# CHAPTER : IV

## CHANGES IN FREE AMINO ACIDS DURING LARVAL GROWTH.

# INTRODUCTION

Within the egg as a whole there is no growth. Only a kind of "inner growth" occurs in the egg representing a transformation of stored yolk components into active protoplasm. The growth during insect development is restricted to the larval development and during this feeding period there will be deposited all the mass necessary for the final adult. Dipterous larvae are known to accumulate lipids, glycogen and proteins during development (Pennncoft, 1960; Coupland, 1957). The reason for storing these constitutents in larvae is fairly obvious, namely as a built up of material which can later be used at the time of metamorphosis when adult tissues are being assembled.

Studies on the metabolism of amino acids in insect larvae have yielded a wealth of data. Especially since the advent of such new techniques as microbiological assays, paper and ion-exchange chromatography and isotopic labelling, it has become possible to follow quantitative changes in these amino compounds which are present even in extremely few concentrations. It has also been demonstrated by many authors that the amino acids, in addition to their function as protein constituents, enter into diverse metabolic pathways and participate in many other physiological activities. In view of the fact that a large part of our knowledge about amino acids in insects derives from studies dealing with larval development, In the present study, the alterations in total free amino acids and the individual free amino acids in the larvae of Chrysomyia rufifacies have been worked out during larval growth and moulting.

## MATERIAL AND METHODS

Alterations in the free amino acids were worked out in the whole larvae during growth and moulting. The rearing of the Chrysomyia was carried out as described in chapter II. The larval growth stages selected for study as mentioned in the chapter on material and methods. The extraction of free amino acids, chromatographic separation and quantification 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.6. Occurance of various free amino acids in the larval tissue during growth and moulting is shown in table No.3. Alteration occuring in the total free amino acid content per 100 mg. of wet weight of the larval tissue in growth and moulting are shown in plate No.7. The alterations occuring in the individual free amino acids are shown in the plate number 8, 9. The quantitative alterations in the total and individual free amino acids have been in Table No.4.

1. The pattern of free amino acids during larval growth.

As shown in the plate No.6 during the larval development of Chrysomyia a total of at least eighteen free ninhydrine-positive compounds could be distinctly separated on paper chromatogram; these include cystine, histidine, lysine, β-alanine serine, aspartic acid, threonine, glutamine, glycine, glutamic acid, proline, β-amino-butyric acid, valine, tyrosine, methionine, isoleucine, phenylalanine, leucine. At a comparative level glutamic acid, glycine, histidine occur in the highest concentrations. Proline could not bedetected within the amount of materials used during early larval growth but occur in 3rd day and 4th day of larval development. Cystine and leucine occur in very low concentrations. In general, except proline

