CHAPTER : V

CHANGES IN FREE AMINO ACIDS DURING METAMORPHOSIS.

INTRODUCTION

Metamorphosis includes a series of ontogenetic events through which the insect transforms from the larva to the adult. The transformation process involves mainly the destruction of most larval tissues and organs (histolysis) and the differentiation of imaginal structures (histogenesis). The biochemistry of insect metamorphosis has been the object of many investigations and the results also allow some comparisons with structural changes.

Considering the profound changes which the developing insect undergoes at the time of metamorphosis, it is perhaps surprising that only minor changes in the pool size of free amino acids during this period have been detected. As Levenbook has pointed out, it must be admitted that no direct evidence has so far been brought forward to show that variations in free amino acids are really in association with the process of metamorphosis (Levenbook, 1962). It is true that the developing pupa represents a closed system and thus its concentration levels of free amino acids could be considered as indicating the balance between histolysis and histogenesis. In general, however,

the fluctuation is too small to account for such drastic morphological changes, unless it is assumed that the amino acids produced by histolysis are immediately used for synthetic purpose. In many cases the variation of the amino acid concentration also does not fit the time axis of the major morphogenetic events. As matter of fact there is no experimental proof that the decomposition of larval proteins actually proceeds as far as the production of amino acids prior to their being utilized for the formation of adult proteins.

As noted by Agrell (1952), some tissue proteins of the larval structures in Calliphora may be decomposed only to the level of peptides which are bound with phospholipids and enter probably directly into cellular components of the adult. A close parallelism between the peptide fraction and the morphological changes has been emphasized (Agrell, 1964). It is true that tracer studies on both Sphinx ligustri (Brieteux-Gregoris et.al., 1957) and Hyalophora (Skinner, 1960) indicate a high incorporation of amino acids into pupal tissues. But these results show only the ability of developing pupa to take up free amino acids and do not necessarily mean that it is the major pathway of protein synthesis during histogenesis. It

is also clear that besides histolysis and histogenesis variations due to inter-conversion and other metabolic connections of amino acids cannot be neglected. Thus the causal relationship between changes in free amino acids and metamorphosis are still open to question. This chapter reports the results of the study in which the alterations in total and individual free amino acids in Chrysomyia pharate pupal development and pharate adult development have been worked out.

MATERIAL AND METHODS

Alterations in the free amino acids were worked out in the whole pharate pupae and pharate adult developmental stages. The rearing of Chrysomyia was carried out as described in chapter II. The pharate pupae and pharate adult developmental stages as well as freshly emerged adult selected for study as mentioned in the chapter on material and methods. The extraction of free amino acids, chromatographic separation and quantitative estimation of free amino acids were carried out as described in Chapter II.

OBSERVATIONS

The stage specific pattern of free amino acids is illustrated in plate No.10. Occurrance of various free amino acids in the pharate pupa and pharate adult developmental stages and freshly emerged young adult

are shown in Table No.5. Alterations occuring in the total free amino acid content per 100 mg of wet weight of insect tissue during pupation and of freshly emerged young adult are shown in plate No.11. The alterations occuring in the individual free amino acids are shown in the plate number 12 and 13. The quantitative alterations in the total and individual free amino acids have been shown in Table No.6.

1. <u>The pattern of free amino acids during Metamorphosis</u>.

As shown in the plate No.10 pharate pupa, pharate adult developing stages and the freshly emerged young adult of <u>Chrysomyia</u> show the presence of at least seventeen free ninhydrin positive compounds. These include cystine, histidine, lysine, β-alanine, serine, aspartic acid, proline, valine, tyrosine, methionine, isoleucine, phenyl-alanine, leucine. At a comparative level, glutamic acid, glycine, histidine, lysine, proline were in high concentrations and formed the major components of the total free amino acids. Phenyl alanine and leucine joined them near about a ecdysis. β-amino butyric acid could not be detected within the amounts of material used. Cystine occur in very low concentration. In general except β-amino butyric acid there is no qualitative difference in

