where all of the ROS can be seen to be present as phagosomes within the PE cells.

A possibility that cannot be excluded by these experiments is that the PE cells are actually phagocytizing only shed packets of ROS disks, which is known to occur in vivo. I tried to minimize this possibility by preparing the ROS after exposing the rats to light for only 1/2 hour. Previous investigations with rats (*2*) and frogs (*3*) suggested that maximum shedding of ROS tips occurs after 1 to 2 hours of light exposure. The assumption made in choosing 30 minutes of light exposure was that activation of the ROS plasma membrane has occurred in this time, but only minimal shedding has taken place. Furthermore, the few tips that have been shed tend to remain with the eyecup, rather than with the excised retina. If shedding has not already occurred under the conditions of illumination used in these studies, it is possible that the activation affects the entire outer segment plasma membrane of light-exposed photoreceptors, any part of which may then be phagocytized. However, it is not possible to deduce from the results reported here which of these two alternatives is correct.

The nature of the activation required to make the ROS palatable to the PE is unknown. The chemical composition or the molecular architecture of the plasma membrane must be modified sufficiently for the PE to recognize it as foreign. Current theories suggest that it is the oligosaccharide side chains of surface glycoproteins that are responsible for the recognition of one cell by another (*12*). O'Brien suggested that the addition of galactose and fucose to rhodopsin in the ROS plasma membrane could be the signal that results in the shedding or phagocytosis of ROS fragments (*13*).

Whatever the chemical signal is for shedding and phagocytosis, these processes are synchronized by light, but can also occur in the absence of light, according to a circadian rhythm (*2*). The PE cells in tissue culture retain their ability to distinguish between ROS isolated from animals exposed to light and those from dark-adapted animals—that is, activated and nonactivated ROS. This system will thus prove useful for studying the involvement of surface components of the ROS and the PE in the phagocytic process.

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5. Young [see (*1*)] has shown that the ROS of the rat takes about 9 days to be renewed. Thus radioactive disks synthesized after injection of [^{3}H]leucine would be displaced to the tip of the outer segment after 9 days and would, therefore, be in an optimum position to be phagocytized by the PE monolayer.
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10. In control experiments with PE cell monolayers, puromycin (50 μg/ml) decreased the incorporation of [^{3}H]leucine into PE cell monolayers by 90 percent.
11. This 20 percent decrease in the extent of phagocytosis is probably not entirely due to the inhibition of synthesis of ^{3}H-labeled proteins by puromycin. It is known (*14*) that phagocytosis results in loss of plasma membrane from the cell surface. Continual protein synthesis is required to replace this internalized plasma membrane. Since protein synthesis is inhibited in the presence of puromycin, new plasma membrane cannot be assembled and inserted into the existing plasma membrane. Thus the rate of phagocytosis will gradually decrease under these conditions. It is probable that these constraints result in the leveling off and decrease in phagocytosis normally seen after 4 hours of incubation. The extensive internalization of plasma membrane during phagocytosis may result in a refractory period during which synthesis and assembly of new plasma membrane occurs.
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L-Canaline Detoxification: A Seed Predator's Biochemical Mechanism

Abstract. *The seeds of the Neotropical legume,* Dioclea megacarpa, *the sole food source for developing larvae of the bruchid beetle,* Caryedes brasiliensis, *contain about 13 percent* L-*canavanine (dry weight). Canavanine detoxification and utilization produces* L-*canaline, a potent neurotoxic and insecticidal amino acid. This seed predator has developed a unique biochemical mechanism for degrading canaline by reductive deamination to form homoserine and ammonia. In this way, canaline is detoxified; canavanine's stored nitrogen is more fully utilized and its carbon skeleton is conserved.*

About 13 percent of the seed dry matter of the Neotropical legume *Dioclea megacarpa* consists of L-canavanine (*1*), a structural analog and potent antimetabolite of L-arginine. L-Canavanine undoubtedly contributes to the effective chemical barrier against predation established by this leguminous plant, because the bruchid beetle *Caryedes brasiliensis* is the only known consumer of the seed (*2*). This seed predator possesses an arginyl-transfer RNA (tRNA) synthetase that does not charge canavanine (*3*). Consequently, *C. brasiliensis* avoids a principal cause of canavanine toxicity, that is, the production of canavanine-

$$H_2N\text{-}C(=NH)\text{-}NH\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)CO_2H \rightarrow$$
L-canavanine

$$H_2N\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)CO_2H + H_2N\text{-}C(=O)\text{-}NH_2 \quad (1)$$
L-canaline urea

$$H_2N\text{-}C(=O)\text{-}NH_2 \rightarrow CO_2 + 2NH_3 \quad (2)$$

$$H_2N\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)CO_2H + NADH + H^+ \rightarrow$$
L-canaline

$$HO\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)CO_2H + NH_3 + NAD^+ \quad (3)$$
L-homoserine

Fig. 1. Metabolic reactions of the bruchid beetle *Caryedes brasiliensis* whereby L-canavanine and L-canaline are detoxified and utilized.

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containing, functionally altered proteins (*4*). In addition, the beetle larvae actually use the seed canavanine as a dietary nitrogen source by hydrolyzing it to canaline and urea. Its extraordinarily high urease activity is used to obtain ammonia for its nitrogen metabolism (*1*). Appreciable urease formation is very unusual in insects and represents an important evolutionary adaptation of the bruchid beetle to enable mobilization of the considerable nitrogen stored in the canavanine of its foodstuffs (*1*).

