

## CUTICULAR STRENGTH AND PIGMENTATION OF FIVE STRAINS OF ADULT *BLATTELLA GERMANICA* (L.) DURING SCLEROTIZATION: CORRELATIONS WITH CATECHOLAMINES, $\beta$ -ALANINE AND FOOD DEPRIVATION

T. H. CZAPLA,<sup>1</sup>† T. L. HOPKINS<sup>1,\*</sup> and K. J. KRAMER<sup>2</sup>

<sup>1</sup>Department of Entomology, Kansas State University, Manhattan, KS 66506 and <sup>2</sup>U.S. Grain Marketing Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Manhattan, KS 66502, U.S.A.

(Received 7 February 1990; revised 25 April 1990)

**Abstract**—Five German cockroach *Blattella germanica* strains, wild-type (*VPI*), yellow (*y*), orange (*or*), black (*Bl*), and pale body (*Pb*) which differ in cuticular colouration, were used to investigate possible correlations of catecholamine and  $\beta$ -alanine concentrations with cuticular strength as measured by puncture resistance of adult pronotal cuticle. The cuticular strength of all strains was the same at ecdysis, but that of *VPI*, *or* and *y* increased more rapidly than *Bl* and *Pb* during sclerotization. Cuticle of *VPI*, *y* and *or* reached maximal levels of puncture resistance by day 4, whereas *Bl* and *Pb* cuticles failed to achieve this strength by day 14. Extractable *N*- $\beta$ -alanyldopamine (NBAD) and *N*- $\beta$ -alanyl norepinephrine (NBANE) accumulated rapidly in *VPI*, *y* and *or* cuticles during the first 12 h after ecdysis, but their accumulation was delayed in *Bl* cuticle until 24 h. *Pb* cuticle had the least strength and also the lowest NBAD and NBANE levels.  $\beta$ -Alanine concentrations were also lowest in *Bl* cuticle during melanization. Conversely, dopamine was highest in *Bl* and *or* cuticles during melanization but was very low in *y* and *Pb* cuticles, which lack black pigmentation. Therefore, *N*- $\beta$ -alanyl catecholamine and  $\beta$ -alanine concentrations in cuticle are positively correlated with increasing cuticular strength during stabilization of the exoskeleton, whereas dopamine is correlated with melanization. When dopamine is apparently shunted into the melanization pathway in *Bl* cuticle or when lower concentrations of the *N*- $\beta$ -alanyl catecholamines occur in *Pb* cuticle, cuticular strength is reduced. Cuticular strength and weight were substantially reduced in cockroaches deprived of food after ecdysis and even later after feeding was resumed. However, cuticular thickness and catecholamine content in starved insects did not differ from those in fed insects. Deficiencies in the protein and chitin content of the procuticle as indicated by reduced weight and density likely result from starvation. Therefore, fewer sites for macromolecular cross-linking or other sclerotization reactions would be available and consequently a weaker cuticle would result.

**Key Word Index:** Cockroach; *Blattella germanica*; mutants; cuticle; sclerotization; catecholamines; dopamine; *N*- $\beta$ -alanyldopamine; *N*-acetyldopamine

### INTRODUCTION

Sclerotization and pigmentation of newly secreted cuticle involve a complex sequence of biochemical processes that determine the physical and chemical characteristics of the insect exoskeleton. Secretion of different types and quantities of proteins, chitin, minerals, lipids, and *o*-diphenols; cross-linking of the chitin-protein matrix by quinones along with its dehydration and increasing insolubility all contribute

to the final properties of stabilized cuticle (Andersen, 1979, 1985; Brunet, 1980; Kramer and Hopkins, 1987; Willis, 1987; Kramer *et al.*, 1988; Sugumaran, 1988). The relationships between the chemical constituents of cuticle, their interactions, and the resulting physical properties have been investigated in relatively few cases. The stiffness of honey bee abdominal cuticle has been associated with an increase in the insolubility of cuticular proteins (Thompson and Hepburn, 1978), whereas increasing amounts of acid-extractable ketocatechols in locust cuticle were correlated with greater indentation hardness (Hillerton *et al.*, 1982). Major differences between stiff and flexible cuticles have been related to increased amounts of insoluble proteins and the relative

\*To whom all correspondence should be addressed.

Present address: Department of Biotechnology Research, Pioneer Hi-Bred International, Inc., P.O. Box 38, Johnston, IA 50131, U.S.A.

thickness in the former type of cuticle (Hepburn and Joffe, 1976). Sclerotized puparial cuticle from *Musca domestica* and *Stomoxys calcitrans* had significantly greater relative strength per unit thickness than the mineralized puparia of *M. autumnalis* (Roseland *et al.*, 1985). *M. autumnalis* achieves a cuticular strength similar to that of the other species by increased thickness of the mineralized cuticle. In *Tribolium castaneum*, puncture resistance increased more rapidly in the cuticle of the rust-red wild-type strain than in the *black* mutant strain (Roseland *et al.*, 1987). The greater puncture resistance of wild type cuticle correlated with increased concentrations of *N*- $\beta$ -alanyldopamine (NBAD) and  $\beta$ -alanine, whereas these components were present at much lower levels in the *black* cuticle. Puncture resistance and NBAD concentrations in both types of cuticle became equivalent 6 days after ecdysis.