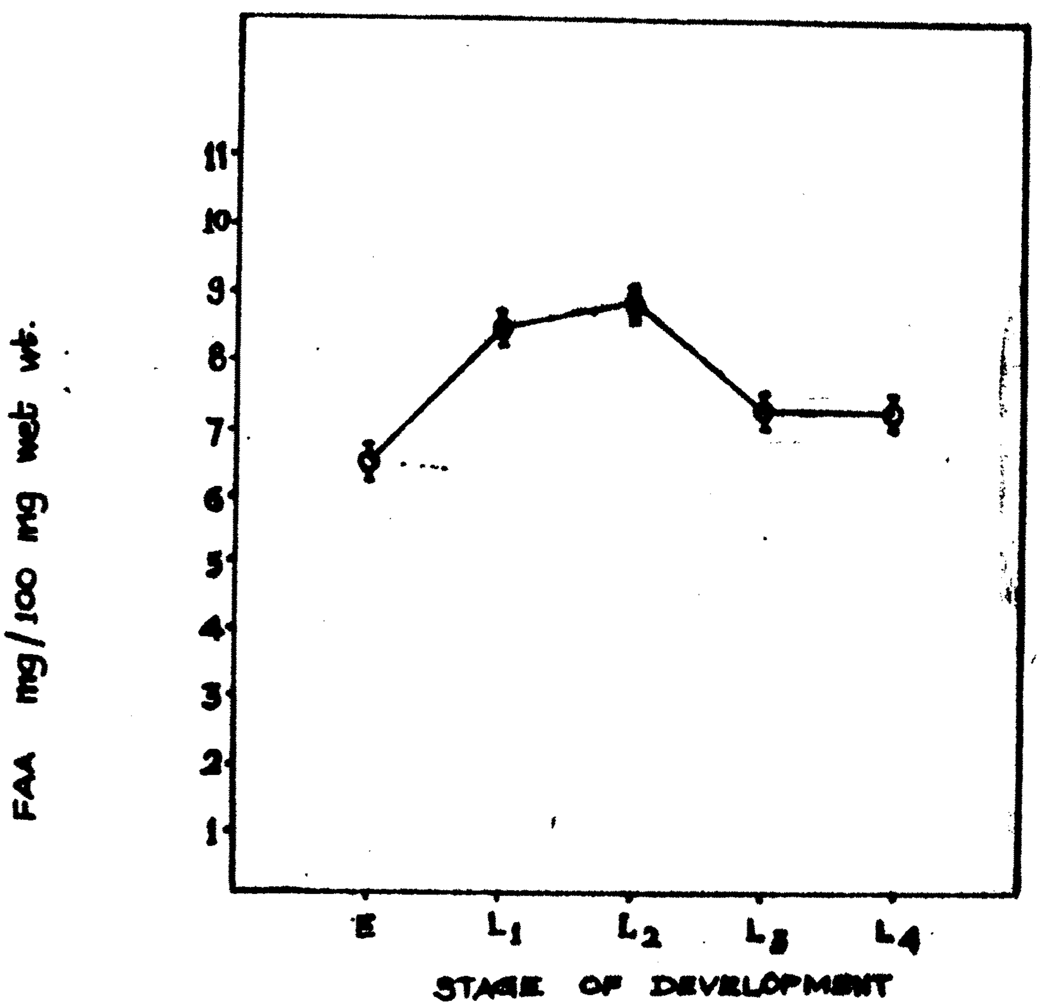
TABLE No. 3

**FREE AMINO ACIDS IN LARVAL GROWTH OF BLOWFLY CHRYSOMYIA RUFIFACIES.**

| | AMINO ACIDS | LARVAL GROWTH | | | |
|---|---|---|---|---|---|
| | | $L_1$ | $L_2$ | $L_3$ | $L_4$ |
| 1 | Cystine * | Trace | Trace | Trace | Trace |
| 2 | Histidine | + | + | +++ | ++ |
| 3 | Lysine | + | ++ | ++ | + |
| 4 | β -alanine | + | ++ | + | + |
| 5 | Serine | + | + | ++ | + |
| 6 | Aspartic Acid | + | + | ++ | + |
| 7 | Threonine | + | + | ++ | + |
| 8 | Glutamine | + | + | ++ | + |
| 9 | Glycine | ++ | ++ | ++ | ++ |
| 10 | Glutamic acid | ++ | ++ | ++ | ++ |
| 11 | Proline | - | - | Trace | + |
| 12 | β -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 - 7


FAA mg/100 mg wet wt.
11
10
9
8
7
6
5
4
3
2
1
E
$L_1$
$L_2$
$L_3$
$L_4$
STAGE OF DEVELOPMENT

