BIOCHEMICAL STUDIES DURING DEVELOPMENT AND AGING IN *CALLOSOBRUCHUS ANALIS* (FAB.) (COLEOPTERA : BRUCHIDAE)

BY

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN DEPARTMENT OF BIOLOGICAL SCIENCES

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

PILANI, RAJASTHAN

INDIA

1976

DEDICATED TO

MY PARENTS,
Sh. B.D. DHAND,
Late Smt. SHARAN DHAND
&
GAURI

ACKNOWLEDGEMENTS

I wish to take this opportunity to express my gratefulness to Dr. S.K. Mitra, Director of B.I.F.S., for kind patronage and financial help throughout the work without which this work would not have been complete. I am thankful to Dr. S.C. Rastogi, Associate Professor, under whose supervision and guidance the present work has been carried out.

My thanks are due to Dr. H.L. Kundu, Head, Deptt. of Biological Sciences, for providing laboratory facilities, to Prof. G.L. Arora and Dr. H.R. Pajni, Punjab University for identification of the specimens.

I am indebted to parents and sisters whose constant encouragement, I have been enjoying throughout this work. My thanks are due to Sqn. Ldr. Mrs. and Mr. O.R. Rao, Lt. C.S. Jagdev, Dr. Rajinder Singh Rai and Mrs. and Mr. R.C. Mohan Rao for their good wishes.

I am thankful to the Institute authorities for awarding me a U.G.C., Junior Research Fellowship throughout my stay.

10/4/'76

(R.K. Dhand)

LIST OF RESEARCH PAPERS

1. Acid and alkaline phosphatase activity in relation to egg laying and aging in Callosobruchus analis (Fab.) (Coleoptera:Bruchidae). Can. J. Zool. 53 : 1500-1504.

2. Trehalase and trehalose in relation to aging in Callosobruchus analis (Fab.) (Coleoptera:Bruchidae) J. Insect Physiology (Communicated).

3. Age related changes in water content, protein, nucleic acids, lipids and sugar content in Callosobruchus analis (Fab.) (Coleoptera:Bruchidae). J. Insect Physiol. (Communicated).

Acid and alkaline phosphatase activity in relation to egg-laying and aging in *Callosobruchus analis* (Fab.) (Coleoptera: Bruchidae)

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Received March 12, 1975

DHAND, R. K., and S. C. RASTOGI. 1975. Acid and alkaline phosphatase activity in relation to egg-laying and aging in *Callosobruchus analis* (Fab.) (Coleoptera: Bruchidae). Can. J. Zool. 53: 1500–1504.

Acid and alkaline phosphatase (EC 3.1.3.2 and EC 3.1.3.1) activities have been studied during aging of male and female *Callosobruchus analis* (Fab.). Acid phosphatase activity was found to be much higher than alkaline phosphatase activity in both sexes throughout adult life. Both the enzymes demonstrate two peaks of activity, one on the 3rd day and the other on the 10th day of the adult life. The first peak coincides with egg-laying activity in the female, where the egg production is found to be at a maximum until the 3rd day. The peak for both enzymes on the 10th day may be responsible for mobilizing of metabolites before the death of insects.

DHAND, R. K., et S. C. RASTOGI. 1975. Acid and alkaline phosphatase activity in relation to egg-laying and aging in *Callosobruchus analis* (Fab.) (Coleoptera: Bruchidae). Can. J. Zool. 53: 1500–1504.

On a estimé l'activité de la phosphatase acide (EC 3.1.3.2) et de la phosphatase alcaline (EC 3.1.3.1) chez le mâle et la femelle de *Callosobruchus analis* (Fab.) durant le vieillissement. L'activité de la phosphatase acide est beaucoup plus grande que celle de la phosphatase alcaline et ce, chez les deux sexes, durant toute la vie adulte. On observe deux sommets d'activité de la part des deux enzymes, un le 3e jour et l'autre le 10e jour de la vie adulte. Le premier sommet coïncide avec la ponte chez la femelle, la production d'oeufs étant maximale jusqu'au 3e jour. Le sommet observé le 10e jour est sans doute responsable de l'accumulation de métabolites qui précède la mort des insectes.

[Traduit par le journal]

Introduction

Aging is accompanied by many biochemical changes in animal tissue at the subcellular level. Of several biochemical mechanisms, alterations in the activity of phosphomonoesterases are believed to explain declining functional efficiency of tissue with increasing adult age. Activity of phosphatases has been studied in relation to aging by many authors. In males of *Musca domestica*, Rockstein (1956) found a steady decline of acid and alkaline phosphatase[1] activity with advancing age. In the same insect, Barker and Alexander (1958) reported a maximum activity of alkaline phosphatase in 2-day-old larvae of housefly and highest acid phosphatase activity in the egg stage. Sex-specific differences for phosphatases were reported by Raychaudhuri and Butz (1965*b*) during the life of *Tribolium confusum*. Lambermont (1960) reported a steady decline of acid phosphatase activity in both the sexes of yellow fever mosquito, *Aedes aegypti*. Rousell (1971) reported an increase in alkaline phosphatase activity in *Musca autumnalis* during the 1st week of adult life followed by a steady decline until the 4th week. Nath and Butler (1971, 1973) recorded that the acid and alkaline activity is age dependent in black carpet beetle, *Attagenus megatoma* and concluded that the enzymes appear to be related to biology of the organism.

Although available literature is stimulating, a satisfactory generalization of the enzyme–aging relationship is not possible unless more data on a variety of insect species are made available. Therefore *Callosobruchus analis* was studied, as it is a short-lived pest on leguminous seeds and does not feed after emergence. The aim of the present study was to determine the acid and alkaline phosphatase activity in relation to aging, sex, and egg production.

Materials and Methods

A homogeneous culture of *Callosobruchus analis* was kept in half-litre culture jars in the laboratory. The jars were half-filled with uninfected peagram seeds, *Cicer*

[1]Acid phosphatase (EC 3.1.3.2) = orthophosphoric-monoester phosphohydrolase (acid optimum); alkaline phosphatase (EC 3.1.3.1) = orthophosphoric-monoester phosphohydrolase (alkaline optimum).

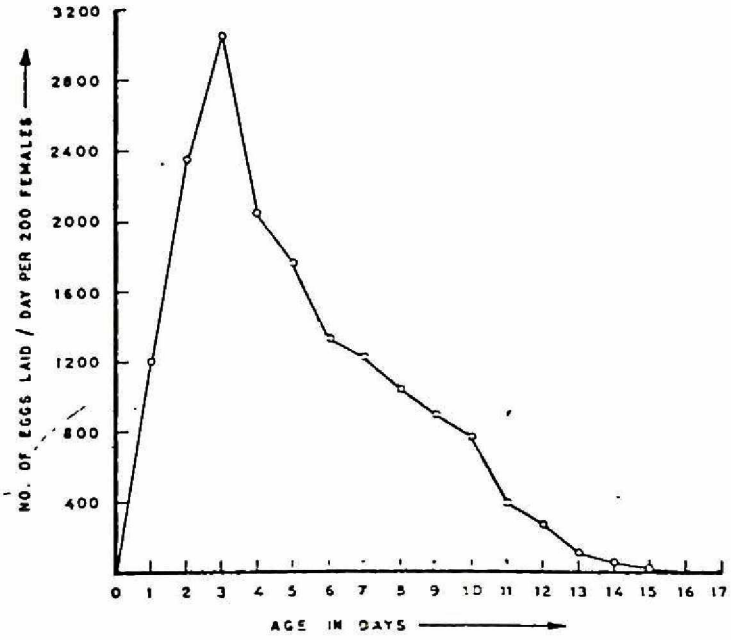

FIG. 1. Egg-laying activity in female *Callosobruchus analis* during aging.

arietinum. Jars were covered with muslin cloth and the culture was kept at 25 ± 3 °C and 60 ± 10% relative humidity. The culture was allowed to go through four generations and each generation was reared on fresh uninfected seeds to obtain a homogeneous culture before enzyme studies were begun.

Insects of both sexes were collected every day at 10 a.m. and were taken to be 24 h old. They were placed in new jars with uninfected seeds, where they were allowed to mate and lay eggs. Insects of different age groups were kept in separate jars at a density of three to five eggs per seed.

Egg-laying Rate

Studies on the rate of egg-laying were made by keeping batches of 50 females with an equal number of males of the same age group in each of four different glass jars. Freshly laid eggs are minute white bodies observable by the naked eye. The eggs were counted at every 24-h interval under the dissecting binocular microscope and the counted ones were marked with India ink to avoid errors. Every 3rd day, old seeds were replaced with new uninfected ones. The same procedure was followed for the next four generations and the average of the number of eggs laid per 200 females was calculated after every 24 h.

Determination of Phosphatase Activity

Acid and alkaline phosphatase activities were studied in either sex at intervals of 24 h from the 1st day of adult life. Activity was studied by the method of Bodansky (1933) with sodium β-glycerophosphate as the substrate. Acid phosphatase activity was studied at pH 5.0 and its alkaline counterpart at pH 9.2. The enzyme activity was measured in micrograms of inorganic phosphorus released at 37 C after 30 min of incubation. Inorganic phosphorus released was measured by the method of Fiske and Subbarow (1925) at 820 nm on a DU-2 Spectrophotometer and the calculations were made per insect.

Enzyme activity was studied in whole-body homogenates, for which males and females were homogenized separately in ice-cold triple-distilled water in a prechilled glass homogenizer. Each time, batches of 30 insects of either sex were weighed and homogenized for 5 min with intermittent cooling, the homogenate was centrifuged at 2500 rpm for 5 min, and the final volume was made up to 20 ml and stored in a refrigerator. Four replicates were taken for each enzyme assay per age group of insects and the average values of the activity of enzymes were calculated per insect.

Results

Average number of eggs laid per day by 200 females was tabulated and plotted as shown in Fig. 1. For the first 3 days, the number of eggs laid showed a progressive increase and thereafter there was a gradual decline until the last day of adult life.

The enzyme activity has been expressed in terms of inorganic phosphorus released per insect during 30-min incubation. From Fig. 2, it is observed that acid phosphatase shows a peak of activity in both sexes on the 3rd day. In males the activity is higher than in females until the 3rd day and declines steadily thereafter until the 6th day; after that there is a gradual increase to another peak on the 10th day. However, in females the acid phosphatase activity is always higher after the 3rd day than is that of males.

Figure 3 indicates an interesting pattern of alkaline phosphatase activity. It is observed that

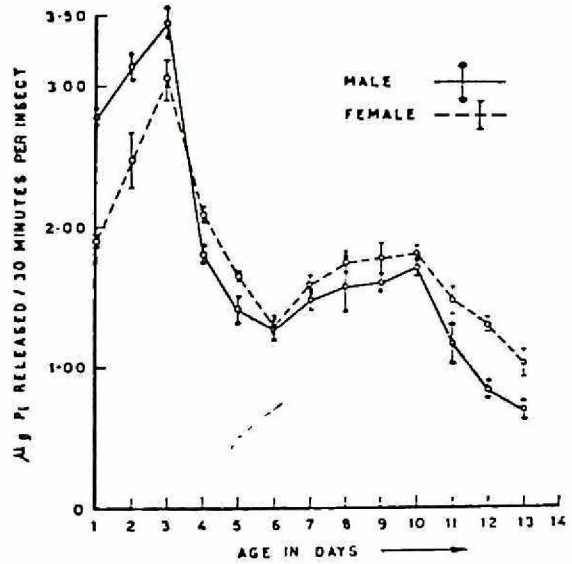

FIG. 2. Acid phosphatase activity in male and female *Callosobruchus analis* during aging. Vertical lines on mean values represent standard deviations.

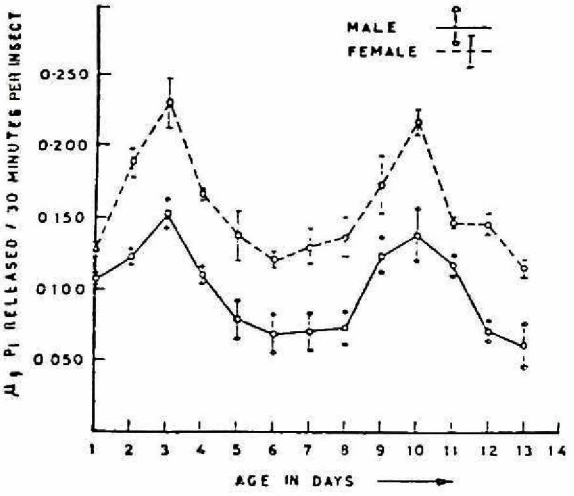

FIG. 3. Alkaline phosphatase activity in male and female *Callosobruchus analis* during aging. Vertical lines on mean values represent standard deviations.

alkaline phosphatase is always at a higher level in females than in males. In females the alkaline phosphatase activity goes on increasing until the 3rd day, thereafter there is a decline up to the 6th day, then an increase to another peak on the 10th day. After the 10th day of adult life the activity decreases sharply on the 11th day followed by a gradual decrease until the 13th day. In males, however, the enzyme shows the characteristic pattern of the female, but always remains at a lower level. The higher level of enzyme on the 3rd day has an interesting correlation with egg-laying (Fig. 1).

From Fig. 4, it is clear that the acid and alkaline phosphatase ratio is always higher in males than females throughout adult life. Here it should be noted that in females the acid and alkaline phosphatase ratio varies from 9 to 16 times from 1st day to 13th day, whereas in males the ratio shows wide fluctuations up to the 8th day of adult life, after which it decreases to the lowest level, remaining almost constant for the rest of adult life, between 10.5 to 25 times. In males, the acid/alkaline phosphatase ratio is almost 1.5 times that of females up to the 10th day of adult life.

Discussion

Considerable evidence now exists to indicate a positive correlation between aging and changes in the activity of acid and alkaline phosphatases in insects. Their role in insect development, especially in relation to nutrition and egg maturation, has been well established (Ludwig *et al.* 1962; Raychaudhuri and Butz 1965*a*, 1965*b*; Nath and Butler 1971, 1973).

Raychaudhuri and Butz (1965*b*) reported in female *Tribolium confusum* two peaks of acid phosphatase, one in early life and the other in the later life of the adult. There is, however, only one peak of alkaline phosphatase in females, soon after emergence. In males, the

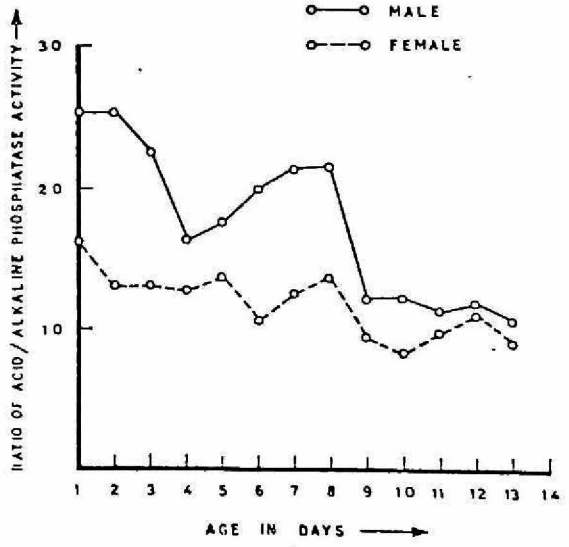

FIG. 4. Ratio of acid phosphatase to alkaline phosphatase activity in male and female *Callosobruchus analis*.

activity of alkaline phosphatase remains almost constant throughout adult life, whereas acid phosphatase shows one peak in early life. In *Tenebrio molitor*, Ludwig *et al.* (1962) were able to record only one peak for both acid and alkaline phosphatase, during early life of the insects. In black carpet beetle, *Attagenus megatoma* (Nath and Butler 1973), it has been shown that the alkaline phosphatase increases steadily up to the 9th day, which is in contradistinction to the observations reported in other species (Rousell 1971; Naqvi and Ashrafi 1968).

The activity pattern of acid and alkaline phosphatase in *Callosobruchus analis* is interesting. Both acid and alkaline phosphatases show a steady increase of activity in both males and females, attaining a peak on the 3rd day of adult life (Figs. 2, 3). Then it declines up to the 6th day and thereafter rises to give another peak on the 10th day of adult life. It is significant to note that after the 3rd day, acid phosphatase is more pronounced in females than in males (Fig. 2). However, alkaline phosphatase activity is always at a higher level in females than in males. High levels of both the enzymes for the first 3 days of adult life appear to be related to their role in nourishing the sperms in males, and in egg maturation and egg-laying in females, since the maximum number of eggs is laid on the 3rd day of adult life (Fig. 1). This observation supports Moog's (1946) hypothesis that acid phosphatase plays an important role in nourishing the sperms. Raychaudhuri and Butz (1965*a*, 1965*b*) also suggested that the sperms produced by the males at the peak activity of acid enzyme may be rich in acid phosphatase, which would perhaps ensure a longer life of the offspring. They further suggested that the eggs produced at the height of enzyme activity by the females are more viable.

As far as the second peak of activity is concerned, two possible explanations could be advanced. First, it may be due to release of acid enzyme from the lysosomes, whose membranes acquire greater permeability before death. Secondly, it is suggested that the spurt of acid enzyme activity on the 10th day may be associated with tissue breakdown and metabolism of stored foods like carbohydrates and fats. Greater transport of metabolites is needed, since the insects do not feed during adult life. Lockshin and Williams (1965*a*, 1965*b*) have suggested that in the last days of adult life of *Antheraea pernyi*, cathepsin and acid phosphatases are released from lysosomes because of increase in permeability of their membranes, resulting in the increase in activity of acid phosphatase as well as cathepsin. By the activity of both of these enzymes, muscle breaks down gradually to ensure biological death. It has also been found that the death rate of the present insect coincides with the high activity of phosphatases (unpublished observations).

It appears that the peak of enzyme activity on the 10th day is concerned with the transport of metabolites to cope with the increased energy needs of the insects, as suggested by Tribe (1966) and Baker and Lloyds (1970). The first peak, however, is concerned with gamete maturation and egg-laying in the insects.

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ABBREVIATIONS

L_1	=	Ist Larva
L_2	=	IInd Larva
L_3	=	3rd Larva
L_4	=	4th Larva
L_5	=	5th Larva
PP	=	Pre pupa
P	=	Pupa
WW	=	Wet Weight
DW	=	Dry Weight
WC	=	Water Content
µg	=	Microgram
Mg	=	Milligram
Pi	=	Inorganic phosphorus

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CHAPTER - 1

INTRODUCTION

INTRODUCTION

In recent years considerable interest has veered round the physiological and biochemical studies during insect development and aging. Consequently, voluminous literature has accumulated on the subject. In insect development, starting from fertilization of the egg to the formation of adult stage, a series of physiological and biochemical changes take place which govern major events like embryonic and postembryonic development including growth, moulting and pupation. Although developmental events are largely under hormonal control (review by Gilbert, 1964), there are enough evidences which suggest involvement of biochemical aspects also. These aspects include changes in the patterns of free amino acid pools, quantitative changes of free amino acids, changes in haemolymph and tissue proteins, lipids, sugars, nucleic acids and enzyme patterns. Since the developing organism represents a most dynamic condition, it is imperative that significant biochemical changes take place throughout the development.

Notwithstanding exciting progress made in this area (reviews by Agrell and Lundquist, 1973, Chen, 1962 and

1966, Gilbert, 1967, and Wyatt, 1967), there are, however, many gaps in our knowledge. A satisfactory correlation cannot be made to explain biochemical changes vis-a-vis morphogenetic events during insect development owing to a wide variety of insect species, their diverse modes of feeding and variable patterns of development.

Another aspect, which has increasingly received attention of the physiologists, is the study of the aging process in insects. Excellent review on aging has been given by Rockstein and Miquel (1973). According to them, aging has been defined as the sumtotal of time dependent reproducible changes, both in structure and function for a given organism during its entire life span.

Therefore, it is logical to presume that aging is accompanied by many biochemical changes in the animal tissue at cellular and subcellur levels. Some isolated studies have been made in this direction on a number of insects concerning total body composition as well as individual tissue composition. Of several biochemical mechanisms, the activities of certain enzymes are believed to explain declining functional efficiency of the tissues with increasing age. Although the available information is stimulating, no general theory of aging could be established in view of the paucity of information on different insect species.

Present study had been undertaken in an attempt to bridge some of the gaps in our knowledge of biochemical changes during insect development and aging. For the purpose, Callosobruchus analis (Fab.) has been chosen. Callosobruchus analis is a serious pest on the stored grains, especially the leguminous seeds. This genus has five species which are cosmopolitan in distribution, out of which at least three species, viz. C. analis, C. maculatus and C. chinensis are common in India.

C. analis lays eggs on the whole seeds, and after completing embryonic life for a few days, the larvae hatch out and bore through the seeds. Larvae are voracious feeders and undergo five successive moultings until they become adult. The adults live for about 15 days and do not feed in this stage.

This insect has been selected for three reasons. Firstly, the life cycle is short, i.e. about 30-32 days. Secondly, pure culture of these insects can be maintained in the laboratory under controlled conditions. Thirdly, there is no information available on the biochemical aspects of development and aging of this insect. The present work arose from all such considerations.

The thesis embodies investigations on the biology, egg laying rate, life tables and survival curves of these

insects under controlled laboratory conditions. Survival curves and life tables lend special support in the study of aging since the life span of aging adults is subjected to the influence of extrinsic and intrinsic factors. Biochemical aspects include activities of certain enzymes, changes in proteins, sugars, lipids, nucleic acids and amino acids, both during development and aging. Besides biochemical studies, changes in wet weight, dry weight and water content have also been determined to find out whether or not there is any correlation between these parameters on one hand and aging on the other hand.

CHAPTER - 2

MATERIALS AND METHODS

2.1 LABORATORY CULTURE

2.2 STUDY OF LIFE CYCLE

2.3 EGG LAYING RATE

2.4 PREPARATION OF LIFE TABLES

2.5 DETERMINATION OF WET WEIGHT, DRYWEIGHT, WATER CONTENT AND TOTAL LIPIS DURING DEVELOPMENT AND AGING.

2.6 ENZYME ACTIVITY DURING DEVELOPMENT AND AGING

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2.10 ESTIMATION OF RNA, DNA AND PROTEIN

2.11 QUANTITATIVE AMINO ACID ESTIMATION

MATERIALS AND METHODS

2.1 LABORATORY CULTURE

Callosobruchus analis (Fab.) was reared in the laboratory on fresh uninfected peagram seeds, Cicer arietinum. In order to obtain a homogeneous culture, the insects were allowed to go through four generations. The culture was maintained in half litre culture jars, half filled with fresh uninfected pea gram seeds. Jars were carefully covered with muslin cloth secured with rubber bands. The culture was maintained in the laboratory at 25 ± 3°C and Relative humidity of 60 ± 10%. The seeds were reshuffled for sufficient aeration and new seeds were added occasionally so as to prevent microbial growth and parasites. Adjustment by manual handling was done in a way so that about 3-5 eggs were laid on a seed. Sexual dimorphism is exhibited by C. analis and the sexes were identified by morphological characters (Raina, 1970 and Arora, 1969). The characters are:

(1) Males are light brown in colour while females are dark brown.

(2) Pygidium in male is directed downward while in females it is directed backward to facilitate copulation.

(3) Males are active, always tracking for females while females are timid and passive.

2.2 STUDY OF THE LIFE CYCLE

Only 24 hour old eggs were selected for the study of development stages. Eggs so obtained were kept under the laboratory conditions and observed daily. After establishment of embryonic period; the time of larval hatching was studied by dissecting the seeds. For the study of different larval stages, the infected seeds were opened daily with sharp, fine needles and the time of sudden increase in the size of larvae and the shedding of old skin was precisely noted. The same procedure was followed for aubsequent four generations and the results were compiled (Table 2.1).

The embryonic period lasts 6-7 days from the time of egg laying. Hatching takes place between 6th and 7th day after egg laying. After hatching has been accomplished, the empty egg shells on the seeds turn creamy white filled with frass and loose their transparency. The newly hatched larvae bore through the seed and remain there until emergence. The larvae undergo through 5 successive moultings inside the seed and develop into a pupa. It takes about 30 days to complete the development until emergence.

Table 2.1 Showing the various larval stages and their duration

S. No.	Stage	Duration of stage (in days)	Age of the developmental stage in hours/days after emergence
1	Embryonic	6-7	144-168 hrs
2	Hatching of larva	Between 6th and 7th	Between 144-168 hrs
3	Ist larva	3-4	Upto 11th day
4	IInd larva	3-4	Upto 15th day
5	IIIrd larva	3-4	Upto 18th day
6	IVth larva	3-4	Upto 22nd day
7	Vth larva	3-4	Upto 24-25th day
8	Prepupa	2	Upto 26-28th day
9	Pupa	3-4	Upto 30th day

From table 2.1, it is obvious that each of the developmental stage lasts for 3-4 days except that of pupal stage which lasts for 5-6 days.

Biochemical studies were done on the developmental stages of definite age group as given below:

I. One day old embryo or 24 hr old eggs

II. Four day old embryo or 96 hr old eggs

III. 1st larval stage or 240 hr after egg laying

IV. 2nd larval stage or 312 hr after egg laying

V.	3rd larval stage	or 384 hr after egg laying
VI.	4th larval stage	or 430 hr after egg laying
VII.	5th larval stage	or 576 hr after egg laying
VIII.	Prepupa	or 624 hr after egg laying
IX.	Pupa	or 672 hr after egg laying

The adult emergence starts 30 days after egg laying.

2.3 EGG LAYING RATE

The egg laying rate was studied by keeping batches of 24 hr old 50 females and an equal number of males of the same age in four different glass jars. Following mating, eggs were laid on the seeds which are visible with the naked eye as small, whitish and shining bodies. The eggs were counted after intervals of 24 hour under the dissecting binocular microscope and the counted ones were marked with India ink to avoid counting errors. Every 3rd day, old seeds were replaced with new uninfected ones. The same procedure was followed for the next four generations and the total count was taken after every 24th hour as mean number of eggs laid per 200 females.