In this study, we investigated the cuticular strength of five strains of the German cockroach, *Blattella germanica*, and possible correlations with catecholamine and  $\beta$ -alanine concentrations during sclerotization. The strains studied were wild-type (*VPI*) and yellow (*y*), which had high *N*- $\beta$ -alanyl catecholamine concentrations; orange (*or*), which exhibited both high dopamine and *N*- $\beta$ -alanyl catecholamine concentrations; black (*Bl*), which initially had high dopamine concentrations but low NBAD and NBANE; and a pale body (*Pb*) strain not previously analysed (Czapla *et al.*, 1990). We also compared  $\beta$ -alanine levels, cuticle thickness and the effect of food deprivation on cuticular strength during the period of sclerotization.

## MATERIALS AND METHODS

### Insects

Colonies of wild *B. germanica* and four mutant strains differing in cuticular pigmentation (Ross and Cochran, 1974) were obtained from Dr Mary Ross, Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, Va. They were reared in plastic containers bedded with wood shavings at  $28 \pm 2^\circ\text{C}$  with a photoperiod 16 h light per day. Water and Purina® 'Lab Chow' were provided *ad lib*. Adults were collected at ecdysis for analysis at various times during a 14-day period, quick frozen in dry ice, and then stored at  $-20^\circ\text{C}$  until analysed.

### Measurement of cuticular strength

Puncture resistance of pronotal cuticle of adult females was used as a measurement of cuticular strength. Each pronotum was dissected, cleaned of adhering muscle and fat body, rinsed, and then held a short time before testing in distilled water to prevent dehydration. Dehydration occurs when pronota are held in air and causes changes in puncture resistance. Groups of pronota, freshly prepared for each experiment, were tested in a completely

randomized design. The apodeme on the inner surface was removed so as not to interfere with the puncture analysis. Resistance to puncture was analysed with an Instron® Tensile Tester Model 1132, equipped with a 2 kg compression load cell and a probe modified for work with insect cuticle (Roseland *et al.*, 1985; Grodowitz *et al.*, 1987). The probe diameter was 0.61 mm and the base plate aperture through which the probe entered after puncture was 0.71 mm. The pronotum with the centre as the puncture point was aligned on the custom stage with the anterior edge away from the operator. The probe speed was  $2.5 \text{ mm s}^{-1}$ . Puncture resistance values represent a combination of compressional, shearing and tensile forces. Although a specific parameter is not measured by this method, it reliably assesses relative cuticle strengths, provided that the same types of cuticle (pronotum) are compared. Freezing and thawing of the cockroaches prior to analysis had no significant effect on puncture resistance when compared to freshly dissected cuticle.

### Measurement of cuticle thickness

Cuticle thickness was determined using an ETEC Autoscan U-1 scanning electron microscope. Pronotal cuticles were dissected, cleaned and immediately frozen in liquid nitrogen. They were then fractured on a medial line. One half was mounted for SEM and sputter-coated with gold. Thickness at two to four locations near the centre and near the edges of each pronotum was measured.

### Extraction of catecholamines from cuticle

Individual pronota did not provide enough material for catecholamine analysis; therefore, whole body cuticle was used. Catecholamine concentrations were similar in both pronotal and abdominal cuticle of *Periplaneta americana* (Czapla, unpublished data). Abdominal and thoracic integuments were dissected, cleaned of adhering muscle and fat body and the inner surfaces scraped to remove the epidermis. Pieces of cuticle were given a rinse with distilled water, blotted dry and weighed on a microbalance. The final reading was taken when weight loss due to evaporation had stabilized at  $<0.001 \text{ mg/s}$ . The cuticle was then homogenized in a ground glass tissue grinder containing 0.15 ml of 1.2 M HCl, 5 mM ascorbate, and 0.12 mg of the internal standard,  $\alpha$ -methyl dopa (AMD). The homogenate was centrifuged at 1300 *g* for 1 min and the supernatant was removed for analysis by HPLC-EC.

### Analysis of catecholamines

Extracts (0.1 ml) were adsorbed on alumina at pH 8.4 and recovered in 1 M acetic acid for HPLC-EC analysis (Czapla *et al.*, 1988). Catecholamines were resolved using a reversed phase, C18 5-micron column at a flow rate of  $1 \text{ ml min}^{-1}$  and detected with a Bioanalytical System LC4B electrochemical detector set at +720 mV. The primary mobile phase consisted of methanol (15% v/v), sodium octyl

sulphate (0.16 mM), sodium EDTA (0.09 mM), and 0.1 M  $H_3PO_4$  adjusted to pH 2.9 with NaOH. A second mobile phase consisted of acetonitrile (26% v/v), 1.1 mM sodium dodecyl sulphate, 0.05 mM sodium EDTA, and 0.1 M  $H_3PO_4$  adjusted to pH 3.3 with NaOH. The retention times of standard catecholamines were compared with those of electroactive compounds in cuticular extracts using both mobile phases. Chemical standards were obtained from commercial sources or were synthesized (Czapla *et al.*, 1988). Quantities of individual catecholamines were calculated by comparing peak areas with that of the internal standard recovered in each extract and then correcting for recoveries established using standard compounds.

#### Amino acid analysis

Whole body cuticle was dissected and cleaned of adhering tissues, homogenized in a ground glass tissue grinder containing 0.15 ml of 1.2 M HCl, 5 mM ascorbate, and then hydrolyzed in 2 ml of 6 M HCl and 5% phenol at 110°C for 20 h *in vacuo*. Samples were then evaporated to dryness under  $N_2$ . The hydrolysates were redissolved in 0.35 ml of distilled water, 0.01 ml of dilution buffer, and 0.04 ml of *p*-toluene-sulphonic acid; centrifuged for 1 min at 13,000 g; and analysed for amino acid composition (Spackman *et al.*, 1958; Liu and Chang, 1971).