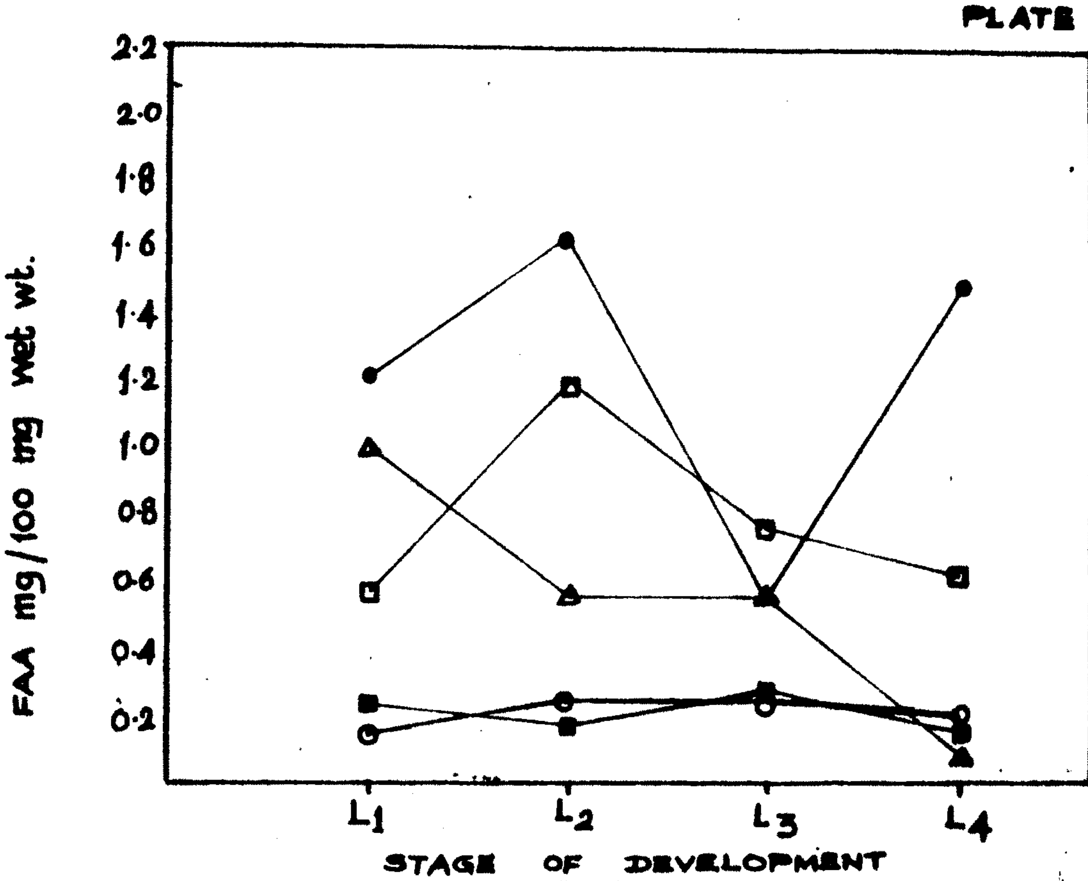

2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
FAA mg/100 mg wet wt.
L1
L2
L3
L4
STAGE OF DEVELOPMENT

