THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Chromatographic Analysis of Free Amino Acid of Acrida Luqubris A. Giqantia and Chrotogonus Trachypterus

Kulvir Sra Dhindsa
Assistant Professor, DAV College, Chandigarh, India
Deepak Saini
Assistant Professor, JCDAV College Dasuya, India

Abstract:
The Presented study deal with paper chromatographic analysis of the free amino acid (FAA) in the the various parts of the bodies of three species of grasshoppers viz. C. trachypterus, A.gigantea and A. lugubris has been made with the view to find out the qualitative and quantitative differences in them. The concentration of the FAA arginine, dihydroxyphenylalanine, DL-alanine, isoleucine, proline, hydroxyproline has been found to be much higher in the nymphs as compared to both the adults males and females of C.trachypterus. Tyrosine and Nor-leucine have been found to be absent in the males but seem to be present in the females of C. trachypterus. However, the 2 amino acids lysine and methionine present in the males could not be detected in the females of this species. An unidentified spot was observed in the females of C. trachypterus. Further, the concentration of FAA glycine, DL-alanine and isoleucine were found to be higher in the females than the males. The concentration of FAA arginine, dihydroxyphenylalanine, glycine and tryptophan to be highr in the females than the males of A.gigantea. Lysine and methionine identified in the males could not be detected in the females. A yellow coloured band has been noticed only in the males but no free amino acid could be identified in it. The two sexes of A.lugubris have also revealed the differences in their FAA pattern .The amino acids histidine, aspartic acid glutamic acid and threonine have been found only in the females whereas the three amino acids lysine, methionine and butyric acid could be detected only in the males of this species. The concentration of FAA arginine, phenylalanine and valine was seen to be higher in the females as compared to the males. The comparison of the FAA pattern in the adults of three species of grasshoppers viz. C.trachypterus, A.gigantea and A.lugubris has shown differences not only in the number and type of FAA in them but also in their concentration. The FAA of the various body parts the adult females of A. lugubris. The amino acids lysine, methionine, butyric acid, tyrosine and nor-leucine detected in the thorax were found to be absent in the head and abdomen. It is the glutamic acid which is absent in the thorax but is present in head and abdomen. The maximum concentration of FAA, arginine, dihydroxyphenylalanine, Dl-alanine and isoleucine detected in the thorax region only.

1. Introduction

Chromatography is a technique which helps in the separation of the chemical ingredients of a biological sample. The importance lies primarily in its use as an analytical tool. Paper chromatography did not become much popular until the work of Consden *et al.* (1944) who not only introduced, but also demonstrated its applications. Bacteriologists, geneticists, botanists, zoologists and a variety of other workers have employed paper chromatography for the study of proteins, amino acids, carbohydrates, steroids, antibiotics, vitamins and many other substances in the biological systems. Entomologists and many others interested in the biochemistry and nutrition of the organisms have employed this method for the purpose of studying the amino acids of the insects because of its simplicity, versatility, reproducibility and the possibility of identifying impure substances. Adriano *et al.* (1953), Kirk et *al.* (1954) & Micks (1956) have applied paper chromatography as a tool in taxonomy and population genetic studies. The various chromatographic techniques have also provided their usefulness in the determination of various organic substances in the biological systems to a standard accuracy by which differences or similarities in the free amino acids can be observed at intraspecific as well as interspecific levels. Buzzarti (1953), Fox (1954, 56) and Kaplan *et al.* (1957) are the few taxonomists who made the use of this technique. Moreover, from the chromatography of the developmental stages, the interrelationships of the species can be well established. Lot of work is available in this field but on other representatives of insects than the order Orthoptera. In the flies and mosquitoes, various workers have analyzed their various developmental stages for FAA in them (Agrell, 1949; Hodorn and Mitchell, 1951; Chen, 1960; Dang and Pant, 1964; Chaput and Lilies, 1969; Singh et *al.* 1971, 1973; Cavalloro and Phillippe, 1974; Sarin, 1975; Sidhu a-nd Kang, 1979 and Gakhar and Nagpal, 1989).

Earlier workers have analyzed the FAA in the whole body of the males and females of various insect species to see the differences in them *(*Auclair and Dubreuil, 1952; Fox, 1954; Kaplan et *al.*, 1957; Chen, 1958; Chen and Diex, 1961; Duffy, 1964 and Thakare et *al.*, 1976).

The concentration of the free amino acids in the insect body is higher as compared to the other animals. The higher concentration of the free amino acids is believed to play an important role in osmoregulation as suggested by Bishop *et al.* (1926) and Beadle & Shaw

(1950). Besides, buffering of the blood, the main function of the free amino acids is in the protein synthesis or in metabolism. Quite a number of amino acids are known which play a role in the synthesis of cuticle i.e. cuticular proteins, chitins, polyphenols etc. as reported by Pant and Aggarwal (1963) According to Pant and Varma (1974), the free amino acids play an important role in metamorphosis and detoxification mechanism in the insects similar to the higher animals. Similar reports have been also given by Shyamala (1964), Chen (1958) and Kaplan et al. (1958), Fox (1956), Duffy (1964), Thayer and Terzian (1970) have reported that there exists a difference between the amount and kind of ninhydrin positive substances in the two sexes of the various insects. Pratt (1950), Micks and Ellis (1951) have also attempted to analyze the free amino acids in the adult insects in order to differentiate the sexes on the basis of chromatographic analysis.

Gakhar and Nagpal (1989) the qualitative and quantitative changes in the free amino acid content in the excreta of different larval instars of Diacirisa obliquia a by 2-dimensional paper chromatography. In all 18 free amino acids, have been observed in the instars I and VI. The amino acids aspartic acid/ GABA, leucine/isoleucine, serine and glycine were common in the excreta of all the larval instars. Ten free amino acids aspargine, glutamine, lysine, ornithine, phenylalanine, pro line, tryptophan, tyrosine, threonine and valine disappeared in the instar II and III. GABA and serine were predominant in the excreta of all the larval instars. The change in these have been related to the various physiologcal events during larval growth.

Mathsushima et al. (1989) reported the changes in the free amino acids concentration in the tissues of the fresh water pulmonate, Helisoma duryi during hypertonic stress. They found that the amino acids alanine, glutamate, aspartate, glutamine, glycine and serine were the major components of the free amino acid pool which increased in response to hypertonic stress. The literature thus reveals that chromatographic analysis of free amino acids in the bodies of various insects constitute a promising field of research. It is believed that the present paper which includes the paper chromatographic analysis of the free amino acids in the whole bodies of the nymphs and the adults and in the different body parts of the three adult orthopteran species viz. Acrida luqubris. A. giqantia and Chrotogonus trachypterus would add to our existing scientific information in this area of research..

2. Materials and Method

2.1. Materials

The following three species of orthopterans (Table I) were collected from their natural environment for the analysis of the free amino acids in them. For the study of the quantitative and qualitative differences in the FAA (Free Amino Acid) pattern in the above mentioned species, the whole body of these insects were analyzed chromatographically. Also the body parts of these copies were subjected to such analysis, like the head, thorax and abdomen. The biochemical differences were also observed in the developmental stages (nymphs and adult) and the different sexes of these species.

