

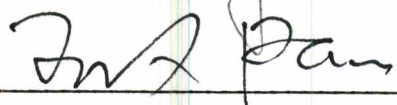
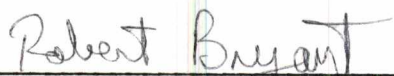
To the Graduate Council:

I am submitting herewith a thesis written by Karen Yvonne Long entitled "Rhodanese Activity in the Southern Armyworm, *Spodoptera eridania* Cramer (Lepidoptera:Noctuidae)." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biochemistry.

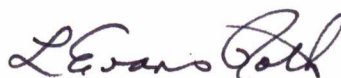


Lena B. Brattsten, Major Professor

We have read this thesis and
recommend its acceptance:



Accepted for the Council:



Vice Chancellor
Graduate Studies and Research

RHODANESE ACTIVITY IN THE SOUTHERN ARMYWORM,
SPODOPTERA ERIDANIA CRAMER
(LEPIDOPTERA:NOCTUIDAE)

A Thesis
Presented for the
Master of Science
Degree
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Karen Yvonne Long
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Finally, she wishes to thank her family and a special friend, Skip Lynch, who have always believed in her and supported her in the pursuit of her dreams.

ABSTRACT

Rhodanese activity in *Spodoptera eridania* Cramer, the southern armyworm, was investigated. The sources of rhodanese included southern armyworm larval midgut, fatbody, and Malpighian tubule mitochondria and isolated midgut bacteria. Several types of experiments were conducted to investigate rhodanese activity in the southern armyworm, these included: activity measurements in all different developmental stages; distribution of activity in several different organs; inducibility of larval midgut activity by dietary cyanide, methionine, cysteine, and thiosulfate; activity in seven species of bacteria isolated from the midgut. The ability of the bacteria to grow in a cyanide containing medium was established. In addition, a small survey of rhodanese activity in several other species of insects showed its presence in all of these.

The results of these experiments show that the southern armyworm has abundant rhodanese activity in all major organs and throughout all life stages apparently without regard to changes in its endogenous physiological conditions or to external dietary factors. There is no apparent relationship between rhodanese activity levels and tolerance to cyanide in the southern armyworm or in any of the other insect species.

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CHAPTER I

INTRODUCTION

The enzyme, rhodanese (thiosulfate:cyanide sulfur-transferase EC 2.8.1.1) was discovered by Lang (1933) as catalyzing the following reaction: $\text{SSO}_3^{2-} + \text{CN}^- \rightarrow \text{SCN}^- + \text{SO}_3^{2-}$. It has since been found to be of ubiquitous occurrence, the highest concentrations in mammals are in the liver, kidney, and adrenals (Lang, 1933) and the lowest concentrations in muscle and blood. Activity has been found in numerous vertebrates and invertebrates and has also been found in several species of bacteria and plants. The major physiological role of rhodanese in any of these systems is not known, but a list of suggested roles was reviewed by Westley (1973). He suggested various synthetic, detoxifying and oxidation-reduction functions of rhodanese. Lang (1933) proposed cyanide detoxification as the role of rhodanese when he discovered the enzyme. Szczepkowski and Wood (1967) proposed disposal of sulfide produced from cysteine by cystathionase action as a second detoxification role for rhodanese; this suggests a coupled cystathionase-rhodanese reaction. A biosynthetic role was suggested by Schneider and Westley (1964, 1969), based on the highly reactive persulfide of thiosulfate as a donor of reduced sulfur for biosynthesis of cysteine from serine without a sulfide intermediate. Wang and Volini (1968) suggested an oxidation-reduction role for rhodanese. This very highly speculative role was based on the finding that a sulfhydryl group in the active site of the enzyme was rapidly oxidized by aromatic oxidants

(i.e., dinitrobenzoic acid) and rapidly reduced by thiols (i.e., dithiothreitol). Oxidation-reduction, synthetic and detoxifying functions for rhodanese in plants and bacteria have also been suggested (Torri and Bandurski, 1967; Vanderbergh et al., 1979). Still, no one has discovered the major role of rhodanese in any of these systems. The purpose of this study was to investigate the possible involvement of rhodanese in cyanide detoxification in larvae of the southern armyworm, *Spodoptera eridania* Cramer, feeding on cyanogenic foliage. The southern armyworm larvae, although very polyphagous, prefer to feed on lima bean, *phaseolus lunatus*, foliage. Although cultivated varieties of the lima bean have been selected for minimal cyanogenesis, the original native lima bean foliage contains very high concentrations of the cyanogenic glycoside linamarin (Baumeister et al., 1975). Upon mastication β -glycosidases are released which hydrolyze the glycoside to glucose and a cyanogenic aglycone, which spontaneously releases hydrogen cyanide at slightly alkaline pH such as in the insect gut. Thus the armyworm or any herbivore feeding on cyanogenic plants are exposed to large amounts of hydrogen cyanide (HCN). This release of HCN is a defense mechanism on the part of the plant and has gained general acceptance as such (Eisner, 1970). The mechanism by which the armyworm tolerates such high concentrations of cyanide (Table 1) compared to other organisms is not known. The LD₅₀ of cyanide to the southern armyworm mature sixth instar larvae is approximately 10 times higher than that to the cockroach and 80 times higher than the injected LD₅₀ to the rat (Hall et al., 1971; Negerbon, 1959). Millipedes are also known to be very tolerant to cyanide (Hall

TABLE 1
LD₅₀ OF INJECTED CYANIDE IN ARMYWORM,
COCKROACH, RAT AND MILLIPEDE

Organisms	LD ₅₀ (mg/kg)
Southern Armyworm	800 ± 200 ¹
German Cockroach	74 ²
Butler's Yellow Footed Millipede	305 ²
Rat	10 ³

¹Brattsten, unpublished.

²From Hall et al., 1971.

³Negerbon, 1959.

et al., 1971). It is frequently suggested in the literature that rhodanese is responsible for the detoxification of cyanide and therefore critical to the survival of insects exposed to cyanide (Rothschild and Parsons, 1962), but this hypothesis has not been unambiguously examined. I have investigated the possible involvement of rhodanese in cyanide detoxification and survival in the southern armyworm.