FIG. 1

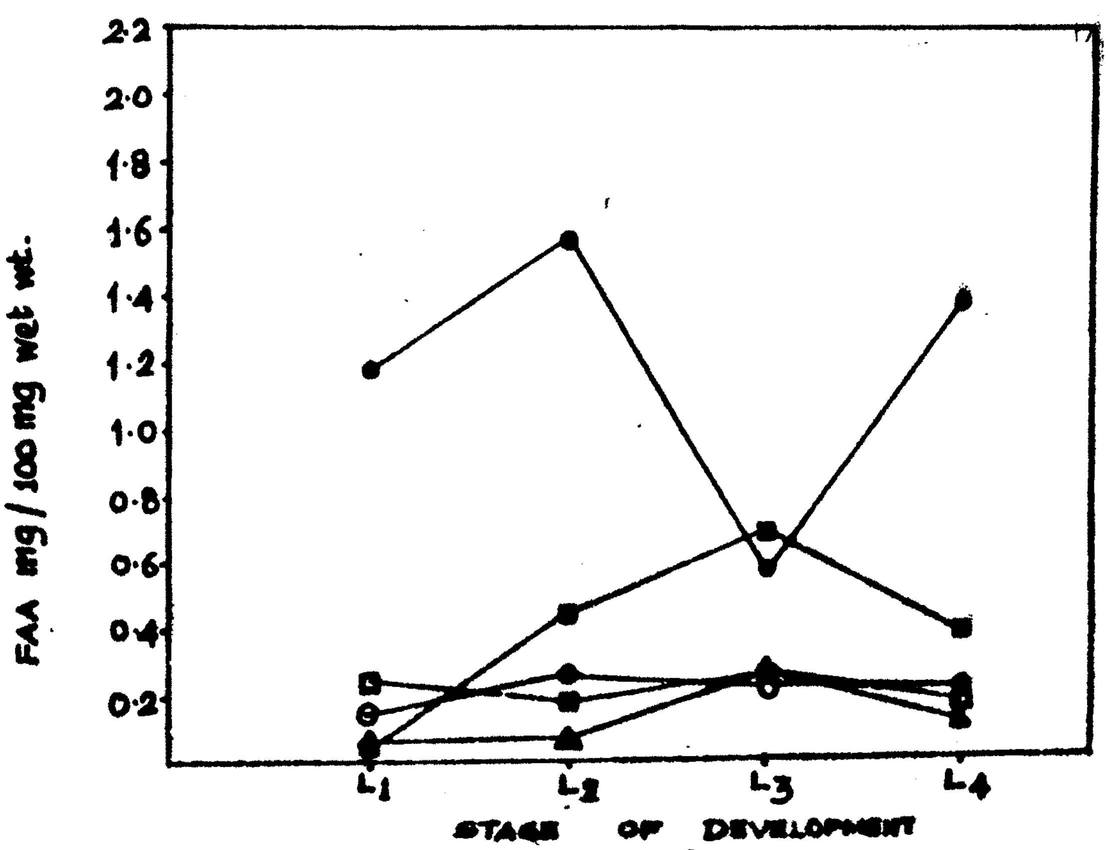

2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
FAA mg/100 mg wet wt.
L1
L2
L3
L4
STAGE OF DEVELOPMENT

FIG. 2

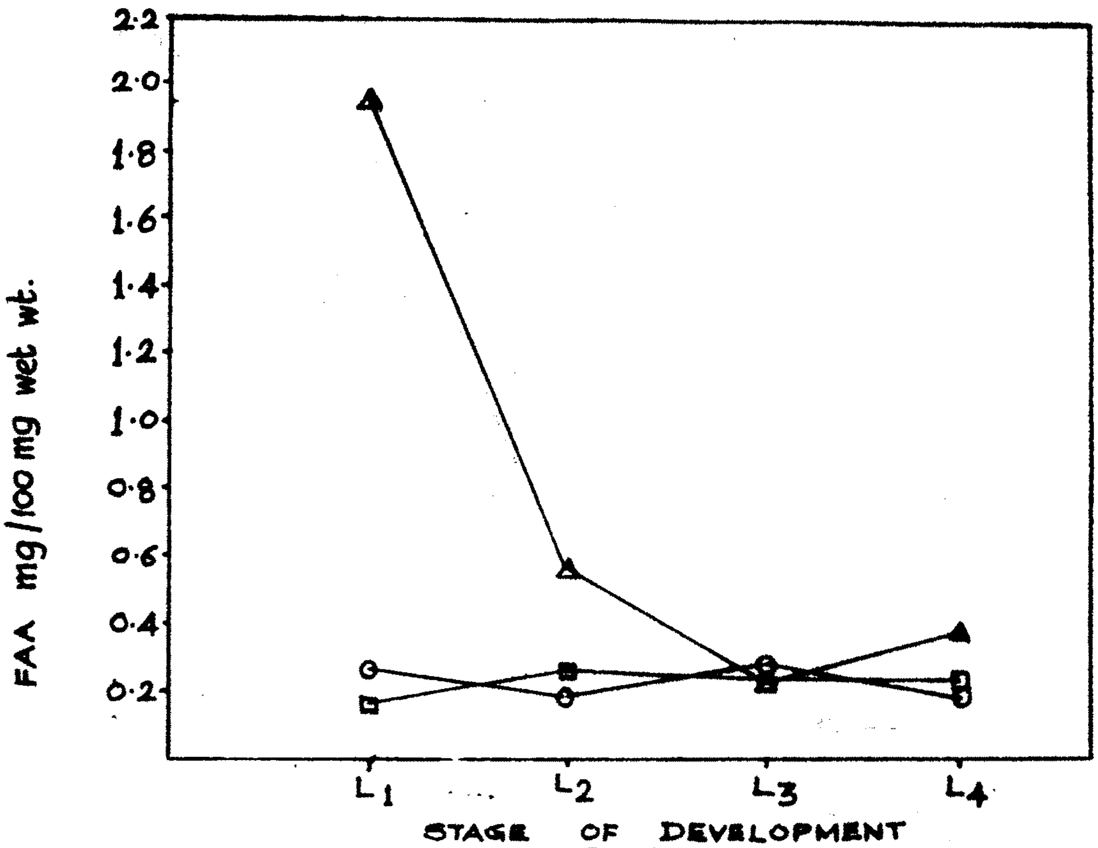

2·2
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
FAA mg/100 mg wet wt.
L1
L2
L3
L4
STAGE OF DEVELOPMENT

