CHAPTER : VI

CHANGES IN FREE AMINO ACIDS IN FAT BODY AND HAEMOLYMPH DURING LARVAL GROWTH AND METAMORPHOSIS.

INTRODUCTION

A critical review of the biochemical changes occurring during insect development, growth and metamorphosis, which have been recently reviewed by Agrell and Lundquist (1973), reveals that significant alterations take place in the amounts of nucleic acids, proteins, carbohydrates, lipids and enzymes during embryogenesis, larval growth and metamorphosis. There is also a large body of literature on the free amino acids in insects, but most studies are limited to one or other phase of insect development i.e. embryogenesis, larval growth or metamorphosis. Secondly most of the aforementioned work has been carried out with the experiments in which homogenates can give useful general information concerning the occurrance, quantity and interconversion of metabolites, they reveal very little about location and transfer within the insect.

During recent years increasing attention has been paid to the possibility that insect fat body may be a site of intermediary metabolism as distinct from its mear passive role of serving as a depot for the storage of proteins, carbohydrates and lipid reserves. A recurrent suggestion is that the fat body may in some respects be analogous to the mammalian liver.

The haemolymph is the only circulating fluid, and fills the body cavity, or haemocoel. It is separated from the cellular tissues by only a thin permeable connective tissue membrane, and is maintained in circulation by a tubular, dorsal heart, that is some times assisted by accessory pulsatile organs in the limbs. Haemolymph comprises about 10% -- 40% of the body's volume. It is collected by dropping from a cut in the cuticle, or when the volume is small, directly into a capillary pipette. The haemolymph is a biochemically rich solution, very different from vertebrate plasma, with widely varying proportions of inorganic ions, high levels of free amino acids, usually a rather high level of trehalose, and some times substantial amounts of other solutes such as organic phosphates, citrate, glycerol, and peptides (Wyatt, 1961; Jeuniaux and Florkin, 1974). In intimate association with the haemolymph is a cellular tissue, the fat body, which combines many of the roles of the liver and adipose tissue of vertebrates. The concentration of soluble components in insect haemolymph is extraordinarily variable. In most insects a substantial fraction of the osmotic activity is made up by free amino acids. This chapter reports, the results of the study of alterations in free amino acids in these two

important organ systems during larval growth and metamorphosis of *Chrysomyia*.

MATERIAL AND METHODS

Changes in the total free amino acids and the individual components were studied in fat body and haemolymph during larval growth and metamorphosis. The isolation of fat body and collection of haemolymph were carried out as described in the chapter on material and methods (Chapter II). The stages of larval growth and pharate adult development were selected for such a study as mentioned in the chapter on material and methods.

OBSERVATIONS

The stage specific pattern of free amino acids of fat body and haemolymph during larval growth and metamorphosis is illustrated in plate No.14 occurrance of free amino acids in the fat body and haemolymph during larval growth and metamorphosis are shown in Table No.7 and 8. Alterations occurring in the total free amino acids per 100 mg of fresh tissue weight in the fat body are shown in the plate No.16. The alterations occuring in the various individual free amino acids in the fat body are shown in plate No. 17 to 20 respectively. Alterations occuring in the

PLATE NO 14

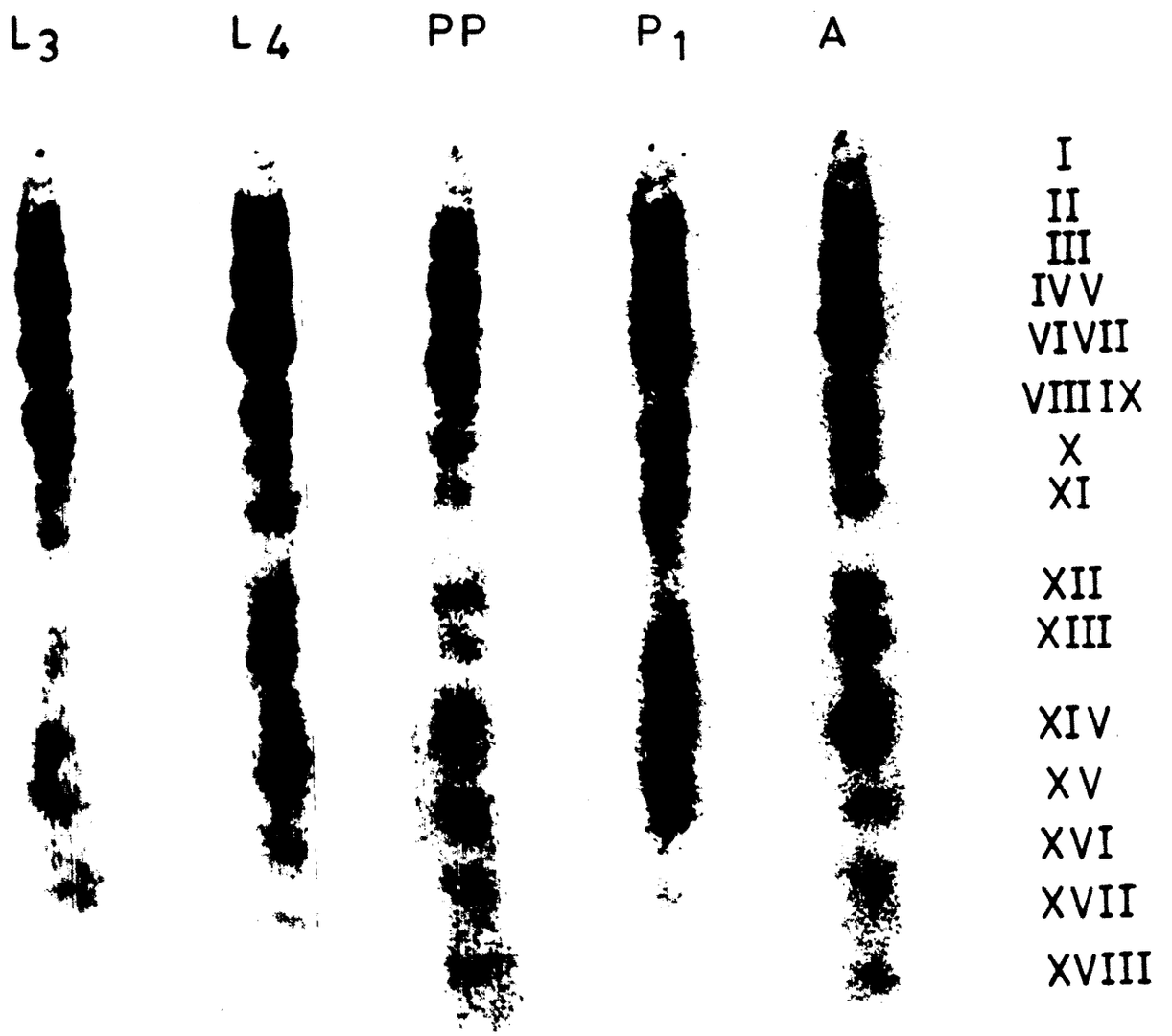
L3
L4
PP
P1
A
I
II
III
IVV
VIVII
VIII IX
X
XI
XII
XIII
XIV
XV
XVI
XVII
XVIII

PLATE NO 15

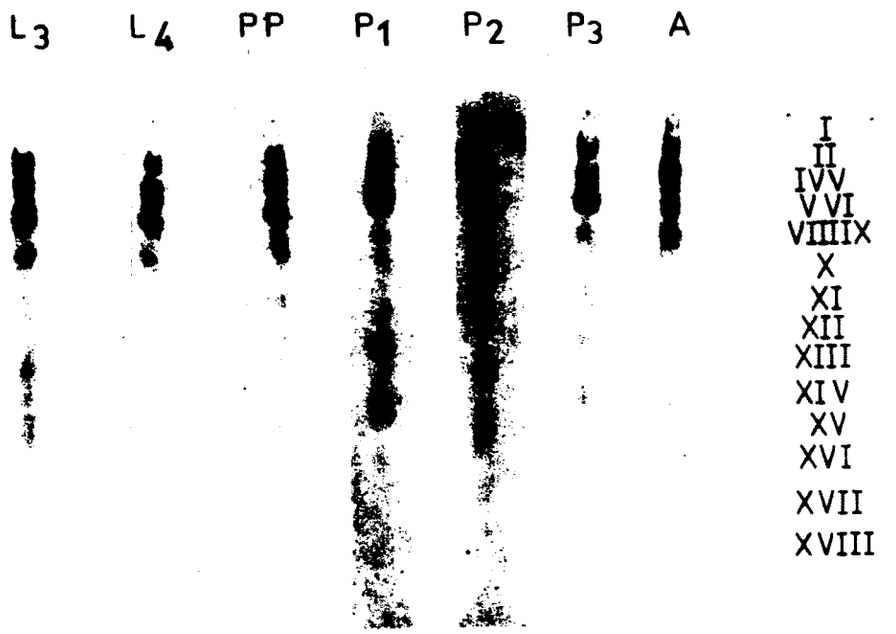

FIG-1

FIG-2

TABLE No. 7

FREE AMINO ACIDS IN FAT BODY OF CHRYSOMYIA RUFIFACIES DURING LARVAL GROWTH AND METAMORPHOSIS.