PLATE NO 10

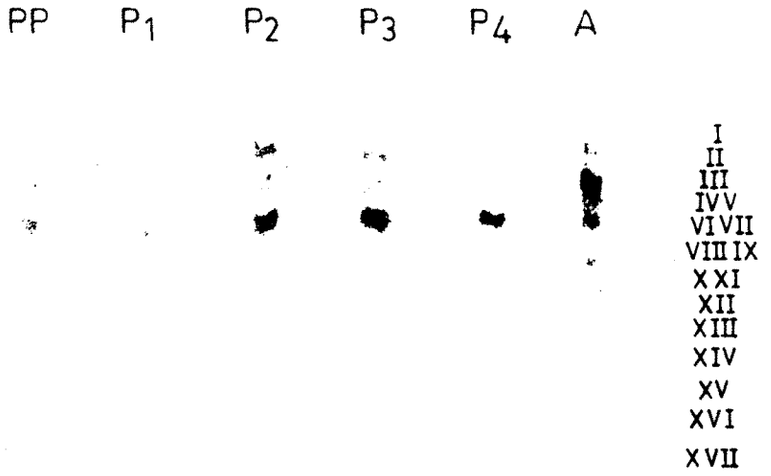


FIG-1

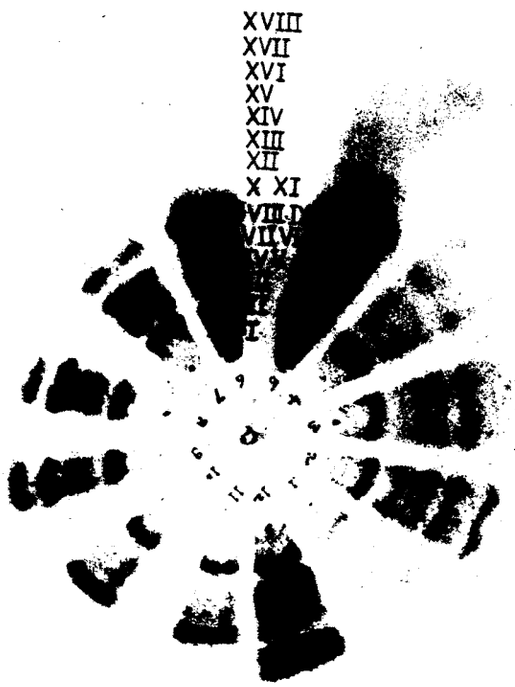


FIG-2

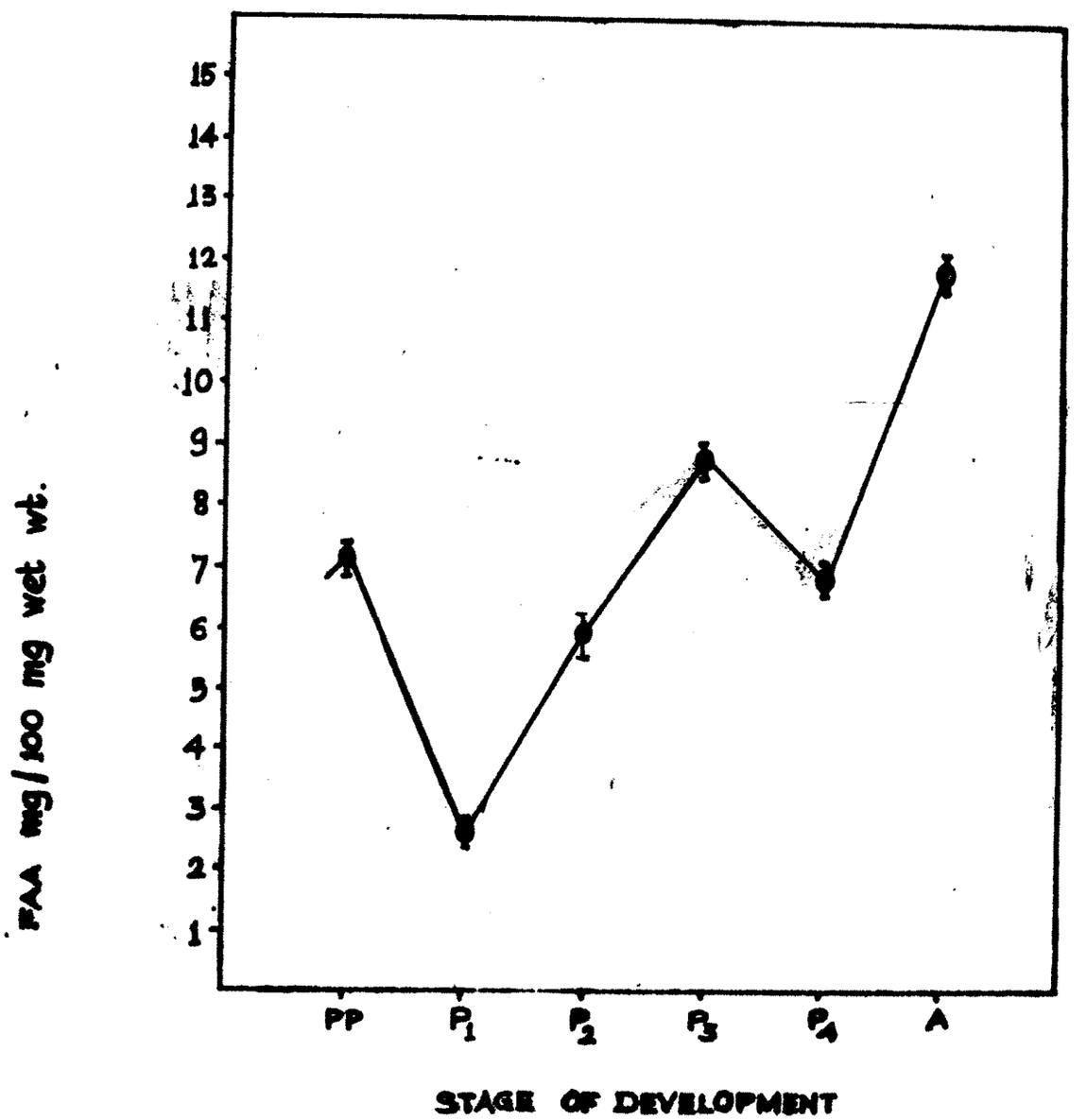
TABLE No. 5

FREE AMINO ACIDS IN METAMORPHOSIS OF BLOWFLY CHRYSOMYIA RUFIFACIES.

	AMINO ACIDS	PHARATE PUPA	PHARATE ADULT DEVELOPMENT				ADULT
		PP	P_1	P_2	P_3	P_4	A
1	Cystine	Trace	Trace	Trace	Trace	Trace	Trace
2	Histidine	++	+	+	++	+	+++
3	Lysine	+	+	+	++	++	+++
4	-Alanine	++	+	+	+	+	+++
5	Serine	++	+	+	+	+	++
6	Aspartic acid	++	+	+	+	+	++
7	Threonine	++	+	+	+	+	++
8	Glutamine	++	+	+	+	+	++
9	Glycine	+	+	+	+	++	++
10	Glutamic acid	+	+	+	+	++	++
11	Proline	++	++	++	+++	+++	++
12	-Amino-N-butyric acid	-	-	-	-	-	-
13	Valine	+	+	++	++	+	++
14	Tyrosine	+	+	++	++	++	++
15	Methionine	+	+	++	++	+	++
16	Isoleucine	+	+	++	++	+	++
17	Phenyl alanine	+	+	+	+	+	++
18	L-leucine	+	++	++	+++	+	++

* Tr - Trace quantity,
\+ Minimum,
++ Moderate,
+++ Maximum.

PLATE - 11


FAA mg/100 mg wet wt.
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
PP
P1
P2
P3
P4
A
STAGE OF DEVELOPMENT