2.4 PREPARATION OF LIFE TABLES

For studying the death rate among adult insects, groups of 80-100 females and equal number of males were taken in 1 litre glass jars within 5 hour of hatching of

adults. Subsequently, the number of dead individuals of either sex were counted after an interval of 24 hour and the dead ones were removed from the jars. This was done until the last survivor also died. The observations and counts were made for 4 generations and the mean value on daily basis was taken. Life tables have been constructed from the mortality rates (Krebs, 1972).

2.5 DETERMINATION OF WET WEIGHT, DRY WEIGHT, WATER CONTENT AND TOTAL LIPIDS DURING DEVELOPMENT AND AGING

Wet Weight (WW). WW per adult of either sex was determined by taking random samples of 100 insects of either sex of the same age by weighing on Mettler balance. The same procedure was followed for the next four generations and the mean WW per insect was calculated. By the same method WW per developmental stage was also calculated.

From the WW of 100 adults, number of insects per gram WW were calculated. Also the number of larvae or pupae per gram WW were also determined.

Dry Weight (DW) and Water Content (WC). WC and DW were determined by gravimetric method. The samples of 100 insects, larvae or pupae previously used for the determination of WW and number of insects, were utilized. The counted and weighted insects were then dried at 60°C on a thermostatic hot plate until DW was constant. Thus the dry

well defined post-embryonic stages and adults of either sex from emergence to 13th day of adult life, so as to find out any correlation between enzyme activity and the different stages of development and aging.

For enzyme activity during aging, insects of both the sexes were collected at 10 A.M. after intervals of 24 hour and the insects so obtained were taken to be as 24 hour old. Similarly, different developmental stages were collected by dissecting the infected seeds at specific times after egg laying.

Preparation of Homogenates. Enzyme activity was studied in whole body homogenate for which males and females were homogenized. Each time 30 insects of either sex were weighed and homogenized separately for 5 minutes with intermittent cooling and the homogenate was centrifuged at 2500 r.p.m. for 5 minutes; the final volume was made upto 20 ml and stored in refrigerator until use.

Similarly, same procedure was adopted for preparing homogenates of larvae and pupae except 1st larval stage, where 60 larvae were used and in the embryonic stage where 400 eggs were taken for each stage.

The homogenate thus obtained was utilized not only for enzyme activity but also for the estimation of RNA, DNA, proteins, total sugars and trehalose.

Acid and Alkaline Phosphatase Activity. Acid and alkaline phosphatase activity was studied by the method of Bodansky (1933), using sodium β-glycerophosphate as substrate. Acid phosphatase was studied at pH 5.0 while alkaline phosphatase activity was determined at pH 9.2

The enzyme activity was measured as μ_gPi released/ 30 minutes at 37°C.

Substrate Preparation. The substrate for acid phosphatase was made by dissolving 0.500 gm of sodium β-glycerophosphate and 0.424 gm of sodium diethyl barbitone in 70 ml of triple distilled water to which 5.0 ml of 1N acetic acid was added. After mixing thoroughly, added a few millilitres of petroleum ether and the solution was made to 100 ml by taking the meniscus in between organic and water layer.

Substrate for alkaline phosphatase was prepared in the same way except that no acetic acid was added in this case. The pH of the solutions so prepared was adjusted with the help of dilute NaOH and 1N acetic acid on Philips PR9405M precision pH meter. The pH was maintained at 5.0 for acid phosphatase and pH 9.2 for alkaline phosphatase. The substrates for both the enzymes were stored in refrigerator.

Determination of Enzyme Activity. For the estimation of phosphatases, six clear test tubes were taken; three each

diluting to a final volume of 100 ml. The pH 5.2 was adjusted on pH meter.

Procedure. For trehalase activity, 4 clear test tubes were taken, marked as incubation tube, sugar blank tube, enzyme blank tube and one blank. The incubation tube showed the experimental enzyme activity, sugar blank gave reducing sugars present in trehalose, enzyme blank tube gave the reducing sugars present in the homogenate, while blank is used to adjust spectrophotometer to null point.

0.6 ml of citrate buffer was pipetted in each tube and then 0.5 ml of trehalose solution was added to incubation tube and sugar blank. The tubes were incubated in a water bath maintained at 32°C to give preincubation for 10 minutes. Then added 0.5 ml of homogenate to incubation tube and enzyme blank tubes, and after mixing the tubes were incubated for 15 minutes at 32°C. After the incubation time was over, tubes were heated in a boiling water bath for 10 minutes and after cooling solutions were made in all the tubes to 2.0 ml with distilled water. Then solutions were centrifuged and supernatant was used for glucose estimation.

Determination of Released Glucose. Glucose released by the trehalase activity was estimated by Folin-Wu method (Oser, 1965). Glucose (BDH ANALAR) was used to make standard curve under exactly the same conditions.

2.7 TOTAL SUGARS

Total sugars in all the stages were estimated by the method of Dubois et al. (1956) using 5% Phenol (ANALAR) and Analar conc. H_2SO_4. The homogenate already prepared was employed for the purpose. Absorbance was measured at 490 nm on DU-2 spectrophotometer. Glucose was employed for standard curve. The total sugars present were calculated as µ gm of sugar/insect WW as well as mg sugar/gm WW tissue and also as percentage of total WW.

2.8 TREHALOSE ESTIMATION

Trehalose content of the total sugars was determined by the chemical method of Wyatt and Kalf (1957). The homogenate was deproteinized by boiling the samples in boiling water for 10 minutes and then used for estimation.

Two test tubes were taken, one as blank and the other for trehalose estimation. To the blank added 0.5 ml of distilled water while in the other 0.5 ml of deproteinized homogenate was added. The solutions were evaporated on a hot plate and the residue was redissolved in 0.2 ml of 0.1N sulfuric acid. The tubes were covered with aluminium foils and heated for 10 minutes in a boiling water bath. Then the solutions in the tubes were made alkaline with 0.15 ml of 6N NaOH and tubes were again kept in the boiling water bath for 10 minutes. After the acid and alkaline treatment

tubes were chilled in ice. In the chilled tubes 2.0 ml of chilled anthrone reagent was added (0.2 gm anthrone/ 100 ml of H_2SO_4). Tubes were again allowed to stand in ice for 10 minutes and then mixed well. After mixing, tubes were again cooled for 5 minutes and then these were heated in a boiling water bath for 10 minutes. The readings were taken at 630 nm on DU-2 spectrophotometer. Standard curve of trehalose was made exactly under the same conditions.

The trehalose content was measured as µ gm of trehalose/insect WW and mg of trehalose/gm WW of insect tissue and also as percentage of the total sugar content.

2.9 SEQUENTIAL SEPARATION OF RNA, DNA AND PROTEIN

RNA, DNA and protein were separated from the aqueous homogenates of different stages by the method of Shibko et al. (1967).

For the sequential separation of RNA, DNA and protein from the single aliquot of homogenates, 5.0 ml of homogenate was taken in a centrifuge tube and chilled in ice. 0.5 ml of perchloric acid (PCA) (60-62%) was added and after mixing, the tubes were cooled in ice for 15 minutes to precipitate protein. Then it was centrifuged at 2500 r.p.m. for 10 minutes. The supernatent was rejected and the precipitated proteins were washed twice with 5% PCA.

RNA Separation. The precipitated protein residue was treated with 10 ml of 0.3N sodium hydroxide and incubated for 90 minutes at 37°C. After incubation 0.5 ml of perchloric acid was added and the tube was ice cooled for 15 minutes. After centrifugation, the supernatant containing RNA was collected. The pellet thus obtained was given two washings with 5% PCA and the whole supernatant was collected and the final volume obtained was 15 ml.

DNA Separation. The pellet was resuspended in 10 ml of 1.5% PCA. After mixing, the tube was heated in a water bath for 30 minutes at 95°C. The tube was removed and 0.5 ml of PCA was added and the mixture was cooled for 15 minutes in ice. It was centrifuged and the supernatant was collected. The pellet so obtained was washed twice with 1.5% PCA and the final volume was made upto 15 ml. This supernatant contains DNA.

Removal of Lipids from the Pellet. The pellet obtained after DNA separation contained lipid and proteins. For removal of lipid, the pellet was suspected in 10 ml of 0.35% PCA in ethanol and the supernatant obtained after centrifugation was collected. The pellet thus obtained was kept for 20 minutes in each of the following; ethanol: chloroform (3:1), ethanol:ether (3:1) and in petroleum ether.

Proteins. The pellet obtained after lipid removal contains proteins. The pellet was dried for 10-15 minutes

Sequential separation scheme for RNA, DNA, Protein

5.0 ml homogenate + 0.5 ml ice cold perchloric acid (60-62%)

Allowed to stand for 15 minutes at 0°C

Wash the pellet twice with 5% PCA (W/V)

combined supernatant (rejected)

can be used for glycogen estimation

suspended the pellet in 10.0 ml 0.3N NaOH

Add 0.5 ml of PCA (60-62%), cool in ice for 15 minutes, wash the pellet twice with 5% PCA (W/V)

combined supernatant RNA (15 ml)

suspended pellet in 10.0 ml of 1.5% PCA (W/V); incubated for 30 minutes at 90°C. Add 0.5 ml of PCA (60-62%); cool for 15 minutes; Wash twice with 1.5 PCA

combined supernatant DNA (15 ml)

suspended and washed the pellet in:

(a) 10 ml of Ethanol/0.35 PCA - 10 min.
(b) 10 ml ethanol/chloroform - 10 min.
(c) 10 ml ethanol/ether - 10 min.
(d) 10 ml petroleum ether - 10 min.

combined supernatant (lipid) rejected

Pellet; dried at 50°C for 10-15 minutes and dissolved in 15 ml of 0.5N NaOH (Proteins)

to remove ether. Then the pellet was dissolved in 15 ml of 0.5N NaOH for the estimation of proteins.

2.10 ESTIMATION OF RNA, DNA AND PROTEIN

RNA and DNA. RNA and DNA were estimated from the supernatant by reading their absorbance at 260 nm. Standard curve for RNA was made by dissolving yeast white powder RNA (Biochemicals unit, Patel Chest) in 0.3N NaOH while for DNA, sodium salt of DNA from calf thymus (Patel Chest) was dissolved in 1.5% PCA and standard curves were made by taking the absorbance at 260 nm.

The RNA and DNA content per insect WW as well as per gm WW of insect tissue were calculated from the absorbancy of separated solutions.

Protein Determination. Proteins were estimated from the protein solutions prepared by sequential separation by the method of Lowry et al. (1951) using sodium citrate at 750 nm and the colour developed was stable for 2 hours. Standard curve of protein was prepared by using bovine serum albumin under the same condition. Calculations were done as µ gm protein/insect WW as well as mgm of protein/gm WW of insect time.

2.11 QUANTITATIVE AMINO ACID ESTIMATION

The qualitative and quantitative estimation of amino

acids was done by paper chromatography. Amino acids were estimated both during development and aging.

Material for Study. For the study of amino acid metabolism during development, five larval and two pupal stages were collected and stored in refrigerator. For aging, 8 stages of either sex were collected from the cultures and the stages employed were; 24 hr., 48 hr., 72 hr., 96 hr., 144 hr., 192 hr., 240 hr. and 288 hr. old adults. For each of the developmental and adult stages, 100 individuals were randomly collected except for 1st and 2nd larval stages for which 200 individuals were used.

Preparation of Amino Acid Samples. Each of the stage was homogenized individually in 5 ml of 70% (V/V) ethyl alcohol and diluted to a final volume of 20 ml. The homogenates were kept overnight and then centrifuged at 2500 r.p.m. for 10-15 minutes each and the supernatants were collected in marked petridishes. The residues were washed twice and the supernatants obtained after centrifugation were collected in separately marked petri dishes. The brownish residue obtained was washed twice in petroleum ether for 30 minutes each time. The petroleum ether washings were rejected and the residue was dissolved in 2.0 ml of 10% isopropyl alcohol and stored in a refrigerator until use.

Chromatography. Descending chromatography was done on Whatman No. 1 paper (18" x 24"). For optimal resolution,

100 µl of amino acid extract was used. The spot applied was in the form of a thin 2.0 cm long line with a micro-syringe by alternate spotting and drying. For identification of spots, 22 known amino acid standard samples were simultaneously applied on the same chromatogram for comparison. Rf values were not calculated.

Butanol:acetic acid:water (4:1:5) solvent system was used and allowed to run for 27 hr. continuously. The chromatograms were dried and passed through ninhydrin solution (0.2% ninhydrin in acetone) and developed by drying in an oven at 55°C for 10 minutes.

Quantitative Estimation. For quantitative estimation, each of the ninhydrin-positive spots were eluted in 4.0 ml of 65% ethanol and constantly shaken for 1 hour for maximum extraction of the reddish blue coloured spots. The absorbance was taken at 570 nm except proline which was read at 440 nm against the blank prepared by extracting a part of spotless chromatogram equal to the size of spots already extracted.

Standard values of known amino acids were obtained by the usual chromatographic technique and the values of the unknown samples compared and calculated as µ gm/insect WW basis.

Note:- All experiments conducted above were given four consecutive repetitions and a mean value was used for calculations. Standard deviation was calculated and drawn wherever necessary.

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CHAPTER - 3

BIOLOGY OF C. ANALIS

BIOLOGY OF C. ANALIS

SUMMARY

This chapter deals with the changes in the wet weight, dry weight and water content during development and aging in either sex of Callosobruchus analis. Besides this, demographic studies and egg laying rate have also been studied. It is found that wet weight as mg/individual declines during embryonic development followed by a constant increase during larval development reaching a maximum value in the 5th larva. During pupation and aging the wet weight/ insect again declines until 13th day of adult life. In relation to changes in the wet weight of insects or their developmental stages, the number of individuals/gm wet weight also varies. Water content is lowest in embryonic stages followed by its constant increase upto 5th larva when it reaches a maximum value. After larval development, water content decreases in pupal stages. During adult life water content is lowest in 24 hour old females which goes on increasing until 13th day. However, in males, it is maximum in 24 hour adult stage and shows three drops in mid life and later attains the original level of water content in last days which is almost equal to 24 hour adult stage.

Dry weight was estimated by subtracting water content from total wet weight and it shows the opposite trend to that of water content.

Number of eggs laid/day increases from Ist day to 3rd day of adult life when it is maximum. Thereafter the egg laying rate declines gradually. As for demographic studies, it has been observed that life expectancy (e_x) of females is higher than males at any stage of adult life. Death rate (1000 q_x) goes on increasing as the age of insects advances. Survival curves obtained are of 'Intermediate type' in which number of deaths/day rises slowly for the first half of insect life followed by an abrupt increase in number of deaths in the 3rd quarter and in the last few days number of deaths/day again slows down.

3.1 INTRODUCTION

A number of reports have appeared on the longivity and life tables (Pearl and Parker, 1924; Pearl and Miner, 1935, Pearl, 1940; Rockstein and Lieberman, 1958, and more recently Nowosielsky and Patton, 1965). Despite these reports, our knowledge regarding the survival and mortality rates in insects is far from satisfactory. Life tables are important since they help us in understanding the "changing force of mortality" or probability of death with advancing age. It is a common knowledge that older organisms are more prone to environmental strains and are liable to show increased death rates which their younger counterparts are able to withstand. Besides this, life tables also help us in the prediction of life expectancy of an individual at any particular age.

Lints and Lints (1969) and Burcombe and Hollingworth (1970) reported that the duration of life in Drosophila is influenced by the temperature at which embryonic and post-embryonic development of the insect takes place. Further, Burcombe and Hollingworth (1970), have also suggested that the speed and the duration of development in Drosophila also modifies the longivity of the adults. Lints and Hoste (1974) recorded that a change in the life span of the offspring depends upon the age of parents. Lamb (1964) studied the effect of radiations on the adult life in insects and

reported that radiations cause a shortening of life span in males whereas in females there is an enhancement of life span which is a consequence of induced sterility and decreased facundity. Berberian et al. (1971) demonstrated that staggered egg laying enhances the life span of females and this enhanced life is due to the manipulation of neurosecretions which control the egg laying and aging. According to Rockstein (1958), longivity is inherited and the life span is the result of combined influence of genetic and environmental factors. Nowosielsky and Patton (1965) noted the effect of mating and grouping on life span. Goodman (1963), reported an increased life span for most fecund, Musca domestica females. Gray and Berberian (1971) showed an increase in longivity of females on increased milk feeding and concluded that the longivity is the result of the effect of enhanced egg maturation and egg laying.

Edward (1958) found variations in the percentage of water content in Chironomous riparius with growth and aging. Lang et al. (1965) and Blevin (1972 and 1973b) have reported a decrease in wet weight and water content with advancing age. On the contrary, Wharton et al.(1965) found an increase of water content with advancing age in American cockroach.

The culture of C. analis was maintained under well-defined laboratory conditions. As according to Lints (1971)

and Rockstein and Miquel (1973) strict control of environmental methods is essential in longivity studies as the amount of variables which influence longivity are enormous and a bad control or the oversight of variables leads to wrong conclusions. The purpose to study the life span, egg laying, wet weight and dry weight and water content under the controlled conditions has been to analyze the biochemical changes during development and aging under the same conditions and also to determine whether any correlation exists between the egg laying rate and life tables accompanied by biochemical changes.

3.2 RESULT OF BIOLOGY, DEMOGRAPHIC STUDIES AND EGG LAYING STUDIES.

3.2.1 Total Wet Weight (WW)

Changes in total wet weight per larva, pupa or adults of either sex are given in Figs. 1, 2 giving observations on the water content during the life cycle. The average weight of the freshly laid egg is 0.0000297 mg, and it decreases slightly until hatching takes place. The larva feeds voraciously and consequently, the WW increases to 0.580 mg until 1st larval stage. The WW of larva increases from 1st to 5th larval stage, when it reaches maximum of 9.55 mg per larva. Fifth larval stage moults into pupal stage between 24-26 days after egg laying. As pupa represents a non-feeding stage having very high metabolic rate, the WW

gradually decreases to 6.93 mg on 28th day after egg laying shows a decrease of 30% loss in WW between 5th larval stage to pupa. This intense loss in WW is obviously due to utilization of stored food during the pupal period.

The adults start emerging from the seeds between 30-31 days after egg laying. The adults further show a decrease in WW to 6.103 mg in females and 5.733 mg in case of males (Fig. 2). As the age of adults of either sex advances, the WW goes on decreasing gradually (Fig. 2A). But the decline in WW is much pronounced during the first 5 days of adult life which is 4.833 mg in females and 3.833 mg in males. Thus there is a decrease of 17% in WW of females and 32% in males. This appreciable decrease in WW in the first few days seems to be a result of intense activity, viz., copulation and egg laying.

By the time the adults are 13 day old, the WW has considerably reduced. The females weigh 3.805 mg/insect and males weigh 2.857 mg/insect.

In order to estimate the difference in the loss of WW of either sex as well as during developmental stages, the number of insects/gm have been calculated and results plotted in Fig. 1B and 2B. It is observed that the WW of females is always higher as compared to the males at any time of the adult life and the loss in WW in males is higher in comparison to females.

3.2.2 Water Content (WC) and Dry Weight (DW)

Variations in WC per WW of larvas, pupae and adults of either sex have been in Figs. 3, 4 and 5. The WC of newly laid eggs 43.5%, which gradually increases to 50% in 5th larva reaching the highest level which gradually decreases to 44% in the pupal stage (Fig. 3). The level of WC per WW of adult insects of either sex has been plotted in Fig. 4. When taken per insect basis, one day males have higher WC but subsequently females have a higher WC than the males (Fig. 4A). Figures 4B, gives percentage of water per WW in either sex. One day old males have 55.7% water. As the age advances from one day onwards, water percentage shows three conspicuous drops, one on the 3rd day, i.e., 52.5%, second on the 6th day, i.e., 53.3%, and third on the 10th day, i.e., 52.5% of the WW. After 10th day percentage of water starts increasing reaching a level of 55.4% on the 13th day. On the other hand, females have lowest water percentage per WW on 1st day of adult life, i.e. 50.4% which increases to 52.1% on 3rd day and thereafter it remains almost constant until 10th day, following which it increases to 55.0% for the remaining period.

As the dry matter is estimated by subtracting WC from the total WW, the dry matter shows exactly the opposite trend as has been shown in Figs. 3 and 5.

3.2.3 Egg Laying Rate

The average number of eggs laid per day by 200 females has been plotted in Fig. 6. For the first three days, the number of eggs laid shows a progressive increase being maximum (3056 eggs) on the 3rd day and thereafter there is a gradual decline until the last day.

3.2.4 Life Tables

Life tables have been worked out to find out whether any correlation exists between enzyme activity and concentration of metabolites on one hand and with the changing "Force of Mortality" on the other hand. Life tables have been prepared from the sample population. The values have been deduced to an initial base value of 1000 individuals of either sex for the purpose of calculations, where

x = age interval

lx = number of survivors at the start of age interval x

dx = number dying during age interval x to $x+1$

qx = rate of mortality during the age interval x and $x+1$

e_x = mean expectancy of life for organisms alive at start of age x

The four different columns which are the results of above calculations, i.e. lx, dx, qx and ex, are just different ways of summarizing one set of data. Thus if we have any one of the columns, we can calculate the rest. For calculating lx, dx, qx and ex, the original number of insects of either sex were calculated to the initial base value of 1000 insects of either sex and columns were made by calculation as

$$lx + 1 = lx - dx$$

where lx+1 is the number of surviving at the start of age with interval x.

$$qx = \frac{dx}{lx}$$

For calculating the expectancy of life (ex) at any given age, we have to obtain average number of individuals alive at each age interval which is called life table age structure ($\mathcal{L}x$);

$\mathcal{L}x$ = Number of individuals alive on the average during the age interval x to x+1

$$= \frac{lx + lx + 1}{2}$$

Then all these different values of $\mathcal{L}x$ from 1st day to the last day of adult life are summed cumulatively from bottom of life table and a set of values expressed in units of individual x time units is obtained (insect days)

which we call Tx;

$$\text{now} \qquad Tx = \sum_{x}^{\infty} lx$$

Finally by dividing Tx by total number of individuals (lx) at each age interval, we can get average age expectation of life;

$$e_x = \frac{Tx}{lx}$$

Thus by the above calculations life tables have been constructed (Krebs, 1972). Fig. 7 gives the number of individuals dying dx where dx is number of individuals dying within the age interval x. Fig. 8 gives the number of individuals (lx) surviving lx is number of individuals surviving to the age interval x. Fig. 9 gives the death rates 1000 qx where 100 qx or $(\frac{dx}{lx})$ is Mortality rate with age interval x. Fig. 10 gives the average exceptancy of life (e_x) where e_x is average life expectancy at any given age x.

The survival curves (Fig.8) obtained are of the intermediate type (Rockstein and Miquel, 1973) showing that the death rate is slow in younger population, gradually increasing during the 3rd quarter of life (between 10-13 days) as evident from Fig. 7 and finally death rate again slows down in the last 2-3 days. Thus the last few survivors attain a longer life span as compared to the average span of other insects.

In case of males, a slight increase in number of deaths/day has been observed between 4th and 5th day, i.e., when the egg laying rate is at its peak (Figs. 6 and 7). The average life expectancy of females is found to be higher at any age (x) as compared to males (Fig. 10).

3.3 DISCUSSION

Changes in the wet weight (WW), dry weight (DW) and water content (WC) have been extensively studied by many authors (Edward, 1958; Warchalowski, 1961; Marek, 1961, 1962; Gere, 1964; Lang et al., 1965; Church and Robertson, 1966; Courture and Huot, 1967; Ring, 1973; Abasa, 1972; Blevins, 1973b; Roonwal and Verma, 1973).

Total WW, DW and WC have been studied in C. analis from 24 hour egg stage to 13 day old insects, i.e., during embryogenesis, post-embryonic and aging periods. The total WW, which is 0.0000297 mg in 24 hr. egg slightly decreases at 96 hr egg stage and afterwards it goes on increasing until 5th larval stage followed by appreciable fall until pupation (Fig. 1A). After adult emergence WW in either sex of C. analis goes on decreasing throughout the adult life (Fig. 2A). From Fig. 2A, it is apparent that WW of females is always higher than males and the loss of WW for the first six days in either sex is very high.

Corresponding to increase or decrease of WW during development and aging, the number of individuals/gm WW also shows some variations. In fact the number of individuals/gm WW shows indirectly the loss or gain of WW at any stage during the cycle (Figs. 1B and 2B). From Fig. 2B, it is evident that the loss of WW during aging in males is much higher compared to females.

The changes in WW of C. analis are understandable when we look at the life of this animal. In the embryonic stage, because of intense metabolic activity, slight decrease in WW is justified as embryonic stage is a closed system where the embryo depends for its energy requirements on the stored materials. After hatching, the larva feeds voraciously and the larval growth takes place in a geometrical progression obeying "Dyars rule". As a consequence of larval growth, the WW also shows a gradual increase in consonance with the growth of the larval tissue (Agrell and Lundquist, 1973). The highest WW in the life cycle of C. analis is gained in the 5th larval stage.

Following pupation, the WW decreases appreciably because pupa is a closed, non-feeding stage, where old larval skin is shed and all the larval tissue are modified to form adult tissue. Excessive metabolic activity required for histolysis and histogenesis explains the loss in WW.

The adults after emergence do not feed and the life

of the individual is dependent upon the stored food materials. This phenomenon was named as "self-destruction" by Gere (1964). The sole function of the males after emergence is to look for the females for mating while the function of the adult females is to lay the fertilized eggs. Both these processes require appreciable amount of energy, hence the loss in WW is incumbent on the individuals.