SSr. No.	Name of species	Area of collection	Time of collection
11	Chrotogonus trachypterus Blanchard.	Backyard of Zoology, Department, P.U.Chandigarh	May-Aug.
22	Acrida giganatea Hbst.	Botanical garden, P.U. Chandigarh	August-Sept.
33	A.lugubris Burr.	P.U. Playgrounds Chandigarh	August-Sept.

Table 1: Name, area and time of collection of the species under study

To keep the accuracy of the spots, the tissue extract was taken from the fixed number of individuals for each analysis (Hodorn and Mitchell, 1951) Therefore, to analyze the FAA from the developmental stages of *C. trachvpterus.* Each time three nymphs, two adult males and one adult female were taken. Also, when analyzing the species for the estimation of free amino acids in different sexes, the number of individuals taken was as follows:

1. *C.trachvpterus* - Males-2 Female-1 2. *A.gigantea*- Males-2 Female-1 3. *A.lugubris* - Males-3 Female-1

The live insects were collected from the grasses either by spreading a net or with hand and taken in test tubes plugged with cotton swabs and brought to the laboratory for extract preparation.

3. Methodology

The outline procedure for the circular or horizontal paper chromatography consists of the following steps.

1. Smple collection 2 Extract preparation 3. Separation (chromatography) 4. Detection

3.1. Extract preparation

Smaller insects could be crushed on the spot after coagulating the proteins in them by boiling. Since the material for the present investigation was of larger size Micks (1956). The individual insects were taken in the watch glass, their hard parts like the legs, antennae, tegmina and the hind wings were removed with the help of scissors. The remaining tissue was crushed in a hand homogenizer with 1-2 ml of distilled water. The material was centrifuged at 2000 RPM for 5 minutes and the supernatant was retained. Protein precipitation was carried out by adding 2-3 drops of 95% ethanol to approximately 2 ml of the supernatant and centrifuging again at 2000 RPM for 3-5 minutes. The supernatant so obtained was then shaken with three volumes of chloroform and subsequent centrifugation done and then the fraction was removed with the help of a clear dropper for free amino acid determination.

3.2. Separation (Chromatography)
The various items required for the experiment are as given below:

3.3. Chromatoqraphic Chamber
This consists of 2 petri-dishes of equal size and diameter. In the lower petridish the solvent' was placed and the second petri dish was used to cover it. The solvent was kept in the chamber for 10-15 mts. before starting the run so that the chamber may get saturated with the solvent vapours.

3.4. Solvent Used
A mixture of butanol, acetic acid and distilled water (60:25:15) was used as- a solvent because it is considered to be the best solvent for the separation of amino acids from the sample.

3.5. Detector/Location Reagent
For the detection of the different amino acids on the chromatogram ninhydrin solution in acetone (0.2%) was sprayed on it using a fine nozzled atomizer as recommended by Toennies and Kolb (1951).
The circular chromatography techniques also called Rutter method or horizontal chromatography. It was carried out in the following steps as recommended by Swarup *et al.* (1981) with slight modifications.
(i) Application of the sample (ii) Solvent run/developing (iii) Detection/Location.

3.5.1. Application of the sample
A circle of a diameter of 1 cm was drawn in the centre of the circular filter paper whatmann no.1 with the help of a sharp lead pencil and a compass. This paper was held with the help of a clean forcep and placed on a watch glass or a petri dish before the sample was applied with the help of a very thin capillary tube. The spotting was done 8-10 times, so that a satisfactory concentrated spot was obtained allowing the previous spot to dry before applying the next one. Much spreading of the spots was avoided keeping the spots confined to a small area.

3.5.2. Solvent run
After the application of the sample, the spots were allowed to dry and then a central hole was made in the paper. A paper wick was cut out from another filter paper in the form of a long triangle and inserted in the hole of the filter paper. This filter paper was then placed in the chromatographic chamber for the solvent run, lifting the upper petri dish of the latter and covering it again, after placing the paper, taking care that the wick was dipping in the solvent. The solvent was allowed to run for 1-1.5 hrs. till it reaches the edges of the petri dish. After this the filter paper was lifted with the help of a foreep, wick removed and the solvent front was marked with the help of a lead pencil. The paper was then dried.

3.5.3. Detection
0.2% ninhydrin in acetone was used as a detector or locating reagent. For this, 200 mg of ninhydrin was dissolved in 100 ml of acetone. Holding the filter paper with the help of a forcep, at a distance, the ninhydrin solution was sprayed on it using an automizer with a fine nozzle. Care was taken to spray the paper uniformly and not in excess. To obtain the coloured spots of the amino acids on the filter paper it was first dried in the open and then warmed in the oven at 100°C for 10-15 minutes. The coloured bands so obtained were studied for the estimation of the various free amino acids in the experimental sample.

3.6. Preservation of the Amino Acid Chromatograms
As the coloured spots fade away within a few hours it is necessary to keep some of the chromatograms without spraying them with ninhydrin for getting the photographs as and when required. For this the chromatograms can be stored at room temperature in clear cardboard or wooden boxes for a few days.

3.7. Photography:
The chromatograms were sprayed with ninhydrin solution, dried in the open and then placed in the oven at 100°C for 1-2 hours. They were then taken out and boundaries of the different spots were marked. The visual colour and t-he colour intensities of the spots were recorded. The following abbreviations were used to record the different colours of the spots. (1) O- orange (2) P- purple (3)V- violet (4) PV purple violet (5) PP-pink purple (6)BV- blue violet. The colour intensities were marked using the signs [-,+,++,+++ indicating the concentration of FAA in the various bands].

3.8. Identification of the Coloured Spots/Arcs/Bands
For this standard map of known 24 amino acids was first prepared under the same experimental conditions as that used for materials under investigation. The R_f (Reference Front) value of the spots was calculated as follow:

$$R_f = \frac{\text{Distance travelled by spot}}{\text{distance travelled by solvent}}$$

The R_f value as defined above is usually infractions but it is convenient to refer it in percentage or numbers (Rf value x 100). Thus the Rf value has been calculated in numbers throughout the present course of investigations.

3.9. Preparation of the Standard Map of Known Amino Acids

1.5 mg of each standard amino acid was weighed separately. Its solution was made by dissolving it in 1 ml of 10% isopropanol. A drop of concentrated HC1 was used to clear the solution, if necessary. The solution of each amino acid was spotted on the circular filter paper whatmann No. l. After the solvent run was made in the chromatographic chamber/ and then it was sprayed with ninhydrin solution and the paper dried in the oven at 100°C for 10-15 minutes. The colour of the spot was noted and its R_f value was calculated. In this way the colour pattern and the R_f values of the 24 known standard amino acids from the kit were obtained.