Urea formation from canavanine also results in the biosynthesis of equal amounts of L-canaline, a highly toxic, insecticidal structural analog of L-ornithine (*5*). The potent neurotoxic properties of canaline were demonstrated unequivocally in the tobacco hornworm moth (*6*). Here, we consider the novel biochemical adaptations of *C. brasiliensis* larvae to avoid the effects of canaline produced as a consequence of canavanine degradation. The bruchid beetle larvae circumvent this predicament by the reductive deamination of canaline to form homoserine and ammonia. The relevant pathways from canavanine to homoserine and ammonia in *C. brasiliensis* are illustrated in Fig. 1.

Homoserine synthesis from canaline, reaction 3 (Fig. 1), was established by demonstrating canaline-dependent production of NAD^+ (nicotinamide adenine dinucleotide) by bruchid beetle larvae (Fig. 2). In this assay, the forward reaction rate has been greatly enhanced by coupling ammonia formation from canaline to the subsequent reduction of α-oxoglutarate (*7*). We identified homoserine as a reaction product with the use of homoserine dehydrogenase, isolated and purified from pea (*Pisum sativum*). In this reaction, homoserine dehydrogenase catalyzed the NAD^+-dependent oxidation of homoserine to form aspartic semialdehyde and reduced NAD (NADH). Homoserine dehydrogenase caused reduction of NAD^+ only when canaline and NADH were present in the original reaction mixture (Fig. 2) (*8*).

Caryedes brasiliensis has the requisite enzymes to achieve integrated reactions 1 to 3 (*9*), and by these reactions not only detoxifies canaline but also gains homoserine and ammonia. The metabolic expenditure for producing the carbon skeleton structure of canavanine is thereby conserved as homoserine (*10*); moreover, the associated production of ammonia from canaline increases the mobilization of canavanine's stored nitrogen by 50 percent.

The results of this study and previous ones form the basis for the hypothesis that this predator has adapted to the toxic constituents of its foodstuff by functioning biochemically much as a canavanine-synthesizing plant. This conclusion is supported by three observations.

First, there is the production of an arginyl-tRNA synthetase capable of discriminating between arginine and canavanine. The formation of an aminoacyl-tRNA synthetase able to distinguish between an amino acid that constitutes a protein and one that does not is a well-recognized basis for prevention of autotoxicity in higher plants (*11*).

Second, the biochemical strategy of forming urea from canavanine prior to urease-mediated ammonia production is not only utilized by the bruchid beetle larva, but it is also the principal plant catabolic pathway for mobilizing canavanine's nitrogen (*12*). A correlation between seed canavanine content and urease activity has been established (*13*).

Third, this study has established that this bruchid beetle utilizes the conversion of canaline to homoserine to detoxify canaline and complete the degradation of canavanine. In the jack bean (*Canavalia ensiformis*), a leguminous plant, canavanine is biosynthesized by a cyclic reaction sequence, termed the canaline-urea cycle, which is analogous to the Krebs-Henseleit ornithine-urea cycle (*14*). The carbon skeleton is introduced into this cycle by conversion of homoserine to canaline, in a manner comparable to the production of ornithine from glutamic acid in the ornithine-urea cycle. Thus, while the biochemical purpose for the interconversion of canaline and homoserine differ in *Caryedes brasiliensis* and *Canavalia ensiformis*, these nonprotein amino acids are an integral part of their canavanine metabolism.

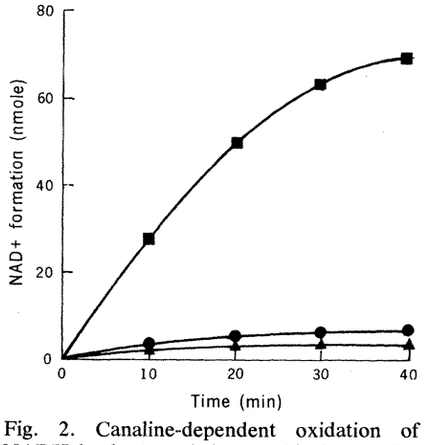

Fig. 2. Canaline-dependent oxidation of NADH by larvae of the bruchid beetle *Caryedes brasiliensis*. A bruchid beetle larval extract was prepared from 27 individuals having a fresh weight of 2.45 g (*3*). The larval extract was freed of low-molecular-weight constituents by gel-filtration chromatography (Sephadex G-25; column, 15 by 350 mm). The standard assay mixture consisted of 100 m*M* tricine buffer (*p*H 7.5), 25 m*M* L-canaline (*p*H 7.5), 1.0 m*M* NADH, 20 m*M* α-oxoglutarate (*p*H 7.5), 5.2 μg of larval protein, and 0.5 mg of glutamic acid dehydrogenase (Sigma, type II; 55 unit/mg). The reaction was monitored at 340 nm with a Gilford recording spectrophotometer; the cuvette chamber was maintained at 37°C by circulating water. The experiment consisted of a complete assay mixture (■), a similar mixture that lacked canaline (●), or one that lacked glutamic acid dehydrogenase and α-oxoglutarate (▲).

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9. It has not been established whether the bruchid beetle produces a distinctive enzyme that mediates reaction 3 (Fig. 1) or simply utilizes an existing protein of limited specificity (such as a dehydrogenase) that is capable of reductively deaminating several amino acids. Isolation of a protein catalyzing reaction 3 would represent discovery of a new class of enzymes characterized by their ability to reductively cleave and form the O-N linkage of the aminooxy group.
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