Similar results were obtained by Marek (1962) on Tenebrio molitor; Gere (1964) on Lymantria dispar; Courture and Hout (1967) on Plodia punctella and Abasa (1972) on Sarcophaga tibialis during the development. Marek (1961), Gere (1964), Lang et al. (1965), Abasa (1972), Blevins (1973) have recorded similar observations on the changes in WW during aging. Recently, Roonwal and Verma (1973) reported a higher WW and DW in the females of Microtermes obesi, compared to males. In contrast to the above results, Marek (1961), reported in Gryllus domesticus, a high WW, DW and WC in males in comparison to females.

The changes in the DW and WC both during development and aging are given in Figs. 3, 4 and 5. From Fig. 3, it is observed that DW as percentage of WW was maximum in 24 hr egg stage which gradually goes on decreasing until 5th larval stage, followed by gradual increase in the pupa. However, WC as percentage of total WW shows exactly the opposite trend of DW. The highest percentage of DW recorded

during embryonic stage is understandable as the eggs of insects have very high yolk content. The decrease in DW during larval stage is due to the accumulation of metabolic water and loss in transpiration. Chen (1951) has suggested that there is a general decrease in metabolic rate following the increase in larval growth of insects except at the time of moultings when the metabolic rate is higher. The increase in DW and decrease in percentage of WC of the total WW in pupa is due to the loss of water by transpiration following the U-shaped respiratory activity and the release of water and CO_2 (Agrell 1947 and 1952).

In C. analis, WC remains almost constant throughout the embryonic development (Fig. 3). Contrary to this observation, Ludwig and Ramazzotto (1965) recorded a loss of WC in embryonic development, while McFarlane (1966) and Moloo (1971) recorded an appreciable increase in WC during embryogenesis. Marek (1962) recorded an increase in the WC in the 1st pupal stage of T. molitor and also reported a constant DW in pupa. Church and Robertson, (1966) noticed the maximum WW of Drosophila melanogaster in 2nd instar larva which declined until prepupa. Edward (1958) also noted variation in WC during growth and moulting. According to Wigglesworth (1970), changes taking place during development are under the control of moulting hormone.

During aging, WC as per cent of WW per insect in females goes in increasing from 1st day to 13th day of adult

life. On the other hand, males show fluctuations in WC in mid-life when it is 52.53%, while after emergence and in the last days of adult life, WC is almost 55% (Fig.4B). From Fig. 4A, it is evident that WC (mg)/insect is higher in females in contrast to males throughout the adult life except on 1st day when males predominate over the females.

In contrast to present observations, Wharton et al. (1965) reported a little change in WC during aging in American cockroach when fully fed, but on starvation, WC goes on increasing with age. As C. analis does not feed after emergence, it can be well compared to the starving insects but only in females WC increases with age whereas males actually show a drop in the WC in mid-life. Pearincott (1960) in Musca and Abasa (1972) in Sacrophoga tibialis, have noticed a constant WC during aging. On the other hand Blevins (1973b) found an actual decrease for 1st two days in Aedes aegypti following which it remains almost constant.

The decrease in the WC in males of C. analis in mid-life can be attributed to high transpiration rate because of high metabolic activity required for mating. It has also been theorised by Calabrese and Stoffolano (1974) in Phormia rigina that high respiratory rate after emergence was due to "Mating Drive". Bursell (1974) gave similar explanation. In case of females, a steady increase of WC can be attributed to the accumulation of metabolic

water. The relatively low WC of C. analis may be due to the presence of very high fat content (Figs. 29 and 30).

DW as per cent of the WW is given in Figs. 3 and 5 and it follows an opposite trend to that of WC. Here the work of Gere (1964) on Lymantria dispar is of considerable significance. Like C. analis, L. dispar also does not feed after adult emergence and the animal depends for its energy needs on stored material by "self destruction". He reported that loss of WW in males is much higher and WC after emergence of the adults decreases and it is 56.5%. He observed that low level of WC was due to the presence of high stored fat content. Females of L. dispar utilize fats for egg laying and males to locate and to compulate with females. He also reported that 80% of stored fats in females are transferred to the eggs.

The number of eggs laid/day by C. analis are given in Fig. 6. From the figure, it is found that the average number of eggs laid/day by 200 females show a progressive increase for the first 3 days, being maximum on the 3rd day after which there is a gradual decline until the last day of adult life. The results of Raina (1970) are at variance with the present observations as regards the number of eggs laid/day by C. analis. Here it would be worthwhile to mention that Raina (1970) studied the egg laying rate of C. analis at 30°C and RH 70%, whereas in

the present study the egg laying rate was studied at 25 ± 3°C and RH 60 ± 10%. In the present work, maximum egg laying is reached on the 3rd day which corresponds to maximum phosphatase activity. The correlation between egg laying rate and phosphatase activity has been shown earlier by Dhand and Rastogi (1975). This observation finds support from the conclusions drawn by Moog (1946) and Raychaudhuri and Butz (1965b).

Life table curves of C. analis are given in Figs. 7, 8, 9 and 10. From Aig. 9, it is observed that number of deaths/day show progressive increase until 8th day followed by a sudden spurt in the number of deaths/day between 9-13th day of adult life of either sex followed by a sudden drop again in the last days of adult life. In case of males, a slight increase in number of deaths/day is noticed between 4-5 days. Survival curve of C. analis is given in Fig. 8 and the curve obtained is of "Intermediate type" (Rockstein and Miquel, 1973; Krebs, 1972) which demonstrates that number of deaths/day for the first few days is less followed by sudden increase in death number/day which again drops on the last days (Figs. 7 and 8). From the figure 8, it is evident that females have higher longivity than males. It is, therefore, logical to presume that animals having "Intermediate type" of survival curve die of real senescence and these types of curves can be employed to correlate the number of deaths/day with

physiological aging (Rockstein and Miquel, 1973). Fig.9 shows the death rate/day. From the figure, it is inferred that the death rate of males is higher than females. Life expectancy of females is given in Fig. 10. It is observed that the expected life of females is always higher than males at any stage of adult life.

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Fig. 1A Changes in wet weight/individual during development.

1B Changes in number of individuals/ gm. wet weight during development.

Fig. 2A Changes in wet weight/insect during aging.

2B Changes in number of insects/gm. wet weight during aging.

Fig. 3 Changes in percent water content and dry weight/wet weight during development.

Fig. 4A Changes in water content (mg.)/insect wet weight during aging.

4B Changes in percent water/wet weight of insect during aging.

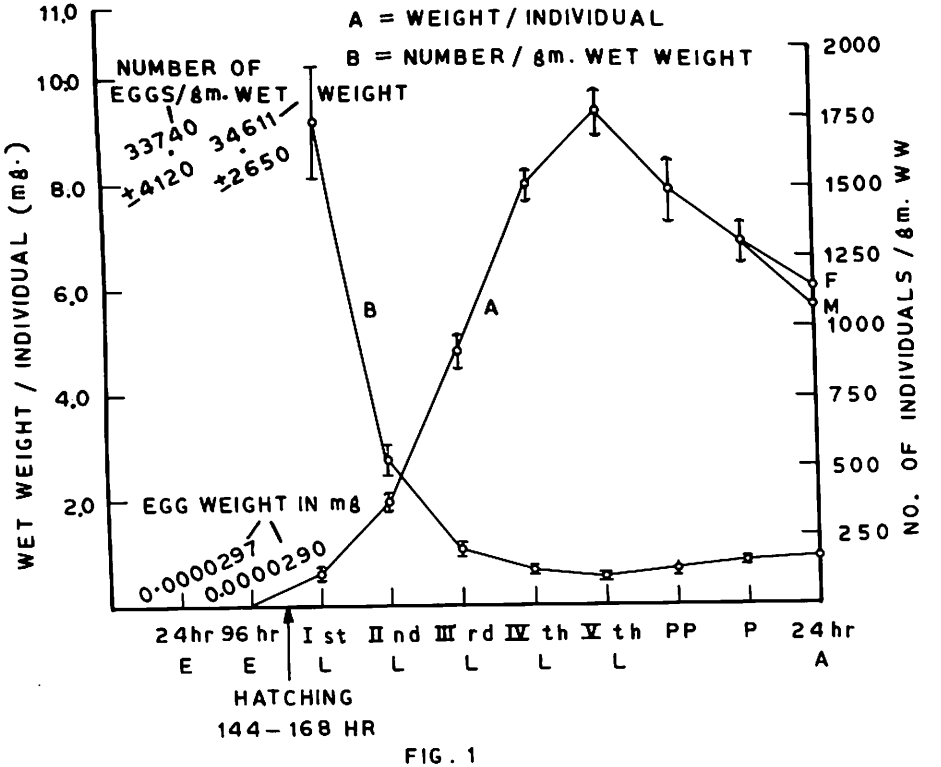

FIG. 1

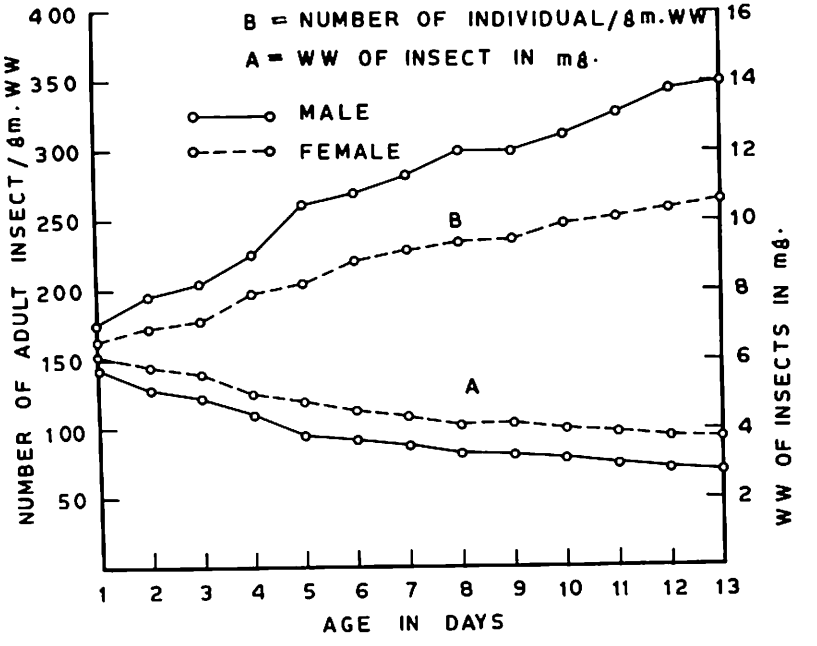

FIG. 2

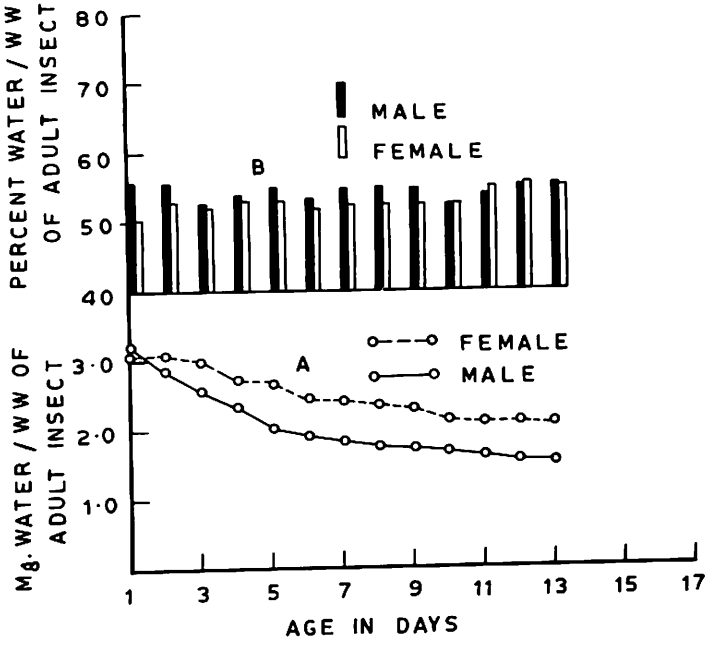

FIG. 4

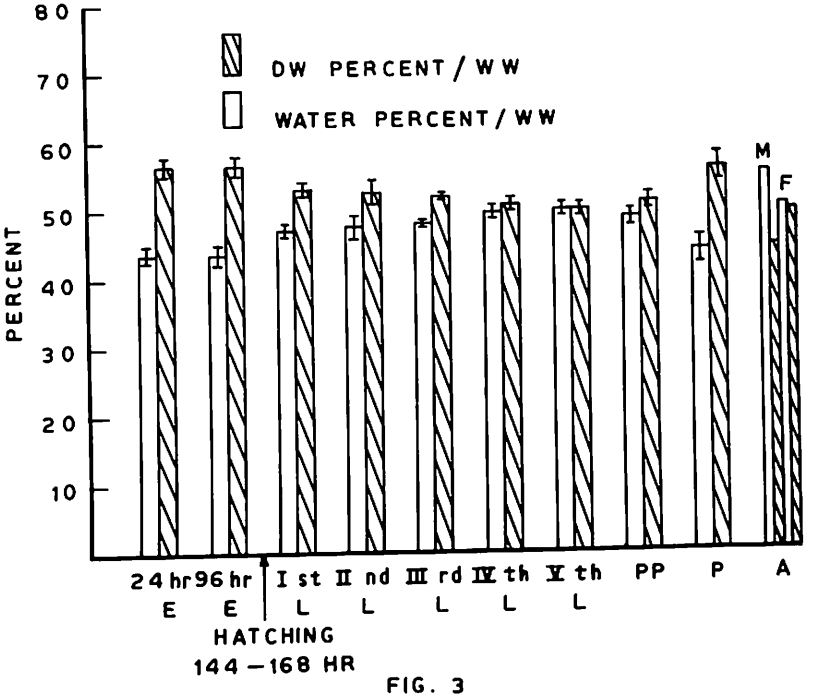

FIG. 3

Fig. 5A Changes in dry weight(mg)/insect wet weight during aging.

5B Changes in percent dry weight/insect wet weight during aging.

Fig. 6 Egg laying during aging.

Fig. 7 Changes in number of deaths/day (d_x) during aging.

Fig. 8 Changes in the nurber of survivors/day (l_x) during aging.

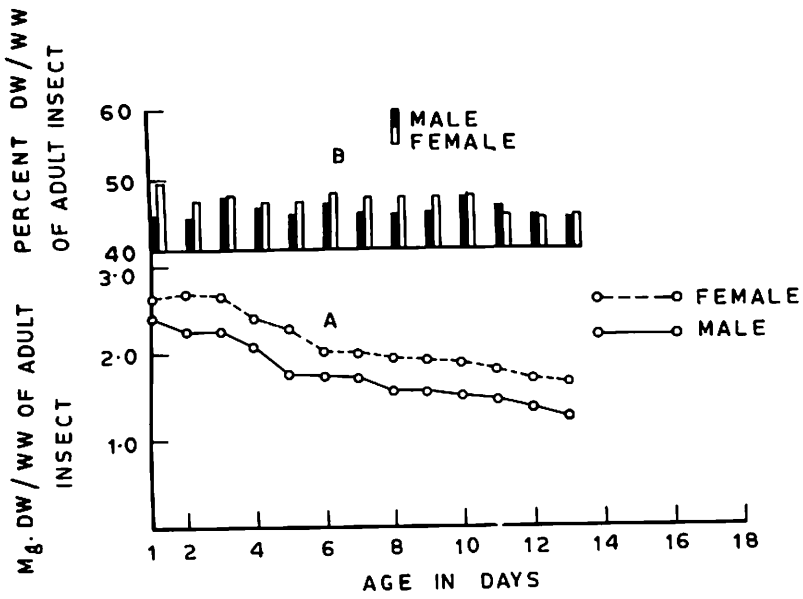

FIG. 5

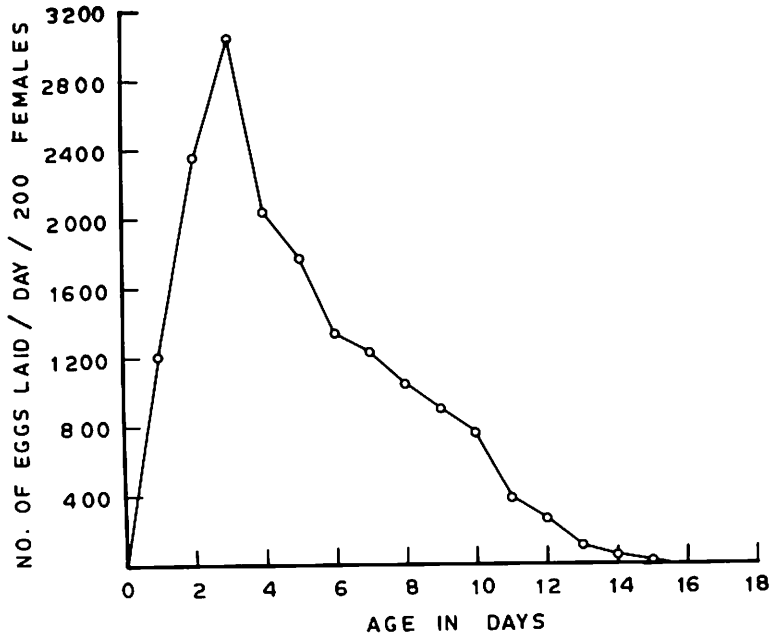

FIG. 6

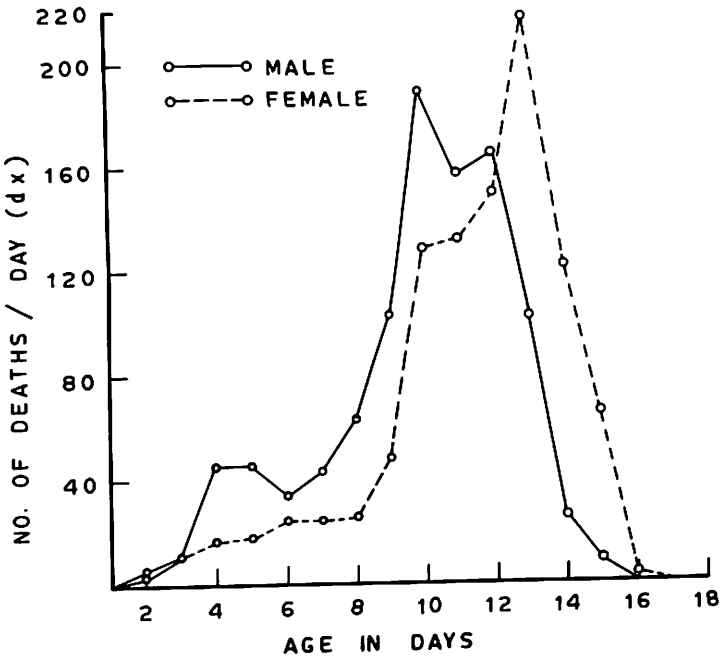

FIG. 7

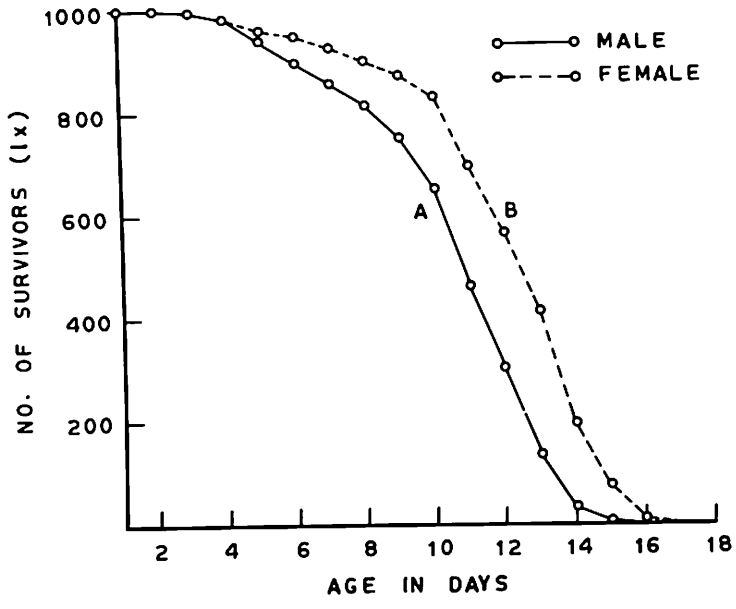

FIG. 8

Fig. 9 Changes in the death rate/day (1000 q_x) during aging.

Fig.10 Changes in expected life in days (e_x) during aging.

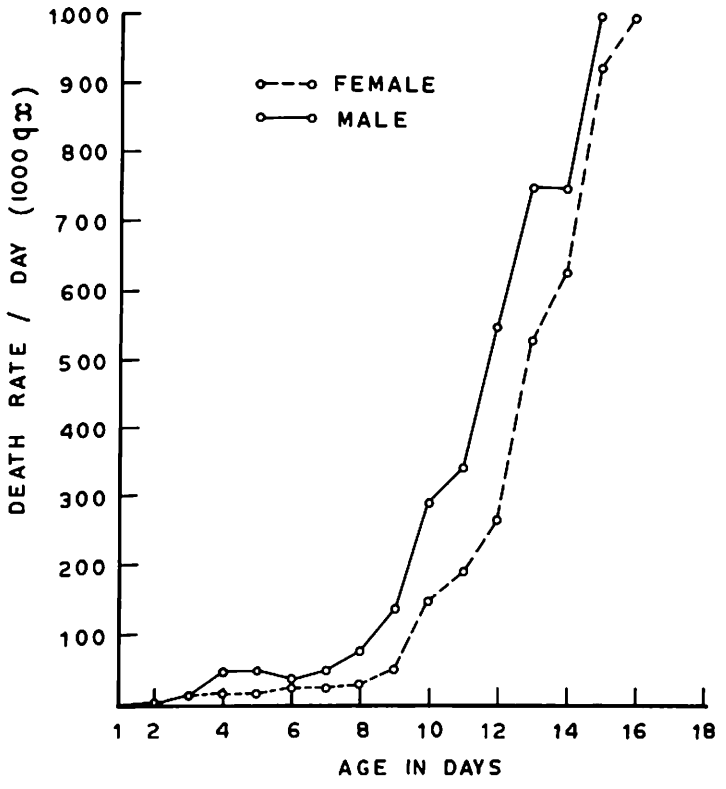
DEATH RATE / DAY (1000 qx)
1000
900
800
700
600
500
400
300
200
100
FEMALE
MALE
1 2 4 6 8 10 12 14 16 18
AGE IN DAYS

FIG. 9

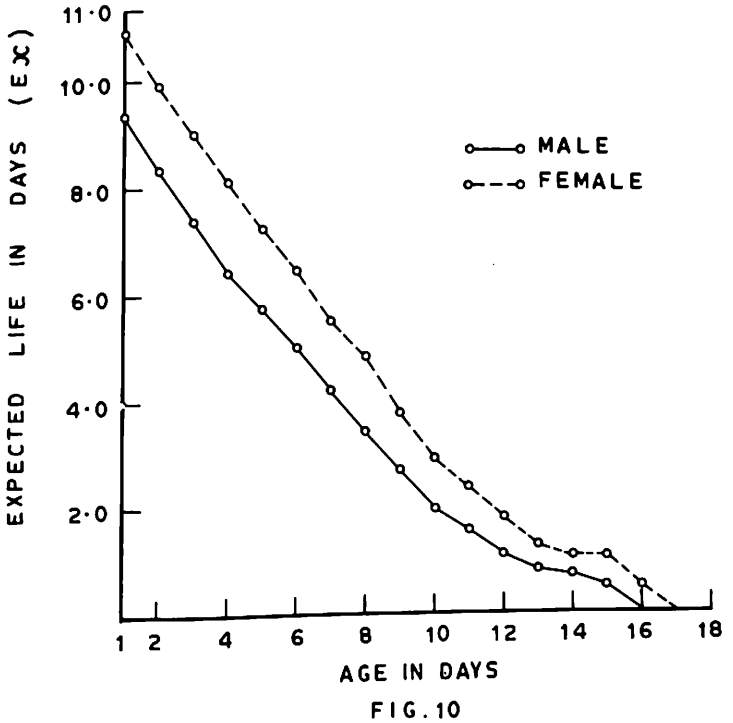
EXPECTED LIFE IN DAYS (ex)
11·0
10·0
8·0
6·0
4·0
2·0
MALE
FEMALE
1 2 4 6 8 10 12 14 16 18
AGE IN DAYS

FIG. 10

CHAPTER - 4

ENZYME STUDIES

ENZYME STUDIES

SUMMARY

In this chapter results of acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1) and trenalase (EC 3.2.1.28) have been presented. Enzyme activities have been studied during embryogenesis postembryonic development and aging of C. analis. It has been observed that the acid and alkaline phosphatase activity shows an increasing trend during embryogenesis while trehalase activity remains almost constant. During postembryonic development all the three enzymes show a peak activity in the 5th larva. In pupa, the activity of all the three enzymes falls markedly. Following adult emergence, phosphatases show two peaks of activity, one on 3rd day and the other on 10th day of adult life. Trehalase activity shows two peaks of activity in females, one on 3rd day and the other on 10th day, while in males this enzyme gives only one peak i.e. on 10th day. During course of investigation, it has been observed that the first peak of activity coincides with egg laying and mating while second peak coincides with the maximum death rate of insects.

The results of the phosphatases and trehalase have been discussed in relation to embryogenesis, larval development, egg laying rate and death rate of the insect.

reported in Drosophila embryo that acid phosphatase shows no change during embryogenesis while alkaline phosphatase appears a little later and concluded that alkaline phosphatase is concerned with histodifferentiation and acid phosphatase plays a role both in synthesis and degradation of yolk. Ashrafi and Fisk (1961) found the highest acid phosphatase activity in egg stage of Stomoxys calcitrans.