#### Food deprivation

Initial experiments involved both sexes of the *VPI* strain, with one group being fed for 4 days and the

other starved for 4 days. A second experiment involved only *VPI* females and was divided into four groups: (1) controls fed for 12 days; (2) fed for 4 days and starved for 8 days; (3) starved for 4 days and then fed for 8 days; and (4) fed the first day and starved for 11 days. Puncture resistance and cuticle thickness measurements were made as previously described.

#### Statistical analysis

Data were collected using a completely randomized design. Statistical tests of significant differences between strains were performed using the SAS least-squares means procedure (LSMEANS) with  $P \leq 0.05$  (SAS, 1982). Regression analysis was also conducted.

## RESULTS

#### Cuticular strength during sclerotization

Puncture resistance for *VPI* (52 g),  $\gamma$  (52 g) and *or* (50 g) cuticles were significantly greater than that for *Bl* (38 g) and *Pb* (31 g) cuticles when their overall means were compared ( $P \leq 0.05$ ,  $n = 180$ ). Cuticles from all strains displayed a similar time course during ecdysis, with the lowest strength occurring at ecdysis and the greatest at 4–14 days (Fig. 1). Although no differences in puncture resistance of newly ecdysed cuticle (approx. 10 g) were observed between strains, *Pb* cuticle was lower in puncture resistance than the other cuticles at 3 h. At 6–12 h, both *Bl* and *Pb* pronotal cuticles had significantly less strength than those from strains *VPI* and  $\gamma$ . However, by 24 h after

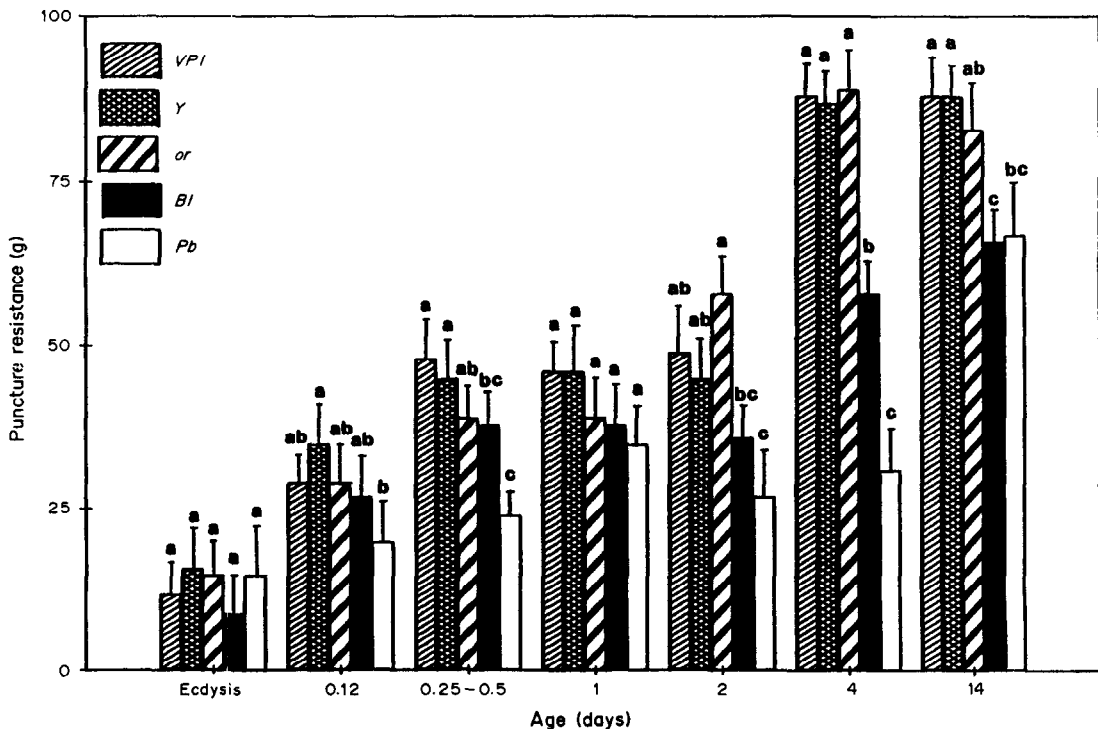


Fig. 1. Puncture resistance of adult female pronotal cuticles of *B. germanica* strains. Data are the mean values  $\pm$  SE. Values within the same letter are not significantly different ( $P \leq 0.05$ ,  $n = 180$ ).

ecdysis, cuticles from all strains had similar levels of puncture resistance (approx. 40 g). Thereafter, *VPI*, *y* and *or* cuticles showed twofold increases in puncture resistance by day 4 (approx. 80 g), whereas *Bl* cuticle had a slower rate, and that of *Pb* showed no increase in strength. By day 14, *Pb* cuticle became equal in puncture resistance to *Bl* cuticle (approx. 70 g), but both had less strength than cuticles from the other strains.

