

# TYROSINE AND TYROSINE GLUCOSIDE TITRES IN WHOLE ANIMALS AND TISSUES DURING DEVELOPMENT OF THE TOBACCO HORNWORM *MANDUCA SEXTA* (L.)\*

R. F. AHMED†, T. L. HOPKINS†‡ and K. J. KRAMER‡§

Departments of Entomology† and Biochemistry‡, Kansas State University, Manhattan, KS 66506 and  
U.S. Grain Marketing Research Laboratory§, Agricultural Research Service, U.S. Department of Agriculture,  
Manhattan, KS 66502, U.S.A.

(Received 5 October 1982)

**Abstract**—Tyrosine glucoside first appeared in two-day-old fifth stadium larvae and reached maximum whole body titres in pharate pupae (2.5 mg/g). A rapid decline occurred shortly before larval-pupal ecdysis as the free tyrosine pool increased to peak titres at ecdysis (1.4 mg/g). Both tyrosine and tyrosine glucoside decreased to low levels (0.5 mg/g) as the pupal cuticle tanned. Tyrosine again increased in one-day-old pupae and remained at high levels (1.5 mg/g) until the beginning of darkening of the pharate adult. Tyrosine glucoside remained at relatively low titres (0.5 mg/g) during pharate adult development. Both tyrosine and tyrosine glucoside increased slightly during adult eclosion and decreased to very low levels after adult tanning. Nearly all the tyrosine glucoside and two-thirds of the tyrosine occurred in the haemolymph where the concentrations during development paralleled those in whole body. Much lower amounts were present in fat body, integument, and gut.

**Key Word Index:** Tyrosine, tyrosine glucoside,  $\beta$ -D-glucopyranosyl-O-L-tyrosine, *Manduca sexta*, tobacco hornworm, insect development

## INTRODUCTION

TYROSINE is the most important precursor for diphenols and quinones that cross-link proteins during tanning or sclerotization of insect cuticle (see reviews by HACKMAN, 1964; ANDERSEN, 1979; BRUNET, 1980). Tyrosine titres rise to high levels by the time of ecdysis and decrease as the new cuticle tans. Accumulation of this amino acid in conjugates or peptides has also been shown to occur in several insect species. Glucosides, phosphate esters and dipeptides of tyrosine or its precursor phenylalanine are released by hydrolysis at the proper time for tanning and pigmentation of new cuticle. Examples of such storage molecules include tyrosine-O-phosphate in *Drosophila melanogaster* (MITCHELL and LUNAN, 1964; LUNAN and MITCHELL, 1969), beta-alanyl-L-tyrosine in *Sarcophaga bullata* (LEVENBOOK *et al.*, 1969), gamma-L-glutamyl-L-phenylalanine in *Musca domestica* (BODNARYK, 1970), and beta-D-glucopyranosyl-O-L-tyrosine (tyrosine glucoside) in *Drosophila busckii* (CHEN *et al.*, 1978), *Manduca sexta* and many other species of Lepidoptera (KRAMER *et al.*, 1980a; ISHIZAKI and UMEBACHI, 1980; ISOBE *et al.*, 1981; LU *et al.*, 1982). The conjugates accumulate during the later stages of larval growth reaching peak titres before pupal ecdysis (or pupariation in Diptera).

Large concentrations of tyrosine storage molecules have been detected in the haemolymph (LUNAN and MITCHELL, 1969; LEVENBOOK *et al.*, 1969; BODNARYK, 1970; JUNNIKKALA, 1976), while other tissues were less well supplied. However, a detailed study of the tissue distribution and titres of such storage molecules in relation to developmental events has not been made. We have previously shown that tyrosine glucoside is the major reservoir of tyrosine in last stage larvae of *M. sexta*, while it was not detected in the penultimate stage (KRAMER *et al.*, 1980a). This suggested that tyrosine glucoside serves as a storage molecule for substrates utilized during pupal and possibly adult cuticle sclerotization. The present study correlates with developmental events the titres of tyrosine and tyrosine glucoside in the whole animal, haemolymph and certain other tissues during metamorphosis of the tobacco hornworm, *M. sexta*.