Rockstein (1956), while studying biochemical changes in males of Musca domestica, found a steady decline of acid phosphatase with advancing age and so was the case with alkaline phosphatase. In the same insect, Barker and Alexander (1958) reported a maximum activity of alkaline phosphatase in 2-day old larva and highest acid phosphatase in egg stage. Activity progressively decreased until pupation and remained constant thereafter. Sex specific differences for phosphatases were reported by Raychaudhuri and Butz (1965b) during adult life of Tribolium confusum. Lambermont (1960) recorded a steady decline of acid phosphatase activity in both sexes of Aedes aegypti. Rousell (1971) reported an increase in alkaline phosphatase activity in Musca automnalis during first week of adult life followed by a steady decline until 4th week. Nath and Butler (1971 and 1973) recorded that the acid and alkaline phosphatase

activity is age dependent in Attagenus megatoma and concluded that the enzyme appears to be related to biology of the organism.

In a comprehensive review, Chen (1966) concluded that phospnatase in animal tissue are associated with; (1) transport of metabolites, (2) metabolism of phospholipids, phosphoprotein, nucleotides and carbohydrates, (3) synthesis of protein. According to the author acid phosphatase has more general distribution and is involved in such processes as yolk and substrate utilization whereas alkaline phosphatase shows stage and tissue specific role indicating its role in differentiation and physiological function digestion in particular.

Trehalase is one of the most important hydrolytic enzymes as it brings about the hydrolysis of non-reducing dissaccharide trehalose which is a major energy reserve in insects. There are very few reports on the activity of trehalase in insects. Alumot et al. (1969) studied trehalase in adult Apis mellifera and reported its high activity in spermathecae. Dahlman (1970) studied age related changes of trehalase activity in different tissues of Tobacco horn worm. Burcombe (1972) reported a decrease in trehalase activity with age in Drorophila melanogaster. Jenses and Buckner (1973) reported in Musca domestica that trehalase activity is about 20 times higher in adults as compared to larva.

Although available literature on the activity of phosphatases and trehalase during embryogenesis, post-embryonic development and aging is stimulatory, yet more information is called for to draw purposeful conclusions.

In this chapter, age related changes of phosphatases and trehalase have been studied during embryogenesis, post-embryonic development and aging in Callorobruchus analis.

4.2 RESULTS OF ENZYME ACTIVITIES

4.2.1 Phosphatases during development and aging

The acid and alkaline phosphatase activity during development and aging is given in Figs. 11, 12, 13, 14. The enzyme activity here has been expressed in terms of inorganic phosphorus (Pi) released per individual (developmental stage) during 30 minutes of incubation at 37°C.

4.2.1.1 Acid and Alkaline Phosphatase during Development

From Figs. 11 and 13, it is observed that acid and alkaline phosphatase activity reaches its peak in 5th larval stage. The acid and alkaline phosphatase activity is lowest in 24 hr. old eggs, which increased appreciably in 96 hr. old eggs. There is 50% increase in acid phosphatase and 75% increase in alkaline phosphatase activity during 4 days of embryonic development.

After larval hatching, the acid phosphatase activity shows 6-7 fold increase in first larval stage, whereas alkaline phosphatase shows a concomitant drop. Thereafter, both the enzymes go on increasing reaching a peak during 5th larval stage. During pupation, the enzyme activity declines. The decline being 40% in case of acid phosphatase and 70% in case of alkaline phosphatase. Newly hatched adults of either sex show a further decline in alkaline phosphatase, whereas acid phosphatase shows a decline in females and an increase in males.

4.2.1.2 Acid and Alkaline Phosphatase during Aging

The results of acid and alkaline phosphatase activity in either sex is given in Figs. 12, 14. From Fig. 12, it is observed that the acid phosphatase shows a peak of activity in both the sexes on 3rd day. In males the activity is higher than the females until 3rd day and declines thereafter steadily by the 6th day, after that there is a gradual increase so as to record a peak on 10th day. However, in females the acid phosphatase activity is always higher after 3rd day than that of males.

An examination of Fig. 14 indicates an interesting pattern of alkaline phosphatase activity. It is observed that alkaline phosphatase is always at a higher level in females than the males. In females the alkaline phosphatase activity goes on increasing until the 3rd day, thereafter

there is a decline upto 6th day and increases to give another peak on 10th day. After 10th day of adult life the activity decreases sharply on 11th day followed by a gradual decrease until 13th day. In the males, however, the enzyme shows the characteristic pattern as that of the female, but always remains at a lower level. Higher level of enzyme on 3rd day has an interesting correlation with the egg laying (Fig. 6).

4.2.2 Ratio of Acid and Alkaline Phosphatase

4.2.2.1 During Development

From Fig. 15, it is observed that acid/alkaline phosphatase ratio goes on increasing from 24 hr. old embryo to 2nd larval stage when it is 8.8, then it decreases upto 5th larval stage to 4.4. From pupa onward, the acid/alkaline phosphatase ratio increases to 10 and continues to rise after emergence, being 16.2 in 24 hr. female and 25.4 in 24 hr. male.

4.2.2.2 During Aging

From Fig. 16, it is clear that acid and alkaline phosphatase ratio is always higher in males than females throughout the adult life. Here it should be noticed that in case of females the acid and alkaline phosphatase ratio varies from 9-16 times from 1st day to 13th day while in

males the ratio shows wide fluctuations upto 3th day of adult life after which it decreases to lowest level remaining almost constant for the rest of life, where it is oetween 10.5-25 times. In case of male, acid/alkaline ratio is almost 1.5 times as compared with females upto the 10th day of adult life.

4.2.3 Trehalase Activity

Trehalase activity has been studied during development and aging of C. analis and the results are given in Figs. 17 and 18.

4.2.3.1 Trehalase activity during development

Distribution of trehalase activity during embryonic and post-embryonic development shows an interesting pattern (Fig. 17) from 24 to 96 hr. egg stages, there is no appreciable change, however, after hatching, trehalase shows a continuous rise reaching its peak at 5th larval instar. From 5th larva to pupa, there is a considerable decline in the activity as much as 1/6 of the 5th larval stage. Following adult emergence from the pupa, there is a sudden spurt in either sex being slightly higher in female.

4.2.3.2 Trehalase activity during aging

Trehalase activity pattern during aging has been

shown in Fig. 18. It is observed that the activity is age-related and the changes of the enzyme in females are more pronounced. In aging males, the enzyme activity goes on increasing giving a peak on 10th day and thereafter a sudden fall is observed. In females also, there is a gradual increase. However, two distinct peaks are observed, one on the 3rd day and the other on the 10th day, following which it declines (Fig. 18).

4.3 DISCUSSION

4.3.1 Acid and Alkaline Phosphatase during Development

Considerable evidence now exists which indicates a positive forrelation of enzymes with development and aging. Regarding the function of acid and alkaline phosphatase, though not much is known, it seems that these enzymes have a significant role to play in the metabolic processes of the insect development and aging. According to Chen (1966) alkaline phosphatase plays an important role in various synthetic processes of cell, whereas acid phosphatase takes an active part in the degradation and synthesis of tissue proteins. The role of acid and alkaline phosphatase in insect development, especially in relation to nutrition and egg maturation has been well established (Baker and Alexander, 1958, Ashrafi and Fisk, 1961, Ludwing et al.,

1962; Sridhara and Bhat, 1963; Lockshin and Williams, 1965b; Raychaudhuri and Butz, 1965b; Nath and Butler, 1971 and 1973).

The activity pattern of phosphatases during development in Callosobruchus analis is given in Figs. 11 and 13. It is found that during embryogenesis, acid and alkaline phosphatase activity is lowest in freshly laid eggs which increases appreciably by the time the embryo is 96 hr old. During this period there is a 50% increase in the activity of acid phosphatase and 75% increase in alkaline phosphatase activity. Indira (1963) recorded similar observations for alkaline phosphatase during embryogenesis. According to her, this enzyme is concerned with protein synthesis. Keeping in view the conclusions drawn by Indira (1963) and Chen (1966), it seems that the increase in activity of phosphatases during embryogenesis may be attributed to a high rate of breakdown of stored materials in egg and synthesis of new products required for the body of embryo. Sridhara and Bhat (1963) reported in B. mori a gradual increase in the acid phosphatase activity during embryogenesis, while alkaline phosphatase also shows a gradual increase which makes its appearance 2-3 days later. However, Yao (1950) could not find any alterations in acid phosphatase activity during embryogenesis of Drorophila.

In the postembryonic development, acid phosphatase increases 6-7 folds in 1st larva, while the alkaline

phosphatase actually shows a slight decrease (Figs. 11 and 13). Thereafter, both the enzymes gradually increase attaining a maximum activity in the 5th larva. In the pupa, acid phosphatase declines by 40% while alkaline phosphatase shows a drop of 70% when compared with the activity in 5th larval stage. In all the stages of development in C. analis, acid phosphatase activity is much higher than alkaline counterpart. Upon adult emergence, the alkaline phosphatase records a further decrease in 24 hr. old males and females. As for the acid enzyme, a slight drop of activity was recorded in females and a concomitant increase in males during 24 hr. after adult emergence. The results of Yoo and Lee (1973) in Dendrolimus spectabilis are almost identical during development as in C. analis.

In this connection, it may be noted that Ashrafi and Fisk (1961), Hedgekar and Smallman (1967), and Nath and Butler (1971) reported maximal activity of acid phosphatase in pupal stage, whereas Barker and Alexander (1958) reported alkaline phosphatase to be maximal in two day old larva of M. domestica. Variations in maximal enzyme activities at certain periods may be of some significance. Nath and Butler (1973) have generalised that the insects with long larval periods show maximal alkaline activity in the last larval instar and those with shorter larval history exhibit the peak of enzyme

activity at a much earlier age. However, Drosophila is an exception to this generalisation, which in spite of a brief larval history, shows maximal phosphatase activity before pupation (Schneiderman et al., 1966).

In the pupa of C. analis, a sharp decline of alkaline phosphatase has been recorded. However, the decline in alkaline phosphatase is much pronounced than the acid enzyme. Barker and Alexander (1958), Sridhara and Bhat (1963), and Rousell (1971) have also observed a decline in alkaline phosphatase in the pupal stage. Barker and Alexander (1953), Ashrafi and Fisk (1961), Sridhara and Bhatt (1963) and Hedgekar and Smallman (1967) have reported the maintenance of a high titre of acid phosphatase in the pupal stage which remains almost unchanged.

In C. analis, the gradual increasing activity of alkaline phosphatase and attainment of maximum activity in 5th larva and its sharp decline in pupal stage has been recorded. As Chen (1966) has concluded that alkaline phosphatase activity is concerned with physiological function, digestion in particular, it seems that this enhanced alkaline phosphatase activity may be due to the increased consumption of food during the larval stage as the enzyme would be largely required for the digestion and the transport of metabolites across the midgut and

further synthesis of materials to be stored in the body. This contention has been further strengthened by Ludwig et al. (1962), Sridhara and Bhat (1963), and Nath and Butler (1973). The sharp decrease of alkaline phosphatase during pupal stage may be due to the cessation of food consumption and histolysis of the digestive tract. According to Sridhara and Bhat (1963), highest alkaline phosphatase and its predominance over acid phosphatase in the 4th and 5th larval stage of B. mori is due to the high alkaline pH in the mid-gut and the absence of alkaline phosphatase in the pupal stage may be due to histolysis of the digestive tract. This conclusion of Sridhara and Bhat (1963) is supported by the present result in pupa.

The increasingly high activity of acid phosphatase upto 5th larva and the maintenance of relatively high titre of acid phosphatase in the pupa accompanied by very low level of alkaline phosphatase is of significance. During larval stages, the high activity of acid phosphatase may be involved in the greater transport of materials from the mid-gut. The pupa represents a closed system in which the larval tissues undergo a transfomation into adult tissue. It is reasonable to assume that the acid enzyme participates in the degradation of larval tissues accompanied by the synthesis of new materials and differentiation of adult tissues. This view has been further supported by Sridhara and Bhat (1963) and Nath and Butler (1971).

4.3.2 Trehalase during development

The activity of trehalase have been traced throughout the period of development (Fig. 17). From the figure, it is observed that the enzyme activity during embryogenesis remains almost unchanged. After larval hatching, the enzyme activity steadily increases upto 5th larval stage followed by a decline in the pupal stage. After adult emergence, there is a sudden increase in trehalase activity.

Trehalase is concerned with the hydrolysis of trehalose to glucose moieties which is utilized for the energy needs of the body tissue. The steady increase in trehalase activity through all the larval stages points to its utilization in hydrolysing trehalase for the increased energy needs of the larva as it grows in size. The decline in the enzyme activity in the pupal stage may be attributed to the fact that the pupa subsits itself more on the fats and to a lesser extent on the carbohydrates (Agrell and Lundquist, 1973). It would be worthwhile to mention here that the total lipid content in the pupa of C. analis rapidly declines, while carbohydrate level records a low rate of decline (Chapter 5). In Calliphora erythrocephala, Agrell (1952) has stated that the main fuel during pupal development is fat supplemented by a small amount of carbohydrates. Crompton and Polakis (1969) also could show very little utilization of labelled glucose in Lucilia during

imaginal development. Wyatt and Kalf (1957) have also shown that trehalose plays a minor role during metaphorphosis as an energy reserve. Thus it is amply clear from the present observation as well as from those of other authors mentioned herein, that larval stages are largely dependent upon sugars, whereas the pupal stage depends more on fats and to a lesser extent on carbohydrates.

4.3.3 Phosphatases and Trehalase during aging

The activity pattern of acid and alkaline phosphatase during aging in C. analis is interesting. Both acid and alkaline phosphatase show a steady increase of activity in both males and females attaining a peak on 3rd day of adult life (Figs. 12 and 14), then declines upto 6th day and thereafter rises to give another peak on the 10th day of adult life. It is significant to note that after the 3rd day, acid phosphatase is more pronounced in females as compared to males (Fig. 12). However, alkaline phosphatase activity is always at a higher level in females as compared to males. High levels of both the enzymes for the first three days of adult life appears to be related to their role in nourishing the sperms in males and egg maturation and egg laying in females, since the maximum number of eggs are laid on the 3rd day of adult life (Fig. 6). This observation lends support to Moog's (1946) hypothesis that acid phosphatase plays an important role in nourishing the

sperms. Raychaudhuri and Butz (1965a and b) also suggested that the sperms produced by the males at the peak activity of acid enzyme may be rich in acid phosphatase that would perhaps ensure a longer life of the offsprings. They further suggested that the eggs produced at the height of enzyme activity by the females are more viable.

Raychaudhuri and Butz (1965b) reported in female Tribolium confusum, two peaks of acid phosphatase, one in the early life and the other in the later life of the adults. There is, however, only one peak of alkaline phosphatase in females soon after emergence. In the males, the activity of alkaline phosphatase remains almost constant throughout adult life, whereas acid phosphatase shows one peak in the early life. In Tenebrio molitor, Ludwig et al. (1962) were able to record only one peak for both acid and alkaline phosphatase during early life of the adult insects. In case of black carpet beetle, Attagenus megatoma (Nath and Butler, 1973), it has been shown that the alkaline phosphatase increases steadily upon 9th day which is an contradiction to the observations reported in other species (Rousell, 1971 ; Naqvi and Ashrafi, 1968).

In so far as the 2nd peak of activity is concerned, two possible explanations could be advanced. First, it may be due to the release of acid enzyme from the lysozomes whose

membranes acquire greater permeability prior to death. Secondly, it is suggested that the spurt of acid enzyme activity on the 10th day may be associated with tissue breakdown and metabolism of stored food like carbohydrates and fats. Greater transport of metabolites is needed since the insects do not feed during the adult life. Lockshin and Williams (1965a and b) have suggested that in the last days of adult life of Antheraea pernyi, cathepsin and acid phosphatases are released from lysozomes due to increase in permeability of their membranes resulting in the increase of the activity of acid phosphatase as well as cathepsin. By the activity of both of these enzymes, muscles break down gradually so as to ensure biological death. At the same time it has been found that the death rate of the present insect coincides with high activity of phosphatases (Figs. 7, 8, 9).

The enzyme, trehalase is of immense importance for, it hydrolyses trehalose to furnish glucose which is utilized for energy needs during copulation and egg laying. Age-related changes in the trehalase activity of C. analis have been found to be quite significant. Trehalase activity in either sex goes on gradually increasing from the day of adult emergence until 10th day and after that it decreases suddenly and continues to decline till 13th day. During the course of study, it has been observed that trehalase

activity is always higher in females in contrast to males (Fig. 18). Secondly, females show a slight increase in trehalase activity on the 3rd day while males do not show any appreciable increase. From Fig. 18, it is clear that females show two distinct peaks, whereas males exhibit only one. The first small increase in activity of trehalase in females on 3rd day is significant as it coincides with the maximum number of eggs laid on this day (Fig. 6). This explains the high energy needs of females for egg laying activity which is met with the glucose released from hydrolysis of trehalose. In contrast to the present observations, Dahlman (1970) reported a decline in trehalase activity with advancing age of Manduca sexta, tissues such as gut, salivary glands, testis and accessory glands in males and an increase in the enzyme activity in the Malpighian tubules and abdominal ganglia. At the same time he was also able to record sex-specific differences in enzyme activity. Similarly, Burcombe (1972) also recorded declining trehalase activity in Drosophila melanogaster with the advancing age. On the other hand Alumot et al. (1969) reported a high activity of trehalase in spermatheca of Apis mellifera and was unable to record any sex specific differences in the enzyme activity in the haemolymph of males and females. Jensen and Buckner (1973) studied both bound and free trehalase in M. domestica and recorded an enhanced trehalase activity with advancing age.

In C. analis, increasing activity of trehalase with advancing age has got an interesting correlation with the death rate. From Figs. 7, 8 and 9, it is observed that number of deaths/day gradually goes on increasing from first day onwards until it is maximum between 9-13th day of adult life in either sex. Similarly, trehalase activity also shows maximum activity in adults on 10th day in either sex. Thus it seems that as the age advances, the energy needs of the insects also go on increasing so as to maintain the physiological integrity as pointed out by many authors (Tribe, 1966; Strehler, 1962 and Comfart, 1964) that there is a progressive decline of physiological performance with age. It is interesting to note here that Rockstein and Bhatnagar (1965) have shown a significant decrease in size and number of mitochondria in aging M. domestica.

For enhanced activity of trehalase with age in either sex, three possible explanations could be advanced. Firstly, with increasing age some inhibitor of trehalase, which are present in the body are destroyed or there is a conversion of enzyme precursors to active enzyme with advancing age. Secondly, the tissue bound trehalase is increasingly, released from the intra-cellular organelles. Lastly, there can be an increased denovo synthesis of enzyme. In the present context, it seems more convincing that trehalase is

increasingly released from tissue-bound trehalase, which holds good for tissue bound phosphatases as well (Dhand and Rastogi, 1975).

From the foregoing account it is evident that the phosphatases as well as trehalase play a significant role throughout the aging processes. Enhanced activity of phosphatases in the early period of adult life is responsible for the breakdown of stored materials at a faster rate and simultaneous synthesis of rapidly metabolisable products to meet the increasing demand for copulation, egg maturation and egg laying. Similarly increased phosphatase during last days of adult life may lead to consequential breakdown of residual stored materials and body tissue, especially muscles as demonstrated by Lockshin and Williams (1965b), and Butler and Nath (1972), and resynthesis of trehalose as is evident from the rising trehalase activity and trehalose as per cent of the total sugar in the last days of adult life (Figs. 18 and 34B). This increased trehalose synthesis is required for the supply of ever increasing energy needs of the insects with advancing age (Tribe, 1966, and Baker and Lloyds, 1970). Since the number of deaths per day is maximum between 9-13th day of adult life in either sex, it is reasonable to presume that increased breakdown of stored materials and tissue causes depletion of energy stores and physiological exhaustion which ultimately leads to the "biological death".

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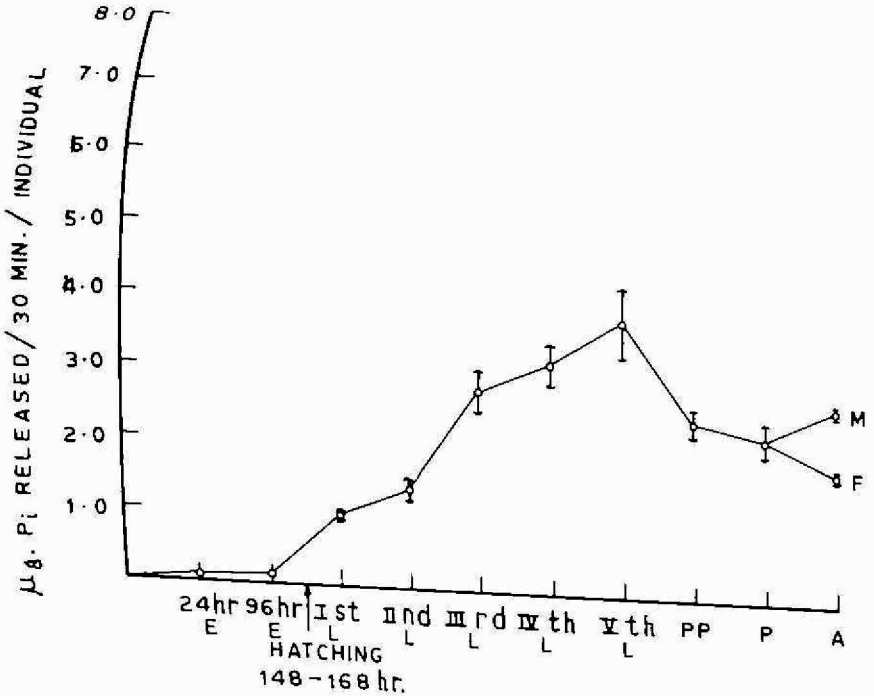

FIG 11

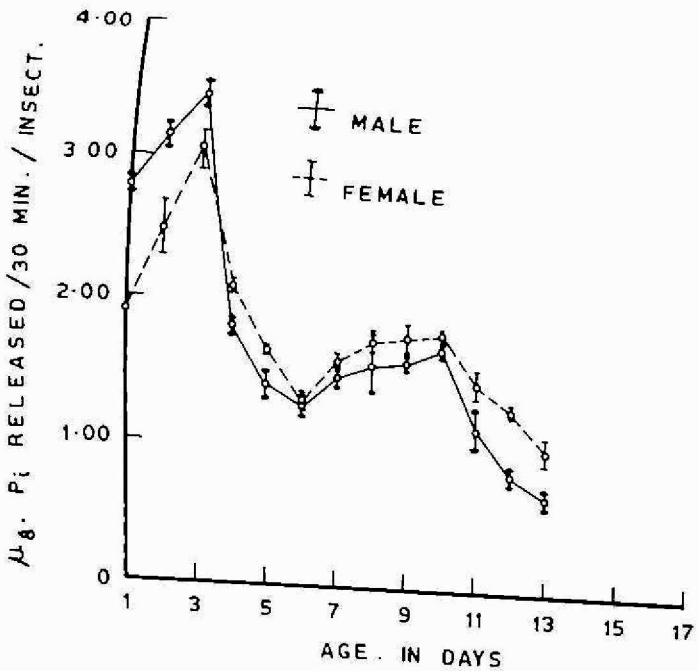

FIG. 12

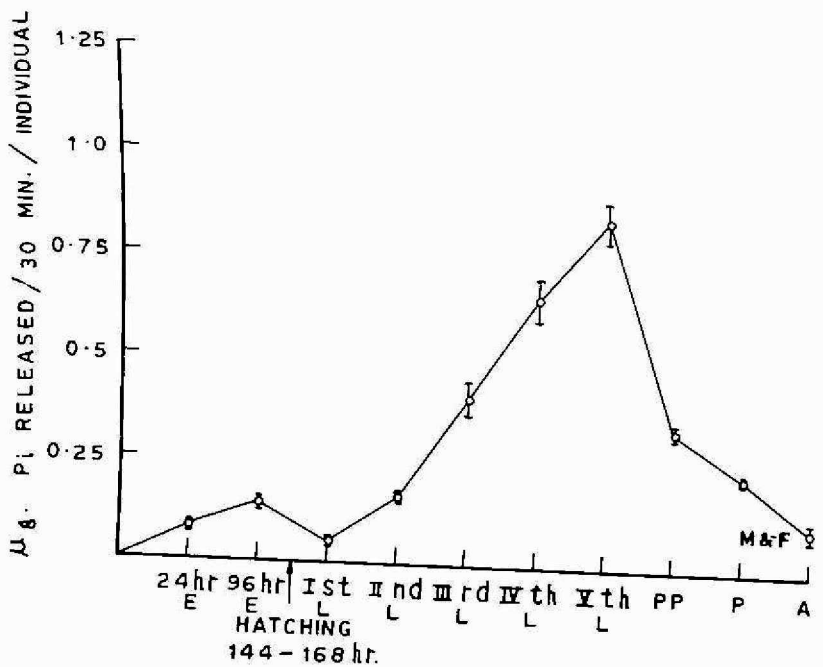

FIG. 13

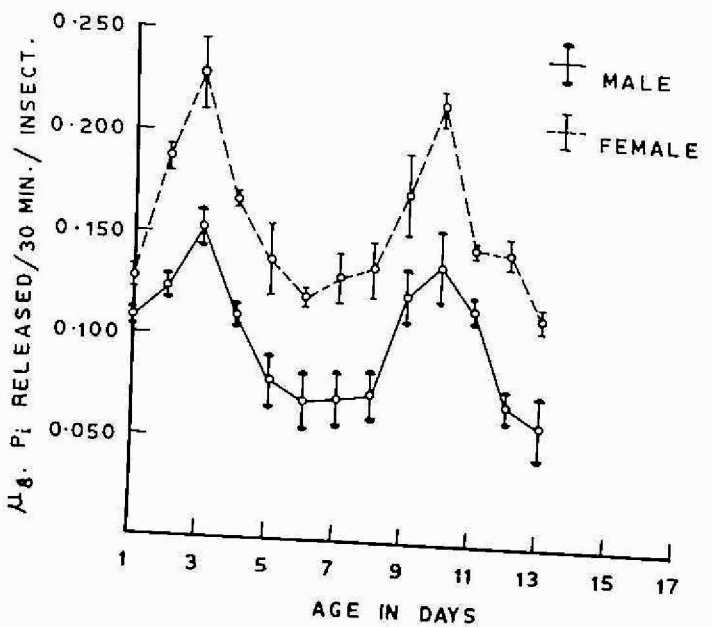

FIG. 14

Fig. 15 Ratio of acid/alkaline phosphatase activity during development.