A survey of armyworm tissues including gut, Malpighian tubules, and fatbody for rhodanese activity showed that activity was rather equally distributed in these major tissues. Since the midgut was the most easily available tissue, it was used for most of the experiments. Also, since it is known that many insects have a population of microorganisms in their guts I isolated the southern armyworm midgut bacteria and investigated their potential contribution to cyanide tolerance of the armyworm through rhodanese activity.

CHAPTER II

MATERIALS AND METHODS

A. Insects

The stock culture of the southern armyworm, originally obtained from C. F. Wilkinson, Cornell University, and supplemented with eggs from Food Machinery Corporation, Rochester, N.Y., was maintained in an environmentally controlled insect rearing room on lima bean, *Phaseolus lunatus*, and kidney bean, *P. vulgaris*, foliage. Newly molted sixth instar larvae were selected from this stock culture for the experiments, except for the age versus activity study in which third instar larvae were selected. These groups were kept in plastic boxes with a layer of soil (Bacto autoclaved potting soil) in the bottom. The larvae were fed semiartificial diets (Brattsten et al., 1980). The insects were kept in a room at 23°C with continuous light until used for experiments.

Other insects used in species distribution study were obtained from various sources as specified: Dr. M. L. Pan and Dr. Jungreis, The University of Tennessee, Knoxville, supplied *Hyalophora cecropia* larvae and *Manduca sexta*, respectively. Dr. C. P. Quimby, Stoneville, MS, supplied the *Arzama densa* larvae. Eggs of *Spodoptera frugiperda* were supplied by Dr. P. B. Martin, Tifton, GA. *Musca domestica* (World Health Organization Insecticide Susceptible CSMA strain) were raised in our laboratory. *Ceratomia catalpae* and *Podisus maculiventris* were collected from the field. Mature male rats of Long-Evans descent were purchased from Blue Spruce Farms, Altamont, N.Y.

B. Chemicals

All chemicals used in these experiments were analytical grade, purchased from Fisher Chemical Company. Potassium cyanide, sodium thiosulfate, methionine, and cysteine were incorporated into diets at the time of mixing all ingredients in a Waring blender.

C. Enzyme Preparation

Midgut mitochondria for rhodanese assays were prepared by removing the midgut, opening it by a longitudinal cut to remove the gut contents, and rinsing the gut tissue in ice-cold, 0.25 M sucrose. Ten to twelve midguts were required to obtain a sufficient amount of protein for rhodanese assays. The tissue was homogenized in sucrose and centrifuged at $12,000 \times g$ for two seconds (excluding acceleration and deceleration time) in a Sorvall high speed refrigerated model RC-2 centrifuge with a SS-34 fixed angle rotor. The supernatant was filtered through glass wool and centrifuged at $39,000 \times g$ for four minutes (modified after Keeley, 1973). The pellet was resuspended in a minimal volume of ice-cold sucrose (0.25 M).

Fatbody tissues were obtained by turning the larval skin, after removal of the gut, inside-out and scraping the inside of the skin with a pair of forceps. Thirty to forty fatbodies were pooled, homogenized and centrifuged in the same manner as the midguts.

Malpighian tubules were obtained by removing the midgut, then with a pair of forceps gently removing the malpighian tubules from the surface of the midgut. Thirty to forty sets of tubules were pooled to

obtain appropriate amounts of protein for assays. Tubule mitochondria were prepared by the same procedure as for midgut mitochondria.

Bacterial rhodanese preparations were made by inoculating 250 ml autoclaved nutrient trypticase soy broth in Erlenmeyer flasks with cultures isolated from southern armyworm midgut and identified by Dr. R. E. Bryant, The University of Tennessee. The bacteria were allowed to grow at 27°C with gentle shaking for four to eight hours, or until the absorbance of culture read at 600 nm was 0.8 which indicated late exponential growth phase of these cells. The suspensions were centrifuged at $12,000 \times g$ for 10 minutes to pellet the bacterial cells. The cells were washed twice by adding 20 ml of ice cold 0.25 M sucrose, centrifuging at $12,000 \times g$ for 10 minutes. The washed bacterial cells were then resuspended in 3 ml of 0.25 M sucrose and sonicated (6.2, 6×15 sec, 0°C) with a Branson sonifier to disrupt the cell walls. The resulting brei was centrifuged for 10 minutes at $12,000 \times g$ and the cell free supernatant was filtered through glass wool and used for enzyme assays.

D. Protein Determination

The concentration of protein in the mitochondrial suspensions and bacterial cell free suspensions was measured by the Lowry method (Lowry et al., 1951). Bovine serum albumin (from Sigma Chemical Co.) was used as a standard. The protein concentration was adjusted to 2 mg/ml with cold 0.25 M sucrose to meet the requirements of the rhodanese assay.

E. Enzyme Assay

Rhodanese was assayed by modifying the procedure described by Sörbo (1953). The enzyme activity incubation mixture consisted of 80 μ moles of glycine buffer, pH 7.8, 600 μ moles $\text{Na}_2\text{S}_2\text{O}_3$, 100 μ moles KCN, in a total volume of 3.2 ml in a 25 ml Erlenmeyer flask. The reactions were started by addition of 1 mg mitochondrial protein in 0.5 ml sucrose and were allowed to proceed at 32°C for 20 minutes in a shaking incubator. The reactions were stopped by addition of 0.5 ml formaldehyde (37% w/w). The concentration of the product (SCN^-) was evaluated colorimetrically after complexation with ferric ions [16% w/v $\text{Fe}(\text{NO}_3)_3$ in NHNO_3] to form the brilliantly red ferric rhodanate complex. The entire incubation mixture was then centrifuged at $12,000 \times g$ for 10 minutes to sediment the precipitated protein. The OD_{460} (λ max for ferric rhodanate) read on a Bausch and Lomb Spectrophotometer 70 against a blank, consisting of all the above ingredients including formaldehyde added at time zero. The supernatants were diluted to give OD reading of approximately 0.5-0.8. Up to a 10 fold dilution of the original supernatant did not affect the accuracy of the measurements although the formaldehyde which apparently improves the stability of the ferric rhodanate complex (Sörbo, 1953) became insufficient by further dilution of the supernatants. The complex was stable for at least 60 minutes. The amount of SCN produced was estimated by comparison to a standard curve constructed with known amounts of potassium thiocyanate added to the entire system, the extinction coefficient, $4417.5 \text{ M}^{-1} \text{ cm}^{-1}$, was then calculated from this curve (Figure 1). One rhodanese unit was defined as the amount of enzyme which forms one nanomole of thiocyanate per minute per milligram protein.