Sl. No.	Name of the Amino acid	Base triplet code*	Colour	Rf Value
1.	Serine	AGU, AGC	Blue-violet	17
2.	Cysteine	UGU, UGC	Purple	20
3.	Cystine	-	Purple	23
4.	Ornithine	-	Blue Violet	23
5.	Arginine	AGA, AGG	Light-blue	29
6.	Dihydroxyphenylalanine	-	Purple	31
7	Glycine	GGGU, GCC, GCA	Brownish	33
8	DL-Alanine	GCU, GCC, GCA	Blue-violet	37
9.	Iso-leucine	-	Brownish	45
10	Proline	XCCU, CCC, CCA, CCG	Yellow	45
11	Hydroxy proline	-	Yellow	46
12	Threonine	ACU, ACC, ACA, ACG	Yellow	47
13	Histidine	CAU, CAG	Blue-violet	47
14	Aspartic acid	GAU, GAC	Purple	47
15	Lysine	AAA, AAG	Blue-violet	50
16	Methionine	AUG	Brownish	50
17.	Glutamic acid	GAA, GAC	Blue-violet	61
18.	Butyric acid	-	Blue-violet	65
19.	Tyrosine	UAA, UAC	Light-purple	69
20.	Nor-Leucine	-	Blue-violet	59
21.	Tryptophan	UGG	Brownish	70
22	Phenylalanine	-	Purple	74
23.	Valine	GUU, GUC, GUA, GUG	Purple	75
24	L-Leucine	UUA, UUG	Blue-violet	83

Table 2: Standard map of 24 known Free Amino Acids

There have been seen some variations in the Rf values when compared to those obtained by earlier workers. It is perhaps because of the influence of various factors like the temperature, grade of the paper, the nature of the mixture etc. (Swarup *et al*., 1981). Hence to minimize the experimental variations, the standard solutions of the compounds were run individually under the same laboratory conditions as the material during the present studies Nirenberg and Mathael (1961).

To identify the amino acids in the various spots or bands of the sample under study, first the R_f values of all the bands were calculated in each chromatogram. For this purpose both the R_f value and the colour of the spots were taken into consideration to identify the spots. Thus comparing the R_f value and the colour of each spot with that of the standard amino acids, the amino acids in each band were identified and recorded.

3.10. Free amino acids in the whole bodies of nymphs and adults of Chrotogonus trachypterus (Tables 3-5).

The sample extracts of the whole bodies of the nymphs and adult males and females of *C.trachypterus* were prepared according to the procedure given by Micks (1956) but with slight modifications. The chromatograms were prepared, developed and sprayed with ninhydrin solution to study the pattern of free amino acids in them and observed the differences in the nymphs and adults and among the two sexes of the species.

3.10.1. FAA in nymphs

(Table III, Fig.5) The circular chromatogram depicting the free amino acids pattern in the whole body of the nymph of *C. trachypterus* has revealed 8 bands varying in colours from purple to blue-violet and the *Rf* values ranging from 26 to 75. The 15 amino acids which could be identified in these bands are arginine, dihydroxyphenylalanine, DL-alanine, isoleucine, proline, hydroxyproline, lysine, methionine, threonine, histidine, aspartic acid, glutamic acid, phenylalanine, butyric acid and valine. Two unidentified bands have also been observed at Rf values 26 and 56 having purple and blue violet colours respectively. The bands I, II, III and IV carried a very high intensity colour whereas the remaining bands had light colour intensity.

Spot No.	Rf value	Colour	Colour intensity	Amino acids present
I.	26	Purple	+++	Unidentified
II	31	Purple	+++	Arginine, Dihydroxyphenylalanine
III	39	Blue Violet	+++	DL-alanine, Iso-leucine
IV	44	Blue-Violet	++	Blysine, Methionine, Threonine Histidine, Aspartice acid
VI	62	Blue-Violet	+	Unidentified
VII	62	Blue-Violet	+	Glutamic acid, Butyric acid
VIII	75	Blue-Violet	+	Phenylalanine, Valine

Table 3: Free amino acids in the whole body of nymph of C. trachypterus

3.10.2. FAA in adult male: (Table 4)

The circular chromatogrm showing the FAA pattern in the whole body of the adult males of *C.trachypterus* revealed 7 bands varying in colours from pink orange to purple violet and their R_f values ranging from 23 to 82. The 16 amino acids which could be identified in these bands are cystine, ornithine, dihydroxyphenylalanine, threonine, histidine, aspartic acid, glutamic acid, glycine, tyrosine, lysine, methionine, iso-leucine, DL-alanine, proline, leucine and L-leucine. The bands III, IV and V depicted liigh intensity. The band II and II carried a very high intensity color with the band V, VI and VII appear very light.

Spot No.	Rf value	Colour	Colour intensity	Amino acids present
I.	23	Pink-orange	-	Cystine, Ornithine
II	32	Purple-violet	+++	Dihydroxyphenylalanine, Glycine
III	39	Purple-violet	+++	DL-alanine, Isoleucine
IV	47	Purple	+	Throenine, Histidine, Aspartic acid, Proine, Hydroxyproline
VI	47	Light-blue	-	Lysine, Methionine
VII	63	Purple-violet	-	Butyric acid, Glutamic acid
VIII	82	Purple-Violet	-	L-leucine

Table 4: Free amino acids in the whole body of adult males of C. trachypterus

3.10.3. FAA in adult female (Table 5)

The circlar chromatograms depicting the FAA pattern in the whole body of adult females of *C .trachypterus* have revealed 7 bands varying in colours from pink-orange to blue-vilet and their Rf values ranging from 24 to 84. The 15 amino acids which could be identified from these bands are cystine , ornithine, dihydroxyphenylalanine, glycine, DL-alanine, isoleucine, proline, hydroxyproline, threonine, histidine, aspartic acid, glutamic acid, tyrosine norleucine and L-leucine. The bands III, IV and V depicted high intensity. The band V was slightly brownish in colour and this band is retained for a very long time unlike the other bands.

Spot No.	Rf value	Colour	Colour intensity	Amino acids present
I.	24	Light-purple	+	Cystine, onithine
II	32	Pink-orange	+	Dihydroxyphenlalanine, Glycine
III	41	Blue-Violet	++	DL-alanine, Isoleucine
IV	47	Brownish Purple	++	Proline, Hydroxyproline, Threonine, Histidine, Aspartic acid
VI	58	Brownish Purple	++	Glutamic acid, some unidentified compound
VII	69	Blue-Violet	+	Tyrosine, Norleucine
VIII	84	Blue-Violet	+	L-leucine

Table 5: Free amino acids in the whole body of adult females of C.trachypterus

3.11. Free amino acid in the whole bodies of adult males and females of Acrida gigantea (Tables 5, 7)

The sample extracts for the chromatography were prepared from the whole bodies of the individual insects of both the sexes. The chromatograms were sprayed with ninhydrin solution after developing in Butanol-Acetic acid-water solvent. The chromatograms were then studied for the FAA in this species.