	AMINO ACID	LARVAL	GROWTH	PHARATE PUPA	PHARATE ADULT DEVELOPMENT.	ADULD
		L_3	L_4	pp	P_1	A
1	Cystine	Trace	Trace	Trace	Trace	Trace
2	Histidine	Trace	Trace	Trace	+++	Trace
3	Lysine	++	Trace	Trace	+++	+
4	β-Alanine	++	++	++	+++	+
5	Serine	++	++	++	+++	+
6	Aspartic acid	++	++	++	+++	+
7	Threonine	++	++	++	+++	+
8	Glutamine	++	++	++	+++	+
9	Glycine	++	+++	+++	+++	+
10	Glutamic acid	++	+++	+++	+++	+
11	β-Amino-N-Butyric acid	+++	-	-	-	-
12	Proline	-	+++	+++	++	+
13	Valine	+++	+++	+++	Trace	+
14	Tyrosine	+++	+++	+++	Trace	+
15	Methionine	+++	+++	+++	Trace	+
16	Isoleucine	+++	+++	+++	++	+
17	Phenyl alanine	+++	+++	++	+++	+
18	Leucine	+++	Trace	Trace	Trace	Trace

* Values are expressed as mg/100 mg wet wt. of Fat body.

TABLE No. 8

FREE AMINO ACIDS IN HAEMOLYMPH OF CHRYSOMYIA RUFIFACES DURING LARVAL GROWTH AND METAMORPHOSIS.

	AMINO ACIDS	LARVAL GROWTH		PHARATE PUPA	PHARATE ADULT DEVELOPMENT			ADULT
		L_3	L_4	PP	P_1	P_2	P_3	A
1	Cystine	Trace	Trace	Trace	Trace	Trace	Trace	Trace
2	Histidine	+	Trace	Trace	++	+++	Trace	Trace
3	Lysine	+++	+	+	+	++	Trace	+
4	-Alanine	+++	+	+	+	++	Trace	+
5	Serine	+++	+	+	+	++	Trace	+
6	Aspartic acid	+++	+	+	+	++	Trace	+
7	Threonine	+++	+	+	+	++	Trace	+
8	Glutamine	+++	+	+	+	++	Trace	+
9	Glycine	++	+	+	+	+++	+++	++
10	Glutamic acid	++	+	+	+	+++	+++	++
11	-Amino-N-butyric acid	+++	+	+	-	-	-	-
12	Proline	++	+	+	+	++	+++	++
13	Valine	+++	+	+	+	++	++	+
14	Tyrosine	+++	+	+	+	++	+++	+
15	Methionine	+++	+	+	+	++	+++	+
16	Isoleucine	+++	+	+	+	+++	+++	++
17	Phenyl alanine	++	+	+	+	++	+++	++
18	Leucine	Trace	+	+	Trace	Trace	+++	Trace

* Values are expressed as mg/100 mg of Haemolymph.

PLATE No. 16

Alterations in total free amino acids in the fat body of Chrysomyia during larval growth and metamorphosis. The amounts of Free amino acids expressed in mg/100 mg fresh weight of fat bodies. L_3, L_4, PP, P_1 and A refer stages of larval growth and metamorphosis 3rd day larva, 4th day larva, pharate pupa, 1st day pupa, and Adult e respectively.

PLATE - 16

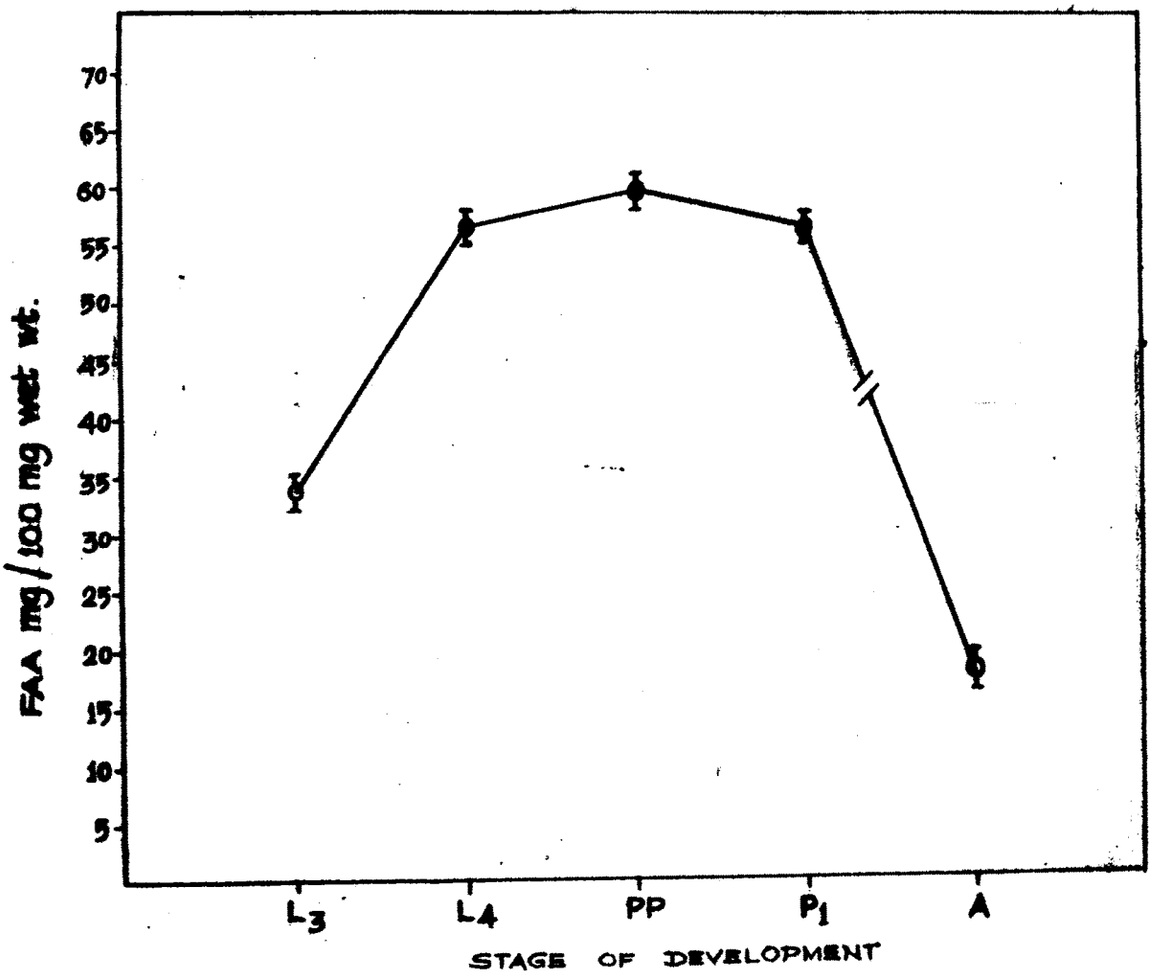
FAA mg/100 mg wet wt.
70
65
60
55
50
45
40
35
30
25
20
15
10
5
L3
L4
PP
P1
A
STAGE OF DEVELOPMENT

PLATE No. 17

Alterations in the individual free amino acids in the Fat body Chrysomyia during larval growth and metamorphosis. The amounts of all the free amino acids are expressed in mg/100 mg fresh weight of Fat bodies. Various stages of larval growth and metamorphosis are as shown in plate No.14.

△ -△ = Serine

● -● = Glutamic acid

□ - □ = Tyrosine

○ - ○ = Isoleucine

▲ — ▲ = Leucine.