Therefore, increases in cuticular strength appeared to proceed at variable rates in the different strains, with the lightest (*Pb*) and darkest coloured (*Bl*) cuticles of *B. germanica* having the slowest rates of increase. Differences also were observed between cuticular strength of *VPI* males and females. Female *VPI* pronotal cuticle was more resistant to puncture than male cuticle by day 4 (98 g vs 66 g,  $P \leq 0.05$ ,  $n = 12$ ). Similar results involving differences in cuticular puncture resistance between sexes also were obtained with other strains (data not shown). By day 4 female *VPI* pronotal cuticle also weighed significantly more than the male pronotum (0.27 vs 0.17 mg, respectively,  $P \leq 0.05$ ,  $n = 12$ ), which could account for the difference observed in cuticle puncture resistance. Male *VPI* cuticular thickness, however, was not significantly different from that of female *VPI* cuticle at 4 days ( $10.4 \pm 0.6$  vs  $11.6 \pm 0.8$  microns, respectively).

#### Changes in cuticular thickness during sclerotization

Pronotal cuticle thickness increased by 50–100% in all strains of *B. germanica* from ecdysis through day 4 (Table 1). Strain *Pb* had the thinnest cuticle by 3 h after ecdysis, whereas *VPI* had the thickest. However, *Pb* cuticle thickness doubled by 24 h, becoming significantly greater than those of the other strains and remaining at that thickness through 4 days. *Bl*, *or* and *Pb* cuticles approximately doubled their cuticular thickness between ecdysis and day 4, whereas *VPI* and *y* cuticles increased by about 50%. Cuticular thickness for all strains was greatest near the centre of the pronotum and diminished by 20–30% toward the edges. Thickness in some cuticles continued to increase after 4 days. For example, *VPI* increased to 16.1 microns by 12 days after ecdysis. The ratios of puncture resistance to cuticle thickness during

Table 1. Cuticular thickness (microns) of the pronota from strains of adult female *Blattella germanica* during sclerotization\*

Time after ecdysis	Strains				
	<i>VPI</i>	<i>y</i>	<i>or</i>	<i>Bl</i>	<i>Pb</i>
3 h	8.6a	8.0ab	8.1a	7.6ab	6.3b
24 h	10.0bc	10.3b	10.7b	9.5c	12.6a
4 days	11.6b	12.0ab	15.0a	14.1a	12.1a

\*Mean values with the same letter are not significantly different between strains of the same age ( $P \leq 0.05$ ,  $n = 10-12$ ).

Table 2. The ratio of puncture resistance to cuticle thickness ( $\text{g micron}^{-1}$ ) of the pronota of adult female *Blattella germanica* strains during sclerotization

Time after ecdysis	<i>VPI</i>	<i>y</i>	<i>or</i>	<i>Bl</i>	<i>Pb</i>
3 h	3.4	4.3	3.6	3.8	3.0
24 h	4.8	4.7	4.1	3.7	2.9
4 days	7.7	7.4	6.1	4.3	3.1

sclerotization are shown in Table 2. Although the ratios of all strains were the same at 3 h, those of *VPI*, *y* and *or* increased about twofold by day 4, whereas *Bl* and *Pb* cuticles exhibited little or no change in their ratios with age.

#### Catecholamine concentrations in cuticle

The  $\beta$ -alanyl catecholamines (NBAD + NBANE) accumulated to peak levels by 12–24 h after ecdysis in all strains. However, *VPI*, *y* and *or* cuticles had concentrations nearly double those of *Bl* and *Pb* cuticles (Fig. 2). Dopamine concentrations also peaked between 12 and 24 h and were 2–4 times higher in cuticles of *or* and *Bl* than in *VPI*, *y* and *Pb* cuticles (Fig. 2). Cuticular *N*-acetyl catecholamines (NADA and NANE) were low in concentration during the first hours after ecdysis, but they reached levels similar to other catecholamines between 4 and 14 days (Fig. 2).

#### $\beta$ -Alanine and other amino acid concentrations in cuticle

*Bl* cuticle had significantly lower concentrations of  $\beta$ -alanine throughout the period of sclerotization and melanization ( $\leq 0.06 \mu\text{mol g}^{-1}$ ), whereas *VPI* cuticle was highest through day 1 ( $> 0.1 \mu\text{mol g}^{-1}$ ) (Fig. 3). The  $\beta$ -alanine concentrations in *y* cuticle were  $> 0.08 \mu\text{mol g}^{-1}$  throughout the 4-day period. Only small differences were observed in the levels of other amino acids extracted from cuticles of the three strains (data not shown). Generally, the amino acids increased in concentration to reach peak levels at day 4, indicating that protein deposition was maximal during that period.

#### Effects of food deprivation on cuticular strength

When *VPI* female and male adult cockroaches were deprived of food for 4 days after ecdysis, they had about 50% less puncture resistance (47 and 38 g, respectively) than those of fed cockroaches (98 and 66 g, respectively,  $P \leq 0.05$ ,  $n = 12$ ). Pronota from fed insects weighed substantially more than those of starved insects ( $0.34 \pm 0.01$  vs  $0.26 \pm 0.01$  mg, respectively; mean values  $\pm$  SE,  $n = 3-4$ ). No significant differences were observed between dopamine, NBAD and NBANE levels in cold acid extracts from cuticles of fed or starved *VPI* adults (Table 3).