## MATERIALS AND METHODS

### Experimental animals

*M. sexta* were reared on an artificial diet according to BELL and JOACHIM (1976) at  $27 \pm 1^\circ\text{C}$  and a non-diapausing photoperiod of 16 hr L: 8 hr D.

### Extraction

Whole animals of different stages or tissues were homogenized in 80% aqueous methanol containing 5 mM ascorbic acid as described by WIRTZ and HOPKINS (1974, 1977). For whole body extraction each insect was homogenized in 15 ml ice cold extraction medium for 2 min at high speed in a stainless

\* Contribution No. 82-544-j. Departments of Entomology and Biochemistry, Kansas Agricultural Experiment Station, Manhattan, KS 66506. Cooperative investigation between ARS, USDA, and the Kansas Agricultural Experiment Station. Supported in part by research grant PCM-8003859 from the National Science Foundation.

‡ To whom correspondence should be sent.

steel vessel (Omnimixer, Sorval). The homogenate was centrifuged at 12,000 *g* for 15 min at 4–5°C, and the sediment extracted two additional times with cold solvent. Pooled supernatants were concentrated on a rotary evaporator to 2 ml for subsequent clean up by ion-exchange chromatography and analysis by gas-liquid chromatography.

To determine the distribution of tyrosine and tyrosine glucoside in various tissues during development, haemolymph, fat body, gut and integument were dissected from different stages. Haemolymph was collected at 4°C from larvae or pupae by clipping the anal horn or the proboscis, respectively, and weighed immediately. The fat body, gut and integument were dissected in cold saline, the gut contents removed, and the tissues rinsed repeatedly with ice-cold saline (160 mM NaCl, 3 mM KCl, 1.8 mM CaCl<sub>2</sub>, 0.2 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.2) saturated with phenylthiourea. The tissues were blotted dry and weighed immediately. The haemolymph and tissues were homogenized with cold extraction solvent in glass tissue grinders (0.1 g/ml), centrifuged, and extracts prepared for analysis as described for whole insects. The efficiency of tyrosine and tyrosine glucoside extraction from whole animals and various tissues was determined according to WIRTZ and HOPKINS (1977).

#### Tyrosine and tyrosine glucoside analysis

Tyrosine glucoside in whole animals and tissue extracts was measured by the following procedure. Aliquots (0.25 ml) from each extract were acidified

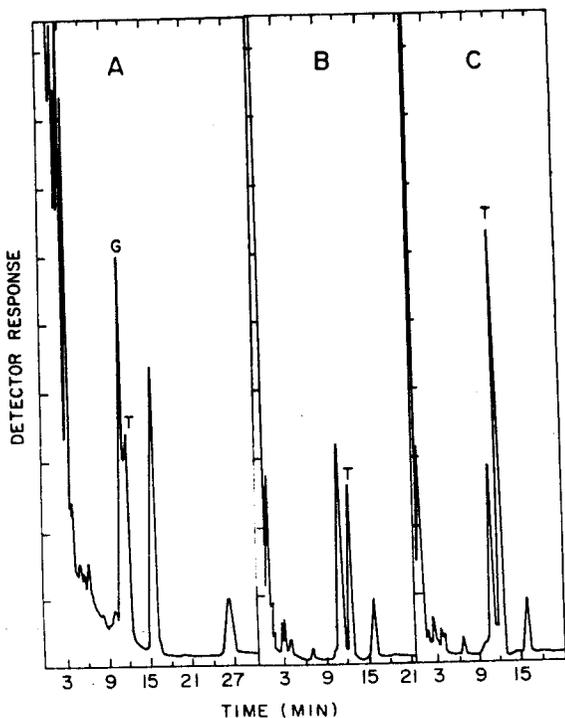


Fig. 1. Gas-liquid chromatography of an 80% methanolic extract of *Manduca sexta*. A. Fifth stadium larva before clean-up showing the interference of glucose (G) with tyrosine (T). B. Pharate pupa after clean up. C. Pharate pupa after clean up and acid hydrolysis. See Materials and Methods for experimental details.

