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### Changes in the free amino acids of the haemolymph of diapause and non-diapause pupae of the cotton bollworm, *Heliothis armigera* Hbn. (Lepidoptera: Noctuidae)

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**Summary.** The concentration of free amino acids in the haemolymph of non-diapausing and diapausing pupae of *Heliothis armigera* was investigated. 20 amino acids were detected in the haemolymph of the studied stages. Asparagine, glutamine, cystine, ornithine, histidine and valine were the predominant free amino acids at all stages. The diapause resulted in increased levels of most of the amino acids.

Insects are known to contain high levels of free amino acids in their haemolymph and tissues<sup>1</sup>. The significance of the high titre of free amino acids in insect haemolymph is still not fully understood. Different studies have shown that the titres of some of these amino acids change during metamorphosis<sup>2,3</sup>. The haemolymph of diapausing pupae of several Lepidoptera was found to contain abnormally high concentrations of several free amino acids as compared to non-diapausing species<sup>4</sup>. Jeffery et al.<sup>5</sup> also reported high amino acids in diapause larvae of *Pectinophora gossypiella*.

This paper presents quantitative data on the changes in the levels of free amino acids of the haemolymph of non-diapausing and diapausing pupae of the cotton bollworm, *Heliothis armigera*.

**Materials and methods.** *Heliothis armigera* was reared on green beans (*Phaseolus vulgaris*) in the laboratory according to Abdallah and Salama<sup>6</sup>. Haemolymph was collected from 2-day-old non-diapausing pupae, 15-week-old diapausing pupae and 18-week-old pharate adults by piercing the integument in the region of the 1st abdominal segment and microcapillary tubes were used to collect the haemolymph.

For the preparation of amino acid extracts, 0.5–1 ml of the haemolymph was deproteinized and freed from fats according to the method of Pant and Agrawal<sup>7</sup>. Free amino acids in the haemolymph extracts were separated and determined quantitatively by 2-dimensional paper chromatography according to the method detailed in a preceding paper by Boctor and Salem<sup>8</sup>. For each sample 6 chromatographic separations were carried out, and the average and the experimental error were calculated (table).

**Results and discussion.** The concentrations of 18 amino acids and 2 amides in the haemolymph of the non-diapausing and diapausing pupae of *Heliothis armigera* are listed in the table. There are some quantitative but no qualitative changes in the composition of the free amino acid pool of the non-diapausing and diapausing *Heliothis* pupae. Asparagine, glutamine, cystine, ornithine, histidine and valine are the predominant free amino acids at all stages, asparagine

is the most abundant amino acid. These data agree with those obtained with *Spodoptera littoralis* which showed that asparagine is characteristic of the studied pupae<sup>9</sup>. Glutamine, asparagine, histidine, ornithine and cystine were also found in high concentrations in *Spodoptera* pupae. In the present investigation diapause in *Heliothis* is associated with great increase of most of the amino acids which results in about 30% increase of the free amino acid pool of the diapausing pupae as compared with the non-diapausing

Free amino acids of the haemolymph of diapausing and non-diapausing pupae of *H. armigera*

Amino acids	Non-diapausing pupae (2-day-old)	Diapausing pupae (15-week-old)	Pharate adults (18-week-old)
Glycine	155.4 ± 5.5	188.2 ± 9.6	220.5 ± 15.9
Alanine	60.0 ± 3.2	122.8 ± 8.4	138.4 ± 8.4
Serine	135.9 ± 7.4	225.8 ± 14.7	220.4 ± 15.0
Threonine	176.3 ± 12.3	282.0 ± 12.1	259.3 ± 17.7
Valine	327.8 ± 16.5	491.1 ± 31.4	553.9 ± 22.4
Leucine	222.4 ± 8.9	324.4 ± 12.7	343.1 ± 17.5
Aspartic acid	29.0 ± 1.8	43.0 ± 2.8	75.1 ± 3.4
Asparagine	1185.3 ± 36.2	1192.8 ± 46.5	1281.6 ± 95.8
Glutamic acid	67.4 ± 3.0	45.8 ± 3.8	35.5 ± 2.6
Glutamine	362.2 ± 19.5	1064.5 ± 80.9	631.0 ± 46.9
Proline	126.7 ± 4.5	175.2 ± 10.3	173.7 ± 12.7
Lysine	281.3 ± 14.7	222.6 ± 15.0	287.2 ± 14.5
Arginine	72.0 ± 2.8	59.7 ± 4.7	53.6 ± 3.6
Histidine	267.9 ± 12.5	568.4 ± 35.2	288.5 ± 12.5
Tyrosine	63.9 ± 4.6	89.7 ± 3.6	73.6 ± 5.7
Citrulline	54.4 ± 3.3	75.3 ± 5.3	54.2 ± 2.4
Ornithine	339.9 ± 16.9	891.8 ± 70.5	346.4 ± 17.5
Methionine	109.5 ± 8.1	129.5 ± 3.8	165.3 ± 9.6
Cystine	934.6 ± 70.9	1047.0 ± 41.8	628.4 ± 30.5
Phenylalanine	112.4 ± 7.3	145.8 ± 9.2	157.6 ± 11.7
Totals	5084.3	7385.4	5987.3

The values are given as  $\mu$ moles amino acids/100 ml of haemolymph.

ones. Glutamic acid, arginine and lysine are the only amino acids which do not increase in the diapausing pupae. The amino acids that increase greatly are glutamine, histidine, ornithine and alanine. The concentrations of these amino acids are at least double or triple those of non-diapausing pupae.