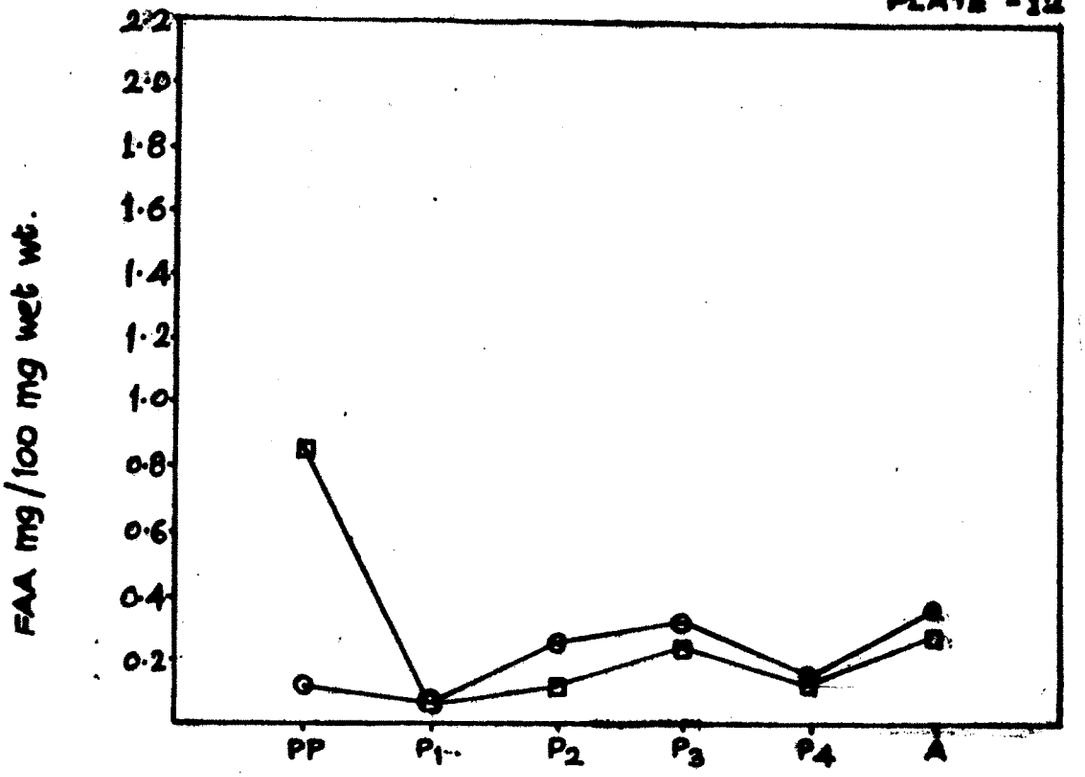

FAA mg/100 mg wet wt.
2·2
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
PP
P1
P2
P3
P4
A
STAGE OF DEVELOPMENT

FIG. 1

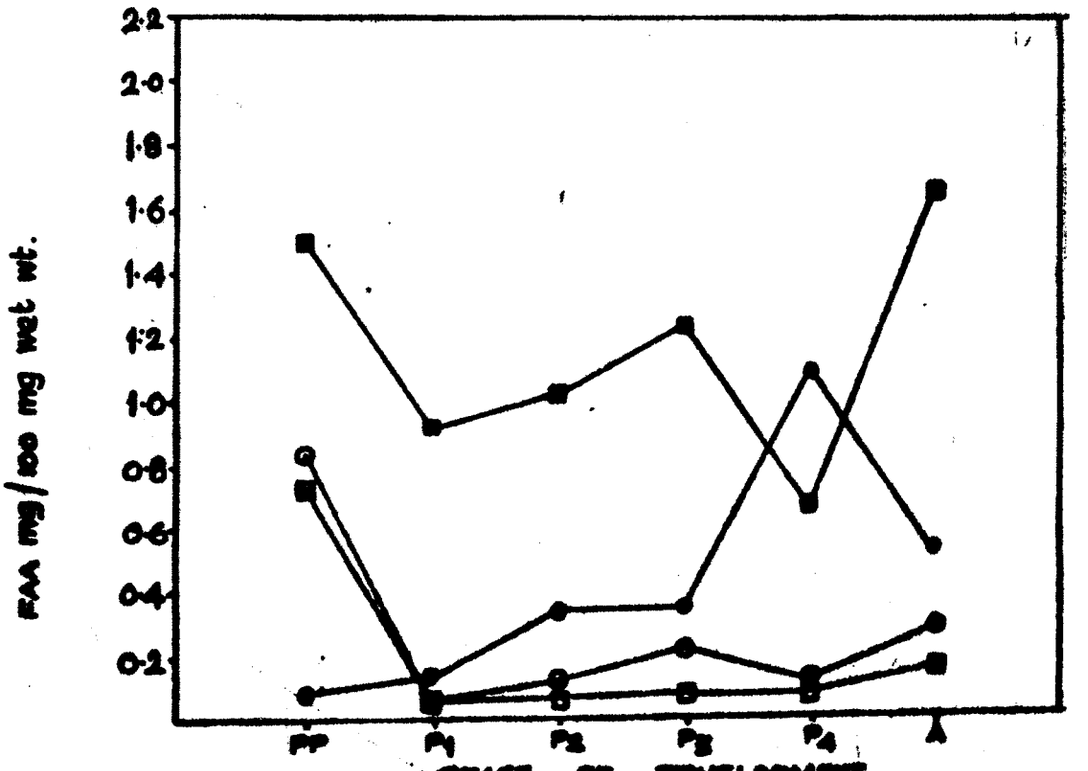

FAA mg/100 mg wet wt.
2·2
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
PP
P1
P2
P3
P4
A
STAGE OF DEVELOPMENT