with 0.75 ml of 25% acetic acid to about pH 3, and were cleaned up as described by ADAMS *et al.* (1977) with slight modifications. The acidified extract was then passed through a small column containing 200 mg of DOWEX 50W-X2 200-400 mesh hydrogen form, (Bio-Rad Co.) and after washing with 1 ml de-ionized water, the amino acids were eluted with 1 ml 2 M NH<sub>4</sub>OH. Aliquots (0.1 or 0.2 ml) of the eluate were taken to dryness in small conical reaction vials (Regis Chemical Co.) under a stream of nitrogen and low heat. One sample of each pair of aliquots was hydrolyzed with 100  $\mu$ l 6 M HCl at 100°C for 15 min and taken to dryness. Both samples were then silylated and analyzed for tyrosine by gas-liquid chromatography (WIRTZ and HOPKINS, 1977). The GLC was equipped with a 190  $\times$  0.4 cm i.d. glass column packed with 7% (w/w) OV-1 on Gas Chrom Q (80–100 mesh, Applied Science), and an argon ionization detector. The argon flow rate was 84 ml/min and temperature was 200°C. Bound tyrosine in the tyrosine glucoside fraction was calculated by subtracting the tyrosine in the unhydrolyzed extract from the total tyrosine in hydrolyzed extract. The increase in tyrosine after hydrolysis originated only from tyrosine glucoside. No other conjugates of tyrosine have been identified in methanolic extracts of *M. sexta* (KRAMER *et al.*, 1980a). Data were analyzed statistically by Duncan's multiple range test at 0.05 level.

## RESULTS

#### Tyrosine and tyrosine glucoside analytical procedures

The extraction efficiency for tyrosine and tyrosine glucoside from whole larvae and pupae of *M. sexta* was more than 90 and 95%, respectively. Similar results were obtained from individual tissues (fat body and haemolymph). The recoveries of tyrosine from *M. sexta* are comparable to those reported for cockroach tissues (WIRTZ and HOPKINS, 1974, 1977).

An ion exchange chromatographic clean up procedure was used to remove glucose and other neutral or negatively charged compounds with retention times during GLC analysis that interfered with tyrosine quantitation (Fig. 1A). Hydrolysis of synthetic tyrosine glucoside with 6 M HCl at 100°C showed that most of the glucose liberated (70%) was destroyed after 15 min and that no additional clean up of the sample was needed after acid hydrolysis.

Tyrosine glucoside was determined using GLC by comparing the tyrosine concentration of unhydrolyzed extracts (Fig. 1B) with total tyrosine due to both free and conjugated tyrosine in the hydrolyzed extracts (Fig. 1C). Our previous research showed that the "bound" tyrosine in methanolic extracts exists mainly as tyrosine glucoside in *M. sexta* (KRAMER *et al.*, 1980a). Protein bound tyrosine was removed during the extraction procedure by precipitation with 80% cold methanol. Thus, the increase in tyrosine in the acid hydrolyzed extracts over that in unhydrolyzed extracts was equivalent to that amount present as tyrosine glucoside.

#### Tyrosine and tyrosine glucoside titres during hornworm development

Tyrosine and tyrosine glucoside titres in whole animals during the last larval stage and during metamor-

Fig. 2. Titres

phosis are shown in Fig. 2. Tyrosine was not detected during the last larval stage but started to increase during the fifth instar. It increased rapidly during the fifth instar larval feeding stage, reaching a maximum at the end of the fourth day of pupation, reaching a maximum of 2.5 mg/g wet weight quite rapidly as compared to the pupa a few hours before ecdysis, but transient increase in tyrosine concentration 6 hr postecdysis, followed by a decrease in concentration (five times lower) about 48 hr after ecdysis. Tyrosine levels low during pupal development, except for a small increase in tyrosine glucoside during the first one-day-old adults.

Tyrosine titre was significantly higher in the fifth instar ecdysis and significantly lower in the first one-day-old adults.

