hemocyanin obtained by DEAE-Sephadex chromatography from the horseshoe crab, *Limulus polyphemus*. As far as the photograph of the electrophoretic pattern presented by them is concerned, only the peak IV they obtained seems to indicate a single monomer. In our experiment, the amino acid compositions of the monomers of *Tachypleus tridentatus* hemocyanin are compared with each other.

Materials and methods. The Japanese horseshoe crab, *Tachypleus tridenatus*, was collected at Kasaoka, Japan. Whole hemocyanin from *Tachypleus* serum was prepared and purified by ultracentrifugation at 150,000 × g for 1 h repeated three times. The hemocyanin monomers were purified by preparative polyacrylamide gel electrophoresis; their purity was shown in the previous study[4]. The amino acid composition of each monomer was analyzed by a JEOL analyzer, model JLC 5AH.

Results and discussion. The amino acid composition shown in the table was obtained by hydrolysis for 72 h in 6 N HCl at 105 °C. The value of the tyrosine content, shown in a bracket, was quoted from the data obtained by hydrolysis for 24 h because it decreased during the hydrolysis time. Values of *Limulus* hemocyanin are calculated from the data for *Limulus polyphemus* presented by Ghiretti-Magaldi et al.[9] and expressed as a percent age of 15 kinds of amino acids shown in the table, to compare them with those of *Tachypleus tridentatus*. From the results of these amino acid analyses, it can be noted, firstly, that the hemocyanins from these 2 horseshoe crabs are very similar. Secondly, the amino acid compositions of the 4 kinds of monomers from *Tachypleus* hemocyanin are similar in the contents of most amino acids, and all monomers have a high content of acidic amino acids. However, significant differences are observed in the contents of histidine and arginine and further, we know each monomer was clearly distinct in both immunological and electrophoretic characters[4]. It seems that differences between the monomers are apparent from the amino acid compositions as shown in the table.

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Proline synthesis from glutamate in the mitochondria isolated from a blowfly, *Aldrichina grahami*

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Summary. Proline biosynthesis from glutamate was demonstrated in a cell free system prepared from blowfly abdomen. The biosynthetic activity was found mainly in the mitochondrial fraction. The biosynthesis of proline from glutamate required ATP, NADPH and Mg^{++} as cofactors.

Proline is known to play a specific role as the energy reservoir in insect flight muscle[1,2]. In a blowfly, *Aldrichina grahami*, the main pathway supplying proline is different at different developmental stages of the insect[3]. The insect has a potent proline synthetic activity from glutamate at the adult stage, and is a good organism for elucidating the proline biosynthetic pathway from the amino acid.

Materials and methods. Animals. Laboratory bred adult flies, *A. grahami* were maintained as described previously[4].

Preparation of cell free homogenate and subcellular fractions. The abdomens (100–120) of blowflies, 5–6-day-old, were isolated and homogenized in a Potter-Elvehjem type teflon-glass homogenizer at 0 °C with 10 ml of 0.25 M sucrose solution containing 1 mM EDTA, 0.2% bovine serum albumin and 5 mM Tris-HCl buffer, pH 7.4. The homogenate was centrifuged at 700 × g for 10 min. An aliquot of the supernatant was removed and used as a cell-free extract. The remaining portion of the supernatant was centrifuged at 10,800 × g for 10 min, then the precipitate was washed with the homogenizing medium, then resuspended in the original volume of the medium (mitochondrial fraction).

The 10,800 g supernatant was centrifuged at 100,000 × g for 1 h and the precipitate was resuspended in the same volume as the mitochondrial fraction, then the suspension and the supernatant of the 100,000 × g centrifugation were used as the microsomal fraction and cytosol fraction, respectively.

Incubation and extraction. The reaction mixture was hypoosmotic, and was incubated at 30 °C in a small test tube. The reaction was stopped by adding 0.1 ml of 10% trichlo-

Table 1. Subcellular distribution of the proline synthetic activity in the abdomen of a blowfly, *A. grahami*. The incubation system contained 0.3 ml of each fraction, 100 µl each of 10 mM NADPH, 10 mM NADH and 100 mM ATP, and 10 µl each of 1 M $MgCl_2$ and 6.5 µCi/ml U-^{14}C-glutamate in a final volume of 0.62 ml

	Radioactivity incorporated (dpm)	
	Proline	CO_2
Cell free extract	3210 ± 115	674 ± 35
Mitochondrial fraction	1595 ± 85	1126 ± 18
Microsomal fraction	73 ± 13	102 ± 4
Cytosol fraction	65 ± 30	39 ± 2

Values represent the mean ± SE for simultaneous triplicate incubations for 100 min.