FIG. 2

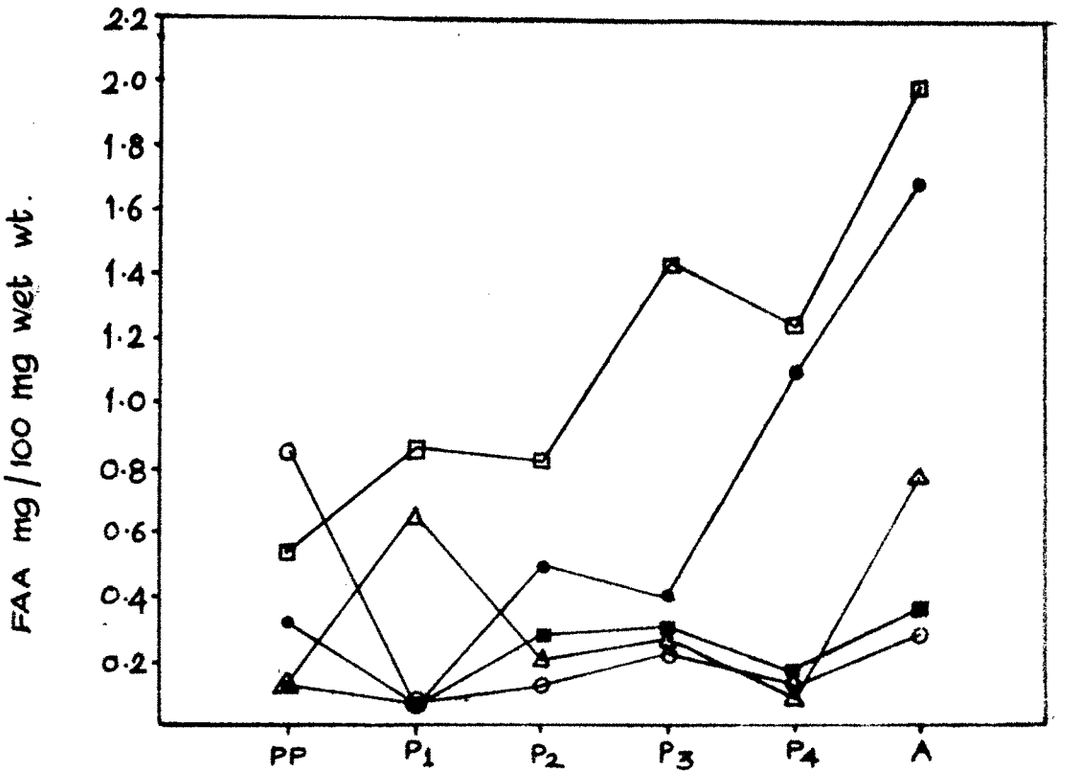

FAA mg / 100 mg wet wt.
2·2
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
PP
P1
P2
P3
P4
A
STAGE OF DEVELOPMENT