Table

Stage	Sample
Larva	Fifth stadium (3–4 days)
Wandering	(1–2 days)
Pupa	Pharate (1–2 days)
	Newly eclosed (6 hr-old)
	12 hr-old
Adult	Pharate (2–10 days)
	(14–17 days)
	Newly eclosed (1-day-old)

\* S.E. =

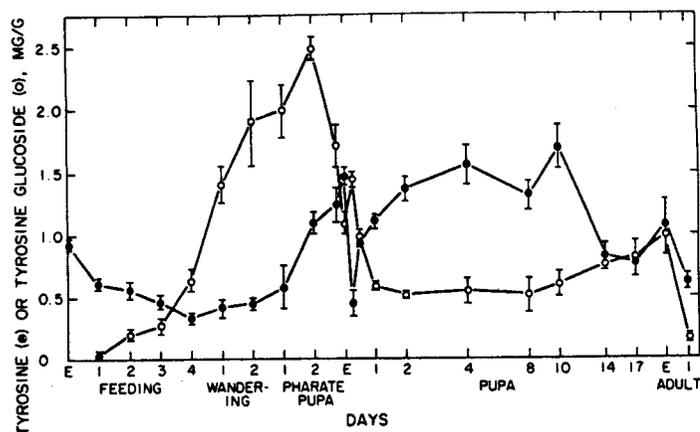


Fig. 2. Titres of tyrosine and tyrosine glucoside in whole body during the development of *Manduca sexta*. Bars =  $\pm$  S.E.M.,  $n = 3$  to 6. E = Ecdysis.

phosis are shown in Fig. 2. Tyrosine glucoside was not detected during the first day of the fifth stadium, but started to increase the second day after ecdysis. It increased rapidly during the last two days of the larval feeding stage and first day of wandering behaviour, reaching a maximum in the two-day-old pharate pupa (2.5 mg/g wet wt). The conjugate then decreased quite rapidly as tanning was initiated in the pharate pupa a few hours before larval-pupal ecdysis. A small but transient increase was observed approximately 6 hr postecdysis, followed by a continued decline in concentration (five fold) reaching 0.5 mg/g wet weight levels about 48 hr after ecdysis. The titre remained low during pupal and pharate adult development except for a small increase near adult eclosion. Tyrosine glucoside decreased to its lowest concentration in one-day-old adults (0.1 mg/g wet wt).

Tyrosine titre was relatively high during fourth to fifth instar ecdysis (0.9 mg/g wet wt), but it declined significantly to reach a low level in the last day of

larval feeding stage (0.3 mg/g wet wt). The concentration remained low during wandering behaviour, then increased rapidly in the late pharate pupa and reached a maximum at the time of larval-pupal ecdysis (1.4 mg/g wet wt). A precipitous decline in tyrosine occurred during the first 12 hr after ecdysis to 0.4 mg/g wet wt. Tyrosine subsequently increased and reached a relatively constant level in two to ten day old pharate adults (1.5 mg/g wet wt). Tyrosine concentrations decreased significantly after ten days at the time of melanization of pharate adults. A small rise in tyrosine concentration occurred during adult eclosion, followed by a decrease as the adult cuticle tanned.

Quantities per insect of free tyrosine, tyrosine glucoside and total tyrosine during various stages of development are presented in Table 1. The wandering and pharate pupal stages accumulated about two times more tyrosine glucoside than free tyrosine on a molar basis. Total tyrosine available from these two

Table 1. Titres of tyrosine and tyrosine glucoside in developmental stages of *Manduca sexta*