The great increase in the free amino acid pool of the haemolymph of the diapausing pupae of *Heliothis* is in general agreement with high aminoacidemia found in several diapause pupae<sup>4</sup>. Somme<sup>10</sup> also reported high ninhydrin-positive compounds in diapause larvae and pupae of 2 Lepidoptera. Mansingh<sup>3</sup> found that diapause was associated with enormous accumulation of certain amino acids which resulted in almost doubling of the free amino acid pool of the diapausing pupae of *Antheraea pernyi*.

The termination of diapause and initiation of pharate adult development of *Heliothis* deplete the amounts of threonine, glutamine, glutamic acid, arginine, histidine, citrulline, ornithine and cystine. The total amount of haemolymph free amino acids varies greatly in the studied pupae. From a minimum level of 5084.3  $\mu$ moles/100 ml of haemolymph in non-diapausing pupae, the total concentration of the free amino acid pool increases to 7385.4  $\mu$ moles/100 ml of haemolymph in diapausing pupae and then decreases to 5987.3  $\mu$ moles/100 ml of haemolymph in pharate adults. Similar patterns of changes in the concentration of individual free amino acids and in the total content of free amino acids of haemolymph have been reported in diapausing and non-diapausing forms of *Antheraea pernyi*<sup>3</sup> and during pupal development of some insects<sup>9,11,12</sup>. Amino acid levels can also be affected by changes in the levels of other substances such as carbohydrates and their metabolic intermediates and derivatives, which also change during metamorphosis<sup>10,13</sup>. Glucose and its metabolic intermediates can provide the carbon skeleton for the synthesis of amino acids in insects<sup>14</sup>. The presence of both ornithine and citrulline in *Heliothis armigera* haemolymph suggests the possible existence of an ornithine  $\rightarrow$  citrulline path in this species. However, there is little evidence for such a pathway or for a complete ornithine cycle in insects<sup>15,16</sup>. The varia-

tion in glutamine concentration in *Heliothis* pupae agrees with that obtained with *Antheraea*<sup>3</sup> and may reflect the role of this amino acid in nitrogen transport during pupal-adult transformation. The histidine concentration which is the highest in diapausing *Heliothis* pupae decreases to almost half in pharate adults. This supports the finding of Mansingh<sup>3</sup> that histidine concentration which was almost unaffected during the metamorphosis of the non-diapausing forms of *Antheraea* increased throughout diapause then depleted to almost half in pharate adults. The results of the present study suggest pronounced changes in the concentration of free amino acids in the haemolymph of the diapausing pupae as compared with the non-diapausing ones. This difference suggests that amino acids are involved in diapause physiology perhaps with respect to energy production.

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## 'Atrichosis', a new hairless gene with cyst formation in rats

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**Summary.** A new hairless gene in the Donryu strain of the rat has been designated atrichosis (*at*). Histological sections demonstrated that atrichotic skin was quite similar to that found in human skin with multiple follicular cysts, which is one type of skin tumor in humans.

Hypotrichosis is known to be a heritable trait. 5 genes in rats, designated hairless (*hr*), naked (*n*), fuzzy (*fz*), hypotrichosis (*hy*) and nude (*nu*), have been shown to influence hair follicle development<sup>1-5</sup>. A new hypotrichosis gene of rats which is reported here causes multiple follicular cyst; this gene may provide more meaningful data for studies of impaired hair growth.

**Genetics.** The hypotrichosis mutant was found at Ehime University School of Medicine. The mutation arose in a closed but not deliberately inbred stock of the Donryu strain. 2 hairless animals, females, appeared in the stock, together with 19 phenotypically normal animals, 7 males and 12 females. The original hypotrichosis females died without producing offspring. The normal-coated pair were

mated and 6 matings produced hypotrichosis animals. In this way 9 heterozygous animals, 3 males and 6 females, were identified and they formed the basis of the atrichosis stock. The segregation of atrichosis from intercross and backcross matings is presented in the table. Atrichosis rats cannot be classified at birth and there is no delay in the

### Segregation of atrichosis

Type of mating	No. of matings	Phenotype of progeny			$\chi^2$	<i>p</i>
		+	<i>at</i>	Total		
+ <i>at</i> ♂ × + <i>at</i> ♀	20	129	49	178	0.0635	0.80
<i>atat</i> ♂ × + <i>at</i> ♀	16	68	78	146	0.0613	0.80