3.11.1. FAA in the adult male: (Table 6)

The circular chromatograms depicting the pattern of FAA in the adult males of *A.gigantea* have revealed 9 bands ranging in the colours from yellow and purple to dark-violet and their Rf values ranging from 18 to 71. The 16 amino acids which could be identified

in these bands are serine, cysteine, DL-alanine, cystine, isoleucine, ornithine, arginine, dihydroxyphenylalanine, glycine, proline, hydroxyproline, threonine, histidine, aspartic acid, glutamic acid and tryptophan. The bands I and II showed a very high intensity. An unidentified band of yellow colour is also present at R_f value 63.

Spot No.	Rf value	Colour	Colour intensity	Amino acids present
I.	18	Pink-Purple	+++	Serine, Cystine
II	24	Pink-Purple	+++	Cystine, Ornithine
III	29	Purple	+	Arginine
IV	32	Purple-Violet	+	Dihydroxyphenylalanine, Glycine
V	37	Violet	+	DL-alanine, Isoleucine
VI	45	Purple-Violet	+	Proline, Hydroxyproline, Threonine, Histidine, Aspartic acid
VII	58	Purple-Violet	+	Glutamic acid
VIII	63	Yellow	++	Unidentified
IX	71	Purple	+	Tryptophan

Table 6: Free amino acids in the whole body of adult males of A.gigyantea

3.11.2. FAA in the adult female (Table 7)

The circular chromatograms depicting the pattern of FAA in the adult females of *A.gigantae* have revealed 9 bands. The 19 free amino acids identified in these bands are serine, cysteine, cystine, ornithine, arginine, glycine dihydroxyphenylalanine DL-alanine, Iso-leucine, proline, hydroxyproline, threonine, histidine, aspartic acid, lysine, methionine, tyrosine, nor-leucine and tryptophan. The bands III and IV carried high intensity while the bands , II, VII, VIII and IX were very light in colour.

Spot No.	Rf value	Colour	Colour intensity	Amino acids present
I.	18	Orange	-	Serine, Cysteine
II	23	Blue-Violet	-	Cystine, Ornithine
III	27	Purple	++	Arginine
IV	32	Purple	++	Dihydroxyphenylalanine, Glycine
V	36	Purple	+	DL-alanine, Iso-leucine
VI	47	Light-Purple	+	Proline, Hydroxyproline Threonine, Histidine, Aspartic acid
VII	49	Violet	-	Lysine, Methionine
VIII	69	Light-Purple	-	Tyrosine, Nor-leucine
IX	71	Light-Purple	-	Tryptophan

Table 7: Free amino acis in the whole body of adult female A.gigantea.

3.12. FAA in the whole bodies of adult males and females of Acrida lugubris (Tables 8, 9).

The sample extracts for the chromatography were prepared from the whole bodies of the adult insects. The chromatograms were spotted, developed and sprayed with ninhydrin solution. They were then studied to analyze the free amino acids in them.

3.12.1. FAA in the adult male (Table 8)

The circular chromatograms depicting the free amino acids in the whole bodies of adult males of *A.lugubris* revealed 8 bands, varying in colours from light orange to purple violet and the R_f values ranging from 23 to 74. The 14 amino acids which could be identified from these bands are cystine, ornithine, aginine, dihydroxyphenylalanine, glycine, DL-alanine, isoleucine, proline, hydroxyproline, lysine, methionine, butyric acid, phenylalanine and valine. The bands III and IV depict very high intensity while the bands V, VI, VII and VIII showed very light cololur.

Spot No.	Rf value	Colour	Colour intensity	Amino acids present
I.	23	Ligh-Orange	-	Cysteine, Ornithine
II	29	Purple-Violet	-	Arginine
III	32	Purple	++	Dihydroxyphenylalanine, Glycine
IV	36	Purple	++	DL-alanine, Iso-leucine
V	44	Yellow	-	Proline, Hydroxyproline
VI	50	Light-Purple	-	Lysine, Methionine
VII	66	Light-Purple	-	Butyric acid
VIII	74	Light-Purple	-	Phenylalanine, Valine

Table 8: Free amino acids in the whole body of adult males of A.lugubris

3.12.2. FAA in the adult female (Table 9)

The circular chromatogram depicting the free amino acids in the whole bodies of adult females *A.lugubris* revealed 8 bands.. The 16 amino acids, which could be identified in these bands are cystine, cysteine, ornithine, arginine, dihydroxyphenylalanine, threonine, histidine, aspartic acid, glutamic acid, phenylalanine, valine, DL-alanine, isoleucine, proline glycine and hydroxyproline. The intensities of the bands II, III and IV are quite high. An unidentified band of blue-violet colour is observed at Rf value 61.

Spot No.	Rf value	Colour	Colour intensity	Amino acids present
I.	22	Pink-Purple	-	Cysteine, Cysteine, Ornithine
II	27	Purple	-	Arginine
III	32	Violet	++	Dihydroxyphenylalanine, Glycine
IV	37	Violet	++	DL-alanine, Iso-leucine
V	46	Violet	+	Proline, Hydroxyproline, Threonine, Histidine, Aspartic acid
VI	56	Violet	+	Unidentified
VII	61	Blue-Violet	+	Glutamic acid
VIII	75	Blue-Violet	+	Phenylalanine, Valine

Table 9: Free amino acids in the whole body of adult females of A.lugubris

3.13. Free amino acids in the head, thorax and abdomen of the adult females of A.lugrubris (Tables 10, 11 and 12)

The sample extracts were obtained from the different parts of the bodies of adult females of *A.luqubris*, like head, thorax and abdomen and were analysed chromatographically for obtaining the free amino acids pattern in them. The chromatograms were developed using Butanol-Acetic acid-Water as the solvent, dried and sprayed with ninhydrin solution. They were then studied to analyze the FAA present in them.

3.13.1. FAA in the head (Table 10)

The circular chromatogram depicting the free amino acids pattern in the head of adult females of *A.lugrubris* have revealed 8 bands, varying in colours from pink-purple to purple-violet and their Rf values ranging from 20 to 73. The 15 ainino acids which could be identified in these bands are cysteine, cystine, ornithine, arginine, dihydroxyphenyialanine, DL-alanine, isoleucine, proline, hydroxyproline, threonine, histidine, aspartic acid, glutamic acid, phenylalanine and valine. The bands III and IV showed high intensity and an unidentified band, purple, violet in colour and Rf value 40 could also be observed.

Spot No.	Rf value	Colour	Colour intensity	Amino acids present
I.	20	Pink-Purple	-	Cysteine
II	25	Purple	-	Cysteine, Cysteine, Ornithine
III	30	Purple-Violet	++	Arginine
IV	35	Purple-Violet	++	Dihydroxyphenylalanine, Glycine
V	40	Purple-Violet	+	DL-alanine, Iso-leucine
VI	46	Purple-Violet	+	Proline, Hydroxyproline, Threonine, Histidine, Aspartic acid
VII	60	Purple-Violet	+	Glutamic acid
VIII	73	Purple-Violet	+	Phenylalanine, Valine

Table 10: Free amino acids in the whole body of adult females of A. lugubris

3.13.2. FAA In the throax (Table 11)

The circular chromatograms depicting the free amino acids pattern in the thorax of the adult females of A.lugubris have revealed 8 bands varying in colours from pink-purple to purple-violet and their Rf values ranging from 21 to 80. The 13 amino acids which could be identified in these bands are cysteine, cystine, ornithine, dihydroxyphenylalanine, arginine, glycine, DL-alanine, iso-leucine, lysine, methionine, butyric acid, tyrosine and nor-leucine. The violet and purple-violet bands at Rf values 26 and .80 respectively could not be identified. The intensities of the bands III and IV were very high while the band VI showed very light colour.