Fig. 16 Ratio of acid/alkaline phosphatase activity during aging.

Fig. 17 Changes in trehalase activity during development.

Fig. 18 Changes in trehalase activity during aging.

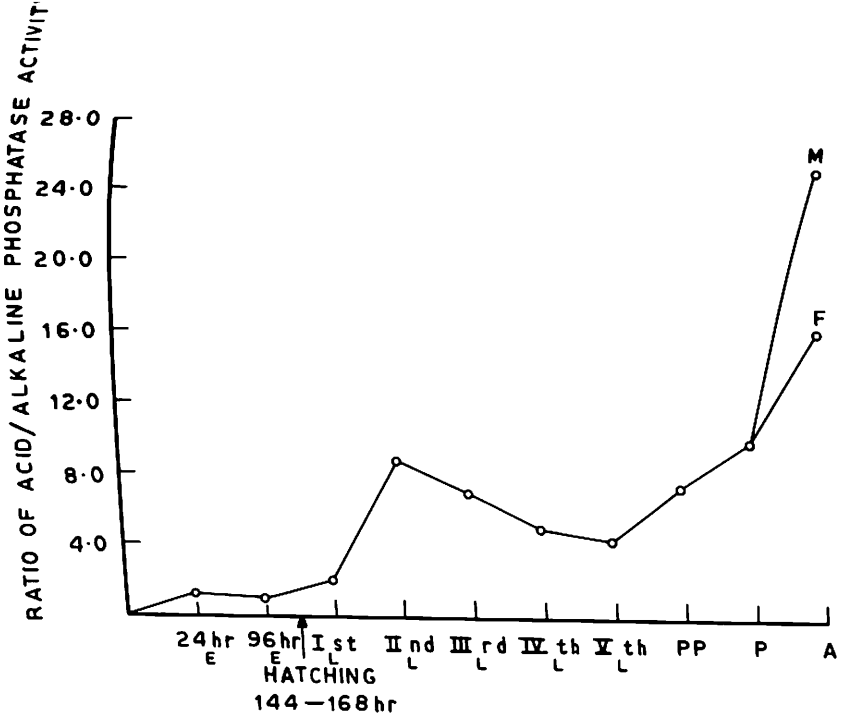

FIG. 15

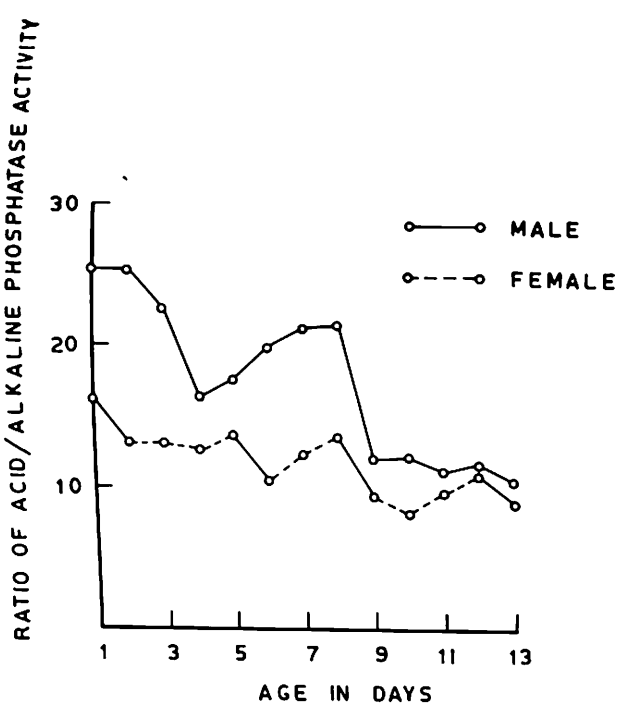

FIG. 16

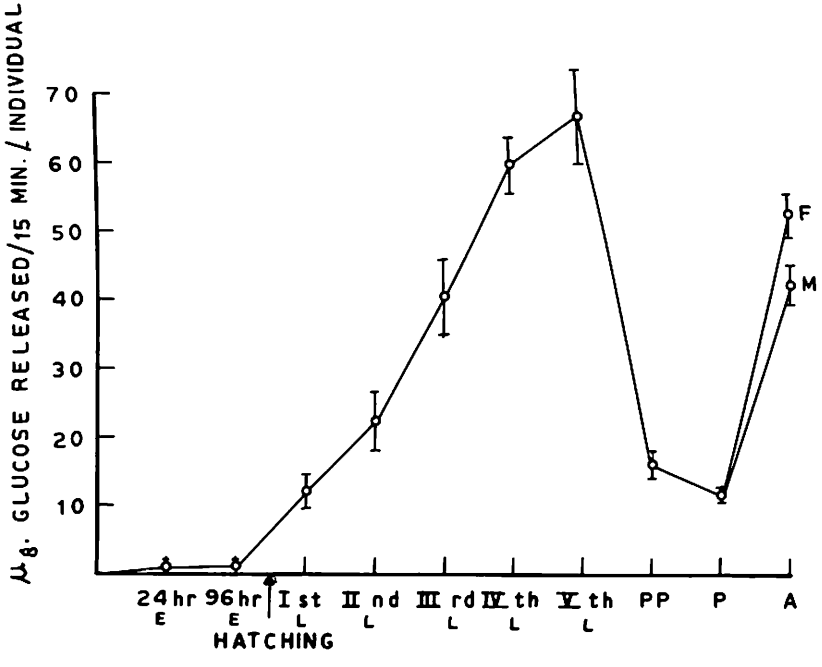

FIG. 17

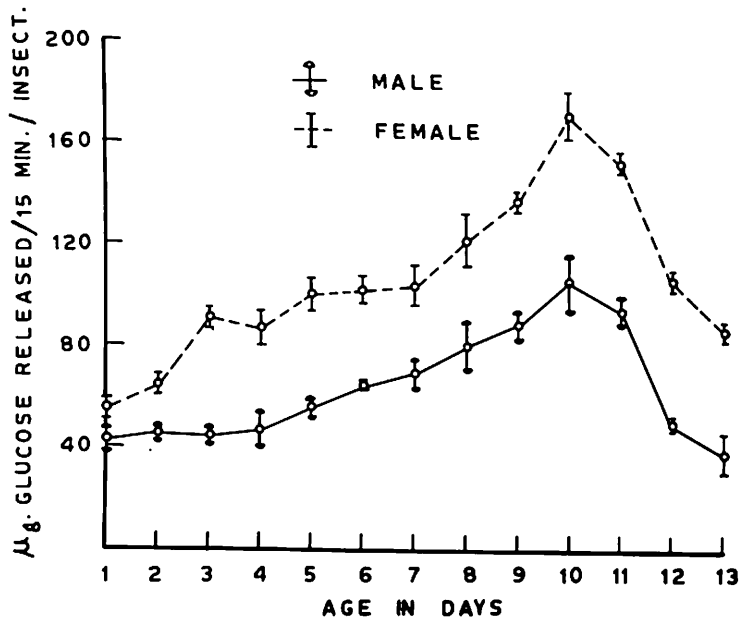

FIG. 18

CHAPTER - 5

STUDY OF DNA, RNA, PROTEIN, LIPIDS, TOTAL SUGARS AND TREHALOSE

STUDY OF DNA, RNA, PROTEIN, LIPIDS, TOTAL SUGARS AND TREHALOSE

SUMMARY

Age related changes in DNA, RNA, Proteins, lipids, sugars and trehalose content of total sugars have been studied during embryogenesis, postembryonic development and aging in Callosobruchus analis (Fab.). DNA content/individual shows a gradual increase from 24 hr. embryo to 5th larva, then records a slow decline in prepupa, again increases in pupa followed by a decline until emergence. After emergence, DNA/insect shows a slight increase for first 2-3 days followed by a decline with age. RNA/individual follows exactly the same course during larval development and shows a constant decline until emergence of adults. After emergence, RNA/insect in females declines, although in males a slight increase is observed, for first 2-3 days followed by a continuous decline. Protein content per individual follows the same trend during larval development until emergence. After emergence, proteins also decline in either sex with advancing age, although in females a slight increase is observed for first 2-3 days. At the same time, ratios of RNA/DNA, Protein/RNA, Protein/DNA as well as Dry weight/DNA, Dry weight/RNA and Dry weight/protein

have also been calculated during development and aging. It is suggested that DNA, RNA and protein synthesis is most active during embryonic development, subsequent to that the "synthesis potential" decreases. Lipids have been estimated as percentage of the dry weight as well as wet weight during development and aging, and at the same time lipid/insect in either sex has also been studied. It is observed that lipid as percentage/wet weight and dry weight declines during embryogenesis followed by a constant increase until prepupa. From pupa onwards lipids declines in either sex until 10th day of adult life. Lipid content of females is higher than males. On 11th day lipid content almost increases 1.5 times of that found on 10th day in either sex followed by a decline until 13th day. This suggests that lipids are used up during embryogenesis and stored during larval development. During pupation and adult life, lipid forms the most important source of energy. Total sugars also show a constant increase until 5th larva followed by a decline until 13th day of adult life. Sugars as percent of wet weight have also been studied. Trehalose has been studied as percent of total wet weight, and percent of total sugars during development and aging. During aging it has been observed that trehalose percent of total sugar shows a decline for first 2-3 days followed by a constant increase with age in either sex. All these above parameter have been discussed in relation to developmental stages, aging, egg laying rate and mating as well as with life tables.

5.1 INTRODUCTION

It is a well-known fact that embryogenesis, post-emoryonic development and aging is followed by many biochemical changes at cellular, subcellular and molecular levels. In Chapters III and IV, an account of the changes in water content, wet weight, dry weight and enzyme activity has been considered in relation to egg laying and life tables during development and aging. Besides these, changes in gross content of total sugars, trehalose content of total sugars, total lipid, total protein, RNA and DNA have also been studied during development and aging of C. analis.

It has been suggested that morphogenesis and aging are under the control of hormones which bring about profound changes in the total body composition and initiate transformation of larva to pupa leading to adult stage (Gilbert, 1964; Wigglesworth, 1954, 1964, 1970; Rockstein et al. 1971; Rockstein and Miquel, 1973).

There have been quite a few studies on the total body composition of insects with an objective to study the protein synthesis, hormonal control of differentiation and genetic changes. It may not be necessary to review the entire literature here since extensive reviews on the subject have been done by Chen (1962 & 1966), Gilbert (1967), Wyatt (1967), Agrell and Lundquist (1973) and Rockstein and Miquel(1973).

Lang et al. (1965) found that during growth total body weight, total DNA, RNA and protein increase while during aging total DNA and protein as well as RNA/DNA ratio remain constant. Further, they concluded that physiological aging in the mosquito Aedes aegypti is not accompanied by significant drop in protein synthesis. Balazs and Haranghy (1965) reported in Drosophila melanogaster that there is an increase of DNA and RNA content in females, and increase of DNA content of the male during the first days of imaginal life, following which DNA content remained constant with advancing age. Krueger and Ballard (1965) determined DNA content of heart and associated tissue of adult Musca domestica of either sex and reported that DNA content remains constant with age. Krishnakumaran and Schneiderman (1964) found that there is no DNA synthesis in adult Cecropia and other saturniid moths while RNA synthesis occurs at a very reduced rate. Recently, Ring (1973) has studied DW, protein and nucleic acids throughout the life of Lucilia sericata and reported that during normal development protein/DW fluctuates markedly during larval and pupal stages whereas in adults protein levels remain relatively high during senescence. RNA/DW ratio was highest in Ist larva which decreases through larval stages and adult life except a slight increase prior to pupation and adult emergence. DNA/DW is also highest in early larva and then decreases until death. RNA/DNA ratio is highest during

embryogenesis showing highest rate of synthetic activity and decrease in adult life of both the sexes.

According to Clarke and Maynard-Smith (1966) there is increased protein synthesis rate with age in D. subobscura. On the contrary Levenbook and Krishna (1971) in Phormia regina, Baumann and Chen (1963) in D. melanogaster, Bleven (1973a) in Aedes aegypti and Baumann (1969) in D. melanogaster have found decreasing capacity for protein synthesis with advancing age.

Lipids, especially neutral lipid, are important source of energy during metamorphosis and aging (Gilmour, 1961; Gilbert, 1967). Lipid metabolism during development and aging of insect have been reviewed by Gilbert (1967) and according to him sexual dimorphism in lipid content which exists in adult, pupa and even larva are under the genetic control. There are a number of factors which influence the lipid content such as stages of development, nutrition, environmental temperature, sex, starvation, diapause, and cold hardiness (Scoggin and Tauber, 1950).

Ishii (1955) recorded 33.2% lipids of the WW in mature larva of Callosobruchus chinensis. Rao and Agarwal (1970) have studied lipid in Trogoderma granarium and observed that lipids increase until 6th larva and decrease rapidly in pupa and lipid content in adults is the lowest.

Mukherjee and Gerere (1960) reported an increased accumulation of lipids and sugars during development that are utilized at a higher rate during pupation and adult life of Musca domestica.

Gilbert and Schneiderman (1961) recorded in Hyalophora cecropia that during embryogenesis 50% of the lipid material is utilized, while lipids during larval growth increase until 5th larva due to rapid synthesis. The increased lipid contents in pupa are either due to synthesis, loss of water or release of lipids from liporotein. Lower lipid content in the adult females is due to the fact that lipids are transferred to egg yolk and she does very little flying. On the other hand, high lipid content of male is due to the fact that males have to do a lot of flying to find a virgin female. Gere(1964), who recorded almost 55% lipid of the DW in adult of Lymantria dispar, observed that lipids continuously decrease with age and 30% of lipids in female are transferred to egg yolk.

Regarding the metabolism of total sugars and trehalose content of total sugar, Wyatt (1967) has reviewed the metabolism of trehalose in insects. According to Friedman (1967), Dutrieu and Gourdoux (1974), trehalose content is under the control of hormones, whereas, Egorova and Smolin (1962), Saito (1963) and Jungaris and Wyatt (1972)

maintain that there is a homeostatic mechanism to maintain a specific blood trehalose level in haemolymph.

Egorova and Smolin (1962) observed in Antheraea pernyi that trehalose as per cent of wet weight goes on increasing until 5th larva. In pupa and adult it declines. Liu and Feng (1965) recorded in Leucaria separata that trehalose increases till mature larva which decreases in pupa. Blood level of trehalose is higher in females. The trehalose forms 66.3 to 94% of the total sugar. Nettles et al. (1971) in Anthonomous grandis and Moreau (1969) in Pieris brassica reported that trehalose increases until mature larva and then it declines until death. According to Dutrieu and Gourdoux (1971), trehalose is utilized at a higher rate in adults of Tenebrio molitor than glycogen.

In order to study the synthesis and utilization of different metabolites, total sugar, trehalose content of total sugar, lipid, protein, RNA and DNA have been estimated from 24 hr eggs to 13th day old adults of Callosobruchus analis.

5.2 RESULTS

5.2.1 Deoxyribonucleic Acid (DNA)/Individual Basis

Development: Changes in the total DNA content during development and aging are given in Figs. 19A and 20A. From Fig. 19A, it is observed that in 24 and 96 hr old embryos, the amount of DNA present is very low (0.093 to 0.096 µg/individual)

After hatching, it increases almost 23 times in the 1st larval stage. Following 1st larva, the DNA content goes on increasing until it attains a maximum value in the 5th larval stage. In the prepupa, there is a decline in the total DNA followed by an increase in the pupa.

Aging: After adult emergence, the total DNA present in both the sexes declines to almost half of that present in the pupa (Fig. 20A). In the aging males and females, the total DNA/insect has been found to rise until 3rd day, followed by a decrease until the 13th day with little fluctuations in the intervening period.

5.2.2 DNA/gm WW Basis

Development: When total DNA is considered per gm WW tissue, it is found to be highest in 96 hr embryo (Fig.19B), followed by a gradual decrease until 4th larva. Then it again starts increasing until the pupa.

Aging: In 24 hr old adults, DNA/gm WW is lowest in both the sexes (Fig. 20B) which goes on increasing slowly until 13th day of adult life. It is significant to observe that males have a higher DNA/gm WW as compared to females at any stage of adult life.

5.2.3 Ribonucleic Acid (RNA)/Individual

Development: The amount of RNA/individual shows a slight increase from 24 hr old to 96 hr old embryo(Fig.21A).

After hatching, the RNA/individual goes on increasing from 1st larva to 5th larva, following which the values decline considerably until emergence of adult.

Aging: During aging the RNA/insect in males goes on declining upto 7th day beyond which it remains almost constant (Fig. 22A). However, in females the pattern is different. Here the RNA/insect increases till the 3rd day, thereafter it declines until 9th day, following which it remains constant for the rest of life.

5.2.4 RNA/gm WW Tissue

Development: When RNA is taken/gm WW tissue, it is highest in 96 hr embryo, which goes on decreasing until prepupa followed by a slight increase in pupa (Fig. 21B).

Aging: After adult emergence, there is an appreciable fall in RNA content/gm WW in males and females (Fig. 22B) at 24 hr adult stage. Then RNA content goes in increasing until 13th day of adult life in males while females show a gradual increase until 8th day, followed by a slight decrease on 9th and 10th day. After 10th day, it however, again increases upto 13th day.

5.2.5 Protein/Individual

Development: From Fig. 23A, it is observed that the amount of total proteins go on increasing from 24 hr embryonic stage until 5th larva, where the proteins are maximum. From

5th larva onwards, the protein content declines and continuous to be so beyond emergence. This is due to utilization of stored proteins as feeding does not take place after 5th larval stage.

Aging: After adult emergence, protein content per adult wet weight further decreases in either sex (Fig. 24A). Total proteins continue to fall in both the males and females until 13th day of adult life.

5.2.6 Proteins/gm Wet Weight

When considered on gram wet weight basis (Figs. 23B and 24B), the total protein shows an increase from one day old embryo to pupal stage. On adult emergence, it further shows an increase in both the sexes until the adults are 13 day old.

5.2.7 Ratio of RNA/DNA, Protein/RNA and Protein/DNA

To have an overall picture of protein synthesis during the life cycle of C. analis, the RNA/DNA, Protein/RNA and Protein/DNA ratios have been calculated.

RNA/DNA Ratio

Development: From Fig. 25A, it is observed that RNA/DNA ratio is highest in the 96 hr embryos, when it is 11.5, which goes on decreasing until 2nd larva. After

showing a slight increase in 3rd larva, the ratio gradually decreases to 4.0 in pupal stage.

Aging: Following emergence, it further decreases to 1.75 and 1.50 in 24 hr males and females (Figs. 26C) and by the time the adults are 13 day old, it decreases to 1.48 and 1.40 in males and females respectively.

Thus it is clear that RNA synthesis is most active in the embryonic development which goes on decreasing as the larval development proceeds.

Protein/RNA Ratio

Development: From Fig. 25B, it is observed that Protein/RNA ratio is least in 96 hr embryo (0.90) and goes on increasing until pupal stage (11.1).

Aging: After adult emergence the ratio increases to 20.0 in males and 25.0 in females (Fig. 26B), and thereafter it decreases and remains almost constant throughout the aging process.

From the protein/RNA ratio study, it is clear that protein synthesis is actively carried on in the development stages. A higher protein/RNA ratio in the adults is indicative of a low rate of protein synthesis.

Protein/DNA Ratio

It is well known that the amount of DNA per cell in

an individual is constant (Davidson, 1969). In the present study, the increase in DNA/larva upto 5th larval stage is a result of increase in the number of cells consequent upon histogenesis. But decrease of DNA in pupa and adult of either sex are indicative of histolytic processes. This is corroborated by protein/DNA ratio as well (Figs. 25C and 26A).

Development: Protein/DNA ratio is lowest at 24 hr (0.91) embryo and goes on increasing until prepupa (51.9) suggesting active protein synthesis. After prepupa it declines in pupa to 43.4 (Fig. 25C).

Aging: After emergence, it further declines to 35.3 in males and 38.0 in females (Figs. 26A). Following this, protein/DNA ratio shows wide fluctuations upto 5th day of adult life and ultimately decreases to lowest ratio of 30.5 in male and 31.0 in female on 13th day.

5.2.3 Ratio of Dry Weight/DNA, Dry Weight/RNA and Dry Weight/Protein.

The information on the DW/DNA, DW/RNA and DW/protein ratios are important since these give us an idea of the DNA, RNA and protein synthesis much clearly, because of the fact that WW at any stage may vary due to water metabolism, but DW is almost constant.

DW/DNA ratio: From Fig. 27A, it is observed that

DW/DNA ratio is 133.1 in 24 hr old eggs which decreases to 167.3 in 1st larva followed by a gradual increase to 231.1 in the 4th larva. In the 5th larval stage, it again decreases to 202.4. Maximum ratio is observed at the prepupal stage (244.0) and thereafter it declines. After emergence the ratio goes on decreasing in either sex (Fig. 28A).

DW/RNA ratio: From Fig. 27B, it is observed that DW/RNA ratio is 19.7 in 24 hr embryo which decreases to 15.5 in 96 hr embryo and after larval hatching, it goes on gradually increasing until pupation. Immediately after adult emergence (Fig. 28B) from pupa, the ratio suddenly increases to 111.2 and 133.1 in male and female respectively, but with advancing age, it gradually decreases in male while in female the pattern is irregular.

DW/Protein ratio: DW/Protein ratio is maximum in 24 hr embryo (eggs) (Fig. 27C), after which it follows a gradual decline until pupation. The ratio once again increases when adult emergence takes place, but after this the decline is constant but slow throughout the aging adults (Fig. 28C).

5.2.9 Lipids

The lipids signify the stored food material in an individual and account for the utilization during egg laying

and copulation. Changes in the lipid content are given as percentage of DW and WW in Figs. 29 and 30.

Lipids as percentage/WW: From Fig. 29, it is observed that lipid per gm WW is 17.4% in 24 embryo which shows a slight decrease at 96 hr embryonic stage. After hatching, lipid percentage/WW goes on increasing until it reaches 30.2% of the WW in prepupa followed by a decrease to 26.0% in pupal stage.

Following adult emergence, lipid percentage/WW decreases to 15.0% in male and 13.0% in female at 24 hr stage (Fig. 30B). This further decreases to 4.7% and 4.9% of WW in male and female, when insects are 10 day old. Interestingly, on 11th day, lipid per cent increases to 7.4% and 7.3% in both the sexes. However, 11th day lipid %age again decreases rapidly to 3.7 and 5.2 of male and female until 13th day.

Lipids as percentage of DW: When lipids are taken as percentage of DW, it is found that lipid form 35.5% of the DW at 24 hr embryonic stage (Fig. 29) which decreases to 29.3% in 1st larval stage. Subsequently, the lipid percentage/DW goes on increasing and reaches a maximum of 59.0% in prepupa. In pupa, it shows a decline to 50.4% of DW.

After adult emergence, lipid percentage/DW decreases to 35.59% and 41.79% of DW at 24 hr stage (Fig. 30C) and

thereafter decreases rapidly to 10.5% and 10.43% in male and female respectively by 10th day of adult life. On 11th day, it rises to 15.2% and 17.1% in male and female followed by decrease to 9.32% and 13.16% on 13th day of adult life in males and females.

Fig. 30A, gives the lipid content per WW of males and females from one day to 13th day of adult life. Here again, the variation in the amount of lipid follows the same pattern as observed in Figs. 30B and 30C. It is obvious that the total lipid percent and their utilization in females is always higher than males (Figs. 30A, B and C).

5.2.10 Total Sugars

Total sugars have been estimated during the life cycle of C. analis, and the results are presented in Figs. 31 and 33.

From Fig. 31A, it is observed that total sugars are lowest in 24 hr eggs and subsequently reaches the highest value of 172 µg in 5th larva. Then the total sugars fall appreciably to 110 µg in pupa.

After emergence, sugar content per insect WW further decreases to 103 µg and 97 µg in females and (Fig. 33A). The females always have higher sugar content as compared to males. The total sugars/WW of adult insect goes on decreasing

gradually from 1st day to 13th day when it is 35.2 µg and 13.3 µg in females and males. But here, decrease in sugars for first six days in males is much more pronounced.

When sugar content/gm WW tissue is taken (Fig.31B), it is observed that total sugar/gm WW tissue is highest in 96 hr embryo when it is 23.75 mg, which decreases to 15.75 mg/gm WW in the pupa. After emergence (Fig. 33C), sugar values again increase to 17.90 and 17.37 mg/gm WW in females and males, followed by a sharp decline in males and a gradual decline in females to 9.6 and 7.1 mg/gm WW on 13th day of adult life.

Total sugars when considered as per cent WW, show a maximum value of 2.42% in 1st larva, followed by a decrease to 1.6% in pupa (Fig. 32B). After emergence, the percentage of sugar/WW (Fig. 33B) slightly increases to 1.69% and 1.71% in 24 hr old females and males. Then the decline is gradual until 13th day in both the sexes. It is apparent from Fig.33A that the sugar consumption in males for first six days is higher as compared to females.