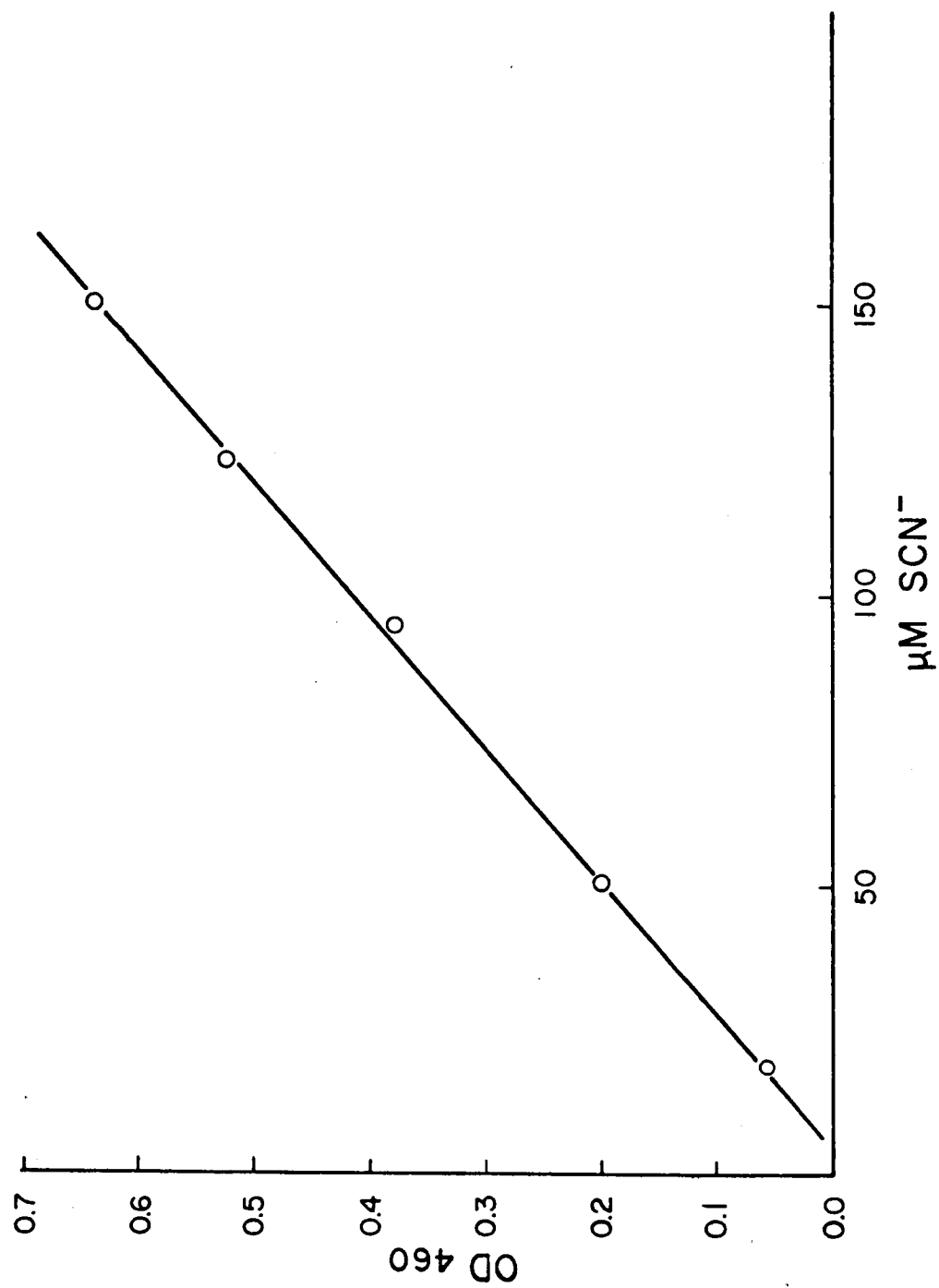


Figure 1. Determination of extinction coefficient of thiocyanate.

CHAPTER III

RESULTS

A. Optimum *in vitro* Conditions for Rhodanese Activity

Optimum *in vitro* conditions for measuring rhodanese activity were established. The mitochondrial protein concentration was linear up to 2 mg in the 3.2 ml incubation mixture. A routine concentration of 1 mg/incubation was selected as it gave sufficiently high activity, was well within the linear portion of the activity profile (Figure 2) and saved considerable effort in collecting enough protein from the tissues. Rhodanese was active over a broad range of pH values (Figure 3). Since the pH of the armyworm midgut is about 7.8, the glycine buffer in the reaction mixture was adjusted to this pH. The use of potassium phosphate buffer gave considerably lower activities over the entire pH range. The optimal concentrations of the two co-substrates were 100 μ moles KCN and 600 μ moles of $\text{Na}_2\text{S}_2\text{O}_3$ (Figure 4) per incubation. The reaction rate was linear for at least 25 minutes at 32°C (Figure 5). An incubation time of 20 minutes was selected for the routine assays so that as much product could be formed as possible while the reaction rate was still linear with time. A survey of rhodanese activity in various southern armyworm tissues showed that all tissues had about the same activity (Table 2).

B. Developmental Stages versus Activity

An age versus activity study was done to determine the relation of rhodanese to the physiological status of the insects. Larvae as they