Stage	No. of insects	Weight (g $\pm$ S.E.*)	mg/insect $\pm$ S.E.*		
			Free tyrosine	Tyrosine glucoside	Total tyrosine
<b>Larva</b>					
Fifth stadium feeding (3-4-day-old)	8	7.50 $\pm$ 0.44	2.77 $\pm$ 0.26	3.30 $\pm$ 0.54	4.51 $\pm$ 0.38
Wandering (1-2 day old)	6	6.94 $\pm$ 0.72	2.77 $\pm$ 0.29	10.46 $\pm$ 1.20	8.31 $\pm$ 0.77
<b>Pupa</b>					
Pharate (1-2-day-old)	6	4.35 $\pm$ 0.44	3.41 $\pm$ 0.45	9.05 $\pm$ 0.86	8.21 $\pm$ 0.95
Newly ecdysed	3	4.18 $\pm$ 0.51	6.45 $\pm$ 0.51	4.72 $\pm$ 1.61	8.95 $\pm$ 1.04
6 hr-old	3	4.04 $\pm$ 0.13	1.79 $\pm$ 0.33	5.98 $\pm$ 0.58	4.96 $\pm$ 0.59
12 hr-1 day old	6	3.50 $\pm$ 0.45	3.33 $\pm$ 0.30	3.09 $\pm$ 0.67	4.97 $\pm$ 0.72
<b>Adult</b>					
Pharate (2-10-day-old)	13	3.13 $\pm$ 0.18	4.64 $\pm$ 0.40	1.59 $\pm$ 0.25	5.48 $\pm$ 0.45
(14-17-day-old)	5	2.43 $\pm$ 0.18	1.88 $\pm$ 0.21	1.82 $\pm$ 0.23	2.84 $\pm$ 0.21
Newly ecdysed	3	2.17 $\pm$ 0.12	2.33 $\pm$ 0.41	2.05 $\pm$ 0.61	3.41 $\pm$ 0.16
1-day-old	3	2.46 $\pm$ 0.18	1.62 $\pm$ 0.22	0.40 $\pm$ 0.05	1.83 $\pm$ 0.20

\* S.E. = standard error.

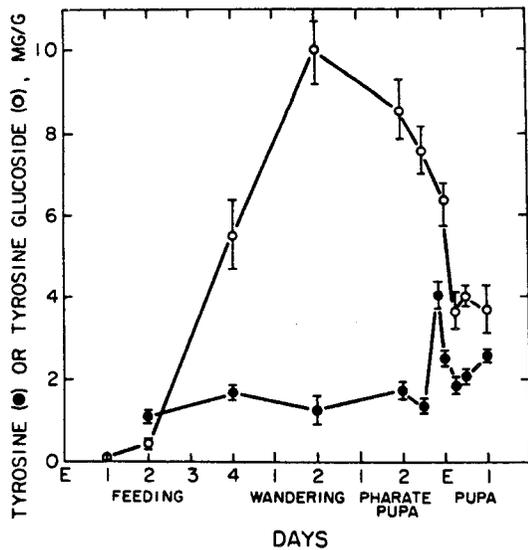


Fig. 3. Tyrosine and tyrosine glucoside titres in haemolymph during the larval-pupal transformation of *Manduca sexta*. Bars =  $\pm$  S.E.M.,  $n = 3$ . E = Ecdysis.

sources for pupal tanning was estimated to be between 8 and 9 mg/insect. During pupal ecdysis, tyrosine glucoside decreased sharply by about one-half, while free tyrosine doubled in concentration. Tyrosine reached its lowest concentration 6 hr after ecdysis while tyrosine glucoside exhibited no significant change.

Tyrosine glucoside decreased in concentration during pharate adult development, while free tyrosine more than doubled during the same period. Tyrosine then dropped to very low levels a few days before adult eclosion during the time when the body and wing scales become pigmented or melanized. Total tyrosine again accumulated from both sources during adult eclosion, but was much less than the levels present during pupal ecdysis. Total tyrosine from both sources declined by about 50% one day after adult eclosion as the adult cuticle tanned.

#### Tyrosine and tyrosine glucoside titres in haemolymph and other tissues

Tyrosine glucoside and free tyrosine titres in haemolymph during the fifth stadium larval and pupal stages are shown in Fig. 3. Fluctuations in tyrosine glucoside were similar to those observed in the whole animal. The highest titre was found during the late wandering stage and early pharate pupa (8 to 10 mg/g wet wt or 30 mM), while levels during the same period in fat body, integument, and gut were 0.50, 0.17, and 0.29 mg/g wet wt, respectively (Fig. 4).