Spot No.	Rf value	Colour	Colour intensity	Amino acids present
I.	21	Pink-Purple	-	Cysteine, Cysteine, Ornithine
II	26	Violet	-	Unidentified
III	31	Purple-Violet	+++	Dihydroxyphenylalanine, Glycine
IV	38	Purple-Violet	+++	DL-alanine, Iso-leucine
V	51	Purple-Violet	++	Lysine, Methionine
VI	56	Purple-Violet	-	Butyric acid
VII	69	Purple-Violet	+	Tyrosine, Nor-leucine
VIII	80	Purple-Violet	+	Unidentified

Table 11: Free amino acids in the thorax of adult females of A.lugubris

3.13.3. FAA in the abdomen (Table 12):

The circular chromatogram prepared from the abdomen of the adult females of *A.lugubris* revealed 8 bands varying in their colours, from pink-purple to purple-violet and their Rf values ranging from 20 to 73. However, only 9 amino acids could be identified in these bands namely cystine, cysteine, ornithine, glycine, DL-alanine, isoleucine, glutamic acid, phenylalanine and valine. The bands IV and V showed high intensity whereas all the other bands appeared very light. The ninhydrin positive compounds in the bands II and IV at Rf values 26 and 44 respectively could not be identified.

Spot No.	Rf value	Colour	Colour intensity	Amino acids present
I.	20	Pink-Purple	-	Cysteine
II	22	Purple	-	Cysteine, Cysteine, Ornithine
III	26	Purple-Violet	+	Unidentified
IV	34	Purple-Violet	++	Glycine
V	39	Purple-Violet	++	DL-alanine, Iso-leucine
VI	44	Purple-Violet	-	Unidentified
VII	61	Purple-Violet	-	Glutamic acid
VIII	73	Purple-Violet	-	Phenylalanine, Valine

Table 12: Free amino acids in the abdomen of adult females of A. lugrubris

Paper chromatography technique has been extensively used by various workers in the past for the analysis of free amino acids in the body parts or entire body of various organisms, to observe their variation among the various developmental stages or in various sexes or among the various species and genera at the biochemical level.

To the best of the author's knowledge very little work of this type has been done earlier on the orthopterans. Pant and Aggarwal (1963b) studied the FAA compostion of the haemolymph of *Acrida exaltata* (Orthoptera), *Poecilocerus pictus* (Orthoptera) and *Bellastoma* (Hemiptera). 17 amino acids namely alpha-alanine, Beta-alanine, aspartic acid, cysteic acid, glutamic acid, glutamine, histidine, lysine, ornithine, tyrosine, valine, phenylalanine, proline, ser-ine, glycine, isoleucine, and tryptophan were shown to be present in the adults of *A.exaltata.* They detected 17 FAA in the nymph and 19 FAA in the haemolymph of *Poecilocerus pictus.* They discussed the presence of these free amino acids in relation to the role played by them in various metabolic functions. However, Bellamy (1958) in *Schistocerca greqaria* noted that glutamic acid is more concentrated in the head *than* in any other tissue. Benassi and Columbo (1961) had shown that the total concentration of amino acids remain constant during the larval period in *S. gregaria.*

Pratt (1950), Micks and Ellis (1951), Bail (1952), Scossiroli and Rasmussen (1954), Kirk *et al.* (1954), Micks (1956) and Chen (1958a) made the studies on FAA in various insects in order to observe their variation in the different species or different genera. The others like Kodani (1948), Buzzarti-Traverso (1953), Bellamy (1958), Rakshpal and Singh (1973) and Dhillon and Sidhu (1977) analysed free amino acids in various parts of the organisms.

The present study carried out the analysis of FAA in the whole body or various body parts of three Orthopteran species to observe their differences in:

a) Nymphs and adults b) Adult males and females c) Different body parts viz head, thorax and abdomen.

3.14. Free Amino Acids in Nymphs and Adults

Agrell (1949) reported a slight reduction in the total concentration of amino acids at the initiation of the pupal stages of Calliphora and this concentration rises in the early mid period and again during the latter half of the purple period.

Hodorn and Mitchell (1951) in D.melanogaster observed that maximum concentration is attained at the third instar larval stage.

Micks and Ellis (1951) while working on the larval and adults of Culex molestus, C.fatigans and Aedes quadrimaculatus observed quantitative differences in their ninhydrin positive materials. Chen (1960) demonstrated that a number of free amino acids such as arginine, cystine, glycine, proline, tryptophan, tyrosine and phenylalanine are indispensable in D.melanogaster for moulting, differentiation, pupation and adult emergence. Benassi and Columbo (1961) reported that the total concentration of amino acids remain constant during larval period of Schistocerca gregaria. Dang and Pant (1964) qualitatively analysed the FAA in the developmental stages of Tribolimn castaneum Hbst. Callasobruchus maculatus (Fabr), Corcyra cephalonica St., Cadra cautella (Wlk), Dacus cucurbitae Coquillet and CX.etus sp. They found that alanine, leucine/isoleucine/norleucine, tryptophan, methionine/valine aspargine, and threonine are present in all stages of the six species. The remaining amino acids- show variability in insects. Chaput and Lilies (1969) found that in Aedes the total concentration of amino acids is maintained at a constant level during the larval development of Leucinodes orbanalis. Cavalloro and Phillippe (1974) identified 19 amino acids in the larvae and adults of Dacus olea. Sarin, K. (1975) reported that various amino acids present in the adults also occurred in the adults of Alphatobius diaperines (Panzer). Gakhar and Nagpal (1989) qualitatively and quantitatively studied the changes in FAA content in the excreta of different larval instars of Diacrisa obliquia and observed 18 amino acids in the instars I and VI. While aspartic acid, GABA, leucine/isoleucine, serine and glycine are common in the excreta of all the larval instars, ten amino acids namely asparagine, glutamine, lysine, ornithine, phenylalanine, pro line, tryptophan, tyrosine, threonine and valine disappear in the instars II and III and GABA and serine are predominant in the excreta of all the larval instars. The changes in these have been related by the authors, to the various physiological events during larval growth.