FIG. 1

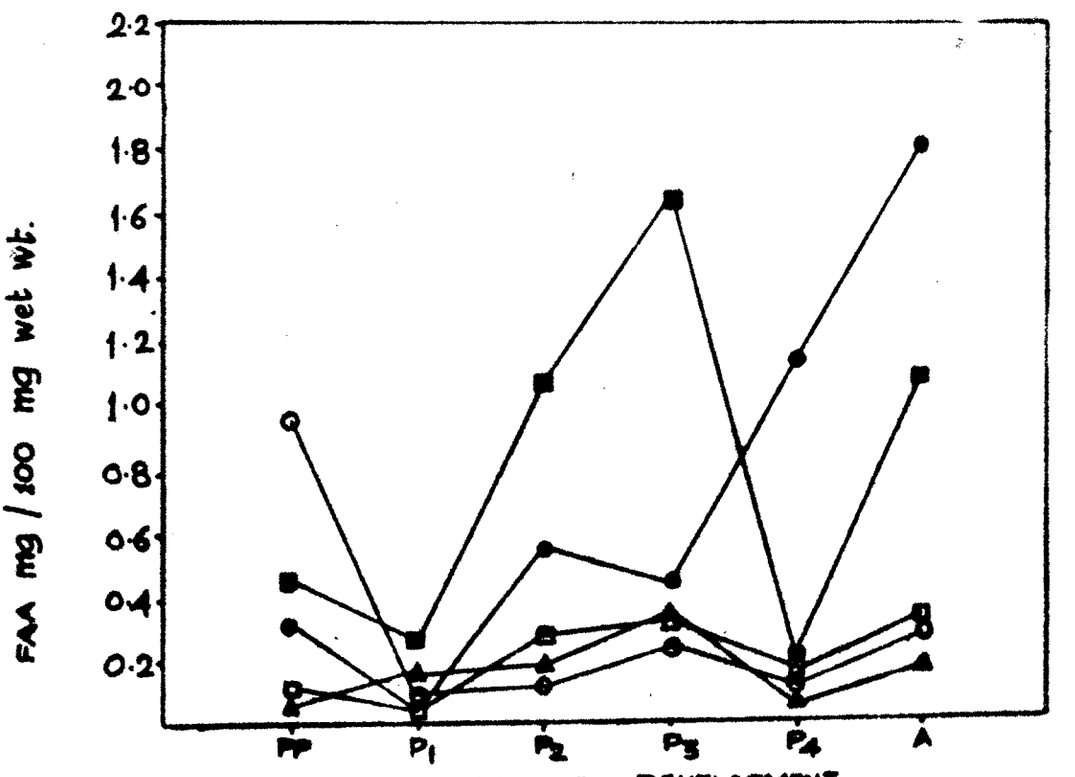

FAA mg / 100 mg wet wt.
2·2
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
PP
P1
P2
P3
P4
A
STAGE OF DEVELOPMENT

FIG. 2

TABLE No. 6

FREE AMINO ACIDS IN METAMORPHOSIS OF BLOWFLY CHRYSOMYIA RUFIFACIES.

	AMINO ACIDS	PHARATE PUPA	CHARATE ADULT DEVELOPMENT				ADULT
		PP	P_1	P_2	P_3	P_4	A
1	Cystine	Trace	Trace	Trace	Trace	Trace	Trace
2	Histidine	1.5030	0.9281	1.0140	1.2680	0.6583	1.6730
3	Lysine	0.5470	0.8894	0.8222	1.4360	1.237	2.07
4	β-Alanine	0.8686	0.0741	0.1239	0.2339	0.1183	0.2947
5	Serine	0.8580	0.0746	0.1239	0.2334	0.1183	0.2962
6	Aspartic acid	0.8650	0.075	0.1249	0.2315	0.1211	0.2898
7	Threonine	0.7410	0.050	0.0968	0.1814	0.084	0.1640
8	Glutamine	0.8510	0.073	0.1198	0.2275	0.1241	0.2911
9	Glycine	0.3080	0.0580	0.5694	0.4320	1.111	1.7170
10	Glutamic acid	0.3120	0.057	0.8711	0.4422	1.134	1.8110
11	Proline	0.0929	0.1160	0.3321	0.3456	1.111	0.5284
12	B-Amino-N-Butyric acid	-	-	-	-	-	-
13	Valine	0.1141	0.0800	0.2741	0.3066	0.1646	0.3610
14	Tyrosine	0.1162	0.083	0.2711	0.3122	0.1596	0.371
15	Methionine	0.1124	0.079	0.2680	0.321	0.148	0.364
16	Isoleucine	0.4500	0.2784	1.290	1.659	0.1900	0.7927
17	Phenyl alanine	0.1228	0.6626	0.1084	0.2968	0.0955	0.7927
18	Leucine	0.0800	0.1857	0.1898	0.3456	0.071	0.1720
	TOTAL FAA (Excluding NH_3)	7.9420	3.7739	6.2917	8.2801	6.6465	12.3079

* Values are expressed as mg/100 mg of wet weight of insect tissues.

the free amino acid content of the pharate pupa and pharate adult development stages as well as in freshly emerged adult. The empty puparia contained very negligible quantity of free amino acids.