Table 2. Requirements for proline synthesis in the crude mitochondrial fraction of the blowfly. The complete incubation system contained 0.5 ml of crude mitochondrial fraction (1.318 mg protein/ml), 100 µl each of 10 mM NADPH, 10 mM NADH and 100 mM ATP, and 10 µl each of 1 M $MgCl_2$ and 6.5 µCi/ml U-^{14}C-glutamate in a final volume of 0.82 ml

Omission	None	NADPH	NADH	ATP	Mg^{++}	All cofactors
Radioactivity (dpm)	2464 ± 116	316 ± 32	1758 ± 78	56 ± 12	46 ± 8	32 ± 8

Values represent the mean ± SE for simultaneous triplicate incubations for 100 min.

roacetic acid (TCA). For determination of ^{14}C labeled CO_2 evolved during incubation, and on the addition of the acid, the gas was trapped with 0.1 ml of approximately 1 M methyl benzethonium hydroxide in methanol[5]. For the preparation of the sample for ^{14}C amino acid analysis, the reaction mixture containing 0.1 ml of 10% TCA was centrifuged. The precipitate was washed with 1 ml of 3% TCA 3 times, and the washings were combined with the supernatant. After extraction of TCA with ether, the remaining mixture was applied to a column (4 × 0.5 cm) of Dowex 50 (H^+ form). Washed with 1 ml each of 10 mM and 1 mM HCl and water, the amino acids were eluted with 2 N ammonia solution. The eluate was evaporated to dryness and dissolved in 0.5 ml of 80% ethanol. One half or one fifth of the solution was applied to a TLC plate after addition of 2 µl of a standard amino acid solution.

Thin layer chromatography. To separate proline from other amino acids, 2-dimensional TLC was performed[6], and the amino acids were localized with ninhydrin[7]. The amount of ^{14}C in the silica gel was determined by solubilizing the sample with 0.5 ml of methanol per 10 ml of toluene base scintillation fluid.

Protein determination. Protein was determined by the method of Lowry et al.[8].

Results and discussion. Table 1 shows the subcellular distribution of the decarboxylation and synthetic activity of proline from labeled glutamate in the homogenate of the abdomen. In the case of cell free extract the radioactivity incorporated into proline for 100 min corresponded to about 2.2% of that of ^{14}C glutamate incubated. It is clear that proline synthesis occurs only in the mitochondrial fraction. The amount of ^{14}C-incorporation into proline by the mitochondrial, microsomal and cytosol fractions accounted for only 54% of the total incorporation by the cell free extract, but the reasons remain obscure. The incorporation of ^{14}C into proline needs ATP, Mg^{++} and NADPH as the cofactors (table 2). Unlike the situation in the rat[9], NADH can partially replace NADPH in the blowfly. From the evidence obtained, we concluded that proline biosynthesis from glutamate in the blowfly occurs in the mitochondria through the reactions almost the same as those of *E. coli*[10].

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Lack of conversion of C_{29}-phytosterols to cholesterol in the khapra beetle, *Trogoderma granarium* Everts

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Summary. Metabolic studies in which ^{3}H-sitosterol, ^{3}H-stigmasterol, and ^{14}C-desmosterol were administered by feeding and injection to the khapra beetle, *Trogoderma granarium* Everts, provided strong evidence that this insect is unable to dealkylate phytosterols and convert them to cholesterol.

We recently reported the sterol composition of the khapra beetle, *Trogoderma granarium* Everts, reared on a diet consisting of a mixture of wheat and brewers' yeast[1]. The relative percentages of cholesterol in the pupae and adults were 1.2 and 1.3%, and the campesterol levels were 67.0 and 59.4%, respectively. Cholesterol comprised about 0.5% of the total sterols present in the diet, whereas the phytosterols campesterol (25.0%), stigmasterol (5.1%), and sitosterol (69.4%) together accounted for 99.5% of the dietary sterols. These data indicated that little, if any, of the C_{28}- or C_{29}-phytosterols ingested by the insect were converted to cholesterol and that the increase in cholesterol and campesterol content of the insect tissues, compared to the diet sterols, may have occurred through selective uptake from the diet. Previous studies had shown that 2 phytophagous insect species, the Mexican bean beetle, *Epilachna varivestis* Mulsant[2], and the milkweed bug, *Oncopeltus fasciatus* (Dallas)[3], are unable to dealkylate phytosterols such as sitosterol or campesterol and produce cholesterol. The Mexican bean beetle saturates the dietary C_{28}- or C_{29}-phytosterols before dealkylating and produces mainly stanols and some lathosterol (Δ^7-cholestenol)[2]; The milkweed bug directly incorporates unchanged dietary sterols into its tissues[3].

The present studies were carried out to determine whether *T. granarium* larvae converted either of the radiolabeled C_{29}-phytosterols, ^{3}H-sitosterol or ^{3}H-stigmasterol, incorpo-