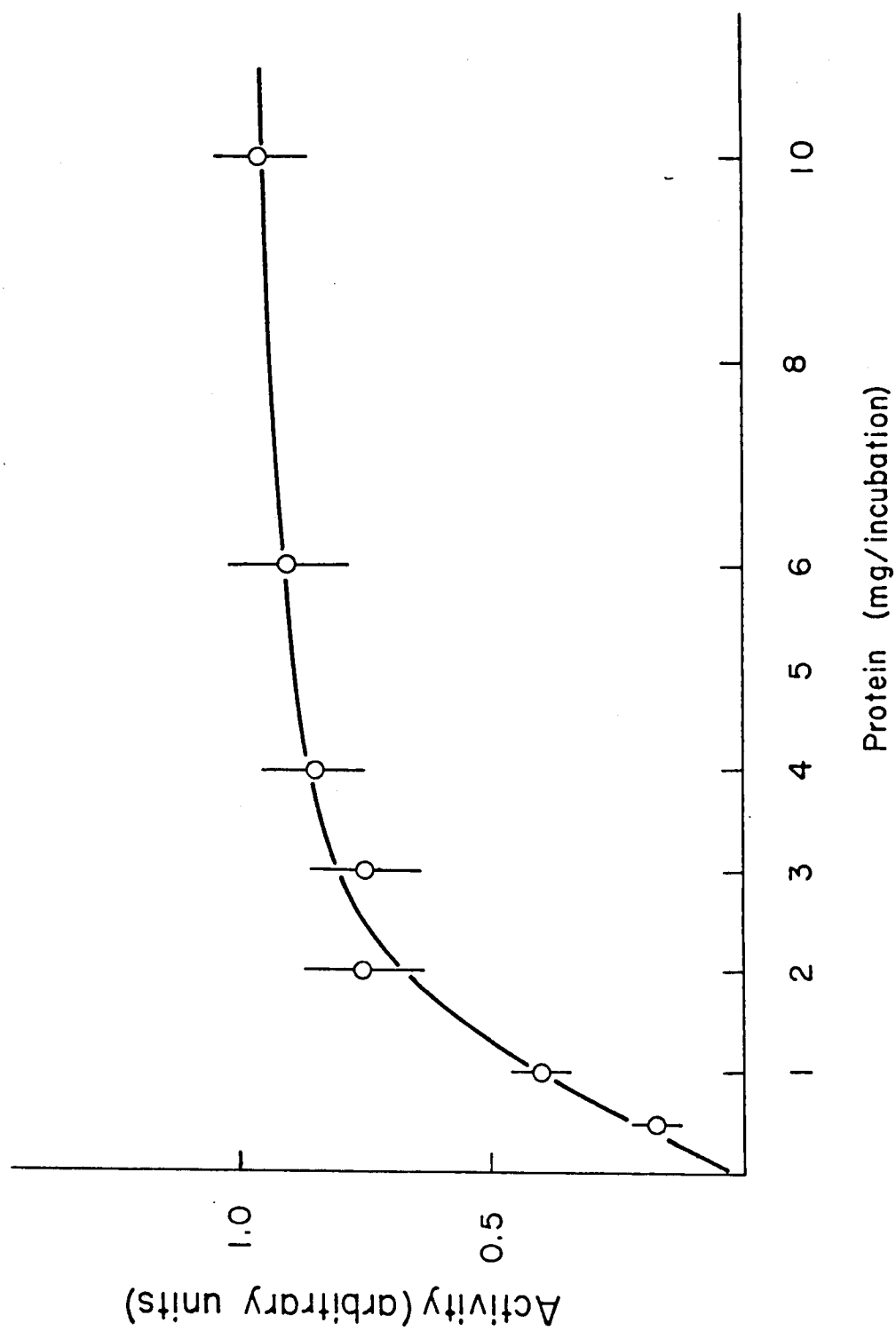


Figure 2. Determination of concentration of mitochondrial protein required for rhodanese reaction. Vertical bars represent standard deviations.

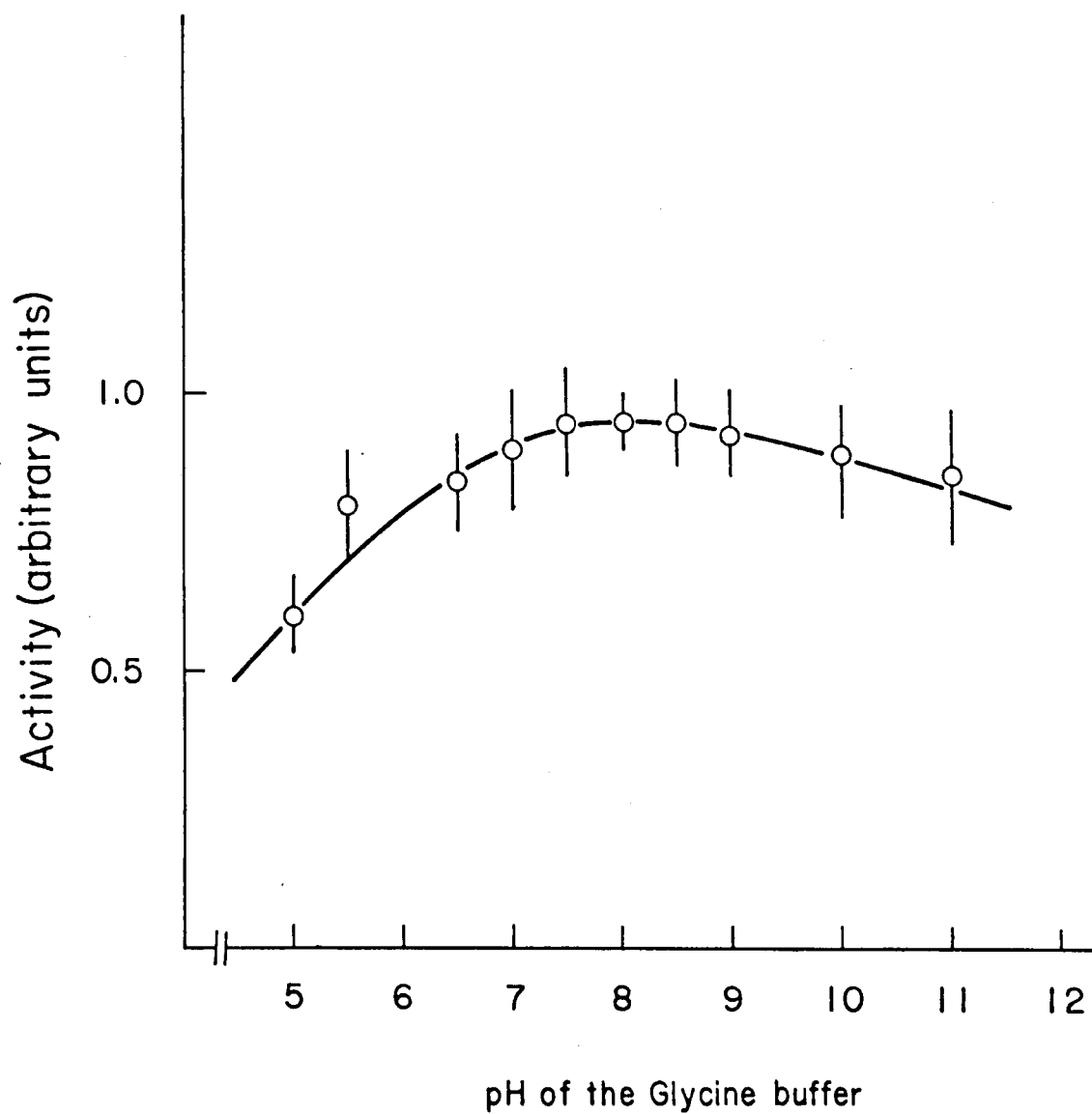


Figure 3. Determination of appropriate pH for rhodanese reaction. Vertical bars represent standard deviations.