Tyrosine glucoside began to decrease in the haemolymph of pharate pupae and reached a significantly low level shortly after larval-pupal ecdysis (3.6 mg/g wet wt). Free tyrosine remained relatively low in concentration (1–2 mg/g wet wt) throughout the last larval and pharate pupal periods, but increased sharply a few hours before pupal ecdysis. Both tyrosine and tyrosine glucoside diminished as the pupal cuticle tanned.

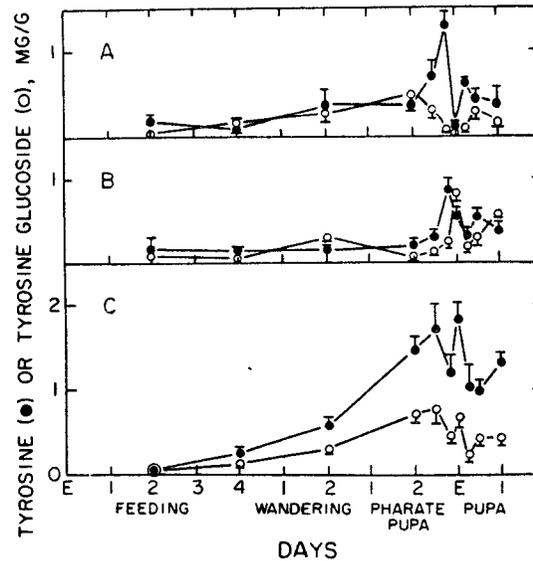


Fig. 4. Tyrosine and tyrosine glucoside titres in gut (A), integument (B), and fat body (C) during the larval-pupal transformation of *Manduca sexta*. Bars =  $\pm$  S.E.M.,  $n = 3$ .

Tyrosine increased significantly 2–3 hr before ecdysis in the gut and integument, followed by a rapid decline thereafter (Fig. 4A and 4B). Unlike the haemolymph, however, tyrosine glucoside did not accumulate during larval development in these tissues, and remained at a low level similar to tyrosine. The fat body had somewhat higher titres of both tyrosine and its glucoside than the integument and gut during the wandering and pharate pupal stages as well as during pupal ecdysis (Fig. 4C). Tyrosine increased more rapidly than its glucoside particularly in pharate pupae and reached a peak concentration (1.8 mg/g wet wt) by the time of ecdysis. Both fat body tyrosine and its glucoside declined as the pupal cuticle tanned 6–12 hr postecdysis. Thus, these levels differed from those in the haemolymph, where tyrosine increased significantly only a few hours before pupal ecdysis.

The distribution of total tyrosine and tyrosine glucoside in haemolymph and tissues was calculated from total tissue weights (WILLIAMS-BOYCE and JUNGREIS, 1980) and are shown in Table 2. Essentially all of the tyrosine glucoside and two-thirds of the tyrosine was found in the haemolymph from mature feeding larvae. The fat body and integument contained

Table 2. Distribution of tyrosine and tyrosine glucoside in haemolymph and tissues of 4-day-old feeding larvae (fifth stadium) of *Manduca sexta*

Tissue	Tyrosine (%, <sup>a</sup> )	Tyrosine glucoside (%, <sup>a</sup> )
Haemolymph	68.5 $\pm$ 3.2*	97.7 $\pm$ 0.7*
Fat body	23.2 $\pm$ 1.7	2.0 $\pm$ 0.6
Integument	7.7 $\pm$ 1.6	0.0
Gut	0.6 $\pm$ 0.1	0.3 $\pm$ 0.2

\* Standard error of the mean,  $n = 3$ .