5.2.11 Trehalose Content

Trehalose has been estimated as per cent of total sugars as well as WW. Trehalose percentage per total sugar is highest at 96 hour embryo stage (72.4%) which decreases to 45.9% in prepupa (Fig. 32A). In the pupa there is a slight

increase to 50%. After emergence, trehalose percentage increases to 75.0% and 71.6% in 24 hour old female and male respectively (Fig. 34B) and subsequently in females it shows a decrease upto 3rd day. After 3rd day, the percentage again shows a rise reaching 95.3% on the 9th day and remains almost constant thereafter. In males, however, besides a slight increase initially upto 2nd day, the trehalose percentage falls on 3rd day and ultimately goes on increasing until 9th day reaching a maximum of 93.7%. After 9th day there is no appreciable change.

Trehalose as per cent WW: When trehalose is estimated as percentage of total WW (Figs. 32C and 34A), it is observed that trehalose percentage is highest in 96 hr embryo when it is 1.73%, which decreases appreciably to 0.3% of WW in pupal stage. After emergence, trehalose percentage again increases to 1.23% and 1.22% in females and males respectively (Fig. 34A). In case of females, it further decreases to 0.996% on 3rd day followed by a slight increase to 1.163% on 10th day and beyond that it again starts decreasing until it reaches 0.946% on the 13th day. Simultaneously, in males trehalose percentage of WW decreases to 0.777% upto 6th day and after showing slight increase on 7th to 10th day, decreases to a minimum of 0.650%.

5.3 DISCUSSION

The developing organism represents a dynamic system undergoing physiological and biochemical changes as morpho-

genesis proceeds. During the course of development, profound changes in the gross content of body metabolites take place in relation to development and aging. These processes are mutually controlled by hormonal, genetic and physical factors (Review by Gilbert, 1964). The growth of an insect can be followed on a number of factors such as physical, physiological and biochemical. Quite a few authors have studied the growth in relation to DNA, RNA, protein content (Lang et al., 1965; Church and Robertson, 1966; Balazs and Haranghy, 1965; Krishnakumaran and Schneiderman, 1964; Ring, 1973, etc.). In the present work an attempt has been made to explain the biochemical changes in relation to embryonic, postembryonic development and aging in C. analis by taking DNA, RNA, protein, lipids, total sugars and trehalose content of total sugars as the parameters.

5.3.1 Nucleic Acid

According to Lang et al. (1965), RNA content is an index of protein synthesis capacity and DNA content is an index of cell number, and RNA/DNA ratio is an index of protein synthesis capacity of the cell. From Figs. 19A and 21A, it is observed that DNA and RNA are synthesized during embryogenesis as evidenced by its increase from 24 hr to 96 hr embryo stage. Similar synthesis and increase of DNA during embryogenesis is recorded by Devi et al. (1963) in Tribolium Confusum; Lockshin (1966) in beetle eggs; Harris

and Forrest (1967) in Oncopeltus eggs and Painter and Kilmare (1967), in housefly eggs. The increased RNA synthesis have been reported by Lockshin (1966), Harris and Forrest (1967). Thus the increased DNA and RNA content during embryogenesis obviously shows an increase in the number of cells as development proceeds.

After the larval hatching, DNA and RNA go on increasing until 5th larva. This shows that DNA and RNA are increasingly synthesized as the larval development proceeds and follow the increase in wet weight of larva. Thus, it clearly demonstrates that as the development proceeds the number of cells/larva also go on increasing. According to Agrell (1964), the increase in DNA content in many species doesnot show increase in cell number, but as the cell size increases, nucleus also shows polyploidy, hence the increase in DNA. Similar trend of DNA and RNA increase during development has been reported by many authors (Lang et al., 1965; Blevins, 1972 and 1973b; Ring, 1973).

Of all the stages of development, changes in the DNA and RNA content during metamorphosis are spectacular. The decrease of DNA during prepupa may coincide with the loss of larval skin and the breakdown of some of the larval tissue leading to the decrease of DNA. The increased DNA during pupa may reflect the new synthesis of DNA for the formation of new tissues required for the adult body. The rapid decline

of DNA from pupa to adult emergence seems to be due to loss of pupal skin, moulting fluid and the loss of wet weight by pupa. Blevins (1972) has also given similar explanation in A. aegypti. On the other hand, RNA content/individual gradually decreases from 5th larva until emergence. The decreasing RNA content during metamorphosis is related to the loss of wet weight, pupal skin and moulting fluid and this also indicates that RNA is synthesized at a lower rate. According to Agalykov et al. (1972), nucleic acid content during metamorphosis depends upon histolysis and histogenesis.

There are a number of conflicting reports on the DNA and RNA content during metamorphosis. The results obtained by Blevins (1972 and 1973b) in A. aegypti and Price (1965) in Callipora have shown results similar to the present work. On the other hand, Devi et al (1963) in Tribolium, Lang et al. (1965) in Aedes and Takahashi (1966) in Philosamia and Yasuo and Tojo (1972) in B. mori have reported a little change in nucleic acid content during metamorphosis.

After adult emergence, a slight increase of DNA content in either sex until 3rd day is significant as the maximum number of eggs are also laid during this period. Thus it seems that slight increase of DNA for first 3 days may have a correlation with egg laying and sperm production.

On the other hand, RNA content shows a slight increase until 3rd day in males followed by a constant decrease until 13th day, whereas in females RNA content constantly goes on decreasing with advancing age. Thus it seems that in the beginning, males show a slight increased synthesis of RNA. Similar to these results, Balazs and Haranghy (1965) in D. melanogaster and Blevins (1972) in A. aegypti have also reported a slight increase in DNA in the first few days which afterwards remains almost constant with advancing age. Lang et al. (1965); Krueger and Ballard (1965) have reported a constant DNA with advancing age. Krishnakumaran and Schneiderman (1964) have also observed no synthesis of DNA with age in Cecropia and in other saturniid moths. According to Davidson (1969), DNA content/cell is constant. So the slight decrease of DNA after 3rd day may be due to slight decrease of cell number with age.

When DNA/gm WW is taken, it is observed that relative DNA content/gm tissue of 96 hr embryo is very high. After larval emergence the decreasing trend until 4th larva suggests that synthesis of DNA upto 4th larva is not following the increase of wet weight. The relative increase in DNA content/gm from 5th larva to pupa may be reflecting the rapid synthesis of DNA during 5th larva, while the increasing trend during pupa may be due to the decrease of wet weight of individuals. The rapid fall of DNA/gm wet weight from pupa to emergence may be due to loss of pupal skin. After the emergence of

adults, the increasing trend of DNA in either sex from 24 hr stage to 13th day, is probably due to increase in number of insects/gm WT or the decrease in weight/insect (Fig. 1).

Similarly the RNA content/gm WT is highest at 96 hr egg stage which decreases until prepupa, followed by a slight increase during pupa but again falls until emergence. After adult emergence, RNA content/gm WT also goes on increasing. Thus, from this it is evident that like DNA, RNA synthetic rate is highest during embryogenesis which consequently decreases until emergence except a slight increased rate of synthesis during pupa. The same explanation may hold good for increase in RNA/gm wet weight during aging for DNA.

5.3.2 Protein

From Fig. 23A, it is observed that protein synthesis during embryonic and larval development follows a trend similar to DNA and RNA synthesis. The rate of protein synthesis goes on increasing until 5th larva. The small decrease of protein from pupation to emergence may be related to a sharp decrease in the wet weight of individuals, although the relative protein level is very high. The rapid rate of protein synthesis during embryogenesis has been reported by many authors (Lockshin, 1966; and Harris and Forrest,

1967. The results obtained by Blevins (1973b) and Chen and Levenbook (1966) for protein synthesis during post-embryonic development are similar to the present work.

After the adult emergence, protein content of females is higher than the males at any stage (Fig. 24A). After emergence females show a slight increase of protein content for the first 2-3 days followed by a constant decrease until 13th day, while males from the day of emergence show a decreasing tendency.

Thus the decrease of protein content during metamorphosis and aging suggests that metamorphosis and aging are accompanied by a decrease in protein synthesis potential in either sex. The higher protein content of females during adult life suggests their ability to synthesize and retain more protein. The slight increase of protein for first 2-3 days in females may be having some relation to egg laying. This conclusion gets support from the results of Blevins (1973b).

Clarke and Maynard-Smith (1966) have reported in D. subobscura that protein synthesis increases with age. On the contrary, Baumann and Chen (1968) in D. melanogaster, Baumann (1969) in D. melanogaster and Levenbook and Krishna (1971) in Phormia regina have reported decreased potential to protein synthesis with age. While Maynard-Smith et al.

(1970) have reported in D. subobscura a rapid decline in protein synthesis activity for the first few days after emergence and thereafter remaining constant with age.

When protein is taken on per gram wet weight basis, it is observed that protein content/gm wet weight is lowest in the 24 hr embryo which gradually goes on increasing until death. Thus keeping in view the results of protein/individual, it seems that protein synthesis is going on until 5th pupal after which the protein synthesis potential declines. Although during pupal life and aging there is excessive loss in wet weight of individuals, the protein content remains relatively high and does not show sizable fluctuations.

5.3.3 Ratio of RNA/DNA, Protein/RNA and Protein/DNA

RNA/DNA: According to Lang et al. (1965), RNA/DNA ratio shows the capacity of organisms for the protein synthesis. In the present case RNA/DNA ratio is maximum at 96 hour embryonic stage which continuously falls until emergence (Fig. 25A). After the adult emergence RNA/DNA ratio is almost constant (Fig. 26C). Thus from the ratio it may be concluded that the protein synthesis potential of the 96 hr embryo is the highest and the capacity of protein synthesis goes on decreasing as growth proceeds. In the adult insects the ratio is almost constant showing that protein synthesis is almost nil or if there is slight

synthesis going on that is being balanced by protein degradation. This observation is supported by the protein content/individual (Figs. 23 and 24). The results of King (1973) on Lucilia sericata for the RNA/DNA ratio are similar to the present observations. The results of Clevins (1973b) in A. aegypti are also partly supported since he reported a rise of RNA/DNA ratio in the 1st larva and just before pupation. Lang et al. (1965), and Balazs and Haranghy (1965) have reported a constant ratio of RNA/DNA throughout aging.

Protein/RNA: The ratio of protein/RNA is given in Figs. 25B and 26B and it is observed that the ratio gradually goes on increasing from 24 hr embryo to pupa followed by a sharp increase until emergence. After adult emergence, the ratio is almost constant with slight fluctuation for first 2-3 days. From Fig. 25B and 26B, it is clear that as the growth advances the RNA content goes on decreasing at a higher rate showing a gradual increase in ratio while the sharp increase of RNA while protein decrease is very less. In the adults RNA and protein contents decreases slowly showing that the ratio is almost constant. Thus from this ratio it is clear that protein synthesis depends upon RNA synthesis so that as RNA synthesis decreases, protein synthesis also decreases until it is almost standstill in the adult life or the protein synthesis rate is very slow.

Protein/DNA: From Figs. 25C and 26A, it is observed that this ratio goes on increasing until prepupa, followed by its gradual decline until emergence. After emergence protein/DNA ratio show slight fluctuations for first 2-3 days and afterwards stabilizes. The ratio is always higher in females than males. The ratio of protein/DNA denotes the relative quantities of protein and DNA at any stage and shows that protein synthesis is dependent upon DNA content, and so to say the cell number of body.

Thus from the ratios of RNA/DNA, protein/RNA and protein/DNA, it is clear that the aging in C. analis is followed by the decreased potential of DNA, RNA and protein synthesis as the development progresses and adult life advances.

5.3.4 Ratio of DW/DNA, DW/RNA and DW/Protein

Since the life cycle of C. analis is peculiar due to the fact that it stores as much food as it can during development for its future consumption as this insect, after adult emergence, does not feed. It has to depend for energy needs on the stored food. Due to this habit ratio of DNA, RNA and protein were determined with DW so as to estimate the synthesis and degradation of DNA, RNA and protein with increase or decrease of dry weight.

DW/DNA: This ratio gives the relative amount of synthesis of DNA as the DW increases or decreases. From

Figs. 27A and 28A, it is observed that DW/DNA decreases from 24 hr egg to 1st larva showing that DNA synthesis during this period is very rapid followed by an increase in the ratio until 4th larva showing that the dry weight increase is more rapid than DNA. The decrease in the ratio in 5th larva shows rapid synthesis of DNA in relation to increase of DW. While the increase in DW/DNA ratio is owing to the increase in dry weight of the pupa due to loss of water while DNA synthesis is decreasing. After pupa the ratio goes on gradually decreasing until the 13th day. It is found that during this period the DW content/insect goes on decreasing while the decrease of DNA is not as rapid so as to lower the ratio of DW/DNA. This is similar to DNA/insect suggesting a rapid rate of DNA synthesis until pupa, after which the rate of synthesis is very slow.

DW/RNA: The ratio of DW/RNA is lowest in 96 hr embryo followed by its gradual but slow increase until pupa. After pupa, the ratio rises very steeply until emergence (Fig. 27B). After emergence the ratio declines slightly from 24 hr to 13th day. This curve clearly shows that RNA content and RNA synthesis were highest at 96 hr stage followed by rapid increase in dry weight, while RNA synthesis does not follow pace with DW increase so that there is an increase of this ratio until pupa. Following

pupa, RNA declines very sharply than dry weight showing a sharp increase in ratio. In the adult life the DW decreases sharply until 13th day while RNA content also shows a decline with age leading to a slight decrease in ratio.

Thus from this ratio it is clear that RNA synthesis is very rapid during embryogenesis followed by its decrease in synthesis rate. After pupa RNA content decreases constantly except for a slight increase for first 2-3 days in males.

DW/Protein: From Figs. 27C and 28C, it is observed that DW/protein ratio is highest in 24 hr embryo which goes on decreasing until prepupa, showing that due to rapid rate of protein synthesis until prepupa there is rapid fall of DW/protein ratio. Following prepupa, the ratio rises slightly until emergence and records a slow decrease from 1st day of emergence until 13th day. The explanation for this is the same as for DW/RNA.

5.3.5 Lipids

Lipids are an important source of energy during embryogenesis, postembryonic development, metamorphosis and aging (Gilmour, 1961; Gilbert, 1967). According to Fast (1964) three quarters of the insect species studied contain less than 10% lipids. But in the present case

lipid content forms a maximum of about 59% of the dry weight in prepupa. This high lipid content may be regarded as adaptation for C. analis as this insect does not feed after emergence.

From Fig. 29, it is observed that lipids both as percent of dry and wet weight decrease from 24 hr to 1st larval stage, followed by it gradual increase until PP. After PP, lipids again decrease until emergence. Thus, it is obvious that during embryogenesis, lipids are being consumed at a higher rate. According to Gilbert and Schneiderman (1961) 50% of lipids of eggs are utilized during embryogenesis whereas Allais et al. (1964) have reported a loss of 32% lipids during embryogenesis in Locusta migratoria. After larval emergence, lipids are synthesized and stored at a rapid rate during postembryonic development until it is maximum in prepupa reaching to a peak of 59% of DW and about 30% of the WW. After the prepupa, lipid contents decline from 59% in PP to 31.6% and 41.8% in males and females respectively until 24 hr adult. Thus there is a loss of about 50% and 30% in males and females during metamorphosis. The increase of lipid during PP may be due to the loss of water, lipid synthesis or release from lipoprotein as has been reported by Gilbert and Schneiderman (1961). The high rate of lipid decline during metamorphosis is of great significance since lipids are the major supplied of energy and sugars to a small extent (Agrell and Lundquist,

1973; Crompton and Birt, 1967; Crompton and Polakis, 1969). Gilbert and Schneiderman (1961) in Hylophora cecropia, George and Nair (1964) in Anthrenus vorex, Kalra et al. (1967) in Culex pipien fatigen have reported similar results during larval development and pupa. According to Russo-Caia and Corere (1960) in M. domestica, both lipid and sugars are accumulated during development and utilized at a higher rate during pupa. Niemierko et al. (1956) have reported in B. mori that females utilize about 50% of stored lipid while males utilize about 30% during pupation. Mukerji and Gupta (1973) have reported the utilization of 22.2% of lipid during metamorphosis in Pseudoletia unipunctata.

After the adult emergence, lipid content are shown in Figs. 30A, B and C. From Fig. 30A two points emerge. Firstly, lipid content is always higher in females than in males. Secondly, lipids gradually go on decreasing from 1st day to 10th day in either sex. Following 10th day a curious phenomenon is observed, i.e., total fat content on 11th day suddenly increases to 1.5 times the value obtained on 10th day. After 11th day lipid again goes on decreasing gradually until 13th day. Figs. 30B and 30C show the lipid as per cent of WW and DW respectively. The course followed by these figures is the same as followed by Fig. 30A.

From Fig. 30C, it is observed that males and females utilize 75% and 65% of lipids on DW basis, in 13 days of adult life. Thus it is clear that males utilize more of fats showing that they have a higher need of energy in adult life. This contention gets support from Bat-Mirian and Galun (1962) who have concluded that males have higher energy needs than females in A. aegypti. Sex dimorphic changes in adult males and females have been reviewed by Gilbert (1967) and according to him sex dimorphism in lipid content is a genetic factor. The higher lipid content of females seems to be because lipids are an important source of energy for egg maturation, and egg laying as has been suggested by D'Costa and Birt (1966), Gere (1964) and Gilbert and Schneiderman (1961). From Figs. 30A, B and C, it is observed that the decrease in lipid for first 7-8 days is much more higher and this is also the period of intense egg-laying activity (Dhand and Rastogi, 1975). The lipid increase noted on 11th day in either sex is a peculiar phenomenon not reported heretofore. It may be due to rapid lipid synthesis from sugar, amino acid and protein as has been reported by Clements (1959) in locust and Van-Handel and Lum (1961) in Aedes.

Abasa (1972) in Sarcophage tibialis has reported decrease of lipids with advancing age. According to Gere (1964), Lymantria dispar which does not feed after emergence of adult and depends for its energy needs by "self-destruction",

has a maximum of 55% lipid of DW during development. According to him, about 30% of lipids in females are transferred into eggs while males utilize their lipid exclusively to find out virgin females and mating. As the adult life of C. analis is similar to L. dispar, the explanation given by Gere (1964) is supported.

5.3.6 Total sugars

From Fig. 31A, it is observed that sugars/individual go on increasing from 24 hr egg stage to 5th larva followed by its gradual decline until emergence of adults. Thus it is clear that sugars are being synthesized and stored until 5th larva. The decrease of sugars during metamorphosis until emergence may be consumed for a part of energy supply and a part may be synthesized into the lipid, protein and the chitin of the adult body.

After the adult emergence, sugar content is highest in 24 hr old insects which continuously goes on decreasing as the age advances (Fig. 33A). Here also, females have higher sugar content than the males. Secondly the decrease of sugar content for the first 3 days is much higher and more so in the males. This clearly shows that as the age of C. analis advances, the sugars are being utilized at a higher rate for energy supply. It is also obvious that male consume more of sugars than the females.

From Figs. 31B and 33C, it is found that when sugars/gm WW are highest in 96 hr embryo forming about 24 mg/gm WW which decreases until pupa. Thus it is clear that as the WW of insect rises, the sugar synthesis rate falls. The slight rise of sugar between pupa and 24 hr old adults shows an increased synthesis at this time. In 24 hr adults sugars form about 17.2 mg/gm WW in males and 17.3 mg/gm WW in females. As the age advances the sugar content/gm WW goes on decreasing, more so in males. Thus sugar consumption in males is much higher giving support to the observation of Bat-Mirian and Galum (1962) that males have a higher metabolic rate.

From Figs. 32B and 33B, it is observed that total sugar percent of the WW is highest in 1st larva being about 2.4% which continuously decreases until pupa. The adult 24 hr male and female have 1.7% sugar of the WW which goes on decreasing as the age advances. This also supports the conclusion that sugar content, although increases during development, does not follow the increase of WW of individuals. These sugars are utilized at a higher rate during aging.

5.3.2 Trehalose as percent of Total Sugars

From Fig. 32A, it is observed that trehalose forms about 72% of total sugars in 96 hr embryo which continuously goes on decreasing until prepupa forming about 46% of the total sugars. In pupa until emergence, there is increase of

trehalose content reaching 71.5% in males and 76.0% in females. Thus it seems that trehalose content from embryo to prepupae goes on decreasing. It may be due to the fact that trehalose is being continuously utilized for the energy needs of larvae as the trehalose activity goes on increasing in larval development. Sharp decrease of trehalase activity during metamorphosis may lead to increased trehalose content during pupation (Chapter 4). It may also be possible that trehalose synthesis is decreased during pupation.

Fig. 32C gives trehalose content as percent of the WW. This follows almost the same course as that of trehalose content of total sugars.

Figs. 34A and B show that trehalose as percent of WW and trehalose as percent of total sugar respectively. From Fig. 34B, it is observed that trehalose content of total sugar is 71.5% in males and 76% in females which decreases in either sex until 3rd day reaching a level of 63% in males and 60% in females. Following 3rd day, trehalose content of total sugars increases until 9th day reaching the maximum and after that the level is maintained at 95-98% in either sex. Thus it seems that the rapid decrease of trehalose percent of total sugar for first 3 days may be related to high energy needs of insects for egg laying and mating. In support of this, it is found that trehalase activity also

shows a small peak in females on 3rd day (Chapter 4). Following 3rd day, gradual increase of trehalose percent of total sugars may reflect the synthesis of trehalose at a higher rate to attain a high level of 95-98% during the last days of adult life. This increased trehalose may be synthesized from lipids or protein as suggested by Agrell (1953) and Ludwig et al. (1964).

The increased trehalose percent of the total sugar may be an adaptation, for during the last days, trehalase activity goes on increasing giving a peak, suggesting that as the age of C. analis is advancing the energy needs of insects goes on increasing. This is supported by the observations of Tribe (1966) and Baker and Lloyds (1970). The increased energy needs may be supplied by the increased breakdown of the trehalose. In turn, due to this stress of high energy needs, trehalose is increasingly synthesized from other stored products.

Fig. 34A, shows trehalose as percent of total WW during aging. From the figure it is observed that in males trehalose as percent of WW goes on decreasing as the age advances, whereas in females there is a slight decrease in trehalose percent of total sugars for first 3 days and subsequently it is maintained at the same level.

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Fig. 19A Changes in DNA content (µg)/individual during development.

19B Changes in DNA content (µg)/gm wet weight of developmental stages.

Fig. 20A Changes in DNA content (µg)/insect during aging.

20B Changes in DNA content (µg)/gm wet of insects during aging.

Fig. 21A Changes in RNA content (µg)/individual during development.

21B Changes in RNA content (µg)/gm wet weight developmental stages.

Fig. 22A Changes in RNA content (µg)/insect during aging.

22B Changes in RNA content (µg)/gm wet weight of insects during aging.

Fig. 23A Changes in protein content (mg)/ individual during development.

23B Changes in protein content (mg)/gm wet weight of development stages.

Fig. 24A Changes in protein content (mg)/insect during aging.

24B Changes in protein content (mg)/gm wet weight of insects during aging.

Fig. 25A Ratio of RNA/DNA during development.

25B Ratio of protein/RNA during development.

25C Ratio of protein/DNA during development.

Fig. 26A Ratio of protein/DNA during aging.

26B Ratio of protein/RNA during aging.

26C Ratio of RNA/DNA during aging.

Fig. 27A Ratio of dry weight/D.A during development.

27B Ratio of dry weight/R.A during development.

27C Ratio of dry weight/protein during development.

Fig. 28A Ratio of dry weight/D.A during aging.

28B Ratio of dry weight/R.A during aging.

28C Ratio of dry weight/protein during aging.

Fig. 29 Changes in percent lipids/wet weight and dry weight during development.

Fig. 30A Changes in lipid content (mg)/insect during aging.

30B Changes in percent lipids/wet weight during aging.

30C Changes in percent lipids/dry weight during aging.

Fig. 31A Changes in sugar content (µg)/individual during development.

31B Changes in sugar content (mg)/gm. wet weight of developmental stages.

Fig. 32A Trehalose as percent of total sugars during development.

32B Percent total sugars/wet weight of developmental stages.

32C Percent trehalose/wet weight of developmental stages.

Fig. 33A Changes in sugar content (µg)/insect during aging.

33B Changes in percent total sugars/insect wet weight during aging.

33C Changes in total sugars (mg)/gm. wet weight during aging.

Fig. 34A Trehalose as percent of wet weight during aging.

34B Trehalose as percent of total sugars during aging.