FIG - 1

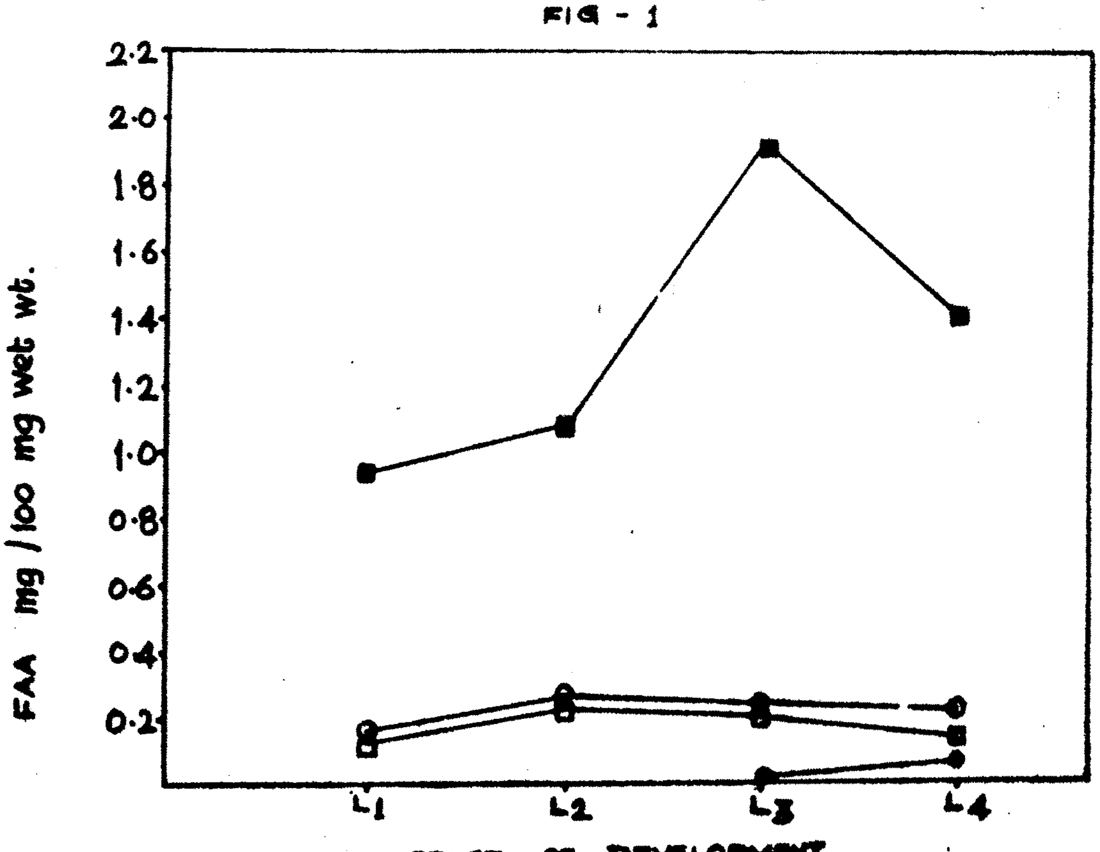

2·2
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
FAA mg / 100 mg wet wt.
L1
L2
L3
L4
STAGE OF DEVELOPMENT