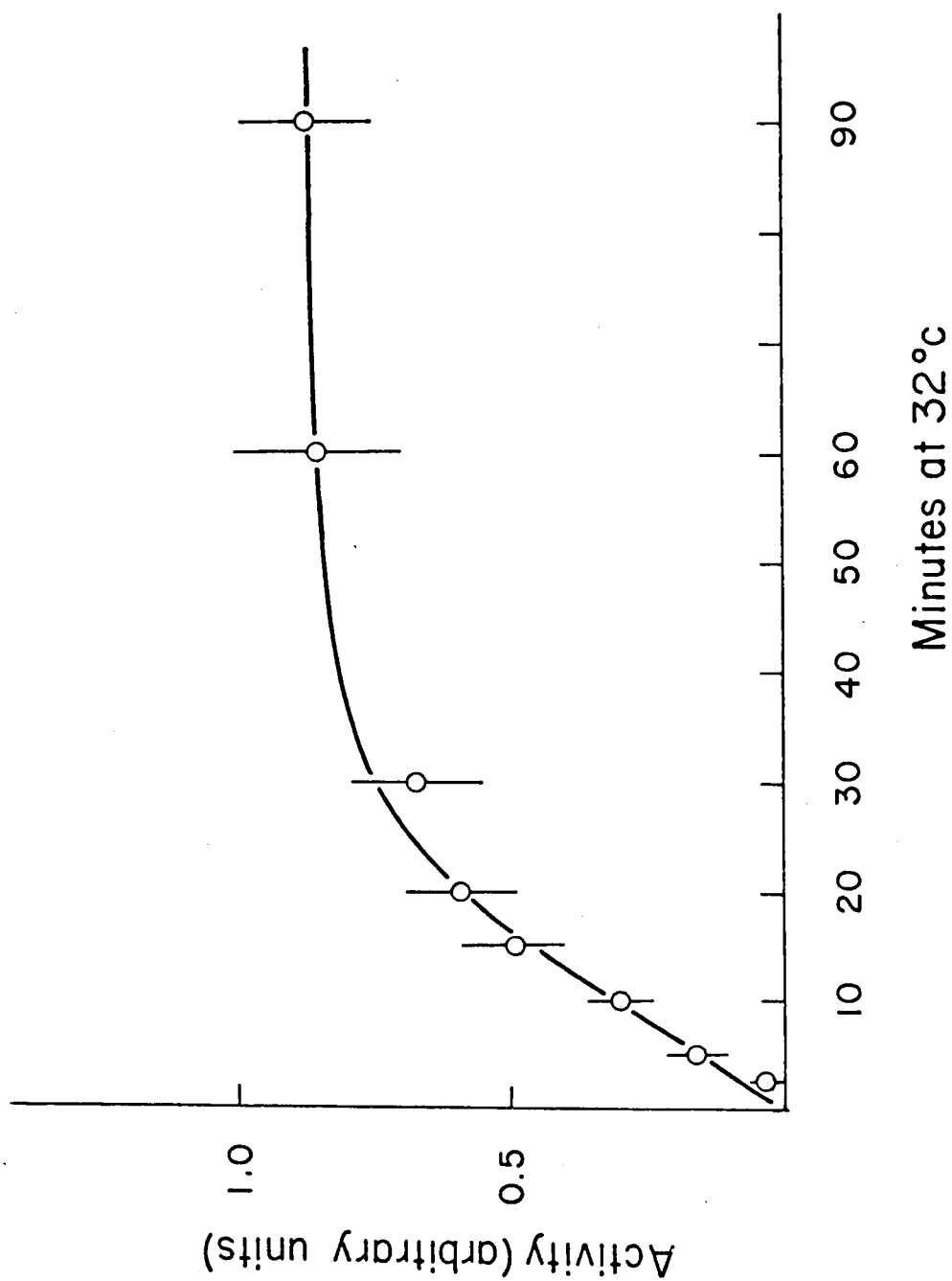


Figure 5. Determination of incubation time required for rhodanese reaction. Vertical bars represent standard deviations.

TABLE 2
RHODANESE ACTIVITY IN VARIOUS SOUTHERN ARMYWORM TISSUES

Tissue	Rhodanese Activity* $\pm$ S.D.
Malpighian Tubules	50.02 $\pm$ 3.18
Fat Body	51.57 $\pm$ 1.44
Midgut	57.18 $\pm$ 2.37

*Units = nmoles SCN produced/min/mg protein.

developed from third instar through to the adult moth stage were monitored for midgut activity. Activities of the microsomal mixed-function oxidases decrease just prior to and during molting then return to their original level during actively feeding stages (Krieger and Wilkinson, 1969; Benke and Wilkinson, 1971). In sharp contrast, rhodanese activity remains essentially constant throughout the developmental stages of the southern armyworm (Figure 6).

C. Species Distribution Study

If rhodanese is responsible for the southern armyworm's tolerance to cyanide one would expect to see higher rhodanese activity in the southern armyworm than in species not feeding on cyanogenic foliate. Two species of insects feeding on cyanogenic foliage were assayed for rhodanese activity, the southern armyworm and the cecropia silkworm, which feeds on black cherry leaves known for their high content of prunasin, amygdalin and other cyanogenic glycosides. Several species of insects not particularly associated with a cyanogenic diet and the rat were also assayed for rhodanese activity. Those include the tobacco hornworm larvae, *Manduca sexta*, which feeds on tobacco and tomato plants; the adult stinkbug, *Podisus maculiventris*, a carnivore; the omnivorous adult housefly, *Musca domestica*; the highly specialized feeders *Arzama densa* feeding on water hyacinth; the catalpa spinx, *Ceratomia catalpae* feeding exclusively on the catalpa tree foliage, and the generalist herbivore the fall armyworm larvae, *Spodoptera frugiperda*. The results (Table 3) showed that all species investigated had very similar activities in the range of 30-70 units, with the exception of