In the present investigations, the whole bodies of nymphs and adult males and females of C .trachypterus have been analyzed for the FAA to study their variations. The FAA pattern from the whole bodies of nymphs has revealed 8 bands varying in colours from purple

to blue-violet and in Rf values from 26 to 75 (Table 3). While the chromatograms of the whole bodies of adult males have revealed 7 bands varying in colours from pink-orange to purple-violet and their Rf values ranging from 23 to 82 (Table 4) and that of the females also carried 7 bands but varying in colours from pink-orange to blue-violet and their Rf values ranging from 24 to 84 (Table 5) . The number, type and concentration of the FAA identified in the nymphs, adult males and adult females have shown variations. While 15 amino acids could be identified in the nymphs and adult males, the number of FAA that could be identified in the adult females is 16. The concentration of the FAA arginine, dihydroxyphenylalanine, DL-alanine/ isoleucine, proline and hydroxypro line is much higher in the nymphs as compared to both adult males and females. The remaining amino acids showed variability.

Two additional unidentified spots have also been observed in the nymphs at Rf values 26 and 56. Hodorn and Mitchell (1951), Kaplan et al. (1957) and Anand (1965) have also detected such spots in the developmental phase of D.melanogaster. They were thought to be the peptides larviae and pupae by these workers. In the authors opinion the presence of the spots at Rf values 26 and 56 in C.trachvpterus may be similar to such peptides. However, nothing can be said with certainity till sufficient data is collected on these lines. The author further feels, depending on the present results, that during the developmental phase there is a change in the number/ type and concentration of FAA in C. trachvpterus. This can be attributed to the fact that a large number of biochemical changes are taking place during metamorphosis. The higher concentration and variation of FAA in the nymphs may be attributed to their requirement for the synthesis of the cuticle i.e. the cuticular proteins.

3.15. Free amino acids in adult males and females of three species of grasshoppers,viz. C.trachypterus, A.gigantea and A. lugubris:

The adult males and females of the same species of insects have been shown to differ in the FAA content in their bodies as reported by various workers.

Auclair and Dubreuil (1952) have shown quantitatively the differences of the FAA and the peptides in two sexes of D.melanogaster but they could not identify the additional spot present only in the males.

Fox (1954) identified 32 FAA in both the sexes of D. melanogaster and also reported the presence of sex peptide only in males. Fox (1956) again investigated the adult entire male and female individuals of D.melanogastr with the view to study chromatographically the role of X and Y chromosomes. According to him, the chromatographic differences between the two sexes are attributed to the number of X-chromosomes and the difference in the balance between the x-chromosomes and the autosomes, which are similar to those involved in the sex determining mechanism. Therefore, the sex determining mechanisms may be itself responsible for chromatographic differences.

The higher concentration of FAA methionine in the females than in the males of D.melanogaster Kaplan et al (1957). The quantitative difference in the FAA between the male and female adult mosquitoes and found that the content of Beta-alanine is distinctly higher in males, whereas the females have higher concentration of methionine sulphoxide Chen (1958a).

Chen and Diem (1961) also reported the presence of free ninhydrin reacting component in the parogonia of adult males in D. melanogaster which corresponds to the sex peptide reported by Fox (1956). This peptide was, however, found to be absent in Culex pipiens and C. fatigans by them. Duffy (1964) observed in the males of three colonized species of mosquitoes, a larger amount of beta-alanine, while in the males of 2 species viz. C.pipiens and C.molestus there is more lysine plus histidine than the females and the males of A.aegypty have more proline and serine than the females. Thakare et al. (1976) reported higher concentration of Beta-alanine in the males than in the females of dragonfly Pentala flavescens. They also reported higher concentration of arginine, leucline, isoleucine, glutamic acid infemales and higher concentration of methionine in males.

The present investigations on the qualitative and quantitative analysis of FAA in the adult males and females of the three species of orthopterans viz. C.trachypterus A. gigantean and A.lugurbis have also revealed differences in their FAA patterns in the two sexes of the these species as reported by the earlier workers but on different group of animals.

3.16. FAA in Adult Males and Females of C.Trachypterus

During the present investigation (Table IV and V), the maximum of free amino acids which could be identified in adult males and females of C.trachypterus is 16 and 15 respectively. A light blue coloured band present in the males oat Rf 49 representingthe amino acids lysine and methionine has been found to be absent in the females of this species whereas, the blue-violet and at Rf value 69 representing tyrosine and nor-leucine is present in the females but is absent in males. Butyric acid also appears to be absent in the adult females. In females the band V observed at Rf value 58 is slightly brownish in colour and its colour is retained for a long time as compared to all other bands. The brownish coloration may be due to the presence of some other unidentified ninhydrin positive compound along with glutamic acid. But nothing can be said about the nature of this compound that has occurred only in females.

The intensities of band II and III in the males of the species are very high when compared to the corresponding bands in the females .Therefore, one can then say that the amino acids dihydroxyphenylalanine, glycine, DL-alanine, isoleucine occur in higher concentration in males as compared to females adults of this species. The band IV, V and VII have higher colour intensity in the females as compared to corresponding bnds in the males.

These findings make one to conclude that the two sexes of C.trachypterus differ in the type and concentration of FAA in them. No sex peptide like the one observed by earlier workers like Fox (1956) in the males of D.melanogaster, have been detected in the adult males of this species.

3.17. FAA in Adult Males and Females of A.Gigantea

From the 9 bands revealed in the chromatograms showing the FAA pattern in males, 16 amino acids could be identified but the female adults of the same species had 19 amino acids in them. The concentration of FAA in the bands I and II is very high in males on compared to that of the corresponding bands in females. The amino acids in these bands are namely serine, cysteine, cystine and ornithine whereas the bands III, IV and IX are more intense in males are compared to the corresponding bands in the females.

The concentration of arginine, dihydroxyphenylalanine and glycine is higher in the females of A.lugubris. This is in the agreement with the work of Duffy (1964) who obtained similar results in C.pipiens. The glutamic acid could not be detected in females of the species under study but has been found to be present in the males. The band corresponding to the band VII in females representing the free amino acids, lysine and methionine has been found to be absent in the males .The concentration of lysine and methionine has been reported to be higher in the famles as compared to males of D. melanogaster by Fox (1956) and Kaplan et al. (1957) and similarly the concentration of lysine to be higher in the males of both C. pipiens and C. molestus (Duffy, 1964).

A yellow coloured ninhydrin positive band at Rf 63 obsrved in the males of A.gigantea during the present investigations could not be analyzed for the presence of any FAA in it. Fox (1954) and Chen and Diem (1961) have also reported the presence of such a spot in the chromatogram of males of D.melanogaster that was present in the females. According to these workers this compound (spot) is a sex-peptide.

The present studies on FAA in the adult males and females of A.gigantea have revealed that the two sexes of this species differ in the number, type and concentration of FAA in them. This may be attributed to the varied biochemical activities and the differences in the number of x-chromosomes in the two sexes.

3.18. FAA in the Adult Males and Females of A.Lugubris

Although the chromatograms of both male and female adults of *A.lugubris* have revealed 8 bands, the number of amino acids which could be identified in these are 14 and 16 respectively. The band V in the male adults is yellow in colour whereas the corresponding band in the females is violet. Therefore, three amino acids identified in this species. The intensities of the bands II and VII are comparatively higher in the females where compared to those in the males. Therefore, the concentration of amino acids arginine, phenylalaline, and valine is higher in females than males. Glutamic acid is present only in females whereas it is butyric acid which is present only in the males of this species. Lysine and methionine are present in the male adults could be not identified in the females adults. An unidentified ninhydrin in positive band has been observed at Rf value 56 in the adult females but nothing can be said about the nature of the compound.