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Our studies sine glucoside after ecdysis tration increa rapidly in th reaching a m ecdysis. KRAM glucoside in M larvae and pup in adult mot *Ephestia caute* (1982). Tyrosin lymph of the pupae of the (1981) but non latter workers lyzed, however tyrosine glucos pharate adults suggested that in the last larv in lepidoptero ently provides to supply subs Larval scleroti of pupae, and requirement. Thesis prior to flect this redu haemolymph alanine in M. stages (KRAM the larval requ Levels of ty declined after ta cuticle, i.e. at the metathorax ated with a sig increase during source of tyros is at a maxim storage protein decrease during stages (KRAM The decreas larval-pupal e increase in ty hydrolysis of pupae during ning). We hav glucoside hydr pupal ecdysis creased rapidl ecdysis to level AL-IZZI (unpu droxylation to peak of activi then declines a (1982) have f

the remaining tyrosine with the former tissue more concentrated. The gut contained less than one percent of either tyrosine or its glucoside.

## DISCUSSION

Our studies showed that the biosynthesis of tyrosine glucoside in *M. sexta* was initiated about 24 hr after ecdysis in fifth stadium larvae. The concentration increased slowly at first in day 2 and 3, then rapidly in the late feeding and wandering stages, reaching a maximum one day prior to larval-pupal ecdysis. KRAMER *et al.* (1980a) reported that tyrosine glucoside in *M. sexta* is present only in fifth stadium larvae and pupae, but not in the penultimate instar or in adult moths. Similar results were reported for *Ephestia cautella* and *Plodia interpunctella* (LU *et al.*, 1982). Tyrosine glucoside was found in larval haemolymph of the armyworm, *Leucania separata*, and in pupae of the silkworm, *Bombyx mori*. (ISOBE *et al.*, 1981) but none was detected in silkworm larvae. The latter workers did not specify the larval stage analyzed, however. ISHIZAKI and UMEBACHI (1980) found tyrosine glucoside in late last stage larvae, pupae and pharate adults of *Papilio xuthus*. All of these studies suggested that the glucoside is synthesized primarily in the last larval stage and prior to the pupal ecdysis in lepidopterous insects. This accumulation apparently provides a sufficiently large reservoir of tyrosine to supply substrates for sclerotization of pupal cuticle. Larval sclerotization is much less extensive than that of pupae, and therefore, would have a smaller tyrosine requirement. The absence of tyrosine glucoside synthesis prior to tanning of new larval cuticle may reflect this reduced need for tyrosine. Manducin, the haemolymph protein rich in tyrosine and phenylalanine in *M. sexta*, is synthesized in various larval stages (KRAMER *et al.*, 1980b) and may supply most of the larval requirement for tanning precursors.

Levels of tyrosine glucoside in whole animals declined after tanning was initiated in pharate pupal cuticle, i.e. at about the time brown bars appear on the metathorax. This fall in concentration was associated with a significant rise in tyrosine, which began to increase during the pharate pupal stage. A possible source of tyrosine while the titre of tyrosine glucoside is at a maximum, is again the tobacco hornworm storage protein manducin. Manducin titres start to decrease during the wandering and pharate pupal stages (KRAMER *et al.*, 1980b).

The decrease in tyrosine glucoside at the time of larval-pupal ecdysis associated with a corresponding increase in tyrosine suggested that enzyme catalyzed hydrolysis of the conjugate is initiated in late pharate pupae during the brown bar stage (pre-ecdysial tanning). We have subsequently found that a tyrosine glucoside hydrolase is induced or activated close to pupal ecdysis (AHMED *et al.*, 1983). Tyrosine decreased rapidly during the first 12 hr after pupal ecdysis to levels found in feeding larvae. HOPKINS and AL-IZZI (unpublished) have shown that tyrosine hydroxylation to DOPA *in vivo* in *M. sexta* reaches a peak of activity shortly before and after ecdysis and then declines as the pupal cuticle tans. HOPKINS *et al.* (1982) have found that N- $\beta$ -alanyldopamine is a

major catechol synthesized for pupal tanning in *M. sexta* and accumulates to very high titres in pharate pupae.

We found that the titre of tyrosine glucoside is very low during the pupal and pharate adult stages. These results agree with those reported for *P. xuthus* (ISHIZAKI and UMEBACHI, 1980). The latter workers attribute this low level of tyrosine glucoside to a low synthetic enzyme activity and not to a low concentration of tyrosine. Our studies showed a high concentration of tyrosine during pupal and pharate adult development which is consistent with such an interpretation.