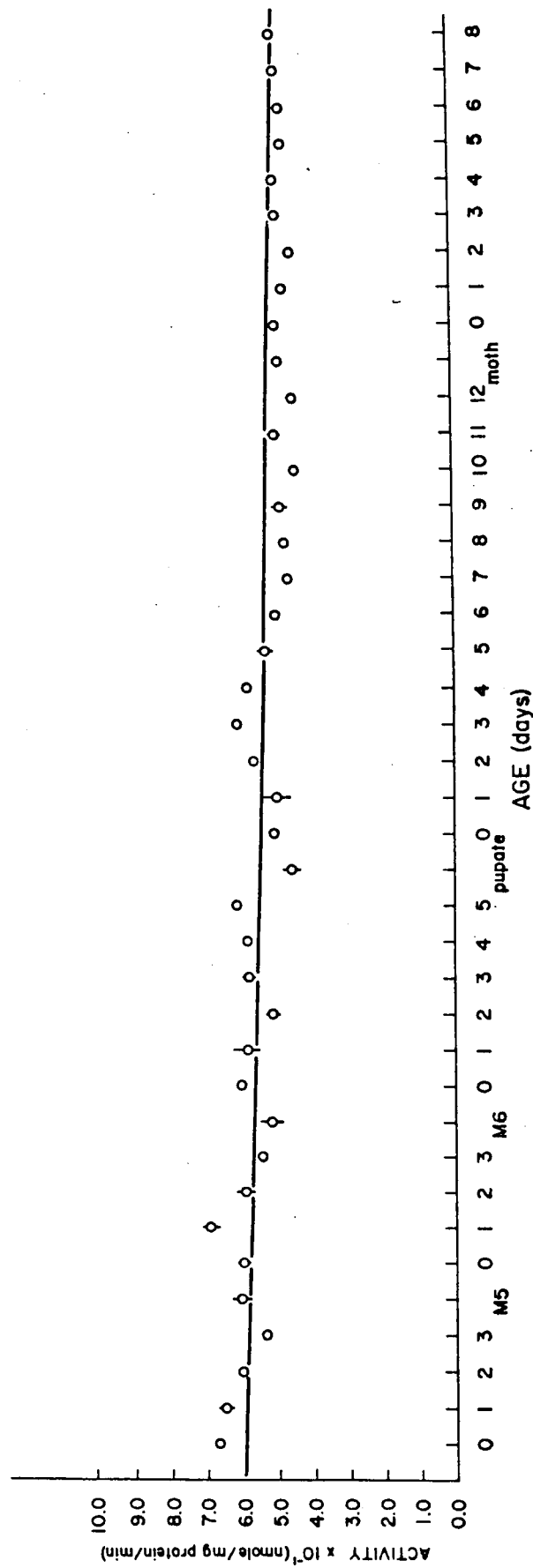


Figure 6. Age of southern armyworm versus rhodanese activity. Vertical bars represent standard deviations.

TABLE 3
RHODANESE ACTIVITY IN SELECTED ANIMALS

Species	Specific Activity*
<i>Arzama densa</i> (gut)	47.99
(fatbody)	52.16
(Malp. tub.)	53.42
<i>Hyalophora cecropia</i>	38.21
<i>Manduca sexta</i>	74.10
<i>Ceratomia catalpae</i>	27.35
<i>Spodoptera eridania</i> (gut)	31.99
<i>Musca domestica</i>	67.90
<i>Podisus maculiventris</i>	52.52
Rat (liver)	520.66
(small intestine)	80.41

*Units = nmole SCN produced/min/mg protein.

the rat liver. The rat, the most sensitive species tested, had the highest amount of rhodanese activity in its liver, although the rhodanese activity level in the rat intestine is remarkably similar to that in the insect gut tissues.

D. Cyanide Induction of Rhodanese Activity

Cytochrome P-450 dependent mixed function oxidases are induced by certain dietary chemicals (Brattsten and Wilkinson, 1973; Brattsten et al., 1977; Brattsten, 1979). This induction increases the enzyme's detoxifying ability. If rhodanese in analogy with the mixed-function oxidases, is involved in the metabolism of dietary allelochemicals as a major cyanide detoxification mechanism, it might also be induced to higher activities by the presence of cyanide in the larva diet. Last (sixth) instar larvae were fed *ad libitum* on cyanide containing diets for 3 days to test this possibility. Larvae freely ate diets containing low cyanide concentrations which even seemed to increase their appetites. At about 1.5% of cyanide in the diet, the larvae ate reluctantly only and refused to eat diets with 2% of cyanide. Figure 7 shows that the larvae fed 0.5% cyanide containing diets gained more weight than the control fed larvae. This was also true of larvae feeding on diets containing up to 1.0% KCN. However, rhodanese activity (Table 4) appears largely unrelated to external cyanide levels in the larval diets. One way analysis of variance showed that the differences in activity in control and cyanide fed larvae were insignificant ($p \leq 0.05$).

