utilize this toxic substance. We report here the first demonstration, to our knowledge, of an insect that converts a poisonous plant substance to a foodstuff, providing nitrogen for primary metabolic pathways.

Analysis of *D. megacarpa* seeds infested with *C. brasiliensis* revealed that developing larvae ingest appreciable canavanine (Table 1). Undigested canavanine occurred in the frass, but nearly 60 percent of the seed canavanine was metabolized (Table 1) (*6*). Enzymatic assay of a homogenate of the larvae revealed the presence of appreciable arginase (*7*), the enzyme that mediates the cleavage of L-arginine or L-canavanine to urea and L-ornithine or L-canaline, re-

Table 1. Metabolism of L-canavanine by larvae of the bruchid beetle *C. brasiliensis* that developed in the seed of *D. megacarpa*. The dry weight of individual intact seeds was calculated by regression analysis of intact seed dry weight on testa dry weight ($y = -7.08 + 5.85x$). Uneaten seed and insect frass were collected from each infested seed, ground by hand, and dried to constant weight at 70°C. Canavanine was extracted by mechanical stirring of the dried samples with 100 volumes of 50 percent aqueous ethanol containing 0.1*N* HCl at 10°C for 18 hours. After the resulting slurry was clarified by centrifugation at 23,000*g* for 15 minutes, the pellet was reextracted with 50 volumes of the solvent for 6 hours at 10°C. Canavanine content was determined by the colorimetric assay method described by Rosenthal (*13*). The term seed denotes cotyledons plus embryonic tissues minus testa.

Dry weight (g)		Canavanine content (μmole)				Canavanine metabolized (μmole)	Ingested seed canavanine metabolized (%)
Intact seed	Uneaten seed	Intact seed	Uneaten seed	Ingested seed	Insect frass		
4.899	0.808	3891.9	641.9	3250.0	1035.4	2214.6	68.1
4.425	0.263	3768.9	224.0	3544.9	978.0	2566.9	72.4
5.999	0.411	4051.9	277.6	3774.3	1466.8	2307.5	61.1
4.766	1.495	3738.5	1172.7	2565.8	1029.7	1536.1	59.9
7.624	1.977	4571.3	1185.4	3385.9	1477.7	1908.2	56.4
6.069	0.816	6184.3	831.5	5352.8	2358.4	2994.4	55.9
4.517	1.320	3436.6	992.4	2444.2	1127.2	1317.0	53.9
4.822	1.654	2807.8	963.1	1844.7	1091.5	753.2	40.8
7.240	0.829	5327.4	610.0	4717.4	2152.9	2564.5	54.4

spectively. Arginase is present in the insects listed in Table 2, and this enzyme is believed to occur commonly in insects (*8*). Further analysis of the bruchid larvae homogenate disclosed extraordinarily high urease activity (Table 2), although urease is rarely reported in insects and its occurrence in these organisms is usually discounted (*9*). Ammonia production by urease-mediated cleavage of urea is also not believed to be of significance in insects (*9*). Nevertheless, *C. brasiliensis* larvae have nearly 30 times the urease content of any of the other tested insect species (Table 2). The specific activity of this enzyme in the bruchid urease pool is greater than that in the majority of canavanine-containing legume seeds tested by Rosenthal (*10*); legume seeds are among the richest sources of this enzyme.

To illustrate the transfer of radioactive carbon by the only biochemical reactions known that can produce $^{14}CO_2$ from [^{14}C-guanidinooxy]-L-canavanine, appropriate atoms are indicated by asterisks in the following equation.

$$H_2N\text{-}(\overset{*}{C}{=}NH)\text{-}NH\text{-}O\text{-}CH_2\text{-}CH_2CH(NH_2)CO_2H \rightarrow H_2N\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)CO_2H + H_2N\text{-}(\overset{*}{C}{=}O)\text{-}NH_2 \rightarrow 2NH_3 + \overset{*}{C}O_2 \quad (1)$$

The experiments summarized in Table 3 assess the quantitative contribution of the hydrolytic cleavage of canavanine to canaline and urea before ammonia formation relative to total canavanine catabolism. To make this assessment, a known quantity of [^{14}C-guanidinooxy]-L-canavanine was reacted with a bruchid beetle larval extract (see Table 3). Under the stated experimental conditions, 3.22 ± 0.11 percent of the canavanine was converted to CO_2 through canaline and urea (*11*). The deproteinized assay mixture contained both unreacted canavanine and possible degradation products resulting from canavanine catabolism by reactions that do not produce CO_2. To distinguish between these two possibilities, the equivalent of 1.000 μmole of radioactive canavanine, from the deproteinized assay mixture of experiment A, was treated so that all unreacted canavanine would be converted to CO_2 (Table 3, experiment B). Such determinations revealed that 98.6 percent of the radioactivity of the deproteinized assay mixture was derived from unreacted canavanine. It is assumed that the remaining radioactivity (1.4 percent) is a measure of canavanine catabolism by reactions other than reaction 1. Thus, 70 percent of the total canavanine degradation [3.22/(100 − 98.6 + 3.22)] resulted from conversion of canavanine to canaline and urea and then CO_2 and ammonia (Table 3).