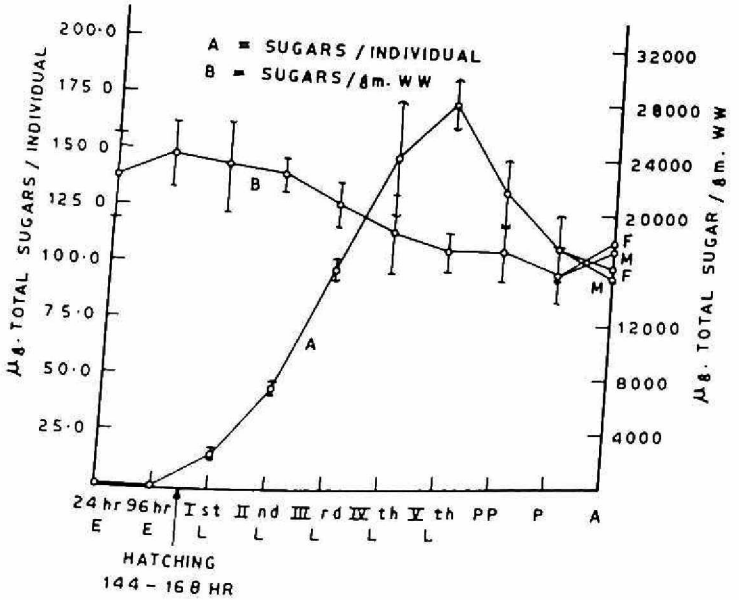

FIG 31

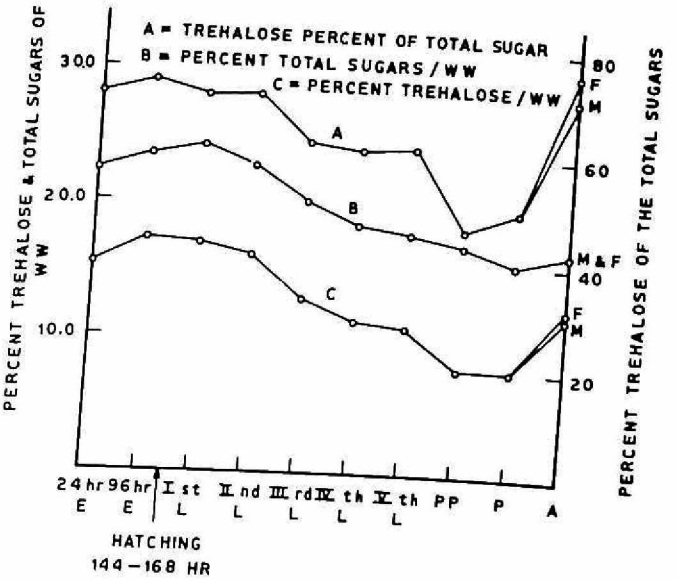

FIG. 32

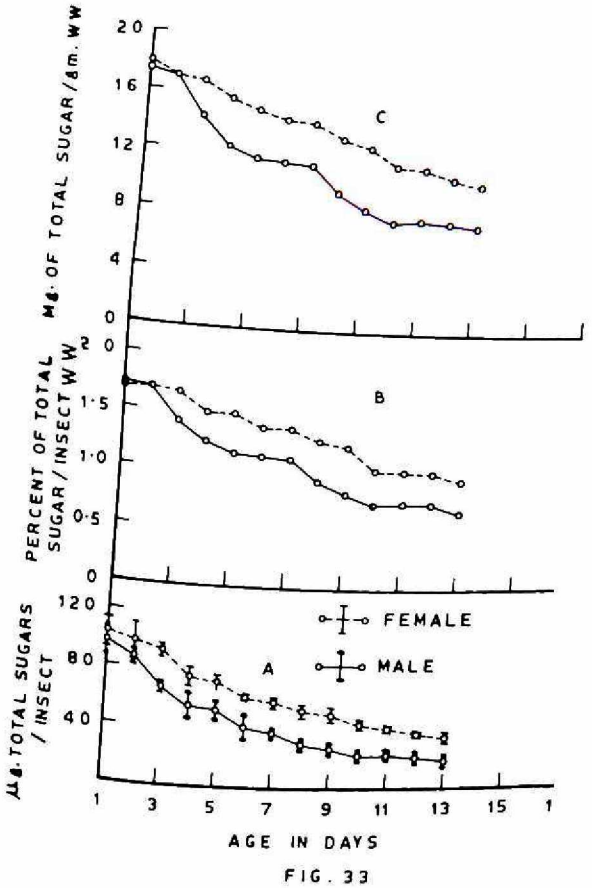

FIG. 33

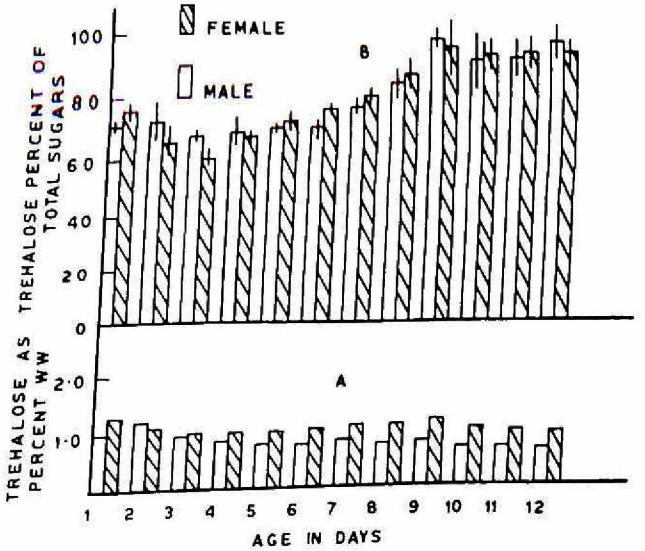

FIG. 34

CHAPTER - 6

QUALITATIVE AND QUANTITATIVE STUDY OF AMINO ACIDS

SUMMARY

Free amino acids have been studied quantitatively by paper chromatography in Callosobruchus analis during postembryonic development and aging. It is found that a total of 17 free amino acids have been detected out of which two are unknown. The identified amino acids are: alanine, arginine, aspartic acid, cystine, glutamic acid, glutamine, histidine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, valine and two unknown (No. 15 and 16). The highest quantities of total free amino acids have been observed in pupa. During development, four amino acids appear at different stages of development and the rest are present throughout the development. Cystine is absent in first two larval stages and present thereafter. Methionine is absent in 1st larval stage and present throughout the remaining life. Phenylalanine is absent in first four larval stages but appears in 5th larva while unknown No. 16 is present only in prepupa and pupa and is absent during larval and adult life. Different amino acids show variations in their quantities during development.

During aging, a total of 16 free amino acids have been detected which are: alanine, arginine, aspartic acid, cystine,

glutamic acid, glutamine, histidine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, valine and unknown No. 15. The total quantity of free amino acids and the quantities of individual amino acids show variations in relation to aging in both sexes which have been discussed in relation to egg laying and mortality rate.

6.1 INTRODUCTION

There has been considerable research concerning the qualitative and quantitative free amino acid pools in the haemolymph of adult insects as well as during post-embryonic development. It has been demonstrated by many authors that free amino acids serve a number of important physiological roles such as protein metabolism (Ussing, 1946; Hackman, 1956; Fakuda et al., 1955; Chen, 1958 and Bricteus-Gregoire et al., 1957), osmoregulation and buffering of the haemolymph (Bishop et al., 1926; Beadle and Shaw, 1950; Pant and Agarwal, 1964; and Gilmour, 1961), energy reserve (Bursell, 1960 and 1963), link between "sugar-free amino acid metabolism" (Price, 1961) and detoxification of haemolymph (Friedler and Smith, 1954; Casida, 1955, and Shyamala, 1964).

The presence of high concentration of Free amino acids in the haemolymph is a characteristic feature of the insects (Wyatt, 1961; Chen, 1962) and the concentration of Free amino acids in haemolymph may be as much as 30 times higher than what it is in other animals (Florkin, 1960). According to Florkin (1937), Drilhon and Busnel (1945), and Gilmour (1961), Free amino acids vary with development moulting, metamorphosis, age, digestion, metabolic activity and nutrition etc. A number of reviews have appeared in literature concerning the pattern of protein and Free amino

acids in the insects (Wyatt, 1961; Chen, 1962 and 1966; Agrell and Lundquest, 1973 ; and Rockstein and Miquel, 1973). In general, almost all the amino acids contained in the proteins have been identified either in tissues or haemolymph. In addition to these, some other amino acids such as β-alanine, taurine, ornithine, α and γ-aminobutric acid have also been reported which are not present in proteins and some amino acid derivatives as S-methyl cystein, thyroxine, phenylalanine, methyl histidine, homoarginine have also been detected (Chen, 1966).

According to Chen (1966), Arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, Isoleucine and Valine are necessary for growth and must be obtained from food. Some of the amino acids as arginine, cystine, glycine, proline, tryptophan, tyrosine, phenylalanine are especially concerned with moulting, differentiation, pupation and emergence of adults (Chen, 1962). Chen (1962, 1966) has reviewed the major part of the work done on free amino acids during embryonic, post embryonic and adult stages of the insects. After that a number of other important reports regarding free amino acids metabolism have appeared, such as, Stidham and Liles (1969), Thayer and Terzian (1970), Garcia-Garcia and Munico (1970), Pant and Lal (1970), and Roberts and Smith (1971). Bawa et al. (1974), have studied the free amino acids in sterile and fertile strains of Callosobruchus maculatus and reported

the presence of histidine, arginine, aspartic acid, serine, glycine, threonine, alanine, proline, tyrosine, methionine, valine, phenylalanine and leucine in fertile strains by paper chromatography.

In order to study the metabolism of free amino acids, these have been studied qualitatively and quantitatively during the period of post-embryonic development and aging in Callosobruchus analis (Fab.), so as to find out if some definite pattern can be deduced in relation to development, metamorphosis, aging and sex in the light of present work.

6.2 RESULTS

6.2.1 Free amino acids during post-embryonic development

The results obtained by (quantitative) paper chromatography technique are given in Plate I. From the fig. it is observed that the total amino acid pool/insect is least in the 1st larva (2.662±0.201 µg) which progressively goes on increasing until 5th larva when it is 19.104±2.010 µg. Following 5th larva, the total quantity of free amino acids decreases slightly in the prepupa. In pupa total quantity of amino acids/insect increases again so as to give a maximum value of 22.762±2.367 µg/insect which is the maximum value during post-embryonic development. After emergence,

free amino acids decrease almost by 40% to give 20.444±1.003 µg in 24 hour old males and 13.479±434 µg in 24 hour old females.

During the course of investigation, it is found that a total of 17 amino acids are present during the development in both sexes, which vary in their amounts as well as in the pattern of appearance during development which is as follows:

During development, four amino acids appear at different stages of development, i.e. cystine, methionine, phenylalanine and an unidentified No. 16. Cystine is absent in the first two larval stages but appears in the third larva and thereafter it is present rest of the life. The highest quantity of cystine is present in 5th larva which decreases in prepupa appreciably to almost 1/4 the value of 5th larva. In pupa, it again rises slightly. Following emergence, cystine shows a decline in the 24 hour old male, whereas in females the quantity increases over that of pupa. Methionine is absent in 1st larva and appears in 2nd larva, also showing the highest quantity followed by its steep fall in 3rd larva to 1/4 the quantity of 2nd larva. After 3rd larva quantity of methionine goes on increasing until pupation. After adult emergence, the quantity of methionine decreases to half in males and to 2/3 in the females of 24 hour adult stage, as compared to the quantity present in pupa. Phenylalanine makes its appearance in 5th larva only and is absent

in first four larval stages. The quantity of phenylalanine is highest in 5th larva, declines in prepupa and again increases in pupa. After adult emergence, phenylalanine declines in either sex of 24 hour adults. The unidentified amino acid (No. 16), is present only in prepupa and pupa and absent in the larval and adult life.

Among other amino acids, proline, unidentified No. 15 show the highest quantities in 5th larval stage. Proline, after showing a gradual decline in prepupa and pupa, again rises in 24 hour old insects of either sex. Whereas unknown No.15 decreases in prepupa to 1/4 the value of 5th larva. In pupa, quantity of unknown No. 15 again increases and following adult emergence again decreases.

Alanine and aspartic acid show maximum quantities during prepupa. Alanine in case of males decreases upto 24 hour males while in females, it declines in pupa and thereafter again rises in 24 hour old females. On the other hand, aspartic acid gradually goes on decreasing from prepupa to 24 hour old adults of either sex.

Remaining amino acids, i.e. arginine, glutamic acid, glutamine, histidine, leucine, lysine, threonine, tyrosine and valine record a gradual increase in quantity from 1st larva onwards reaching maximum concentration during pupal stage. Following adult emergence, the quantities

of these amino acids decrease in either sex except for glutamic acid. Glutamic acid shows an increase in males while in females it shows an appreciable fall. The decrease in the quantity of lysine is appreciable.

In 24 hour adult males, the quantities of arginine, glutamic acid and tyrosine are higher in males as compared to females, while the quantities of all other amino acids are higher in females as compared to males. During development, the quantity of glutamic acid is highest while the relative quantities of alanine, arginine, aspartic acid, glutamic acid, glutamine, lysine, tyrosine, and histidine are much higher than others.

6.2.2 Free amino acids during adult life

During adult life, in either sex, a total of 16 amino acids have been identified out of which one could not be identified. The total quantities of free amino acids in females are higher than males except on 2nd, 3rd and 12th days when males have higher amino acid contents (Plate II). In case of males, the total free amino acids as µg/insect show one distinct peaks, on 2nd day after emergence followed by a gradual decline until 6th day. Following which total quantity of free amino acids goes on declining upto 12th day. On the other hand, in females, total free amino acids decrease from the 1st day after emergence until 4th day followed by an increase on 6th day and subsequently

declining. However, between 8th to 10th day a steady level is maintained.

After adult emergence all the 16 amino acids detected in 5th larva are present i.e. alanine, arginine, aspartic acid, cystine, glutamine, glutamic acid, histidine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, unidentified No. 15 and valine. Both the sexes have same set of 16 amino acids although they differ quantitatively. Glutamic acid is present in highest concentrations in either sex although alanine, aspartic acid, tyrosine, glutamine and glutamic acid are also present in appreciable quantities. All the different amino acids show a highly variable pattern of changes with age in either sex which shall be dealt during discussion.

6.3 DISCUSSION

During the course of development, 17 free amino acids have been identified and quantitatively determined. These are: alanine, arginine, aspartic acid, cystine, glutamic acid, glutamine, histidine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, valine and two unidentified (No. 15 and 16). It is observed that there is variation not only in the total concentration of free amino acids but also in the individual amino acids throughout the development.

The free amino acid pool (µg/individual) shows a

gradual increase from 1st larva to 5th larva (Plate I). Then there is a slight decline during prepupa, and again reaching the maximum value in pupa. Upon adult emergence, the total pool decreases recording a drop by 40%. It is obvious that during larval development there is gradual accumulation of amino acids. Further, along with the increase in total amino acid pool there is a corresponding increase in the total wet weight upto 5th larval stage (Chapter 3 and Fig. 1).

During pupa, histogenesis and histolysis are going on side by side. The slight decrease in free amino acids in the prepupa may be either due to utilization of amino acids for protein synthesis or due to loss in the body fluid during shedding of larval skin. However, quantitative increase of free amino acids during pupa clearly indicates release of free amino acids from proteins. At the same time, it is also observed that during pupation, the loss of wet weight/individual is very drastic (Chapter 3). Since pupa is a closed system, it shows that there is a definite accumulation of free amino acids during this period. According to Chen (1966), pupal free amino acid concentration shows a balance between histogenesis and histolysis. Following pupa, the free amino acid quantities fall appreciably until emergence of adult suggesting utilization of free amino acids for protein synthesis.

According to Ussing (1946), free amino acids tend to be stored during larva, remain relatively at high levels during pupation, and decrease in adults. Ussing (1946) suggested that high levels of free amino acids are utilized for protein synthesis during metabolism and the absence or inactivation of deaminating enzymes accounts for progressive increase of free amino acids in larval and pupal stages. Fukuda *et al.* (1955), Bricteteus-Gregoire et al. (1957), Gilmour (1961) and Chen (1953, 1962 and 1966) have given similar explanations for relativvly high concentration of free amino acids during larval and pupal stages.

Agrell (1949) has shown in Calliphora that free amino acids decline at the initiation of prepupa and the maximum quantity is reached in pupa. According to him, an initial drop of free amino acids during prepupa is due to histogenesis of hypodermal tissue, while the highest concentration in mid-pupa shows the highest rate of histolysis. The appreciable fall in free amino acids in late pupa and adults is due to their utilization for formation of thoracic muscles. Chen and Kuhn (1956) in Ephestia and Chen (1958) in Culex have given similar interpretations. Patterson(1957) however, failed to record any major variations in free amino acid content during pupation of Tenebrio molitor.

After emergence, in either sex, 16 ninhydrin positive spots were identified. These 16 spots are the same 16 free

amino acids which are present in the 5th larva, out of which one is unidentified. Both the sexes have same amino acids but differ quantitatively (Plate 2).

The total quantity of free amino acids in males is maximum on the 2nd day, declines gradually until 6th day and then remains almost constant upto 12th day of adult life (Plate 2). In case of females, the total quantity of free amino acids decreases gradually from 1st to 4th day, then increases upto 8th day and subsequently declines. However, between 8th to 10th day, a steady level is maintained.

Since egg laying and mating are at their peak in the early life of Callosobruchus analis (Chapter 3), it is reasonable to assume that the increase of free amino acids on 2nd day in males may be due to breakdown of proteins for the purpose of sperm formation and their transfer to females. The gradual decline upto 8th day, however, accounts for the continuous utilization of amino acids. On the other hand, in females, rapid decrease of free amino acids in the first few days may be due to transfer of these to the eggs.

Amino acids not only supply nitrogen to eggs and sperms, but they also carry on a host of functions such as; energy reserve, darkening of skin, storage of ammonia released during deamination, buffering action and osmo-regulation etc.

A comprehensive review of free amino acids during development and aging in insects has been made by Chen (1952 and 1966). Since then quite a few interesting reports have appeared in literature on the same subject worth mentioning and among them are those of; Thayer and Fergison (1970) on female Aedes aegypti, Bursell (1963) in Glossina, Mansingh (1964) in Blatella germanica, Ludwing and Jones (1964) in Tenebrio molitor, Stidham and Liles (1969) in female Aedes aegypti, Roberts and Smith (1971) in Melanoplus sanguinipes and Bawa et al. (1974) in Callosobruchus maculatus.

Like the changes observed in the total quantity of free amino acids, the quantities of individual amino acids per individual (µg) also show variations during development. A total of 17 amino acids have been detected in the developmental stages, out of which methioniner is absent in 1st larva although present in the latter stages. Cystine appears only in the 3rd larval stage and continues to be present throughout the rest of life. Phenylalanine is absent in first four larval stages and makes its appearance in the 5th larva and thereafter remains present for rest of life, whereas unidentified No. 16 is present day during pupal stage and remains absent in larval and adult stages. Remaining amino acids, i.e. alanine, arginine, aspartic acid, glutamic acid, glutamine, histidine, leucine, lysine, proline, threonine, tyrorine, valine and unidentified No.15.

are present throughout the larval, pupal and adult stages.

According to Lipke and Fraenkel (1956) and House (1962), arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine and valine are essential amino acids and must be obtained entraneously. According to Chen (1966), α-alanine, cystine, glycine, serine and proline are non-essential. Further, he suggested that arginine, cystine, glycine, proline, tryptophan, tyrosine and phenylalanine are concerned with moulting, differentiation, pupation and emergence of adults.

During development as well as aging, glutamic acid is present in highest quantity, although alanine, arginine, aspartic acid, glutamine, lysine and threonine are also present in appreciable amounts. Appreciable changes in quantities during development and aging are concerned with glutamic acid, Glutamine, alanine, aspartic acid, arginine, tyrosine lysine and Histidine. In the present work it has been observed (Plate 1) that glutamic acid shows a progressive increase from 1st larva to 4th larva, which declines in the 5th larva, thereafter the content of glutamic acid increases steeply during pupa. Following emergence in 24 hour old insects, glutamic acid further increases in males and decreases sharply in females. A further increase is recorded upto 2nd day in males and upto 3rd day in females followed by a gradual decline until 12th day. In case of

Aspartic acid, there is a progressively increase upto prepupa followed by a decline in 24 hour old adults of either sex. Aspartic acid, after emergence shows an increase upto 2nd day in males which declines upto 4th day and then remains almost constant. In females, however, Aspartic acid records an increase upto 6th day followed by a decline upto 12th day. Alanine follows the pattern similar to Aspartic acid upto pupal stage following which Alanine increases in females and decreases in males until the insects are 24 hour old. After emergence, alanine decreases in either sex upto 3rd day which, after showing a slight increase on 4th day in either sex, continuously declines.

Glutamine progressively increases from 1st larva to pupa followed by a steep decline in either sex and more so in males until they are 24 hours old. After emergence, glutamine drops upto 2nd day in females followed by an increase on 3rd day, which then remains almost constant up to 8th day. On 10th day it again increases followed by a decline. On the other hand, in males, glutamine content increases upto 3rd day, remains constant upto 8th day and declines thereafter. Tyrosine also shows a progressive increase upto pupa followed by a constant decline until 4th day after emergence followed by a gradual increase in either sex. Lysine follows a similar trend as that of

cystine until emergence followed by a slow increase of lysine with aging. Remaining free amino acids however, show variable pattern during development and aging.

By using labelled amino acids Price (1961) in M. sexta, Bursell (1963) in Glossina and Mansingh (1964) in B. germanica, were able to show interconversion of some amino acids which could enter Kreb's cycle to meet additional energy demand. Bursell (1963) observed that when oth acetate is given, it is incorporated in α-alanine and glutamic acid which have a role in linking "Suger-amino acid metabolism" resulting in the synthesis of α-alanine and aspartic acid from glutamic acid. According to Bursell (1959), some of the amino acids serve as expendable energy reserve during starvation. As C. analis does not feed after adult emergence, it can be likened to the starving condition. From plates I & II, it is clear that aspartic acid, glutamic acid and alanine are accumulated during development which are later utilized by the adults. Is is likely that these are utilized through the "Sugar-amino acid metabolism" link. Stidham and Liles (1969) have also confirmed this hypothesis. Since these three amino acids are present in highest concentrations, they might also act as nitrogen donors, both during development and aging. Indira . . (1963) and Chen (1958) have also suggested the role of glutamic acid and aspartic acid as energy donors.

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Plate I

Quantitative changes in amino acids during aging

PLATE I

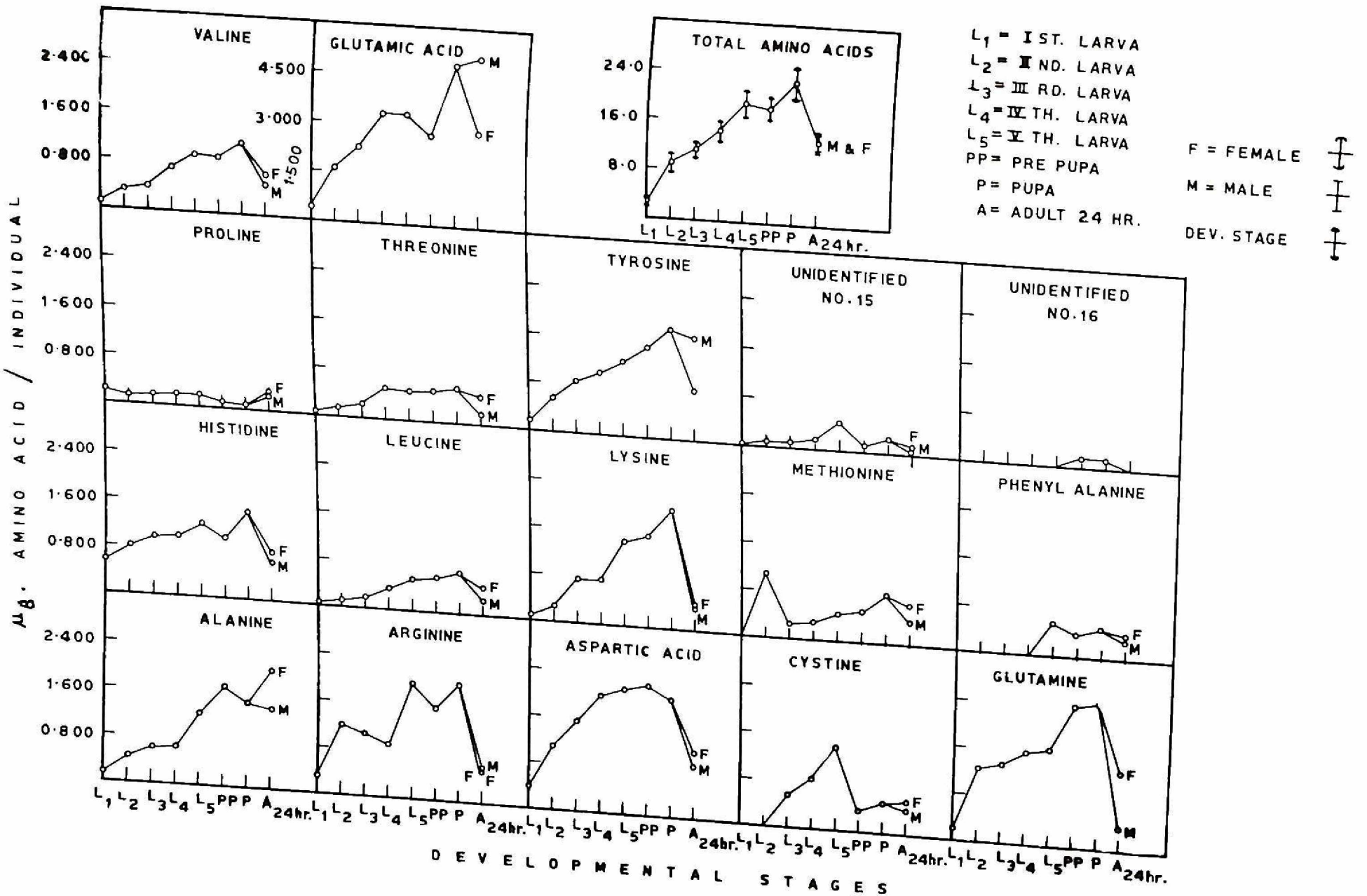
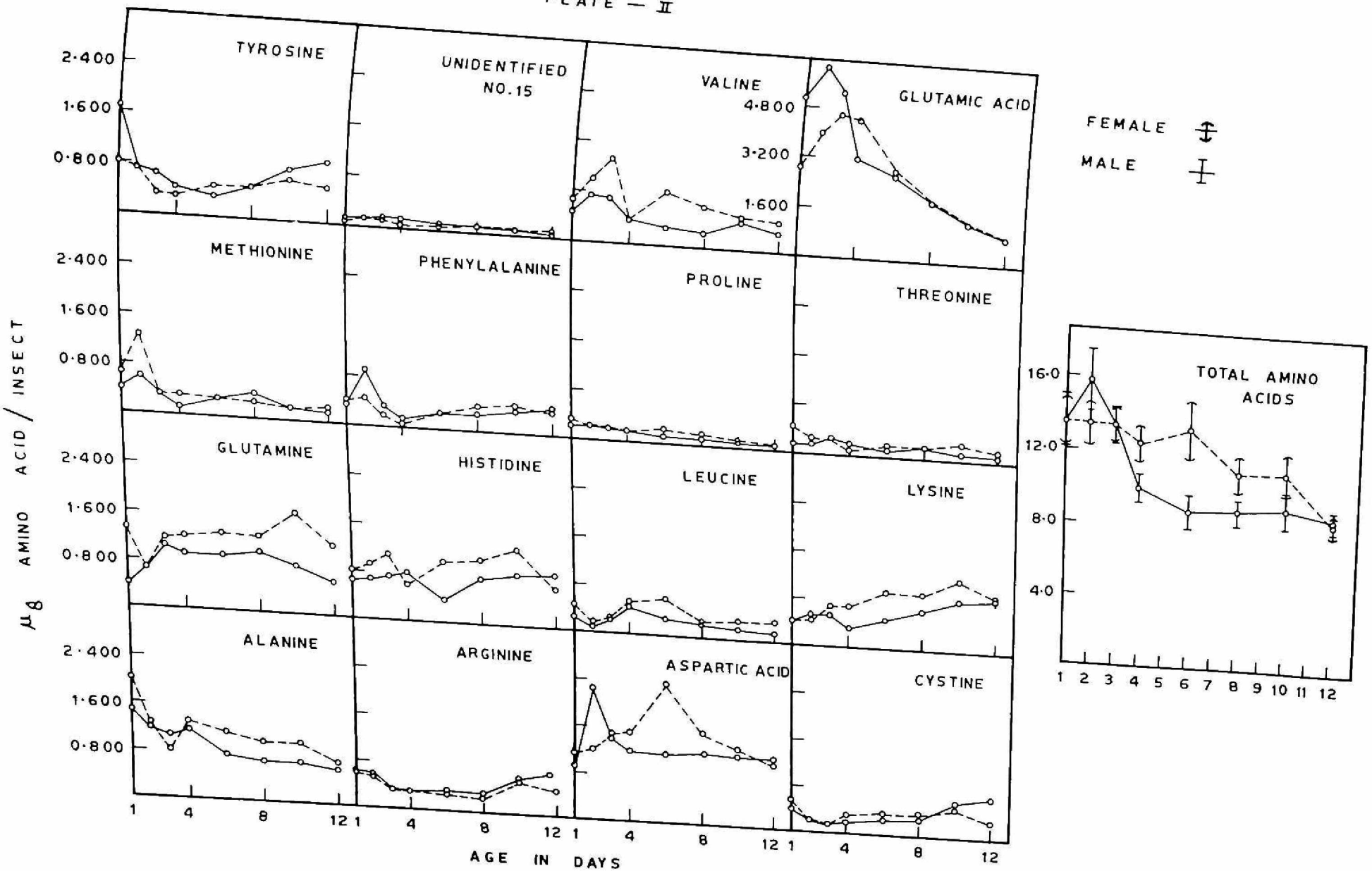