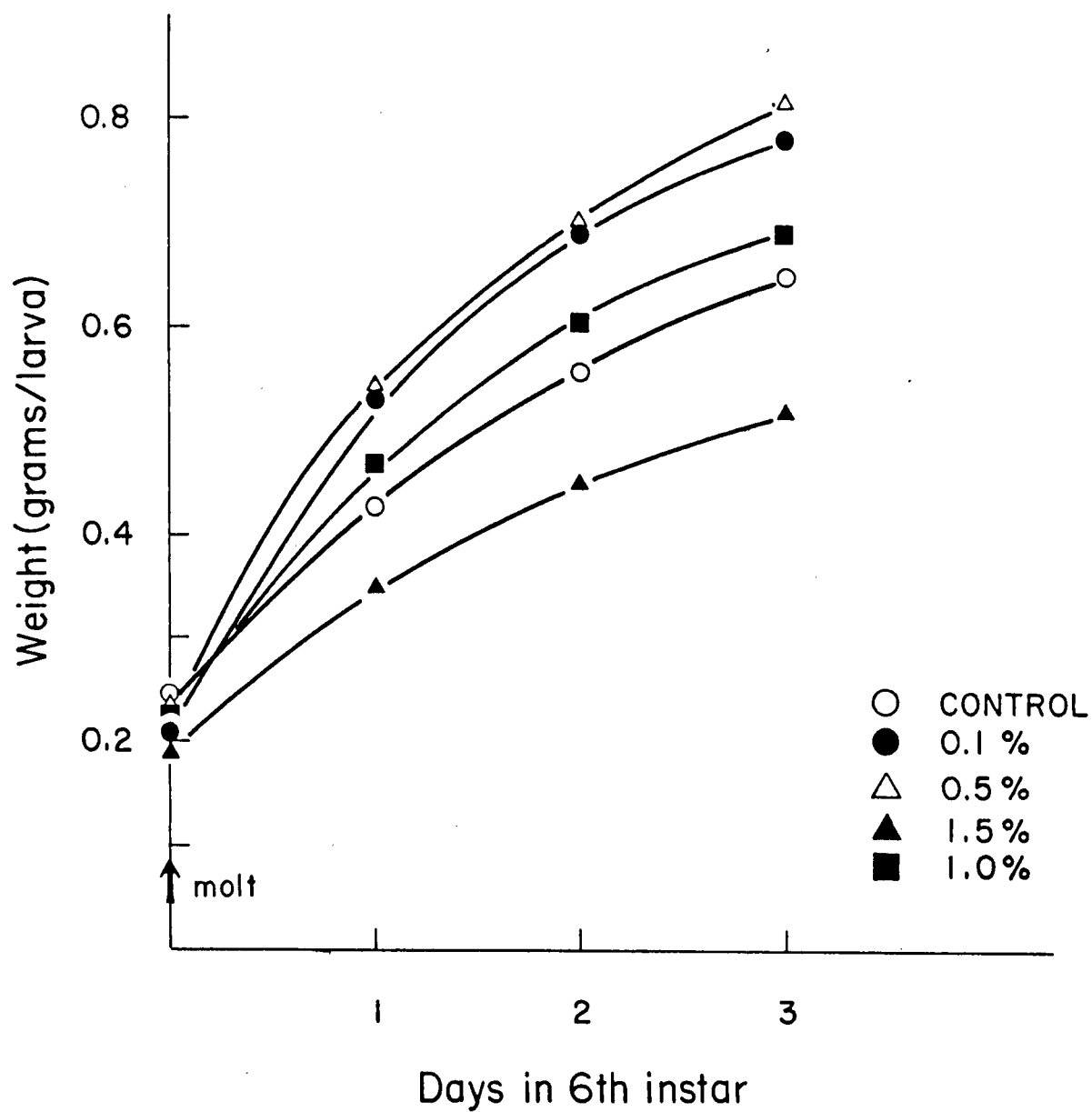


Figure 7. Weight gain of cyanide fed sixth instar southern armyworm.

TABLE 4
RHODANESE ACTIVITY IN CYANIDE FED
SOUTHERN ARMYWORM

Cyanide Supplementation	Rhodanese Activity* $\pm$ S.D.
0.5% CN	52.2 $\pm$ 13.7
1.0% CN	57.04 $\pm$ 15.8
1.5% CN	59.14 $\pm$ 15.53

*nmole SCN produced/min/mg protein.

E. Methionine, Cysteine and Thiosulfate Supplemented Diets

Many investigators believe that rhodanese activity is limited due to lack of an acceptable sulfur donor that can penetrate rapidly enough into the mitochondria. Thiosulfate has been successfully administered as an antidote to cyanide poisoning in sheep (Burrows and Way, 1979). Supplementing the insect diets with sulfur donors might therefore increase rhodanese activity *in vivo*. The results in Table 5 indicate that rhodanese activity is independent of the potential external sulfur donors methionine, cysteine, and thiosulfate. One way analysis of variance showed that the differences in activity in larvae control of sulfur supplemented diets were insignificant ($p \leq 0.05$).

F. Bacterial Rhodanese Activity

The results of the bacterial rhodanese activity study showed (Table 6) that all bacteria isolated from the armyworm larvae fed control diet contained rhodanese activity. Although *Acinetobacter calcoaceticus* had somewhat higher activity, there were no great differences between species. All species had, in fact similar activity as the insect tissues.

G. Cyanide Sensitivity of Bacteria

If one or several of the bacteria was responsible for the cyanide tolerance observed in the southern armyworm larvae, one could expect to see uninhibited growth, perhaps even an increase in growth, in a cyanide containing broth. By using the bacterial growth in control broth as a reference point compared to the amount of growth in cyanide

TABLE 5
RHODANESE ACTIVITY IN SOUTHERN ARMYWORM FED
SULFUR DONOR DIETS

Sulfur Donor	Rhodanese Activity* $\pm$ S.D.
0.5% Methionine	44.3 $\pm$ 10
1.0% Methionine	44.5 $\pm$ 4.0
0.5% Cysteine	45.45 $\pm$ 5.8
1.0% Cysteine	43.7 $\pm$ 1.5
0.5% Thiosulfate	43.35 $\pm$ 0.6
1.0% Thiosulfate	43.6 $\pm$ 1.4

*nmole SCN produced/min/mg protein.

TABLE 6
RHODANESE ACTIVITY IN PURE CULTURES OF BACTERIA
ISOLATED FROM THE MIDGUT OF 6TH INSTAR
S. ERIDANIA

Species	Activity*
<i>Enterobacter agglomerans</i>	40.38
<i>Enterobacter</i> species I	70.63
<i>Enterobacter</i> species II	56.23
<i>Klebsiella oxytoca</i>	52.22
<i>Klebsiella pneumoniae</i>	58.25
<i>Micrococcus</i> species	37.13
<i>Acinetobacter calcoaceticus</i>	108.21

*nmole SCN₂ produced/min/mg protein.

broth, the cyanide sensitivity of the bacteria was tested. The results of the cyanide sensitivity of bacteria showed (Table 7) that all bacteria grew in the cyanide containing broth, but none grew as well or better in the cyanide supplemented broth as in the control broth.

H. Isolation of Bacteria from Control
and Cyanide Fed Armyworms

The isolation of bacteria from control and cyanide fed armyworms showed that the bacteria found in the control and cyanide fed larvae were widely encountered enteric bacteria, with the exception of a few gram positive species. The following species of bacteria were found in cyanide and control fed larvae; *Enterobacter agglomerans*, *Klebsiella oxytoca*, *Kebsiella pneumoniae*, and several other strains of *Klebsiella*. Other species of bacteria found were enteric bacteria and resemble *Enterobacter* but complete identification could not be made.