Results of a second experiment using four different feeding regimes are shown in Table 4. Similar

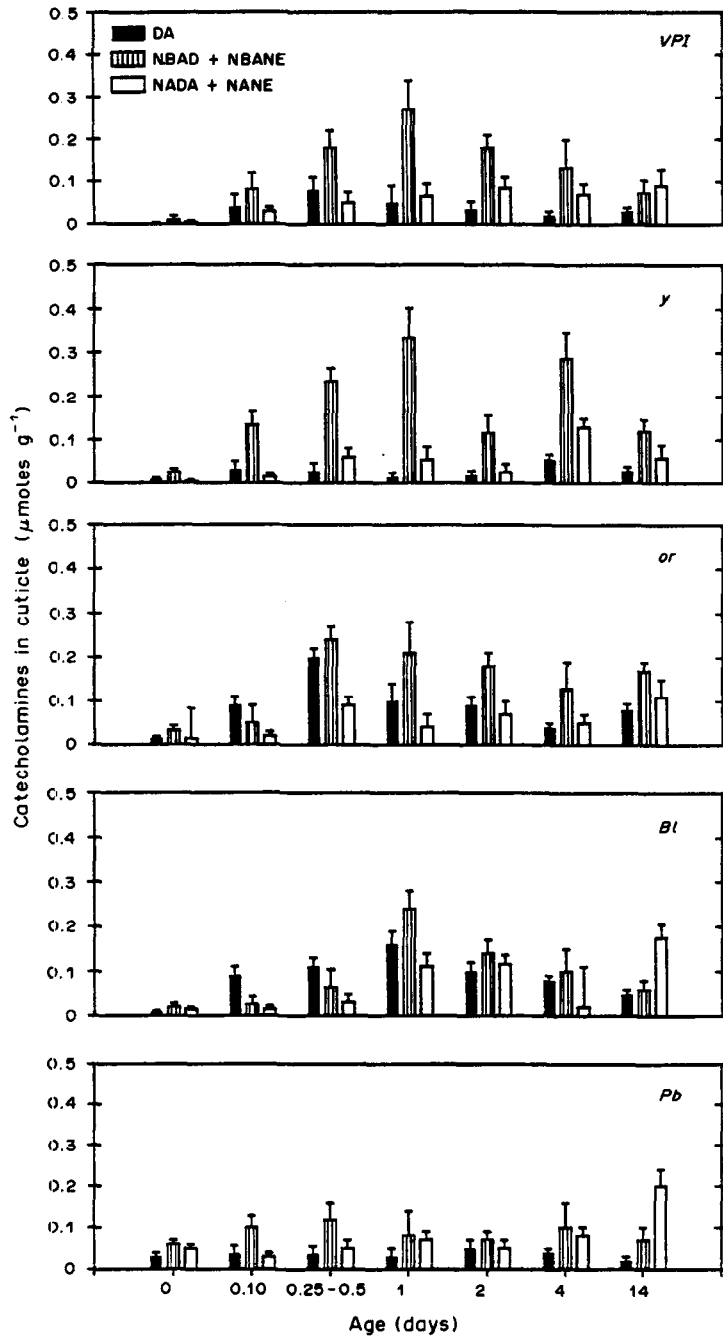


Fig. 2. Concentrations of dopamine, *N*- $\beta$ -alanyl (NBAD and NBANE) and *N*-acetyl (NADA and NANE) catecholamines ( $\mu\text{mol g}^{-1}$  wet wt) extracted from cuticles of *B. germanica* strains. Data are mean values  $\pm$  SE,  $n = 3-7$ .

puncture resistance values (approx. 90 g) were measured for the control group of VPI females fed for 12 days and those fed during the first 4 days after ecdysis, followed by starvation for 8 days. However, females that were initially starved for 4 days after ecdysis then fed for 8 days and those starved for 11 days after feeding for the first day had puncture

resistance values reduced by 35 and 60%, respectively, when compared to unstarved controls. No differences occurred in the thickness of cuticles from any of the groups. Therefore, the density of the pronotal cuticle was reduced when cockroaches were starved during the period of cuticle sclerotization the first few days after ecdysis.

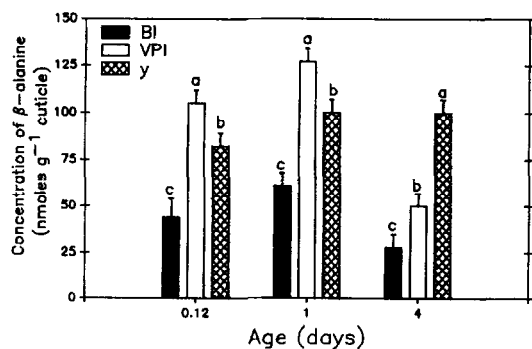


Fig. 3. Concentration of  $\beta$ -alanine ( $\text{nmol g}^{-1}$  wet wt) in cuticles of *B. germanica* strains. Data are the mean values  $\pm$  SE. Values for each strain with the same letter within the same time interval are not significantly different ( $P \leq 0.05$ ,  $n = 17$ ).