PLATE - 17

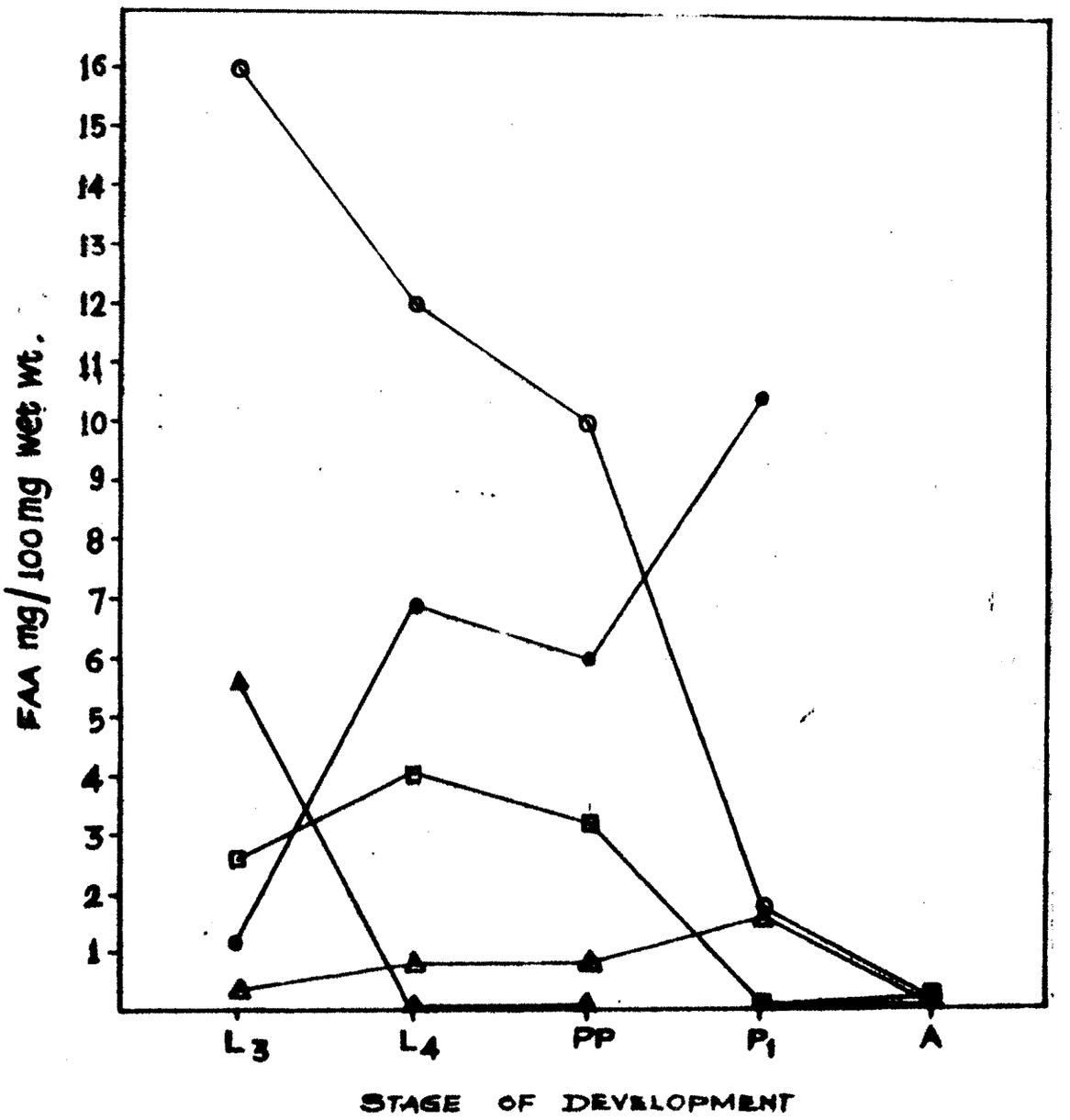
FAA mg/100 mg wet wt.
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
L3
L4
PP
P1
A
STAGE OF DEVELOPMENT

PLATE No. 18

Alterations in the individual free amino acids in the Fat body of Chrysomyia during larval growth and metamorphosis. The amounts of all the free amino acids are expressed in mg/100 mg fresh weight of fat bodies. Various stages of Larval growth and metamorphosis are as shown in plate No.14.

○ - ○ = Glutamine
● — ● = Proline
■ - ■ = Histidine
□ - □ = Threonine.

PLATE - 18

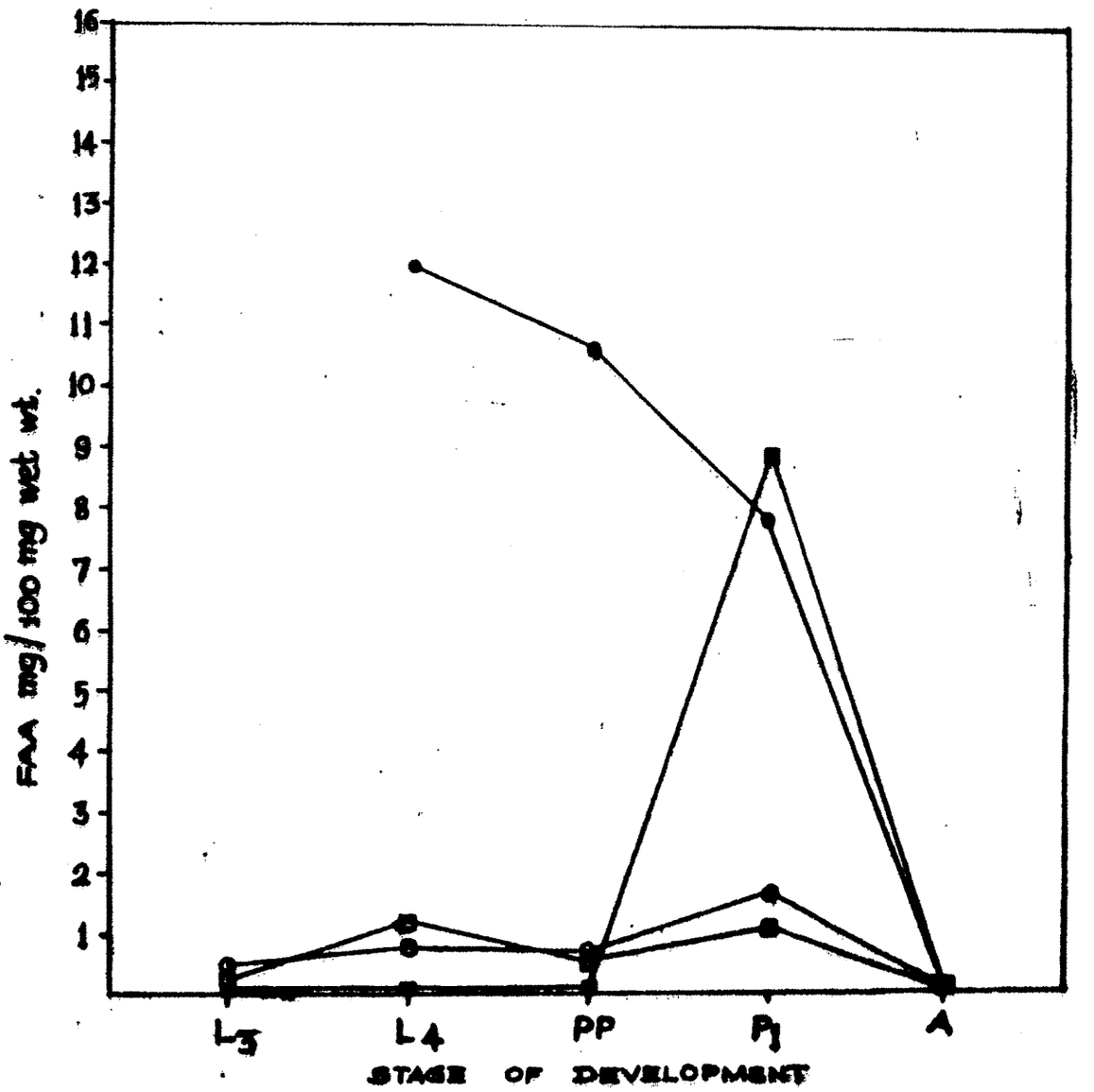
FAA mg/100 mg wet wt.
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
L3
L4
PP
Pj
A
STAGE OF DEVELOPMENT

PLATE No. 19

Alterations in the individual free amino acids in the fat body of Chrysomyia during larval growth and metamorphosis. The amounts of all free amino acids are expressed in mg/100 mg fresh weight of fat bodies various stages of Larval growth and metamorphosis are as shown in plate No.14.