TABLE 7
BACTERIAL GROWTH IN NUTRIENT BROTH WITH
VARYING CONCENTRATIONS OF CYANIDE

Species	Control	0.001 M	0.005 M	0.01 M
<i>Acinetobacter calcoaceticus</i>	+++	+-	-	-
<i>Klebsiella pneumoniae</i>	+++	++	+-	-
<i>Klebsiella oxytoca</i>	+++	++	+-	-
<i>Enterobacter agglomerans</i>	+++	++	-	-
<i>Enterobacter</i> species I	+++	++	+-	-
<i>Enterobacter</i> species II	+++	++	-	-
<i>Enterobacter cloacae</i>	+++	++	+-	-
<i>Micrococcus</i> species	+++	++	+-	+-

++; grew well
+-; slight growth
-; no growth

CHAPTER IV

DISCUSSION

The substrate concentrations required for maximum rhodanese activity was consistent with the data reported in the literature (Himwich and Saunders, 1948) in that at least a three fold excess of thiosulfate is required. Other parameters investigated varied little from the values reported in the same literature. On the other hand, the design of appropriate blanks for the colorimetric measurements is largely ignored or a fairly confused issue in the literature. The zero time blank used in this project provided repeatable values and combined all ingredients of the assay added in a different order so that the enzyme is killed immediately by formaldehyde instead of after a 20 minute incubation.

Rhodanese activity remains essentially constant throughout the development of the southern armyworm in contrast to detoxifying enzymes such as the mixed-function oxidases. A large decrease in activity is seen in this enzyme system in molting larva and pupae stages (i.e., nonfeeding stages). The cause of the decrease seen in cytochrome P-450 has only been speculated upon (Wilkinson and Brattsten, 1973). One possibility is that at molting the insects do not feed so there are no external substances to maintain the enzyme activity by continuous induction. Another possibility is that the endoplasmic reticulum, the site of the cytochrome P-450 activity, is disrupted and reorganized at each molt. Since rhodanese is a soluble enzyme, the activity of the enzyme would not be affected by the disruption and reorganization of

cells and cell organelles. Since no changes in rhodanese activity in the southern armyworm was seen during larval, pupal or adult stages, rhodanese appears unrelated to the physiological status of the insect. Cessation of feeding also appears unrelated to the rhodanese activity level. Furthermore, the species distribution study showed that there seems to be no correlation between rhodanese activity and feeding on cyanogenic or acyanogenic foliage. The rhodanese activity was essentially the same in both organisms feeding on cyanogenic foliage and those feeding on acyanogenic foliage. Perhaps rhodanese is not solely responsible for the southern armyworm's tolerance to cyanide. Since other enzymes such as β -cyanoalanine synthetase can also metabolize cyanide, the enzymes could function together to enable the southern armyworm to tolerate high concentrations of cyanide.

The cyanide induction study showed that rhodanese was unaffected by external cyanide since there was no induction of rhodanese activity seen in the cyanide fed worms. Also, rhodanese does not show a typical dose-response curve that most detoxifying enzymes do (i.e., the mixed-function oxidases). The weight gain seen in the southern armyworms fed on 0.5% cyanide up to 1.0% cyanide (Figure 7, p. 20) could be related to a possible feeding stimulant effect of cyanide. Rhodanese activity was not increased when the southern armyworm diet was supplemented with sulfur donors. The constant levels of rhodanese activity is apparently unrelated to the penetration of the extra sulfur donor into the mitochondria. It is also possible that rhodanese simply can not be induced due to absence of genetic capacity to be induced. Many enzymes

involved in intermediary metabolism characteristically remain at nearly constant activity levels largely independent of external factors.

The presence of rhodanese activity in *Acinetobacter calcoaceticus* was reported (Vandenbergh et al., 1980). Certain species of *Enterobacter* have also been shown to contain rhodanese activity, but zero activity was reported for *Micrococcus* and *Klebsiella* species (Vandenbergh et al., 1979), although I found activity in these species. Perhaps the discrepancies result from differences in species or strains of the bacteria used. It has been suggested that rhodanese could be used to differentiate the various genera of the family Enterobacteriaceae as well as unrelated genera (Vandenbergh et al., 1979).

The cyanide sensitivity study was consistent with the literature (Vandenbergh et al., 1979) for *Klebsiella* and *Enterobacter* strains being cyanide resistant. No information was found in the literature on the sensitivity of the other species that we found to be resistant.

Although no induction of bacterial growth was seen in the bacteria grown in cyanide supplemented broth, histological studies of sixth instar southern armyworm larvae fed 0.5% cyanide for three days (J. H. Samuelian, Thesis, The University of Tennessee, Knoxville, 1981) showed an increase in the bacterial population between the gut wall and the peritrophic membrane. Perhaps the different species of bacteria functioning together synergistically allows an increase in number whereas alone the bacteria do not increase in number. The reason for the increase in bacteria seen in the histological studies could also be due to the localized distribution of the bacteria along the gut wall.

CHAPTER V

CONCLUSIONS

Rhodanese appears unrelated to the observed tolerance to cyanide in the southern armyworm because:

1. The activity of rhodanese remains essentially constant throughout the development of the southern armyworm in comparison to most detoxifying enzymes.
2. Considering the distribution of rhodanese in animal and bacterial species there seems to be no correlation between tolerance to cyanide and rhodanese activity.
3. Rhodanese activity is not induced by cyanide, thiosulfate, methionine, or cysteine.

All of the above show that rhodanese is unrelated to the external factors I used (i.e., cyanide, methionine, cysteine, thiosulfate) which is unusual for an enzyme involved in metabolism of foreign compounds.

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VITA

Karen Yvonne Long was born in Pikeville, Tennessee on November 17, 1956. She attended school in Dunlap, Tennessee and was graduated from Sequatchie County High School as valedictorian in June 1974. The following September she entered Middle Tennessee University, and in May 1978 she received a Bachelor of Science degree in Biology with a minor in Chemistry and English.

After graduation she was employed with Tennessee Valley Authority where she worked until January 1979 when she began teaching at Griffith Elementary School.

In September 1979 she accepted a teaching assistantship at The University of Tennessee, Knoxville and began study toward a Master of Science degree in Biochemistry. This degree was awarded in August 1981.