### DISCUSSION

Sclerotization of insect cuticle involves the stabilization of the chitin-protein matrix by quinonoid metabolites from *N*-acyl catecholamines. Increasing concentrations of these compounds in sclerotizing cuticle results in dehydration and insolubility of the polymeric structure and dramatic changes in its mechanical properties. NBAD and NADA are the primary sclerotizing precursors in different types of insect cuticle (Karlson and Sekeris, 1962; Hopkins *et al.*, 1982), while their  $\beta$ -hydroxylated derivatives, NBANE and NANE, are also involved in sclerotization (Morgan *et al.*, 1987; Czapla *et al.*, 1988, 1989). The quinonoid metabolites of these catecholamines are thought to form adducts and cross-links with cuticular proteins via aromatic ring carbons and aliphatic side-chain carbons (Andersen, 1985; Kramer and Hopkins, 1987; Schaefer *et al.*, 1987; Sugumaran, 1988). However, little information is

Table 3. Catecholamine concentrations ( $\mu\text{mol g}^{-1}$ ) in cuticles of fed and starved adult female *Blattella germanica* VPI of day 4 after ecdysis\*

Catecholamines	Fed	Starved
Dopamine	$0.05 \pm 0.03$	$0.09 \pm 0.04$
NBAD	$0.11 \pm 0.02$	$0.09 \pm 0.02$
NBANE	$0.17 \pm 0.06$	$0.15 \pm 0.04$

\*Mean values are means  $\pm$  SE,  $n = 3-4$ .

Table 4. Puncture resistance (g) and cuticle thickness (microns) of adult female *Blattella germanica* VPI under different feeding and starvation regimes after ecdysis\*

Feeding-starvation regime	Puncture resistance	Cuticle thickness
Fed 12 days (control)	90a	16.1a
Fed 4 days, starved 8 days	91a	14.3a
Starved 4 days, fed 8 days	58b	16.3a
Fed 1 day, starved 11 days	37c	15.1a

\*Mean values with the same letter are not significantly different ( $P \leq 0.05$ ,  $n = 22$ ).

available about the effect of these sclerotizing precursors on the mechanical properties of the cuticle.

We have shown that significant differences exist in cuticular concentrations of catecholamines between wild and mutant strains of *Blattella germanica* (VPI, *y*, *or* and *Bl*) during the first 2 days after adult ecdysis (Czapla *et al.*, 1990). Dopamine was extremely high in *Bl* and *or* cuticles, intermediate in VPI and low in *y* cuticle. Conversely, NBANE was high in cuticles from strains *y* and VPI during the first hours after ecdysis, but lower in *or* and *Bl*. In this study regression analysis of data from all strains revealed that concentrations of *N*- $\beta$ -alanyl catecholamines displayed a positive correlation (non-linear) with puncture resistance during the first day after ecdysis ( $P \leq 0.05$ ,  $R^2 = 0.25$ ), the main period for sclerotization of the newly secreted cuticle. However, analysis of the entire 14-day period showed no significant difference. Regression analysis of data from individual strains (VPI, *y*, *Bl*) also showed that *N*- $\beta$ -alanyl catecholamine concentrations were positively correlated with puncture resistance through day 2.

In time course experiments, *N*- $\beta$ -alanyl catecholamine concentrations increased rapidly in VPI, *y* and *or* cuticles, reaching more than  $0.2 \mu\text{mol g}^{-1}$  within 12 h after ecdysis, during which time puncture resistance increased in these strains. In contrast, *Bl* cuticle did not have high *N*- $\beta$ -alanyl catecholamine levels until 24 h after ecdysis, which correlated with its delayed increase in strength. Concentrations of NBANE and NBAD were lowest in *Pb* cuticle which also had the least puncture resistance. After the first day, cuticular catecholamine concentrations generally decreased in all strains indicating their utilization for sclerotization and pigmentation, and decreased transport from the haemolymph and epidermis.

The higher quantities of the *N*- $\beta$ -alanyl catecholamines in cuticles of strain VPI and *y* potentially increase the number of covalent bonds between metabolites of these compounds and the chitin-protein matrix, which would result in the increased puncture resistance observed. This hypothesis is also supported by both reduced puncture resistance and *N*- $\beta$ -alanyl catecholamine content in the cuticles of strains *Bl* and *Pb*. These differences were also manifested when puncture resistance per unit of thickness was compared. These values for *Bl* and *Pb* cuticles showed little or no increase through day 4, whereas VPI, *y* and *or* values increased twofold or more during the same interval.

The strains that produced the most free dopamine in their cuticles, *Bl* and *or*, also had the darkest pigmentation. Since dopamine apparently serves as the main precursor for melanin (Hopkins *et al.*, 1984; Kramer *et al.*, 1984; Hiruma *et al.*, 1985; Roseland *et al.*, 1987), these mutants may initially shunt more dopamine into the pigmentation pathway rather than into precursors for sclerotization and therefore have a slower rate of strengthening.

Higher  $\beta$ -alanine concentrations in cuticle were also positively correlated with the increased puncture resistance in strains *VPI* and  $\gamma$ . The relatively low level of  $\beta$ -alanine in *Bl* cuticle was consistent with its reduced puncture resistance.  $\beta$ -Alanine is most probably involved in sclerotization as a component of the *N*- $\beta$ -alanyl catecholamines and their quinone-sclerotizing metabolites (Hopkins *et al.*, 1982, 1984; Morgan *et al.*, 1987).

These studies on *B. germanica* strains gave results very similar to those obtained with *Tribolium castaneum*. The black mutant of the red flour beetle had less puncture resistance of the elytra than those of the wild strain type during the first 6 days after adult ecdysis (Roseland *et al.*, 1987). The wild strain reached a maximal puncture resistance level by day 4, the same time that *B. germanica* *VPI*,  $\gamma$  and *or* strains reached their maximal resistance. However, the black mutant of *T. castaneum* did not reach this level until 2 days later. NBAD and  $\beta$ -alanine concentrations also increased more rapidly in the *T. castaneum* wild strain than in the black mutant (Kramer *et al.*, 1984; Roseland *et al.*, 1987). The low concentrations of NBAD and  $\beta$ -alanine in the black mutant therefore correlated with its reduced cuticular strength during the early period of sclerotization. Cuticular thickness was similar in both beetle strains (Roseland *et al.*, 1987). In the locust, *Schistocerca gregaria*, cuticular hardness also reached a maximum about 4 days after ecdysis, and correlated positively with extractable ketocatechol concentrations (Hillerton *et al.*, 1982).