The relatively low level of tyrosine glucoside prior to adult eclosion of the tobacco hornworm does not exclude the possibility that this molecule plays a role in adult cuticle sclerotization. In the late pharate adult stage and during adult ecdysis, tyrosine glucoside increased slightly as was observed in *P. xuthus* (ISHIZAKI and UMEBACHI, 1980) and in *B. mori* (ISOBE *et al.*, 1980). Likewise, the decline in conjugate concentration shortly after hornworm moth eclosion was similar to that found in *P. xuthus* and *B. mori*.

There was no large change in the concentration of tyrosine or its glucoside in gut and integument during the feeding, wandering, and pharate pupal stages. The values were very low and might actually have resulted from contamination with haemolymph where high titres were present. In *M. sexta* WILLIAMS-BOYCE and JUNGREIS (1980) reported that haemolymph was present ("adhered to") in washed tissues depending on the tissue and stage of development, i.e. fat body 13-26%, midgut 6-10%, and integument 2-15%, of the wet weight. Thus, the increase in free tyrosine at the time of ecdysis in these tissues may be due to the increase in tyrosine in the haemolymph. However, the fat body probably plays a major role in tyrosine and tyrosine glucoside metabolism, since free tyrosine concentrations increased to much higher levels than in gut or integument during pharate pupal development. Recently we have demonstrated that the fat body is the main source of tyrosine glucoside hydrolase, the enzyme responsible for free tyrosine buildup (AHMED *et al.*, 1983).

The haemolymph was the primary tissue for storing tyrosine glucoside with 97% in the haemolymph of the late feeding larval stage. Changes in haemolymph titres were very similar to those observed in the whole animal. In all probability the tyrosine glucoside titres in the tissues examined reflect what occurs primarily in the haemolymph.

There have been few reports about the percentage distribution of tyrosine glucoside in haemolymph and tissues from other lepidopterous insects. JUNNIKALA (1976) detected an uncharacterized storage molecule of tyrosine, denoted P1, in *Pieris brassicae* that is most likely tyrosine glucoside (KRAMER *et al.*, 1980a). It was distributed at a ratio of about 1:5 between tissues and haemolymph at the end of the fifth larval stadium. In Diptera, the tyrosine storage compounds were also mainly in haemolymph (LUNAN and MITCHELL, 1969; LEVENBOOK *et al.*, 1969; BODNARYK, 1970). BODNARYK (1970) found that more than 85% of the  $\gamma$ -L-glutamyl-L-phenylalanine in *M. domestica* is present in the blood. LEVENBOOK *et al.* (1969) indicated that sarcophagine ( $\beta$ -alanyl-L-tyrosine) in *S. bulata* is largely, if not exclusively, accumulated in the

haemolymph. LUNAN and MITCHELL (1969) detected tyrosine-O-phosphate primarily in the plasma and not in the blood cells of *Drosophila*. Therefore, we conclude that the haemolymph is the main tissue for storing tyrosine conjugates in insects.

There are several possible reasons for the accumulation of tyrosine glucoside in the haemolymph prior to metamorphosis including: (1) to render tyrosine more soluble so that large reserves can be accumulated in haemolymph for rapid mobilization and transport to sites of metabolism (the conjugate is nearly ten times more soluble than the free amino acid at physiological pH); (2) to prevent premature oxidation of tyrosine by phenoloxidasases by conjugation of the hydroxyl group and (3) to transport tyrosine to target tissues which contain enzymes catalyzing the formation of tanning substrates. This work and other studies in our laboratory suggest that the decrease in tyrosine glucoside during ecdysis is the result of transport of tyrosine glucoside from haemolymph to the fat body where hydrolysis by an ecdysone-induced hydrolase enzyme ( $\beta$ -glucosidase) occurs. We are currently determining the role played by hormones and other factors in the synthesis and hydrolysis of tyrosine glucoside during the course of cuticle morphogenesis.

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