The studies thus point out both the adult males and females of *A.lugubris* vary not only in the number and type but also in the concentration of FAA in them, which accounts for the differences in the two sexes with varied metabolic actitieis in them.

3.19. Comparison of the FAA in the 3 Specieis of Orthopterans Viz C.Trachypterus and A.Gigantea, A.Lugubris

Micks and Ellis (1951) had pointed out that three genera of mosquitoes (Culex, Aedes and Anopheles) could not be readily distinguished by marked quantitative differencs in the amino acid levels. They had also observed that the larvae and adults of Culex molestus, C.fatigans and Aedes quadrimaculates, although look alike exhibited quantitative differences in their ninhydrin positive materials. Ball (1952) analyzed the FAA in the whole bodies of three species of Culex mosquiotoes (fatigans, pipiens, molestus) and reported and quantitatively different chromatograms.

Kirk et al. (1954) chromatographed fresh tissue extracts of the foot muscle of seven species of the land snails for their taxonomic study and observed that each species gives a pattern which can be distinguished clearly from that of the others.

Micks (1956) analyzed the whole specimen as well as extracts of Hemiptera, Orthoptera and Diptera chromatographically for their ninhydrin positive materials and observed that there exists qualitative difference among them. He further reported that the three species of Culex as well as Aedes also possess qualitatively different chromatograms of fluorescent substances.

Comparison of the FAA in the whole bodies of the three species under investigations has revealed that the number of of bands in males of C.trachypterus, A.gigantea and A.lugubris are 7, 9 and 8 respectively. The number of FAA detected in them is 16, 16 and 14 respectively. Serine which is found to be the present in the males of A.gigantea is absent in the males of other two specicies under investigation. The maximum concentration of dihydroxyphenylalanine, glycine, DL-alanine and isoleucine has been found in the males of C.trychypterus. The light blue coloured bands representing the FAA lysine and methionine could be seen in the males of C.trachypterus and A.lugubria but absent in the males of A.trachypterus but showed its absence in the males of A.gigantea and A.lugubris. The chromatograms of the adult females of the three specicies under investigation have also revealed a lot of qualitative as well as quantitative difference in their FAA pattern. Serine has been seen to be present in the adult females of A.gigantea but absent in the females of other two species viz. C.trachypterus and A.lugubris.

The number of coloured bands observed in the females of the three specices under investigation are 7,9 and 8 respectively. The maximum number of FAA is present in the females of A.gigantea. The FAA tyrosine and nor-leucine are present in the females of A.lugubris. L-leucine is present only in the females of C.trachypterus, tryptophan in A.gigantea only and phenylalanine appears to be present only in A.lugubris. The violet coloured band at Rf. 49 representingthe FAA lysine and methionine is present only in A.gigantea.

The studies made thus show that the different species of grasshoppers also very biochemically and these differences correspond to the differences in their taxonomic levels.

3.20. Free Amino Acids in the Head, Thorax and Abdomen of Adult Females of A. Lugubris

Hodorn and Mitchell (1951) studied the various developmental stages, the different body parts and organs of D.melanogaster and observed various significant differences in the size and in colour intensities of the spots, in different tissues and in the genotypes, showing a new field in the study of biochemical genetics. They reported the maximum concentration of the FAA in the thorax and the minimum concentration i.e. only traces in the malphigian tubules. Bellamy (1958) observed in D. melanogaster a maximum concentration of FAA glutamic acid in the head than in any other tissue. Elliot (1958) and Roberts & Baxter (1958) recognized the special metabolic role of gamma aminobutyric acid in nervous tissues like brain in vertebrates.The role of amino acids in buffering, protein and silk synthesis and osmoregulation in the insect blood has been studied by Buck (1953) and Vershtchagan et al. (1961). Shyamala (1964) substantiated the role of glycine in detoxification mechanisms in insects similar to that of higher animals. The present investigations on the FAA composition of the head, thorax and abdomen of adult females A.lugubris has revealed that the 8 bands in the chromatograms of each part probably contain 15,13 and 9 amino acids respectively. The amino acids in the bands III and IV have very high colour intensity in the thorax than that of the corresponding bands of he head and abdomen or there is a maximum concentration of FAA, arginine, dihydroxyphenylalanine, DL-alanine and isolence in the thorax region only. Glutamic acid, phenylalanine and valine, found to be present only in the head and abdomen are absent in the thorax whereas the FAA lysine, methionine, butyric acid, tyrosine and norleucine present in the thorax could not be detected in the head and abdominal parts.

4. Conclusion

The finding thus reveal that not only the different developmental stages, different sexes and different species vary in their FAA content but different body parts of the organisms also differ in the type, number and concentration of FAA in them. This difference s may be attributed to the fact that the different metabolic functions are being carried out in different tissues and organs of the body of an individual. Hence to fulfill this requirement of the synthesis of harmones, enzymes and other structural and functional proteins, the composition of FAA also varies.

5. References

1. Adriano, A. Bazzati-Traverso and Andreas, B.R. (1953). Paper partition chromatography in taxonomic studies. Science, 117 : 58-59.
2. Agrell, I. (1949) . Occurrence and metabolism of the free amino acids during insect metamorphosis. Aceta. Physiol. Scand., 18 :247-258.
3. Anand, M.S. (1965). M, Sc. Thesis. Panjab University, Chandigarh.
4. Auclair, J.L. and Dubreuil, R. (1952). A simple micromethod for the quantitative estimation of free amino acids by partition chromatography. Can. J. Zool., 30 : 109-113.
5. Bail, G.H. (1952). The free amino acids in the whole bodies of culicid mosquitoes. Exptl. Parasit., 1. : 339-346.
6. Beadle, L.C. and Shaw, J. (1950). The retention and the regutation of the non-protein nineteen fraction in the blood of the aquatic larva Sialis tutaria. J. Expt. Biol, 27: 96-109
7. Bellamy, D. (1958). The structure and metabolic properties of tissue preparations from Schistocerca gregaria. J. Biochem. 70 : 580-589.
8. Benassi, C.A. and Columbo, G. (1961). Free amino acids in the haemolymph of Schistocerca gregaria. J. Biochem. 80 : 332-336.
9. Bishop G.H., Briggs, A.P. and Ronzoni, E. (1926). Body fluid of the honey bee larva. J. Biol. Chem., 66 : 77-88.
10. Buzzarti-Traverso, A. A. (1953) Paper chromatographic patterns of genetically different tissues- a contribution to the biochemical study of individuality. Proc. Nat. Acad. Sci., 39 : 376.
11. Cavalloro, R. and Phillippe, R. (1974). Free amino acids in the larva and adults of Dacusolea. Annals. Soc. Entomol. 9 (1) : 141-146.
12. Chaput, R.L. and Lilies, J.N. (1969). Free and peptide bound amino acids during larval and pupal stages of yellow fever mosquito, Aedes aeqypti. (Diptera : Culicidae). Ann. Ento. Soc. America., 62 : 742-747.
13. Chen, P.S, (1958a). Studies on the protein metabolism of Culex pipiens L. Metabolic changes of free amino acids during larval and pupal development. J. Insect. Physiol. 2: 38-51.
14. Chen, P.S. (1960). Changes in free amino acids and protein during larval development of Drosophila and Culex. Verb XIth Int. Kangr. Ent. 53: 201-207.
15. Consden, R., Gordon , A.H., Martin, A.J.P. and Synge, R.L.M. (1944). Quantitative analysis of proteins. A partition chromatography method using paper. Biochea. Jour., 38 : 224-232.
16. Dang, K. & Pant, J.C. (1964). A qualitative analysis of free amino acids in the developmental stages of several insects. Indian. J. Physiol., 12 : 1377--1394.
17. Dhillon and Sidhu D.S. (1977). Free amino acid distribution in certain tissues of Mylabrus pustulata. Thunberg (Coleoptera) Indian. J. Zool., 5 (1) : 29-35.
18. Duffy, J. , (1964). Sex differences in the free amino acids of three colonized mosquito species. Ann. Entomol. Soc. Am., 57 : 24-27.
19. Fox, A.S. (1954). Applications of paper chromatography to taxonomic studies. Science, 123 : 143.
20. Fox A.S. (1956),. Chromatographic differences between males and females in Drosophila melanogaster and role of X and Y chromosomes. Physiol. Zool., 29 : 288-298.