■ - ■ = β -Alanine
△ - △ = Valine
□ - □ = Glycine
○ - ○ = Lysine
● - ● = Phenyl alanine.

PLATE - 19

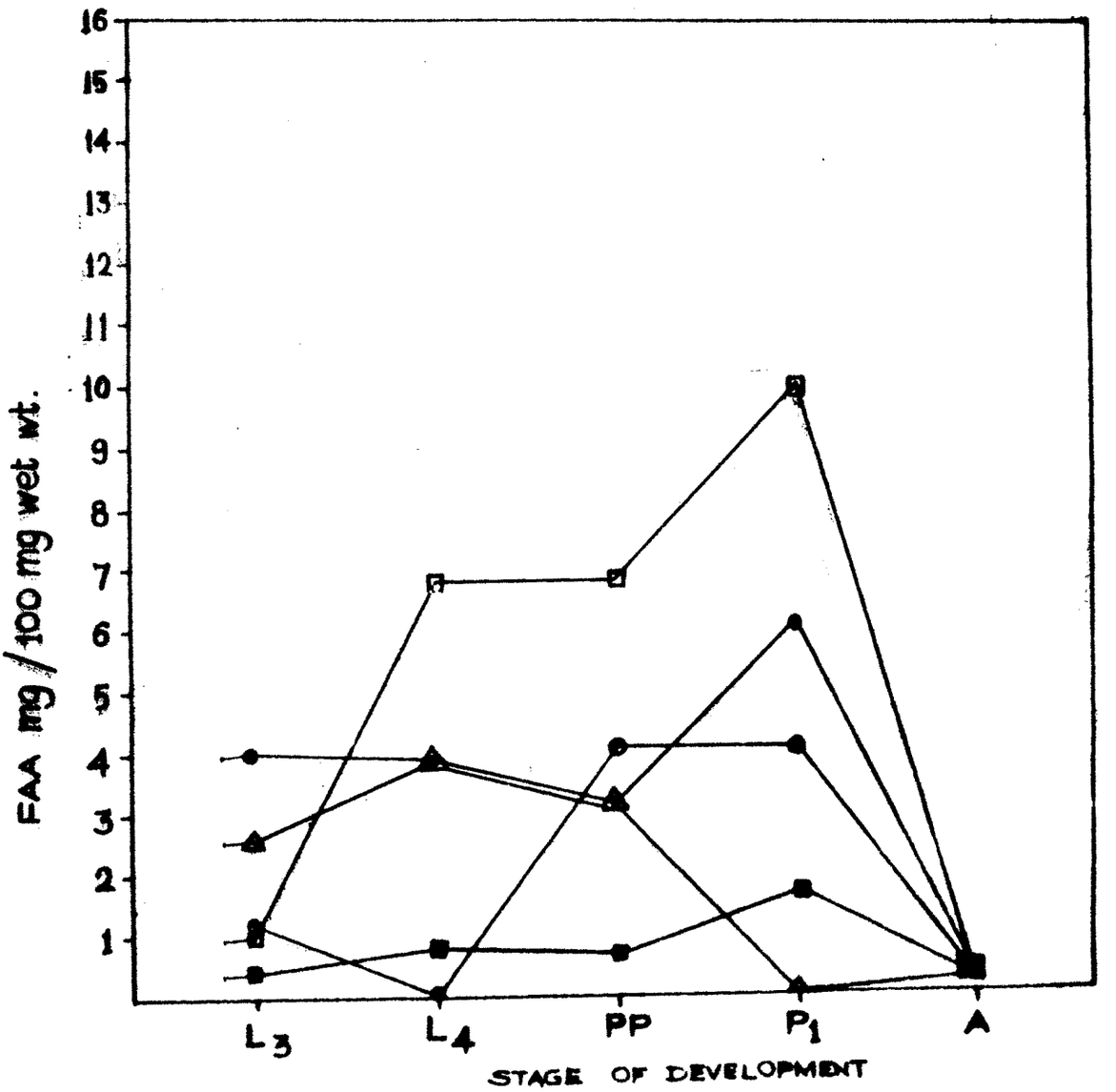
FAA mg/100 mg wet wt.
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
L3
L4
PP
P1
A
STAGE OF DEVELOPMENT

PLATE No. 20

Alterations in the individual free amino acids in the fat body of Chrysomyia during larval growth and metamorphosis. The amounts of all free amino acids are expressed in mg/100 mg fresh weight of fat bodies various stages of Larval growth and metamorphosis are as shown in plate No.14.

□-□ = Aspartic acid
△-△ = β -Amino butyric acid
○-○ = Methionine.

PLATE - 20

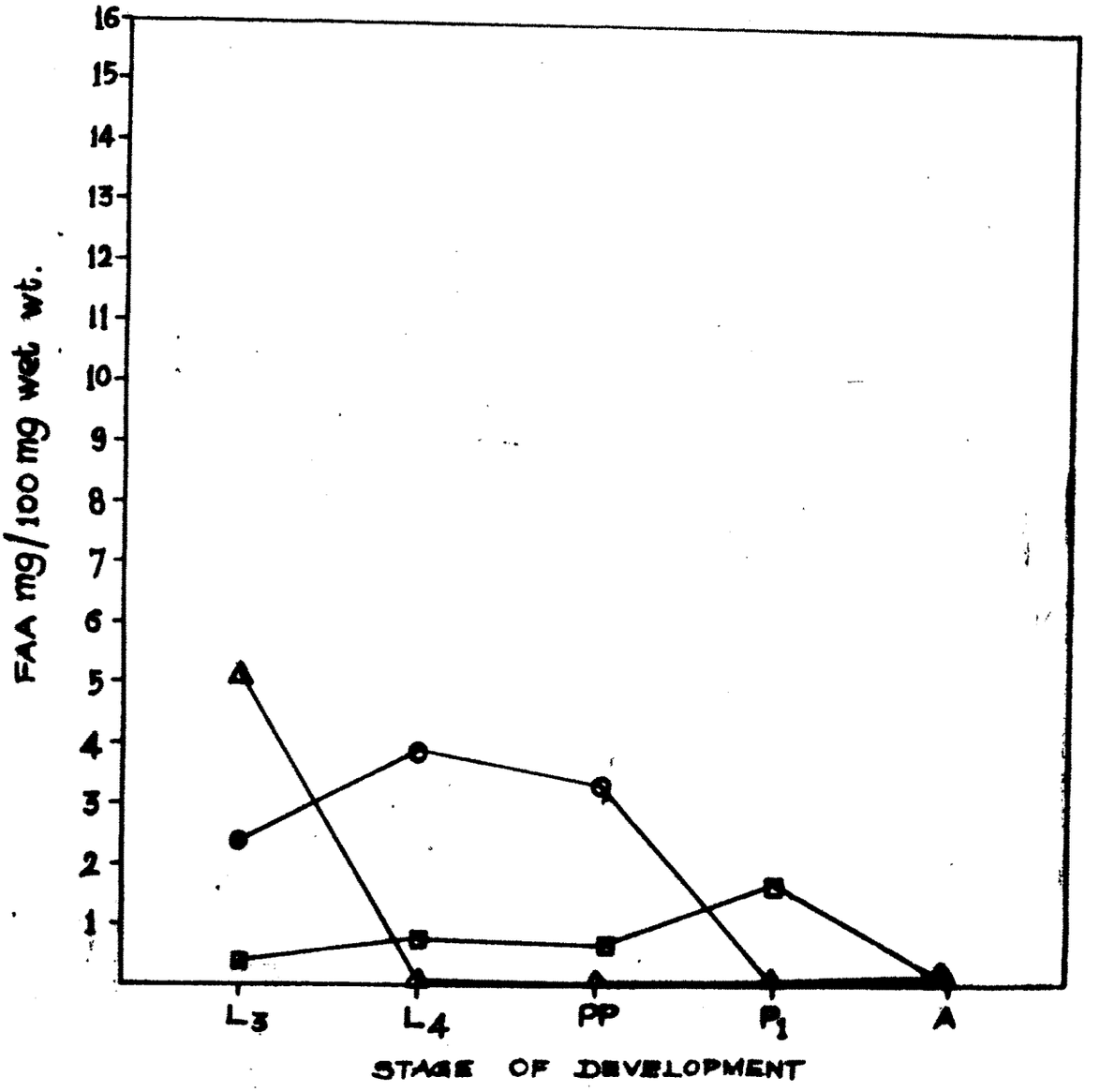
FAA mg/100 mg wet wt.
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
L3
L4
PP
P1
A
STAGE OF DEVELOPMENT

PLATE No. 21

Alterations in total free amino acids in the Haemolymph of Chrysomyia during larval growth and metamorphosis. The amounts of Free amino acids expressed in mg/100 mg fresh weight of haemolymph L_3, L_4, PP, P_1, P_2, P_3 and A refer stages of larval growth and metamorphosis - 3rd day larva, 4th day larva, pharate pupa 1st day, 2nd day, 3rd day pharate adult development and Adult respectively.