FIG. 2

TABLE No. 4

**FREE AMINO ACIDS IN LARVAL GROWTH**
**OF BLOWFLY CHRYSOMYIA RUFIFACIES.**

| | AMINO ACIDS | LARVAL GROWTH | | | |
|---|---|---|---|---|---|
| | | $L_1$ | $L_2$ | $L_3$ | $L_4$ |
| 1 | Cystine | Trace | Trace | Trace | Trace |
| 2 | Histidine | 0.9523 | 0.0980 | 1.9230 | 1.4000 |
| 3 | Lysine | 0.5953 | 1.2200 | 0.7956 | 0.6200 |
| 4 | -Alanine | 0.157 | 0.2500 | 0.2442 | 0.200 |
| 5 | Serine | 0.1571 | 0.2540 | 0.2310 | 0.2100 |
| 6 | Aspartic acid | 0.151 | 0.2600 | 0.2500 | 0.2300 |
| 7 | Threonine | 0.124 | 0.2100 | 0.1800 | 0.1700 |
| 8 | Glutamine | 0.161 | 0.2510 | 0.2410 | 0.224 |
| 9 | Glycine | 1.229 | 1.6400 | 0.5771 | 1.5 |
| 10 | Glutamic acid | 1.19 | 1.5900 | 0.5698 | 1.400 |
| 11 | Proline | - | - | 0.0200 | 0.0500 |
| 12 | -Amino-N- | 1.96 | 0.5500 | 0.2564 | 0.3900 |
| 13 | Valine | 0.23 | 0.1800 | 0.2991 | 0.1869 |
| 14 | Tyrosine | 0.24 | 0.1900 | 0.2818 | 0.1798 |
| 15 | Methionine | 0.25 | 0.195 | 0.02794 | 0.1911 |
| 16 | Isoleucine | 0.04 | 0.4548 | 0.6800 | 0.3900 |
| 17 | Phenyl alanine | 1.00 | 0.5520 | 0.5679 | 0.0900 |
| 18 | Leucine | 0.05 | 0.058 | 0.2704 | 0.1600 |
| | Total FAA (Excluding $NH_3$) | 8.5167 | 8.9528 | 7.6669 | 7.5878 |

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

there is no qualitative difference in the free amino acid content of the larval tissue during growth and moulting.

2. <u>Quantitative changes in total concentrations and in the different groups of amino acids during larval growth.</u>

As can be seen from the curve in plate No.7, the total concentration of free amino acids remain essentially unchanged during the whole period of larval development. The individual amino acids themselves can be roughly divided into three broad groups: Those like Methionine, isoleucine, leucine and glycine which increase to a maximum concentration during the early larval growth stages (1st day and 2nd day old larvae) and then decline, those which continue to increase in amount throughout larval growth (Phospho-ethanolamine, Phosphoserine, proline) and those which show minimal changes during larval life (Aspartic acid, valine, alanine).

No general pattern can be recognized in the variation of individual amino acids: Lysine, isoleucine, $\beta$ -amino butyric acid and histidine decline steadily as development proceeds, while glycine, glutamic acid, glutamine and threonine exhibit a temporary increase. Tyrosine and proline are however two exceptions: they show a continuous increase when the values are expressed per unit body weight. This is especially true during the time approaching puparium formation.

## D I S C U S S I O N

Despite the extensive literature on free amino acids in insects, reviewed by Chen (1962) only relatively few studies have been concerned with quantiatâtive changes during development.

The maximum concentration of free amino acids during larval growth of Chrysomyia occurs at the 2nd day of development. It would seem that insects can be divided into two categories, namely those like Drosophila (Hadorn & Stumm-Zollinger, 1953; Chen & Hadorn, 1954), the blowfly Calliphora augur (Hackmann, 1956) and P.regina (Levenbook, 1966), in which the concentration of free amino acid declines during later stages of larval life, and others like the flor moth Ephestia (Chen and Kuhn, 1956), the Silk worm Bombyx mori (Wyatt et.al., 1956) and the mosquito Culex pipiens (Chen, 1958) and Chrysomyia (Present data) where the concentration remains approximatly constant.

The profiles of free amino acids of individual Chrysomyia larvae differ widely, but three general groups can be recognized. In the first group, the levels are low at earlier stages, increase to a maximum at about 2nd day and then declines, in the second the concentration remains relatively constant during larval life and in the third it continously rises. Free proline is the best example of the latter, which is understandable in view of its importance in the hardening and darkening of the puparium (Hackman, 1953). However, a similar increase in free tyrosine reported for P.regina (Levenbook, 1966) was not so significant in Chrysomyia. The accumulation of these two amino acids suggests therefore the preparation of the larvae for the synthesis of cuticular proteins and the associated tanning.

It is certainly an over simplification to interpret the amino acids pattern merely in terms of protein synthesis and degradation. The amino acids are interrelated from the metabolic view point. Moreover, in contrast to the egg and pupa, which can be considered as closed systems, the larva cannot survive and grow without external nutritional sources which may influence to a great extent it's composition and level of the free amino acid pool. In relating the changes in the free amino acids during larval growth to any particular process involving protein synthesis and degradation, this fact must be taken into consideration. There are evidences indicating that nutrition has a direct effect on the amino acid pattern of insect larvae (Chen and Hadorn, 1955; Chen 1958).

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