Starvation of adult *B. germanica* during the main period of sclerotization the first few days after ecdysis was found to reduce cuticular strength. Cockroaches (*VPI*) that were starved for 4 days immediately after ecdysis had substantially lower puncture resistance values even after 8 days of resumed feeding than did cockroaches that were fed *ad lib.* for the entire period. However, no differences were observed when cockroaches were fed for 4 days after ecdysis then starved for 8 days. Cuticular thickness was similar in all treatments and cuticular concentrations of *N*- $\beta$ -alanyl catecholamines were not different between the 4-day starved vs fed insects. Since the pronotal wet weight of the fed insects was approx. 25% greater at day 4 than that of starved insects, a deficiency in the secretion of proteins and chitin, the main constituents of the procuticle must be caused by starvation. This in turn would lead to fewer sites for cross-linking or other interactions with diphenols and quinones. When feeding of starved cockroaches was resumed, the cuticles increased in puncture resistance, but they did not attain the values of unstarved animals. These results demonstrate that maximal cuticular strength occurs after the insects have fed for 4 days following ecdysis. If, however, they are starved during this early period, when sclerotization normally takes place, the cuticle cannot attain its normal strength probably due to deficiencies in the amounts of protein and chitin.

In *M. sexta* larvae that have a relatively unsclerotized cuticle, starvation for 96 h caused a tenfold decrease in cuticle extensibility and an increase in intrinsic stiffness (Wolfgang and Riddiford, 1987). These effects were reversible if the starved animals were subsequently fed. However, *M. sexta* larvae that were fed with 2% agar had similar cuticular mechanics when compared to the control group. Therefore, the changes seen in the starved larvae appear to have been caused by dehydration and not by starvation. Since the cockroaches in our experiments had a supply of water, dehydration probably did not play a major role in causing a reduction in the puncture resistance of the starved insects. More likely the starved cockroaches were unable to secrete the normal quantities of proteins and chitin into the procuticle during the critical period of sclerotization. The reabsorption of constituents from the new cuticle during starvation is another possibility. In either case, cuticular thickness is unaffected but weight and density are reduced. In the female tsetse fly, *Glossina austeni*, total protein content and thickness of the ventral abdominal cuticle increased during the early part of the pregnancy cycle, reached peak levels 2 days after ovulation, and then decreased to a minimal value just before larviposition (Solowiej and Davey, 1987). Virgin females did not display either of these cycles. Water-soluble proteins appear to be deposited in the cuticle and are then reabsorbed when needed for reproductive purposes. Whether carbohydrate content also changes in cuticle that is reabsorbed during the pregnancy cycle of the fly is not known. The differences observed between pronotal weight and puncture resistance of starved vs fed cockroaches in this study support the hypothesis of a deficiency of protein and perhaps chitin caused by either reabsorption or reduced secretion of these components during starvation.

The different mechanical and chemical properties displayed by the wild-type and mutant strains of *B. germanica* are providing information about the relationship of sclerotizing precursors and the resulting pigmentation and mechanical properties of the exoskeleton. Further studies are needed to define the molecular interactions between cuticular constituents that contribute to these properties.

*Acknowledgements*—The authors thank Dr Deborah Rodgers for assistance with the Instron experiments, Wayne Schope for  $\beta$ -alanine and amino acid analysis, John Krcma for SEM assistance, Sharon Starkey for technical assistance and Dr Mary Ross, Virginia Polytechnic Institute and State University, Blacksburg, Va, for supplying starter colonies of *B. germanica* strains. Contribution No. 90-87-J from the Kansas Agricultural Experiment Station, Manhattan, Kan. Cooperative investigation between ARS, USDA and the Kansas Agricultural Experiment Station. KJK is a research chemist and adjunct professor at the U.S. Grain Marketing Research Laboratory and the Department of Biochemistry, Kansas State University, respectively. Supported in part by National Science Foundation Grant DCB 86-09717 to TLH and KJK, and a Sigma Xi Research Grants-in-Aid to THC.