PLATE - 21

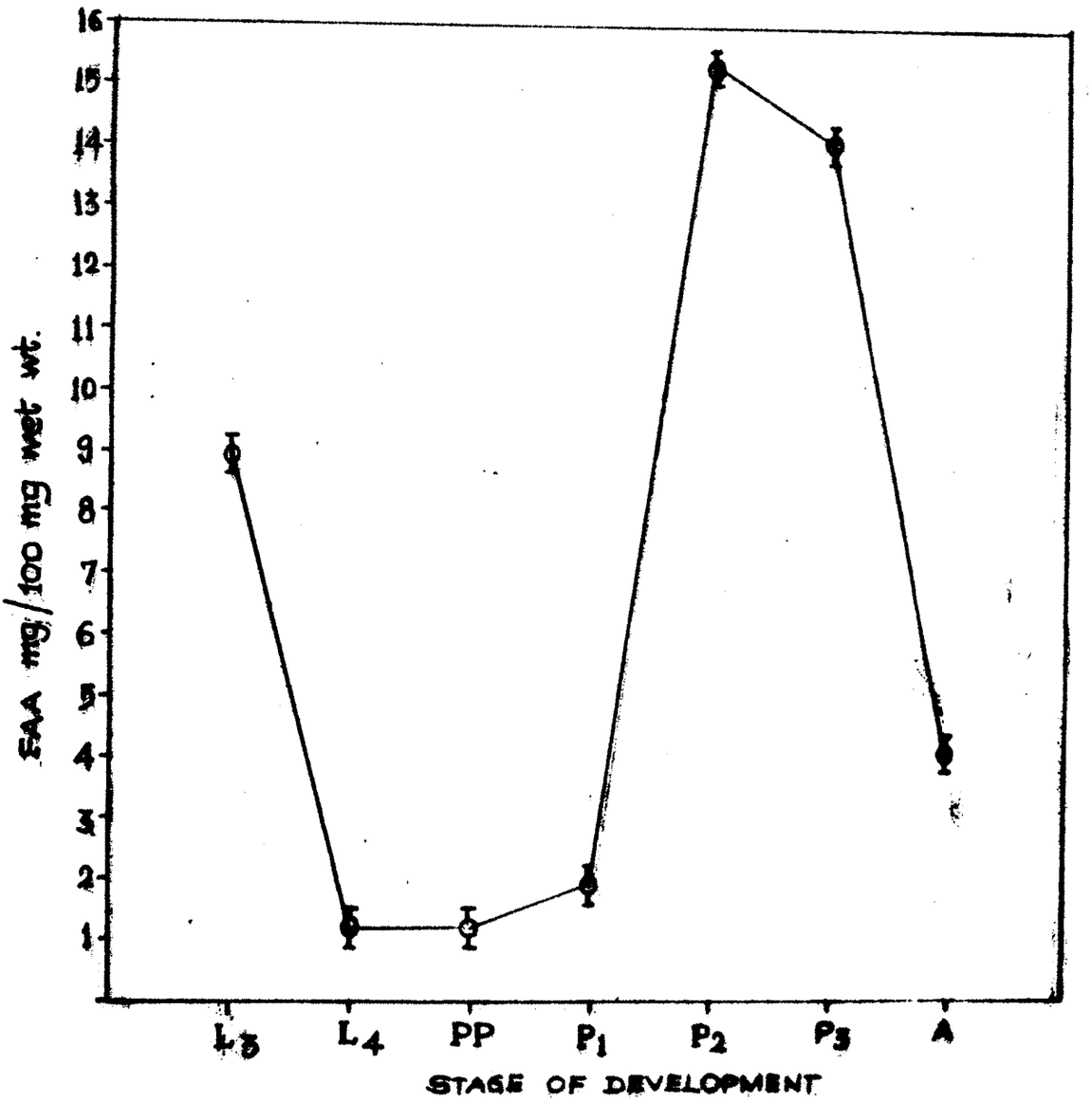
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
EAA mg/100 mg wet wt.
L3
L4
PP
P1
P2
P3
A
STAGE OF DEVELOPMENT

PLATE No. 22

Alterations in the individual free amino acids in the haemolymph of Chrysomyia during larval growth and metamorphosis. The amounts of all the free amino acids are expressed in mg/100 mg fresh wt of haemolymph. Various stages of larval growth and metamorphosis are as shown in plate No. 15 Fig.1.

■ – ■ = β -Alanine
□ - □ = Glycine
△ - △ = Valine
○ – ○ = Lysine
● – ● = Phenyl alanine.

PLATE - 22

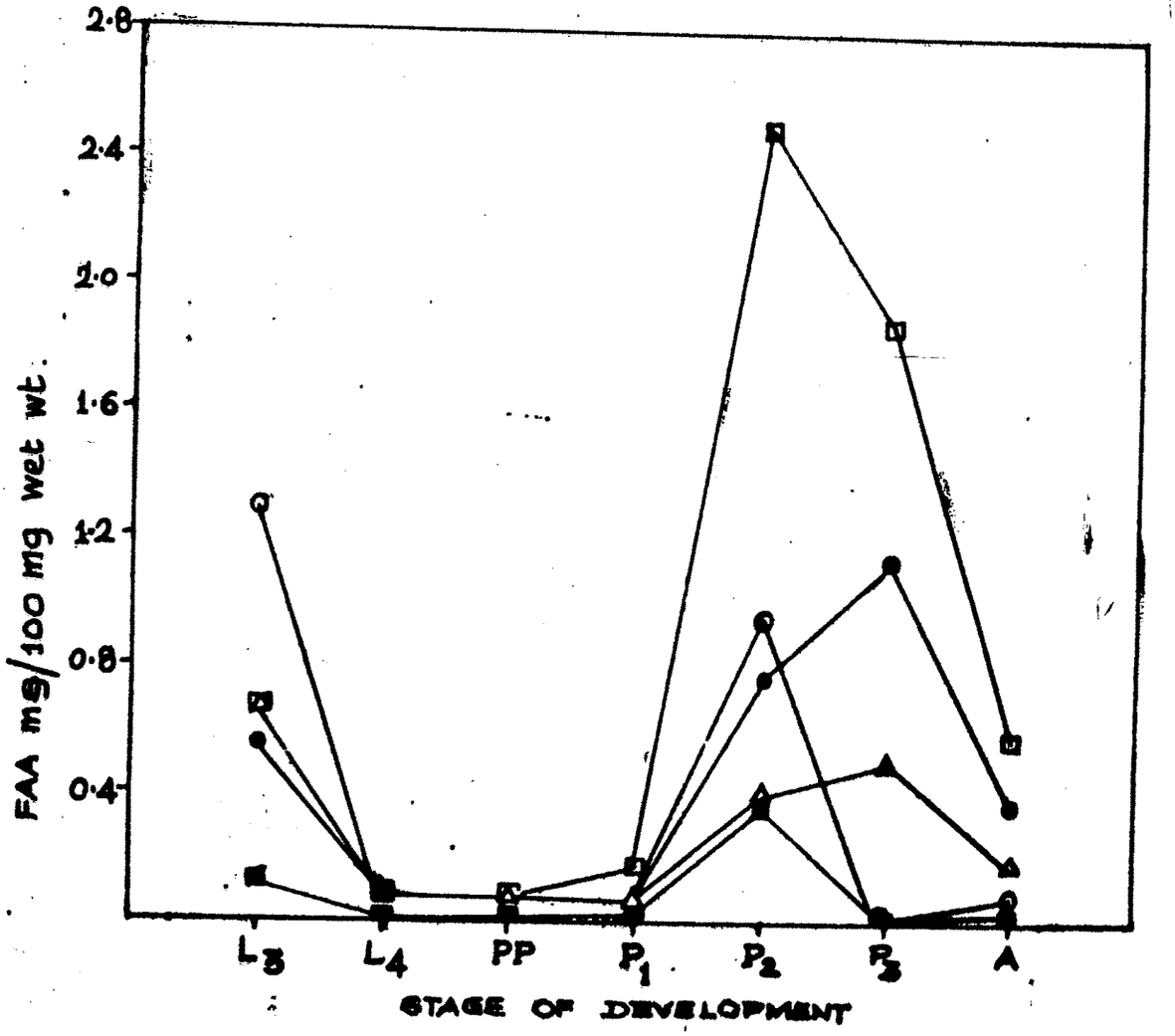
2·8
2·4
2·0
1·6
1·2
0·8
0·4
FAA mg/100 mg wet wt.
L3
L4
PP
P1
P2
P3
A
STAGE OF DEVELOPMENT