These experiments reveal that bruchid beetle larvae do not simply excrete the nitrogen-rich canavanine. Applebaum (*12*) reported that the bruchid beetle larval gut exhibits very low proteolytic activity; this protects the insect against the inhibitors of proteolytic activity found in many legumes. Applebaum's finding suggests that the bruchid beetle larvae would rely on sources other than seed proteins for nitrogen. Of major importance in understanding bruchid-*Dioclea* interaction is the fact that canavanine accounts for 55 percent of every nitrogen atom of the seed and about 94 percent of the free amino acid nitrogen. Thus, it is difficult to imagine how this seed predator can totally avoid utilizing canavanine as a source of dietary nitrogen.

The bruchid larvae respond to the high canavanine concentration of their food by converting canavanine to canaline and urea. If the resulting urea is excreted directly, it would represent a significant loss of usable nitrogen. The pronounced larval urease activity ensures a supply of nitrogen for fixation into organic linkage. One must wonder as to the nature of the bruchid mechanism for processing or excreting the extraneous toxic ammonia. Since the usual seed sources of dietary nitrogen are unavailable to this insect,

Table 2. Urease content of various insect larvae. Terminal stadium larvae (fresh weight, 1 to 4 g) were washed exhaustively to remove any adhering plant debris and then ground with five volumes of 100 m*M* *N*-tris(hydroxymethyl)methylglycine (Tricine) buffer (*p*H 7.0) containing 0.1 percent (by volume) 2-mercaptoethanol and saturated at 4°C with phenylthiourea. The larvae were ground either mechanically with a Sorvall Omni-Mixer for 30 seconds or thoroughly by hand with a mortar and pestle. After the resulting slurry was centrifuged at 20,000*g* for 15 minutes, the pellet was reground as above. Floating debris was removed from the supernatant solutions by filtering through cheesecloth. Insect homogenate (1 ml) was pipetted into a 25-ml erlenmeyer flask, sealed with a rubber septum supporting a plastic center well (Kontes 882300) containing four drops of hydroxide of Hyamine, and brought to 37°C. The enzyme assay was initiated by injecting 1 ml of 100 m*M* urea containing 0.5 μc of [^{14}C]urea. Zero-time samples served as the controls. Sets of duplicate samples, each incubated for three different time periods, served to establish the initial velocity rate. The enzyme assay was terminated by injecting 2 ml of 2*N* HCl; 60 minutes later the center wells were removed and the absorbed $^{14}CO_2$ was measured by liquid scintillation spectroscopy (*14*). One microunit (μU) is that amount of urease which forms 1 pmole of $^{14}CO_2$ per minute at 37°C. Soluble protein values were determined by the method of Lowry *et al.* (*15*). Urease content is expressed as microunits per milligram of soluble protein; ND denotes organisms lacking detectable urease activity.

Organism	Urease content (μU/mg)
Diptera	
Drosophila melanogaster	15
Musca domestica	95
Hymenoptera	
Caliroa sp.	ND
Lepidoptera	
Manduca sexta	ND
Hyalophora cecropia	ND
Hyphantria cunea	ND
Heliothis virescens	ND
Pseudoaletia unipuncta	110
Galleria mellonella	ND
Ephestia kühniella	70
Coleoptera	
Tribolium castaneum	ND
Anthonomus grandis	ND
Hypera postica	480
Leptinotarsa decemlineata	1,405
Callosobruchus maculatus	1,245
Caryedes brasiliensis	38,570

Table 3. In vitro degradation of [^{14}C-guanidinooxy]-L-canavanine by the larvae of the bruchid beetle *C. brasiliensis*. Bruchid larvae (72 individuals) were ground mechanically and processed as described in Table 2, utilizing 0.5 volume of 100 m*M* Tricine buffer (*p*H 7.6) containing 2 m*M* $MnCl_2$. One milliliter of the resulting homogenate (6.4 mg of soluble protein) was incubated at 37°C with 100 μmole of L-canavanine containing 0.5 μc [^{14}C-guanidinooxy]-L-canavanine in a final volume of 2 ml as indicated in Table 2. After 60 minutes, the reaction was terminated by injecting 2 ml of 30 percent (weight/volume) trichloroacetic acid (TCA). Hydrolytic cleavage of canavanine through urea formation was determined by measuring $^{14}CO_2$ formation as described in Table 2. The deproteinized assay mixture was centrifuged at 21,000*g* for 15 minutes and TCA was removed from the supernatant solution by extraction with anhydrous ether. After dilution of the unreacted canavanine with the buffer, the equivalent of 1 μmole of labeled canavanine was treated with commercially prepared arginase (5 mg; 26 unit/mg) and urease (5 mg; 1250 unit/mg) in a final volume of 2 ml for 10 hours at 37°C. Evolved CO_2 represents unreacted canavanine. Each value presented is the mean of three determinations ± the standard error.