## REFERENCES

- Anderson S. O. (1979) Biochemistry of insect cuticle. *Ann Rev. Entomol.* **24**, 29–61.
- Andersen S. O. (1985) Sclerotization and tanning in cuticle. In *Comparative Insect Physiology Biochemistry Pharmacology* (Edited by Kerkut G. A. and Gilbert L. I.), Vol. 3, pp. 59–74. Pergamon Press, Oxford.
- Brunet P. C. J. (1980) The metabolism of the aromatic amino acids concerned in the cross-linking of insect cuticle. *Insect Biochem.* **10**, 467–500.
- Czapla T. H., Hopkins T. L. and Kramer K. J. (1989) Catecholamines and related *o*-diphenols in the hemolymph and cuticle of the cockroach *Leucophaea maderae* (F.) during sclerotization and pigmentation. *Insect Biochem.* **19**, 509–515.
- Czapla T. H., Hopkins T. L. and Kramer K. J. (1990) Catecholamines in the cuticle of four strains of the German cockroach, *Blattella germanica* (L.) during sclerotization and melanization. *Archs Insect Biochem. Physiol.* **12**, 145–156.
- Czapla T. H., Hopkins T. L., Morgan T. D. and Kramer K. J. (1988) Diphenols in hemolymph and cuticle during development and cuticle tanning of *Periplaneta americana* (L.) and other cockroach species. *Archs Insect Biochem. Physiol.* **7**, 13–26.
- Grodowitz M. J., Roseland C. R., Hu K. K., Broce A. B. and Kramer K. J. (1987) Mechanical properties of mineralized and sclerotized puparial cuticles of the flies *Musca autumnalis* and *M. domestica*. *J. exp. Zool.* **243**, 201–210.
- Hepburn H. R. and Joffe L. (1976) Hardening of locust sclerites. *J. Insect Physiol.* **21**, 1741–1746.
- Hillerton J. E., Reynolds S. E. and Vincent J. F. V. (1982) On the indentation hardness of insect cuticle. *J. exp. Biol.* **96**, 45–52.
- Hiruma K., Riddiford L. M., Hopkins T. L. and Morgan T. D. (1985) Roles of dopa decarboxylase and phenoloxidase in the melanization of the tobacco hornworm and their control by 20-hydroecdysone. *J. Comp. Physiol.* **155B**, 659–669.
- Hopkins T. L., Morgan T. D. and Kramer K. J. (1984) Catecholamines in haemolymph and cuticle during larval, pupal and adult development of *Manduca sexta*. *Insect Biochem.* **14**, 553–540.
- Hopkins T. L., Morgan T. D., Aso Y. and Kramer K. J. (1982) *N*- $\beta$ -Alanyldopamine: major role in insect cuticle tanning. *Science* **217**, 364–366.
- Karlson P. and Sekeris S. E. (1962) *N*-Acetyldopamine as sclerotizing agents of the insect cuticle. *Nature* **195**, 183–184.
- Kramer K. J. and Hopkins T. L. (1987) Tyrosine metabolism for insect cuticle tanning. *Archs Insect Biochem. Physiol.* **6**, 279–301.
- Kramer K. J., Hopkins T. L. and Schaefer J. (1988) Insect cuticle structure and metabolism. In *Biotechnology for Crop Protection* (Edited by Hedin P., Mann J. J. and Hollingworth R. M.), pp. 160–185. Symposium Series No. 379. American Chemical Society, Washington, D.C.
- Kramer K. J., Morgan T. D., Hopkins T. L., Roseland C. R., Aso Y., Beeman R. W. and Lookhart G. L. (1984) Catecholamines and  $\beta$ -alanine in the red flour beetle *Tribolium castaneum*. *Insect Biochem.* **14**, 293–298.
- Liu T. Y. and Chang Y. H. (1971) Hydrolysis of proteins with *p*-toluenesulfonic acid. *J. biol. Chem.* **246**, 2842–2848.
- Morgan T. D., Hopkins T. L., Kramer K. J., Roseland C. R., Czapla T. H., Tomer K. B. and Crow F. W. (1987) *N*- $\beta$ -Alanylnorepinephrine: biosynthesis in cuticle and possible role in sclerotization. *Insect Biochem.* **17**, 255–263.
- Roseland C. R., Kramer K. J. and Hopkins T. L. (1987) Cuticular strength and pigmentation of rust-red and black strains of *Tribolium castaneum*. *Insect Biochem.* **17**, 21–28.
- Roseland C. R., Grodowitz M. J., Kramer K. J., Hopkins T. L. and Broce A. B. (1985) Stabilization of mineralization and sclerotized puparial cuticle of muscid flies. *Insect Biochem.* **15**, 521–528.
- Ross M. H. and Cochran D. G. (1974) The German cockroach, *Blattella germanica*. In *Handbook of Genetics* (Edited by King R. C.), Vol. 3, pp. 35–62. Plenum Press, New York.
- SAS Institute Inc. (1982) *SAS Users' Guide: Statistics*. SAS Institute, Cary, N.C.
- Schaefer J., Kramer K. J., Grabow J. R., Jacob G. S., Stepkal E. O., Hopkins T. L. and Speirs R. D. (1987) Aromatic cross-links in insect cuticle: detection by solid state  $^{13}\text{C}$  and  $^{15}\text{N}$  NMR. *Science* **235**, 1200–1204.
- Solowiej S. and Davey K. G. (1987) Variation in thickness and protein content of the cuticle of the female of *Glossina austeni*. *Archs Insect Biochem. Physiol.* **4**, 287–296.
- Spackman D. H., Stein W. H. and Moore S. (1958) Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* **30**, 1190–1206.
- Sugumaran M. (1988) Molecular mechanisms for cuticle sclerotization. *Adv. Insect Physiol.* **21**, 179–231.
- Thompson S. and Hepburn H. R. (1978) Changes in chemical and mechanical properties of honey bee (*Apis mellifera adonsonii* L.) cuticle during development. *J. comp. Physiol. B* **126**, 257–262.
- Willis J. H. (1987) Cuticular proteins: The neglected component. *Archs Biochem. Physiol.* **6**, 203–215.
- Wolfgang W. J. and Riddiford L. M. (1987) Cuticular mechanics during larval development of the tobacco hornworm, *Manduca sexta*. *J. exp. Biol.* **128**, 19–33.