PLATE No. 23

Alterations in the individual free amino acids in the haemolymph of Chrysomyia during larval growth and metamorphosis. The amounts of all the free amino acids are expressed in mg/100 mg fresh wt of haemolymph. Various stages of larval growth and metamorphosis are as shown in plate No. 15 Fig. 1.

○ - ○ = Glutamine
● - ● = Proline
■ - ■ = Histidine
□ - □ = Threonine.

PLATE - 23

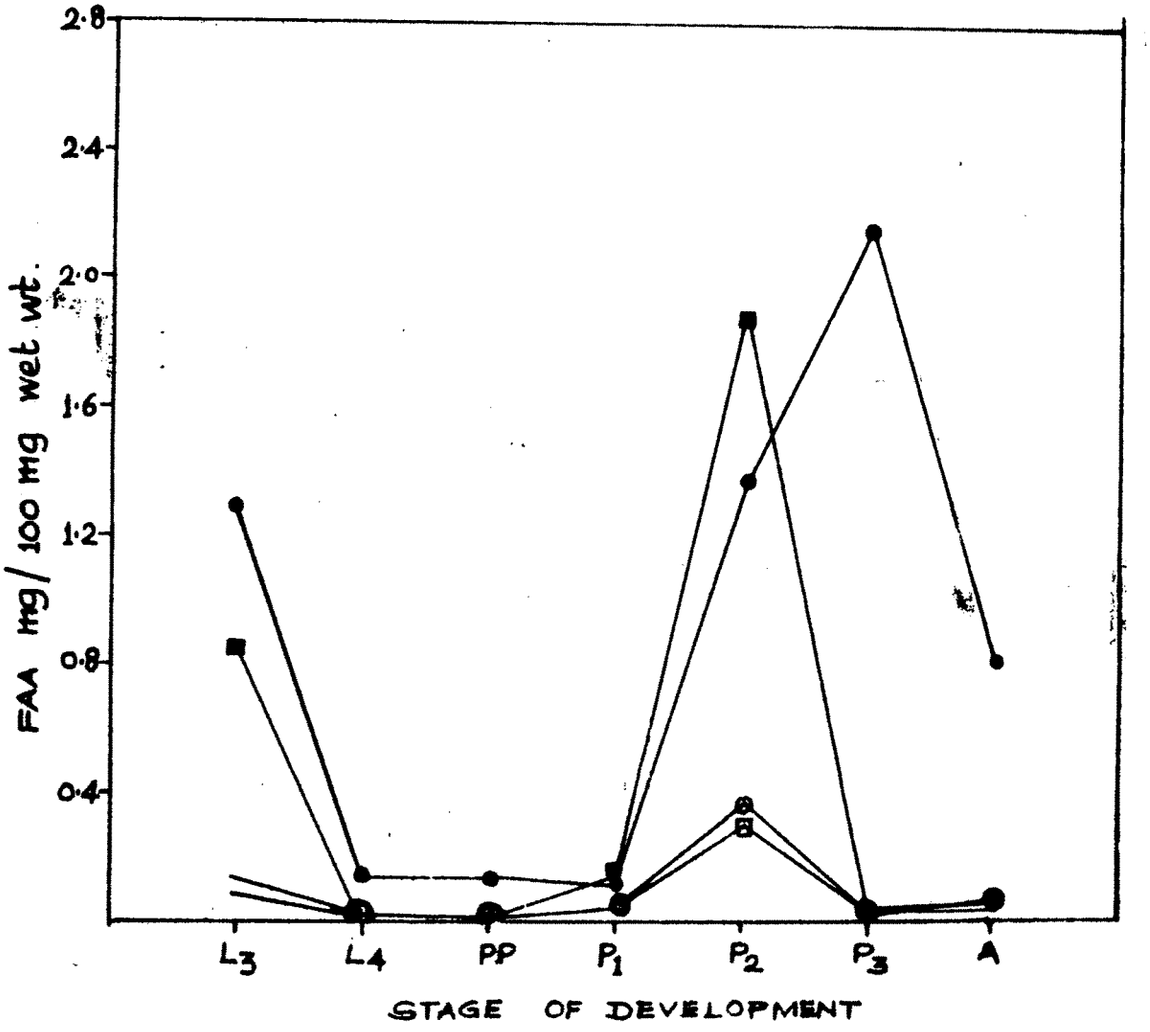
2·8
2·4
2·0
1·6
1·2
0·8
0·4
FAA mg/100 mg wet wt.
L3
L4
PP
P1
P2
P3
A
STAGE OF DEVELOPMENT

PLATE No. 24

Alterations in the individual free amino acids in the haemolymph of Chrysomyia during larval growth and metamorphosis. The amounts of all the free amino acids are expressed in mg/100 mg fresh wt of haemolymph. Various stages of larval growth and metamorphosis are as shown in plate No.15 Fig. 1.

△ –△ = Serine
● – o = Glutamic acid
□ –□ = Tyrosine
○ – ○ = Isoleucine
▲ – ▲ = Leucine.

PLATE - 24

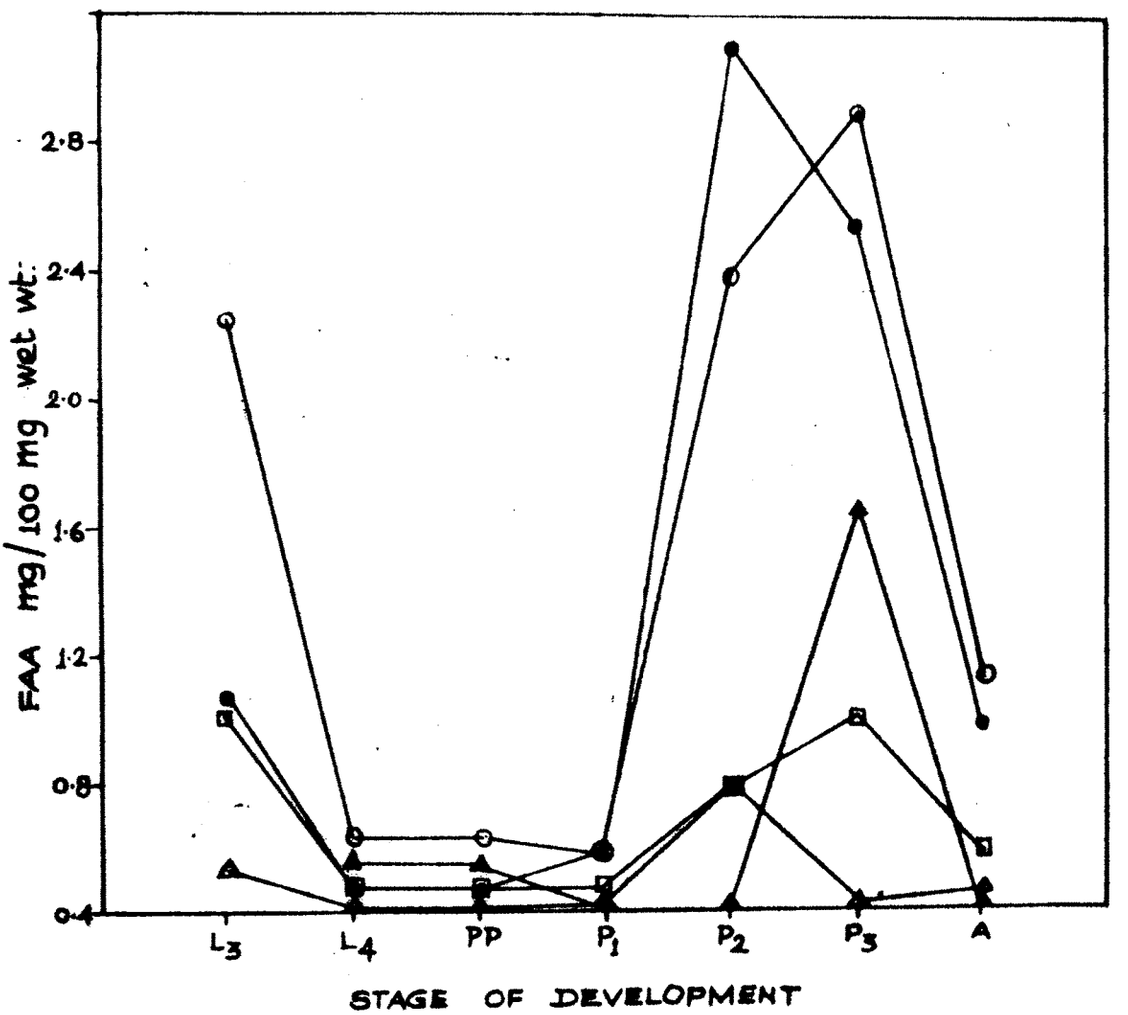
FAA mg/100 mg wet wt.
2·8
2·4
2·0
1·6
1·2
0·8
0·4
L3
L4
PP
P1
P2
P3
A
STAGE OF DEVELOPMENT