21. Gakhar, S.K. & Vandana Nagpal (1989). Changes in the free amino acid in the excreta during the larval growth of Diacrisa obliquia Walker. (Lepidoptera: Insecta). Biol Sci. 55 (5/6) : 377-380.
22. Hodorn, E. and Mitchell, H.K. (1951). Properties of mutantsof Drosophila melanoqaster and changes as revealed by paper chromatography, Proc. Nat. Acad. Science, 37 : 650-665.
23. Kaplan, W.D., Hackman, B. and Holden, J.T. (1957). Patterns of free amino acids in Drosophila melanogaster. Genetics, 42 : 381.
24. Kaplan, W.D., Hackman, B. and Holden. J.T. (1958). Occurrence of unequal amounts of free methionine in males and females of D. melanogaster. Science, 127 ': 473-474.
25. Kirk, R.L., Main, A.R. and Beyer, F.G. (1954) . The use of paper chromatography for taxonomic study of land snails. Biochem. J., 57 : 490-492.
26. Kodani, M. (1948). The proteins of salivary bland secretions in Drosophila. Proc. Noi. Acad. Sci. (USA), 34: 131-135.
27. Matsushima, Osamu, Hamidpur R. Khan and A.S.M. Saleuddin (1989). Changes in the free amino acids concentration in the tissues of the fresh water pulmonate, Helisoma drug during hypertonic stress. A. Comp. Physiol., 94. (4) : 653-658.
28. Micks, D.W. and J.P. Ellis (1951) . Free amino acids in the adult mosquitoes. Proc. Soc. Exptl. Biol. and Mod. 78: 69-72.
29. ¥icks, D.W. and J.P. Ellis (1952). Free amino acids in the developmental stages of the mosquito. Proc. Soc. Exptl. Biol. and Med.,Z9 : 191-193.
30. Micks, D.W. (1956). Paper chromatography in insect taxonomy. Ann. Entomol. Soc. Am., 49 : 576-581.
31. Nirenberg, M.W. and J.H. Mathaei (1961). The dependence of . cell-free protein synthesis in E.coli upon naturally occur ing or synthetic polyribonucleotides. Proc. Natl. Acad. Sci., USA. 47; 1588-1602.
32. Pant, N.C. and Kapoor, S. (1963a). A qualitative analysis of free amino acids in the developmental stages of normal and aposymbiotic Iasioderma serricorne F. Indian. J. Enotomol., 25 (1) : 9-16.
33. Pant, R. and Aggarwal, H.C. (1963b). Free amino acids of some insect tissue homogenates. Curr. Sci., 32 : 20-21.
34. Pant, R. and Agarwal, H.C. (1963). Free amono acids of the haemolymph of some insects. J. Insect. Physiol. 10 : 443-446.
35. Pant, R. and Varma, R. (1974). Variation of amino acids in Musca nebulo Fabr. during metamorphosis. Indian. J. of Exptl. Biol., 12 : 412-414.
36. Pratt, J.J. (1950). A qualitative analysis of the free amino acids in insect blood. Ann. Ent. Soc. Am., 43 : 573-580.
37. Rakshpal. R and Singh A. (1973). Free amino acids in tissues of Periplaneta americana . App. Entomol. Zool., 8. (4) : 241-243.
38. Roberts, E. and Baxter, C.F. (1958). Proc. 4th Int. Congr. Biochem. Vienna, 3. : 268.
39. Sarin, Kunda (1975). Qualitative analysis of free amino aicds in the developmental stages of Alphetobius diaperinus Panzer. App. Entomol. Zool., 8. (4) : 224-246.
40. Scossiroli, R. E. and Rasmussen, I.E. (1954). Paper chromatography of interspecific hybrids in Drosophila. Drosophil. Inf. Serv. 28 : 155.
41. Shyamala, M.B., (1964. Detoxication of benzoate by glycine conjugation in the silkworm Bombyx mori L.J. Insect Physiol., 10: 385-391.
42. Sidhu.D.S., Kang. Harjit K. (1979). Metabolic reserves and pool size of free amino acids during metamorphosis of Callasobruchus maculatus (F.) Entomon., 4. (1) : 51-59.
43. Singh, K.M., Pandey, T.N. and Teotia, T.P.S. (1971). A qualitative analysis of free amino aciids in the developmental stages of Leucinodes orbonalis Guen. Indian J. Entomol., 33 (2) : 152-157.
44. Singh, K.M, Pandey, T.N. and Teotia, T.P.S. (1973). A qualitative analysis of free amino acids in the developmental stages of Marasmia trapezalis. Guen and Dichochrosis punctiferalis. Indian. J. Entomol., 35 (2): 99-103.
45. Svarup H, Pathak, S.C. and Arora, S. (1981), Laboratory techniques in Modern Biology, Kalyani Publishers, New Delhi, Pages. 241-262.
46. Thakare, U.K., Raina, S.K., Rewatkar, S. and Raghakumar, V. (1976). Qualitative studies of free amino acids and' sex differences of the haemolymph of dragonfly Pantala flavescens. J. Anim. Morphol. Physiol., 22 (I): 16-22.
47. Thayer, D.W. and Terzian, L.A. (1970). Free amino acids and related compound in the tissues of ageing female Aedes aegypti mosquitoes. Jour. Ins. Physiol., 16 : 1-15.