2. Quantitative changes in total concentrations and in the different groups of amino acids during metamorphosis.

As can be seen from the curve in plate No.11 the total concentration of free amino acids remain essentially unchanged when larva transformed into prepupal condition (pharate pupa). With the onset of metamorphosis, a marked decrease in total concentration of free amino acid could be noticed. This decrease in total concentration may be due to the marked decrease in glycine, β-alanine, serine and glutamic acid. However, before adult emergence the levels of all these amino acids showed progressive increase and almost re-attained their initial concentration of larval stage, and traced U-shaped patterns except lysine and β-alanine which depicted a slight increase over the original larval concentration. Glycine and serine levels fell nearly by half and three quarters respectively during the first half of pupal development. The amino acid found to change the most was glycine which decreased to about three fold during transformation of 4th day larva to

pharate pupa and fourfold after formation of the teneral phase (white pupa) but with onset of metamorphosis it increased continuously reaching a peak value in freshly emerged adult. Similarly lysine and glutamic acid and proline increased continuously during metamorphosis. Glutamic acid and glutamine underwent decrease followed by subsequent increase in concentration during the pharate adult development.

DISCUSSION

Considering the profound changes which the developing insect undergoes at the time of metamorphosis, it is perhaps surprising that only minor changes in the total concentration of pool size of free amino acids during this period have been detected. A sharp reduction in the total concentration of these compounds in Chrysomyia occur at the initiation and during late metamorphosis. The first decline may be related to the histogenesis of the hypodermal tissue, especially the formation of imaginal buds, and that the second one coincides with the development of muscles in the thorax. The highest concentration is found at about the 3rd day of pharate adult development, at which stage the breakdown of larval tissues reaches a maximum. Similar results have been obtained for Calliphora (Agrell, 1949) and Ephestia (Chen and Kuhn, 1956) and for Culex (Chen 1958a).

The observed increase in lysine, phenyl alanine and leucine titres following pupation and their decrease during the later stage of metamorphosis are suggestive of their accumulation in the first half and utilization during the rest of the metamorphosis period. Despite this at the time of emergence of fly their titres were at a higher level than those during the larval stage. Histidine decreases on the 1st day of pharate adult development but gradually attains increased concentration on the 2nd & 3rd day of pharate adult development. A second drop in the histidine concentration is observed on 4th day of pharate adult development but its level shot up suddenly to a peak concentration in the body of freshly emerged adult. Glutamic acid exhibited unique pattern in that, after getting utilized initially, it's level increased showing a sudden peak during the late metamorphosis. The variation patterns usually obtained for glutamic acid and glutamine during development of insects have been found to be diverse for each insect investigated.

On transformation of the larva to the white pupa the titres of lysine, phenyl alanine, increased and those of glycine, glutamic acid, serine methionine, glutamine and proline is decreased. The concentration of β-alanine which is very low in the 4th day larva

abruptly increased in the white pupa to a maximal concentration and this could be related to the cuticular protein formation which occurs at this stage.

The changes in the individual free amino acids distinctly indicate their trend to compensate one another and this consequently results in the maintenance of an overall constant level in the total free amino acids during metamorphosis.

The changes in the ethanolamine and phosphoethanolamine, serine and phosphatidyl serine may be related to breakdown and biosynthesis of phospholipids. Phosphatidyl ethanolamine is found to the major phospholipid component of Chrysomyia (Mankapure and Sawant, 1980).

High level of proline has been attributed to its special role in flight metabolism (Bursell, 1963). Methionine/Methionine sulphoxide has been suggested to be involved in the redox potential of biological systems (Dent, 1947). Explanations forwarded by earlier investigatérs for observed changes and functions of free amino acids are mostly speculative and lack experimental evidence. Recently, Chen and Levenbook (1966) demonstrated in Phormia regina with

labelled compounds a very low rate of protein degradation in the haemolymph of white pupa and pre-pupal larva of this insect. Moreover, since the pre-pupal tissues evidenced an insignificant rate of free amino acid participation in adult tissue formation in the pupa, it was considered that haemolymph proteins were being used infact after partial degradation (perhaps to peptides) by the tissue cells for adult tissue formation. Thus, it is probable that in insects, during metamorphosis, free amino acids are involved in pathways other than protein synthesis.

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