PLATE No.25

Alterations in the individual free amino acids in the haemolymph of Chrysomyia during larval growth and metamorphosis. The amounts of all the free amino acids are expressed in mg/100 mg fresh wt of haemolymph. Various stages of larval growth and metamorphosis are as shown in plate No.15 Fig. 1.

□-□ = Aspartic acid

△-△ = β-Amino butyric acid

○-○ = Methionine.

PLATE - 28

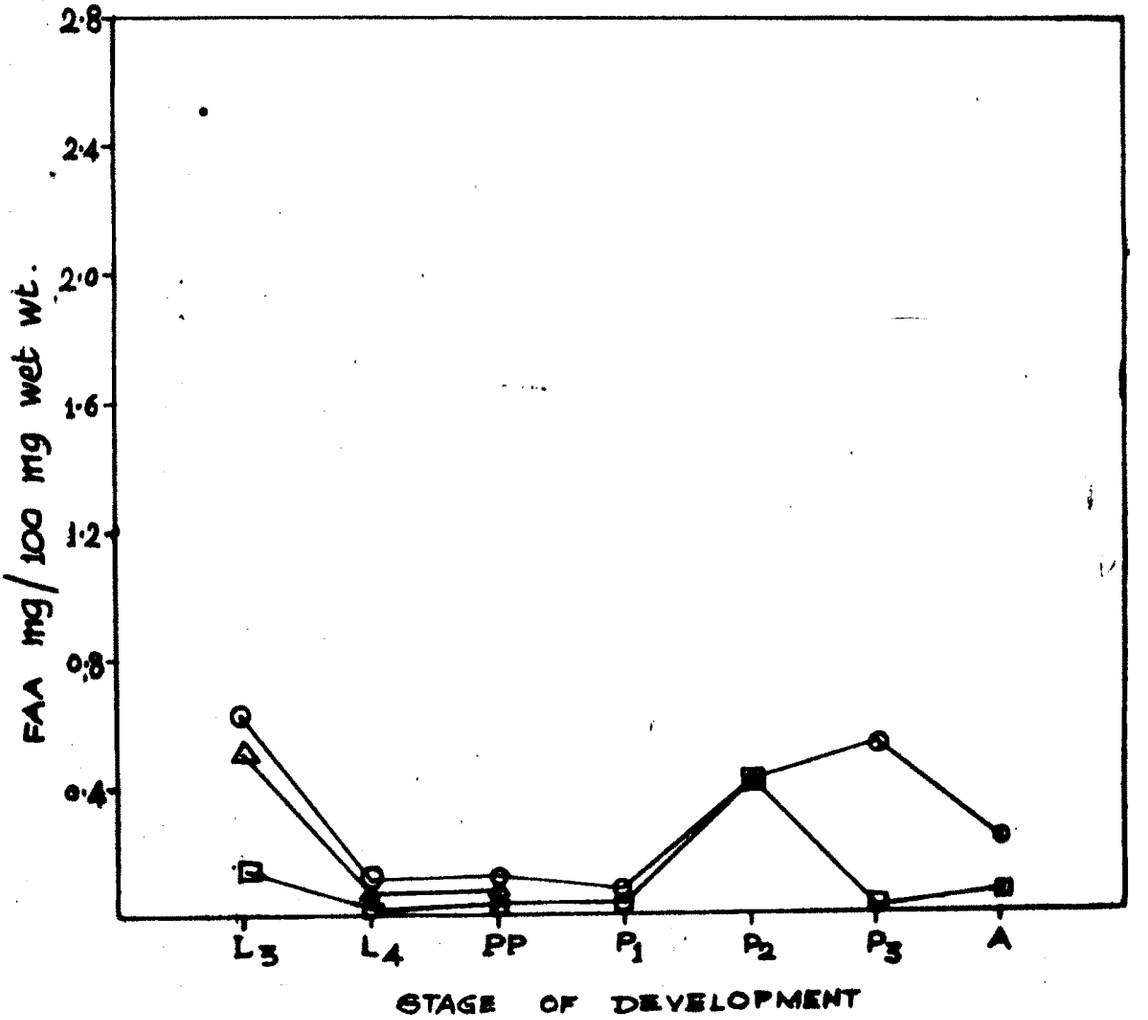
2·8
2·4
2·0
1·6
1·2
0·8
0·4
FAA mg/100 mg wet wt.
L3
L4
PP
P1
P2
P3
A
STAGE OF DEVELOPMENT

TABLE NO. 9

FREE AMINO ACIDS IN FAT BODY OF CHRYSOMYIA RUFIFACIES DURING LARVAL GROWTH AND METAMORPHOSIS.

	AMINO ACID	LARVAL GROWTH		PHARATE PUPA	PHARATE ADULT DEVELOPMENT.	ADULT
		L_3	L_4	PP	P_1	A
1	Cystine	Trace	Trace	Trace	Trace	Trace
2	Histidine	Trace	Trace	Trace	9.063	Trace
3	Lysine	1.2	Trace	Trace	4.126	0.5903
4	β -Alanine	0.41	0.83	0.7452	1.781	0.02290
5	Serine	0.44	0.86	0.7152	1.681	0.2300
6	Aspartic acid	0.42	0.87	0.7358	1.881	0.02410
7	Threonine	0.32	0.64	0.61	1.2	0.020
8	Glutamine	0.4	0.81	0.7052	1.781	0.02290
9	Glycine	1.00	6.8	6.07	10.00	0.1606
10	Glutamic acid	1.1	6.9	6.08	10.5	0.1506
11	β -Amino-N-Butyric acid	5.281	-	-	-	-
12	Proline	-	12.00	10.71	7.812	0.1506
13	Valine	2.506	3.960	3.214	Tracer	0.2120
44	Tyrosine	2.681	4.00	3.214	Tracer	0.24
15	Methionine	2.401	3.960	3.414	Tracer	0.22
16	Isoleucine	16.00	12.00	10.00	1.75	0.3374
17	Phenyl alanine	4.00	3.960	3.214	6.127	0.1856
18	Leucine	5.6	Trace	Trace	Trace	Trace
	Total FAA (Excluding NH_3)	33.679	57.49	59.4274	57.2502	18.1073

Values are expressed as mg/100 mg wet wt. of Fat body.

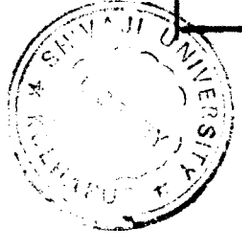

TABLE No. 10

FREE AMINO ACIDS IN HAEMOLYMPH OF CHRYSOMYIA FURIFACIES DURING LARVAL GROWTH AND METAMORPHOSIS.