Initial substrate (μmole)	Evolved CO_2 (μmole)	Canavanine converted to urea (%)
Experiment A: Canavanine conversion to CO_2		
100.0	3.22 ± 0.11	3.22 ± 0.11
Experiment B: Unreacted canavanine		
1.000	0.986 ± 0.0015	98.6 ± 0.15

and since so much nitrogen is sequestered in canavanine, the bruchid larva is probably using canavanine as a food resource.

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Thyrotropin-Releasing Hormone: Stimulation of Colonic Activity Following Intracerebroventricular Administration

Abstract. *Intraventricularly administered thyrotropin-releasing hormone in rabbits elicited an increase in intraluminal pressure changes, a response commonly associated with muscular activity of the colon. The response appears to be central in origin with peripheral expression relying primarily on cholinergic receptors.*

For many years thyrotropin-releasing hormone (TRH) was considered to play a very specific role in the hypothalamicohypophyseal axis, as evidenced by the name. Recently, however, it has been found capable of producing physiological actions apparently independent of its pituitary influences. Clinical trials with parenterally administered TRH have led some researchers to attribute antidepressant capabilities to this compound (*1*), although others have questioned this conclusion (*2*). The results have been more consistent in animals than in humans, and they indicate stimulation of the central nervous system by TRH with or without the influence of anesthesia (*3*). Identification of TRH accumulations in various brain areas (*4*) has lent greater credence to the possibility that TRH and other neuropeptides actively participate in the modulation of various central integration processes.

Recent reports describing the clinical side effects of intravenously administered TRH often mention symptoms that are commonly associated with actions on the gastrointestinal tract, that is, nausea, vomiting, hunger, and poorly defined epigastric sensations (*5*, *6*). Although it has been proposed that these effects are not physiological but reflect a direct pharmacological action of TRH on smooth muscle (*5*), we believe that central mechanisms may be involved since we have been able to demonstrate an increase in gastrointestinal activity in rabbits injected intraventricularly with TRH. This phenomenon might represent an example of central modulation of a peripheral event by a neuropeptide; therefore, we felt that a more precise characterization of the response was warranted.

In this study we used male New Zealand rabbits (2.3 to 3.0 kg) that had been fasted for at least 24 hours and then anesthetized with sodium pentobarbital (35 mg/kg, intravenously). The intraventricular injection site was prepared by surgical techniques described by Jacob *et al.* (*7*). The abdominal area was shaved, topical anesthetic was applied, and a midline incision was made. The edges of the wound were sutured around a ring clamp suspended above the animal, creating a "well." Mineral oil was applied to the exposed viscera, and the ascending colon was located. A small incision was then made about 30 mm distal to the caecum, and an open-tipped cannula was introduced into the lumen and directed distally about 15 mm. The "well" was then covered with clear plastic, and a heating lamp was positioned above the animal to maintain a "well" temperature between 38° and 40°C. Recordings were begun approximately 30 minutes after the pentobarbital injection. Two different cannula-transducer systems were employed. One system consisted of a polyethylene cannula (PE 350) attached by a Y-tube to a Statham pressure transducer (0 to 5 cm-H_2O) and a Harvard infusion pump set to deliver at a rate of 194 μl/minute. The other system consisted of a glass cannula (2 mm inner diameter) attached directly to a Narco BioSystems pressure transducer (0 to 300 mm-Hg).

A typical response elicited by an intraventricular injection of TRH consisted primarily of an increase in magnitude of the intraluminal pressure changes associated with activity of the large intestine (Fig. 1). This response was observed in 80 percent of the animals (N = 20). Of the four animals not responding, three had received a dose of 10 μg of TRH, which was the lowest dose tested. We were unable to demonstrate a dose-response relationship using doses of 10, 20, 30, 50, and 100 μg. Pressure changes elicited by 10 μg and 20 μg of TRH often exceeded responses achieved at higher doses. This observation might be explained by the fact that the responsiveness of the large intestine is influenced by a large number of parameters, and that these parameters are impossible to control completely in a short-term preparation in situ such as ours.

Although there was a marked variation in the pressure patterns (*8*) recorded from individual rabbits, the patterns were unaltered by TRH administration in all but one animal. The frequency of the pressure changes also appeared to be unaffected in all but two animals. However, in the two apparent exceptions, changes that prior to TRH administration had been below the sensitivity limits of our instruments may have been unmasked. Base-line pressure (tone) responded variably to TRH, with most animals showing no change or a slight increase (0.8 cm-H_2O).

Because we had administered TRH in-