	AMINO ACIDS	LARVAL GROWTH		PHARATE PUPA	PHARATE ADULT DEVELOPMENT			ADULT
		L_3	L_4	pp	P_1	P_2	P_3	A
1	Cystine	Trace	Trace	Trace	Trace	Trace	Trace	Trace
2	Histidine	0.88	Trace	Trace	0.1416	1.98	Trace	Trace
3	Lysine	1.034	0.038	0.03896	0.04935	0.95	Trace	0.07747
4	-Alanine	0.1334	0.0067	0.0064	0.02546	0.38	Trace	0.07436
5	Serine	0.1434	0.0061	0.007	0.02446	0.39	Trace	0.07236
6	Aspartic acid	0.1364	0.0064	0.0061	0.02490	0.4	Trace	0.0715
7	Threonine	0.1120	0.0048	0.0041	0.0211	0.3	Trace	0.061
8	Glutamine	0.1384	0.0069	0.0067	0.02746	0.36	Trace	0.07136
9	Glycine	0.66	0.08	0.08116	0.1917	2.5	1.875	0.5868
10	Glutamic acid	0.69	0.085	0.084	0.1817	2.7	2.344	0.5968
11	-Amino-N-butyric acid	0.50	0.060	0.0714	-	-	-	-
12	Proline	1.3	0.1419	0.1428	0.1159	1.37	2.188	0.8215
13	Valine	0.68	0.0903	0.0909	0.05588	0.44	0.55	0.1837
14	Tyrosine	0.61	0.09	0.091	0.05678	0.41	0.8250	0.1737
15	Methionine	0.65	0.94	0.100	0.0558	0.45	0.6450	0.1937
16	Isoleucine	1.86	0.2324	0.2337	0.188	2.00	2.5	0.7311
17	Phenyl alanine	0.56	0.09679	0.0974	0.06609	0.77	1.126	0.3756
18	Leucine	Trace	0.1419	0.14288	Trace	Trace	1.250	Trace
	TOTAL FAA(Excluding NH_3)	9.0876	1.1812	1.2043	1.84138	15.2	13.103	4.5095

* Values are expressed as mg/100 mg of Haemolymph.

total free amino acids in the haemolymph are shown in the plate No.21. The changes occuring in the various individual free amino acids are shown in the plate number 22 to 25 respectively. The quantitative variations in the individual and total free amino acids in the fat body and haemolymph are shown in table 9 and 10 respectively.

1. <u>The pattern of free amino acids in the fat body during larval growth and metamorphosis</u>.

The paper chromatographic separation of the free amino acids in the fat body during larval growth and metamorphosis shown in plate No.18 indicates the presence of cystine, histidine, lysine, β-alanine, serine, aspartic acid, threonine, glutamine, glycine, Glutamic acid, proline, valine, tyrosine, methionine, isoleucine, phenyl alanine, leucine. β-amino butyric acid specifically found in the fat body of 3rd day larvae only. At a comparative level amino acids lysine, glycine, glutamic acid, valine, tyrosine, methionine, isoleucine and phenyl alanine were in higher concentrations. Cystine and histidine (except in 1st day of pharate adult development) were present only in trace amounts.

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

As can be seen from the curve in plate No.16, the total concentration of ninhydrin-reacting components in the fat body on the 3rd day of larval growth is comparatively higher, there is a rapid increase in the free amino acids in the fat body of 4th day larva. This increase continues gradually in the fat body of pharate pupa. The free amino acid concentration is found to be highest in the fat body of pharate pupa. There is a slight decrease in the free amino acid concentration in the fat body of 1st day of pharate adult development. As the quantity of fat body was not sufficient to estimate the free amino acids in the further developmental stages of pharate adult, values of free amino acids from the fat body of freshly emerged adult are compared with those of early metamorphosis stages.

The various individual amino acids in the fat body of Chrysomyia during larval growth and metamorphosis can be divided in to three broad groups; those which increase in concentration throughout larval growth such as glycine, glutamic acid valine

and serine; those which decrease during larval growth upto 4th day of larval growth then increase such as lysine, phenyl alanine and there which change minimally during larval period as well as during metamorphosis such as leucine, β-alanine, aspartic acid. An unexpected finding is the absence of β-amino-butyric acid in the fat body of pharate pupa as well as pharate adult development stages and in the freshly emerged adult. High concentration of histidine is only evidenced in the during early metamorphosis i.e. in the lst day of pharate adult development.

3. <u>The pattern of free amino acids in the haemolymph during larval growth and metamorphosis</u>.

The paper chromatographic separation of the free amino acids in the haemolymph during larval growth and metamorphosis shown in plate No.15 indicates the presence of cystine, histidine, lysine, β-alanine, serine, aspartic acid, threonine, glutamine, glycine, glutamic acid, -amino butyric acid, proline, valine, tyrosine, methionine, isoleucine phenyl alanine and leucine. Amino acid β-amino-butyric acid occur only in the haemolymph of 3rd day and 4th day old larvae as well as in the pharate pupae but not in the pharate adult developmental stages as well as in the haemolymph of freshly emerged adult. At a comparative level

glycine, glutamic acid, valine and tyrosine are found to be present in higher concentrations in the haemolymph of larvae while haemolymph of pharate pupae and pharate adult developmental stages shows the high concentrations of glutamic acid, proline, glycine and histidine.

4. Quantitative changes in total concentrations and in the different groups of amino acids in the haemolymph during larval growth and metamorphosis.

As can be seen from the curve in plate No. 21 the total concentration of free amino acids in the haemolymph of 3rd day larvae is higher, it drops rapidly on the 4th day of larval growth and there is only slight change in the concentration of total free amino acids during development of pharate pupa. The concentration of total free amino acids increases gradually during pharate adult development. The concentration of amino acids suddenly increases on the 2nd day of the pharate adult development and it remains at the high level in the further stages of metamorphosis. The haemolymph of freshly emerged adult shows considerable drop in the total concentration of free amino acids. Individual amino acids follow more or less the same pattern of changes with some statistical variations. Alterations in the concentrations of glycine, glutamic acid, isoleucine are more significant as compared to the rest of the amino acids.

D I S C U S S I O N.

The significant feature of the behaviour of free amino acids in the fat body and haemolymph is the increase in the total concentration during last phase of larval growth, in the pharate pupa and in the early metamorphosis in the former and decrease in the concentration in these stages of development in the later tissue. Following the metamorphosis these concentrations decrease in both these tissues of freshly emerged adult.

Despite the extensive literature on free amino acids in insects, reviewed by Chen (1966) only relatively few studies have been made, concerned with quantitative changes in haemolymph and fat body during largal growth and metamorphosis. The unequal distribution of individual free amino acids between the haemolymph and fat body of Chrysomyia larva and pharate adult in accord with the findings for other species such as Phormia larva (Levenbook 1966), the silkworm (Brieteux Gre'geire and florkin, 1959), the southern armyworm (Levenbook, 1962) and sarcophaga (Pant and Lal, 1972). Too little is known about the physiological significance or mechanisms of amino acids compartmentalisation in insects to warrant useful discussion, but it is worth noting that the values for Chrysomyia

confirm Levenbook's (1962, 1966) generalization that glutamine is concentratial in insect haemolymph, and glutamate in the tissues.

In insects greater structural changes take place over shorter periods of time than are seen in almost any other group of animals and these morphological changes are the result of underlying biochemical and cytological changes. The change in the concentration of free amino acids can very well be correlated with protein content and their metabolism during growth and metamorphosis in blowflies. One can get better coordination with alteration of protein values in individual organ systems such as fat body or haemolymph during larval growth and metamorphosis.

It has been evidenced by several workers (Price, 1973) that early in the last instar the fat body synthesizes proteins and exports them in to the haemolymph while in the later instar proteins are sequestered from the haemolymph in to the fat body. The rate of protein synthesis by the fat body is also high in the early to mid part of the last instar, but then falls off rapidly to a low level, at which it remains until the larva pupates, in diapausing pupae protein synthesis remains at this low level. As a 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.

The tracer technique studies on Sphinx liquatri (Bricteux, Gregoire et. al., 1957) and Myalophora (Skinner, 1966) have indicated "high incorporation of amino acids in to pupal tissues. But these results show only the ability of developing pharate adult to take up free amino acids do not necessarily mean that it is the major pathway of protein synthesis during histogenesis. Besides histolysis and histogenetic variations, due to interconversion and other metabolic conversions of amino acids can not be negleted.

.....