VALINE
GLUTAMIC ACID
TOTAL AMINO ACIDS
PROLINE
THREONINE
TYROSINE
UNIDENTIFIED NO.15
UNIDENTIFIED NO.16
HISTIDINE
LEUCINE
LYSINE
METHIONINE
PHENYL ALANINE
ALANINE
ARGININE
ASPARTIC ACID
CYSTINE
GLUTAMINE
μg. AMINO ACID / INDIVIDUAL
DEVELOPMENTAL STAGES
L1 L2 L3 L4 L5 PP P A 24hr.
L1 = I ST. LARVA
L2 = II ND. LARVA
L3 = III RD. LARVA
L4 = IV TH. LARVA
L5 = V TH. LARVA
PP = PRE PUPA
P = PUPA
A = ADULT 24 HR.
F = FEMALE
M = MALE
DEV. STAGE

Plate II

Quantitative changes in amino acids during aging

PLATE — II


TYROSINE
UNIDENTIFIED NO.15
VALINE
GLUTAMIC ACID
METHIONINE
PHENYLALANINE
PROLINE
THREONINE
GLUTAMINE
HISTIDINE
LEUCINE
LYSINE
ALANINE
ARGININE
ASPARTIC ACID
CYSTINE
TOTAL AMINO ACIDS
FEMALE
MALE
μg AMINO ACID / INSECT
AGE IN DAYS
2·400
1·600
0·800
4·800
3·200
16·0
12·0
8·0
4·0
1 4 8 12

B I B L I O G R A P H Y

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APPENDICES

(TABLES)

Changes in Wet weight, dry weight and water content in adult females during aging

Age in days	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of insects/gm wet weight	163.9	172.4	178.7	197.3	206.9	220.6	223.4	234.3	235.9	247.9	252.1	260.9	262.7
Wet weight/ insect in Mg.	6.103	5.800	5.600	5.067	4.833	4.533	4.377	4.267	4.240	4.033	3.967	3.833	3.805
Percent Water/gm wet weight	50.4	52.9	52.1	53.1	53.0	51.8	52.5	52.5	52.5	52.6	55.1	55.5	55.2
Mg.Water/ Insect	3.077	3.071	2.943	2.773	2.658	2.416	2.400	2.348	2.328	2.124	2.097	2.117	2.110
Percent Dry weight/ gm wet weight	49.6	47.1	47.9	46.9	47.0	48.2	47.5	47.5	47.5	47.4	44.9	44.5	44.8
Mg.Dry weight/insect	2.637	2.707	2.647	2.400	2.235	2.054	2.000	1.958	1.925	1.909	1.816	1.700	1.695

Changes in wet weight, dry weight and water content in males during aging

Age in days	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of Insect/gm wet weight	174.4	194.2	204.1	225.6	260.9	263.0	284.3	300.0	300.0	312.5	328.9	344.8	350.0
Wet weight/ Insect in Mg.	5.733	5.150	4.900	4.433	3.833	3.717	3.517	3.333	3.333	3.200	3.040	2.900	2.857
Percent Water/gm wet weight	55.7	55.5	52.5	53.9	55.0	53.3	54.8	55.0	54.9	52.5	53.8	55.2	55.4
Mg.water/ insect	3.196	2.848	2.555	2.355	2.033	1.917	1.846	1.750	1.749	1.636	1.676	1.609	1.578
Percent Dry weight/ gm Wet weight	44.3	44.5	47.5	46.1	45.0	46.7	45.2	45.0	45.1	47.5	46.2	44.8	44.6
Mg.water/ insect	2.414	2.244	2.255	2.072	1.784	1.779	1.657	1.572	1.563	1.502	1.474	1.361	1.279

Egg laying rate during aging (Per 200 females)

Age in days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Number of Eggs layed/ day	1204	2358	3056	2051	1775	1336	1230	1053	901	770	396	272	105	49	12

Life Table for 375 females of C. analis

(Reduced to 1000 insects)

	Age in days (x)	Actual Death/ day	No. of Deaths/ day/1000 insects (dx)	Survivors (lx)	Mortality rate or Death rate per day (1000 q_x)	Expected life (e_x)
1	0-1	0	0	1000	0	10.91
2	1-2	2	5.4	1000	5.4	9.92
3	2-3	4	10.4	994.6	10.4	9.00
4	3-4	6	16.0	984.2	16.2	8.10
5	4-5	7	18.4	962.8	19.1	7.22
6	5-6	9	24.0	949.8	25.3	6.43
7	6-7	9	24.0	925.8	26.9	5.48
8	7-8	10	26.4	901.8	29.2	4.60
9	8-9	18	43.0	875.4	56.0	3.72
10	9-10	48	128.0	827.4	154.7	2.91
11	10-11	50	133.0	699.4	190.2	2.35
12	11-12	57	152.0	566.4	268.4	1.79
13	12-13	82	218.7	414.4	527.7	1.23
14	13-14	46	122.7	195.7	626.9	1.09
15	14-15	25	66.6	72.0	925.0	1.10
16	15-16	2	5.4	5.4	1000	0.5
17	16-17	0	0	0	0	0

Life Table for 349 males of C. analis

(Deduced to 1000 insects)

	Age in days (x)	Actual Death/ day	No.of deaths per day/ 1000 insects (dx)	Survivors (lx)	Mortality rate or Death rate per day (1000 qx)	Expected life (e_x)
1	0-1	0	0	1000	0	9.34
2	1-2	1	2.9	1000	2.9	8.34
3	2-3	4	11.5	997.1	11.5	7.36
4	3-4	16	45.8	985.6	46.5	6.44
5	4-5	16	45-8	939.8	48.8	5.74
6	5-6	12	34.4	894.0	38.5	5.00
7	6-7	15	43.0	859.6	50.0	4.19
8	7-8	22	63.1	816.6	77.2	3.38
9	8-9	36	103.2	753.5	137.0	2.62
10	9-10	66	189.2	650.3	291.0	1.95
11	10-11	55	157.5	461.1	341.6	1.56
12	11-12	58	166.0	303.6	546.7	1.10
13	12-13	36	103-2	137-6	751.4	0.81
14	13-14	9	25.8	34.4	750.0	0.75
15	14-15	3	8.6	8.6	1000.0	0.50
16	15-16	0	0	0	00	0
17	16-17	0	0	0	00	0

VII

Changes in enzyme activities

during development

Stage of development	24 hour Egg	96 hour Egg	L_1	L_2	L_3	L_4	L_5	PP	P
Acid phosphatase activity (As µg Pi released per 30 min./ individual	0.100 ±0.012	0.150 ±0.020	1.00 ±0.03	1.40 ±0.17	2.32 ±0.30	3.23 ±0.30	3.36 ±0.5	2.51 ±0.2	2.30 ±0.20
Alkaline phosphatase activity(as µg Pi released/ 30 min/indidi-dual)	0.080 ±0.007	0.142 ±0.017	0.050 ±0.005	0.162 ±0.012	0.407 ±0.04	0.657 ±0.05	0.860 ±0.04	0.340 ±0.02	0.230 ±0.01
Ratio of Acid/ alkaline phosphatase activity	1.25	1.1	2.0	8.3	7.0	5.0	4.4	7.4	10.
Trehalase activity (as µg glucose released/30 min/ individual)	0.60 ±0.10	0.64 ±0.10	12.0 ± 2.4	22.2 ±4.0	40.5 ±6.0	50.2 ±4.0	66.7 ±7.1	16.2 ±2.0	11.8 ±0.9

VIII

Changes in enzyme activities during aging in female

Age in Days	1	2	3	4	5	6	7	8	9	10	11	12	13
Acid Phosphatase (as µg Pi released/ 30 min/ insect)	1.902 ±0.05	2.474 ±0.20	3.066 ±0.14	2.078 ±0.03	1.652 ±0.04	1.314 ±0.04	1.594 ±0.05	1.768 ±0.18	1.777 ±0.10	1.907 ±0.04	1.477 ±0.03	1.293 ±0.04	1.022 ±0.044
Alkaline Phosphatase (as µg Pi released/ 30 min/ insect)	0.127 ±0.005	0.138 ±0.006	0.229 ±0.013	0.1648 ±0.0032	0.1375 ±0.013	0.1203 ±0.005	0.130 ±0.013	0.136 ±0.014	0.1727 ±0.019	0.2139 ±0.008	0.1203 ±0.0032	0.1201 ±0.007	0.1124 ±0.0065
Ratio of Acid/ alkaline phosphatase	16.23	13.27	13.26	12.97	13.73	10.63	12.30	13.60	9.69	8.39	11.34	10.93	9.09
Trehalase (as µg glucose released/ 30 min/ insect)	53.3 ±5.3	64.15 ±5.38	91.1 ±3.57	36.6 ±3.33	99.7 ±6.31	101.96 ±6.06	104.07 ±7.44	121.91 ±11.34	137.27 ±4.34	172.70 ±10.70	154.36 ±4.93	106.54 ±4.65	87.2 ±4.55

Changes in enzyme activities during aging in male

Age in days	1	2	3	4	5	6	7	8	9	10	11	12	13
Acid Phosphatase (as µg Pi released/ 30 min/ Insect)	2.772 ±0.04	3.138 ±0.08	3.447 ±0.1	1.315 ±0.05	1.410 ±0.07	1.266 ±0.08	1.497 ±0.05	1.577 ±0.07	1.601 ±0.06	1.721 ±0.05	1.157 ±0.11	0.328 ±0.05	0.628 ±0.05
Alkaline Phosphatase (As µg Pi released/ 30 min/ Insect)	0.108 ±0.003	0.128 ±0.005	0.1534 ±0.0035	0.1097 ±0.0043	0.078 ±0.012	0.066 ±0.013	0.067 ±0.012	0.073 ±0.011	0.1241 ±0.012	0.1366 ±0.013	0.1142 ±0.006	0.0693 ±0.0078	0.0598 ±0.0145
Ratio of Acid/ alkaline phocphatase	25.37	25.42	22.61	16.48	17.76	20.0	21.37	21.55	12.17	9.95	11.93	11.50	10.5
Trehalase (as ug glucose released/ 30 min/ Insect	42.62 ±4.10	45.43 ±2.27	45.54 ±3.74	46.15 ±7.91	55.47 ±3.35	64.53 ±1.41	69.93 ±5.98	79.04 ±10.95	83.51 ±5.05	106.77 ±13.0	94.53 ±5.63	49.37 ±2.56	39.2 ±9.0

Changes in DNA, RNA and Protein during development

Stages of development	24 hr Embryo	96 hr Embryo	L_1	L_2	L_3	L_4	L_5	PP	P
µg DNA/gm. wet weight	3102 ±201	3320 ±170	3174 ±565	3030 ±321	2531 ±203	2190 ±350	2480 ±216	2670 ±105	3390 ±30
µg DNA/ Individual	0.093 ±0.06	0.096 ±0.05	2.3 ±0.4	5.9 ±0.6	12.1 ±1.0	17.5 ±2.3	24.7 ±2.0	21.0 ±3.0	23.5 ±2.0
µg RNA/gm. wet weight	28730 ±2415	36520 ±210	30030 ±300	20300 ±2130	13600 ±2000	14300 ±1500	13330 ±1670	12300 ±1210	13230 ±500
µg RNA/ individual	0.35 ±0.07	1.10 ±0.06	13.30 ±1.80	39.01 ±4.0	90.0 ±3.0	103.0 ±12.0	138.0 ±15.0	97.0 ±10.0	92.0 ±3.0
Mg.Protein/gm wet weight	31.15 ±1.10	32.90 ±1.20	32.13 ±2.47	49.9 ±4.9	67.70 ±6.54	82.5 ±4.15	112.5 ±11.7	139.0 ±4.0	146.7 ±5.0
µg Protein/ individual	0.92 ±0.03	0.99 ±0.03	13.5 ±1.5	96.0 ±9.0	326.0 ±32.0	630.0 ±33.0	1120.0 ±120.0	1090.0 ±30.0	1020.0 ±40.0

Changes in DNA, RNA and Protein during aging in female

Age in days	1	2	3	4	5	6	7	8	9	10	11	12	13
µg DNA/gm wet weight	2223	2290	2390	2440	2590	2690	2700	2690	2850	2920	3003	3096	3130
µg DNA/ Incect	12.70 ±1.39	13.35 ±1.83	13.43 ±2.19	12.60 ±1.06	12.83 ±0.51	12.16 ±1.34	11.79 ±1.29	11.52 ±1.00	12.04 ±0.61	11.73 ±0.73	11.76 ±0.02	11.79 ±0.24	11.95 ±0.35
µg RNA/gm wet weight	3338	3590	3985	3950	4160	4350	4295	4400	4250	4130	4270	4410	4530
µg RNA/ Insect	19.11 ±0.37	19.76 ±2.41	22.58 ±3.67	20.00 ±1.13	20.58 ±2.36	19.27 ±3.69	19.73 ±0.17	18.71 ±1.00	17.93 ±1.32	16.84 ±0.86	16.72 ±1.39	16.83 ±1.35	16.98 ±0.50
Mg.Protein/ gm wet weight	166.70	166.13	170.34	175.39	174.65	176.40	179.62	182.58	176.34	182.29	189.90	191.03	193.70
Mg.Protein/ Insect	0.952 ±0.108	0.966 ±0.088	0.953 ±0.076	0.904 ±0.067	0.863 ±0.100	0.788 ±0.036	0.782 ±0.109	0.779 ±0.041	0.751 ±0.071	0.732 ±0.089	0.744 ±0.088	0.728 ±0.052	0.712 ±0.040

Changes in DNA, RNA and Protein during aging in males

Age in day	1	2	3	4	5	6	7	8	9	10	11	12	13
µg DNA/gm wet weight	2221	2305	2937	2983	3113	3112	3260	3267	3575	3609	3777	3793	4025
µg DNA/ insect	12.4 ±0.20	11.32 ±1.02	14.0 ±1.78	13.33 ±0.52	11.46 ±0.91	11.47 ±0.73	11.37 ±1.10	10.30 ±0.67	11.86 ±0.33	11.30 ±0.43	11.90 ±0.42	10.96 ±0.22	11.50 ±0.40
µg RNA/gm. wet weight	3851	3753	3975	4204	4264	4667	4779	4810	4739	4909	5093	5467	5500
µg RNA/ insect	21.71 ±2.17	19.31 ±1.38	19.11 ±1.60	18.85 ±1.28	16.27 ±2.21	17.25 ±1.97	16.70 ±0.33	16.29 ±0.44	15.31 ±0.95	15.63 ±0.24	15.99 ±0.40	15.30 ±0.36	16.00 ±0.60
Mg.Protein/ gm wet weight	156.05	163.60	163.14	173.99	135.57	192.35	193.33	199.53	207.79	216.39	220.95	223.09	223.5
Mg Protein/ insect	0.875 ±0.072	0.844 ±0.073	0.772 ±0.040	0.780 ±0.043	0.703 ±0.076	0.713 ±0.055	0.679 ±0.043	0.663 ±0.030	0.639 ±0.033	0.639 ±0.024	0.696 ±0.006	0.679 ±0.010	0.650 ±0.021

Ratio of RNA, DNA, Protein and Dry weight during development

Stages of development	24 hr Egg	96 hr Egg	L_1	L_2	L_3	L_4	L_5	Pf	P
Ratio of RNA/DNA	9.1	11.5	7.8	6.6	7.5	6.2	5.6	4.6	4.0
Ratio of Protein/DNA	0.91	1.02	7.6	16.30	27.0	33.9	45.3	51.9	43.4
Ratio of Protein/RNA	1.10	0.90	1.03	2.5	3.6	5.3	8.1	11.2	11.1
Dry weight/ DNA	182.1	172.0	167.3	173.6	205.1	231.1	202.4	244.0	239.0
Dry weight/RNA	19.7	15.5	17.7	25.9	27.9	35.4	36.0	41.6	42.2
Dry weight/Protein	18.2	17.1	16.6	11.0	7.1	6.1	4.4	3.7	4.0

Ratio of DNA, RNA, Protein and dry weight during aging in females

Age in days	1	2	3	4	5	6	7	8	9	10	11	12	13
Ratio of RNA/DNA	1.50	1.48	1.70	1.60	1.60	1.58	1.67	1.63	1.50	1.44	1.42	1.41	1.40
Ratio of Protein/RNA	25.0	24.5	19.0	22.6	21.0	20.5	20.0	20.7	21.0	22.0	22.1	21.5	22.0
Ratio of Protein/DNA	38.0	36.2	35.5	36.0	37.8	32.4	31.1	33.7	31.3	31.3	31.6	31.0	30.5
Ratio of Dry weight/ DNA	207.7	202.0	197.4	190.5	180.0	163.4	169.5	170.0	160.4	163.2	153.4	144.0	143.4
Ratio of Dry weight/ RNA	133.1	136.7	115.4	120.0	110.0	104.4	103.6	104.2	107.5	113.6	108.6	101.2	102.6
Ratio of Dry weight/ Protein	5.54	5.62	5.52	5.30	5.22	5.12	5.02	5.12	5.20	4.58	4.38	4.66	4.76

Ratios of DNA, RNA, Protein and dry weight during aging in males

Age in days	1	2	3	4	5	6	7	8	9	10	11	12	13
Ratio of RNA/DNA	1.75	1.64	1.37	1.40	1.42	1.50	1.47	1.51	1.33	1.33	1.35	1.35	1.48
Ratio of Protein/DNA	35.3	30.75	27.6	29.3	30.75	31.25	30.0	30.8	29.2	30.5	29.25	31.15	31.0
Ratio of Protein/RNA	20.0	21.9	20.2	20.7	21.7	20.75	20.5	20.5	21.8	22.2	22.75	21.5	21.0
Ratio of Dry weight/ DNA	194.6	190.2	161.1	155.6	153.8	153.8	145.4	145.5	132.8	133.0	124.0	123.8	122.0
Ratio of Dry weight/ RNA	111.2	116.2	118.1	110.2	109.5	102.8	99.2	96.5	99.1	96.3	92.1	86.1	82.0
Ratio of Dry weight/ Protein	5.52	5.08	5.84	5.32	5.08	5.0	4.86	4.74	4.60	4.40	4.24	4.00	4.00

Changes in lipids, sugars and trehalose during development

Stages of development	24 hr Egg	96 hr Egg	L_1	L_2	L_3	L_4	L_5	PP	P
Percent lipid/ wet weight	17.4 ±0.5	17.0 ±0.3	17.5 ±1.0	19.4 ±0.7	21.0 ±0.3	24.7 ±1.2	27.3 ±0.9	30.2 ±1.6	26.0 ±2.1
Percent lipid/ Dry weight	35.5 ±1.2	33.0 ±1.5	29.3 ±2.2	36.5 ±1.6	38.3 ±0.9	43.3 ±2.4	55.5 ±1.9	59.0 ±2.9	50.4 ±4.20
Mg.trehalose/gm. wet weight	15.24 ±1.0	17.10 ±1.7	16.32 ±2.1	15.93 ±1.32	12.50 ±2.95	10.72 ±1.1	10.5 ±0.3	7.93 ±2.0	7.35 ±1.0
µg trehalose/ Individual	0.45 ±0.03	0.50 ±0.05	9.8 ±1.2	31.1 ±3.0	61.05 ±15.0	90.20 ±9.0	105.0 ±9.0	62.0 ±9.0	55.0 ±7.0
µg. total sugar/ gm. wet weight	21930 ±3000	23750 ±2200	23100 ±3500	22500 ±1200	20400 ±1600	18520 ±2700	17300 ±1300	17230 ±1970	15750 ±2000
µg total sugar/ individual	0.65 ±0.09	0.69 ±0.06	14.40 ±2.1	44.0 ±2.3	99.0 ±8.0	149.0 ±22.0	172.0 ±13.0	135.0 ±15.0	110.0 ±14.0
Trehalose percent of total sugar	70.0	72.4	70.0	70.5	61.6	60.4	61.0	45.9	50.0
Trehalose percent of wet weight	1.54	1.73	1.70	1.61	1.30	1.13	1.10	0.80	0.80
Total sugar as percent of wet weight	2.23	2.35	2.42	2.28	2.06	1.86	1,80	1.72	1.60

Changes in Lipids, sugars and trehalose during aging in female

Age in days	1	2	3	4	5	6	7	8	9	10	11	12	13
Percent lipid/wet weight	13.1	10.6	16.5	10.4	14.05	11.2	10.1	9.5	7.0	4.9	7.3	6.4	5.2
Percent lipid/dry weight	41.79	35.17	37.72	30.42	29.64	24.77	22.13	20.92	15.00	10.51	17.11	14.37	13.16
Mg.lipid/ Insect	1.105	0.964	0.923	0.330	0.679	0.509	0.443	0.406	0.300	0.200	0.311	0.243	0.200
Mg.total sugars/gm wet weight	17.906	17.000	16.640	15.440	14.610	14.020	13.340	12.700	12.150	10.920	10.640	9.990	9.713
µg total Sugar/ insect	103 ±11.2	98 ±12.6	93 ±4.0	75.5 ±6.7	72.3 ±5.0	62.6 ±1.95	60.2 ±3.08	54.2 ±4.7	51.9 ±4.30	44.1 ±3.0	41.5 ±2.4	33.0 ±0.7	35.2 ±3.0
Percent Sugar/ wet weight	1.69	1.68	1.66	1.49	1.49	1.38	1.37	1.27	1.22	1.09	1.05	0.99	0.92
Trehalose as Percent/ wet weight	1.28	1.10	0.99	1.00	1.07	1.05	1.10	1.10	1.16	1.02	0.99	0.94	0.93
Trehalose as Percent/ total sugar	76.0 ±7.3	65.7 ±4.5	60.0 ±2.6	67.7 ±1.6	72.0 ±3.1	76.2 ±0.3	80.5 ±3.0	86.3 ±5.4	95.3 ±10.0	93.7 ±5.2	94.7 ±6.1	95.6 ±5.0	95.5 ±3.2

Changes in lipids, sugars and trehalose during aging male

Age in days	1	2	3	4	5	6	7	8	9	10	11	12	13
Percent lipid/ wet weight	15.0	13.7	13.1	12.1	11.5	9.7	9.5	8.3	6.05	4.7	7.4	5.1	3.7
Percent lipid/Dry weight	35.59	31.54	28.60	26.76	24.67	20.22	19.31	17.76	13.14	10.47	15.20	11.17	9.32
Mg.lipid/ Insect	0.360	0.703	0.645	0.537	0.440	0.360	0.323	0.279	0.202	0.150	0.224	0.149	0.105
Mg.total sugar/gm. wet weight	17.355	16.959	14.116	12.161	11.338	11.133	10.393	9.175	8.114	7.241	7.430	7.264	7.109
µg total sugar/ Insect	97.2 ±3.8	87.2 ±3.7	67.9 ±2.4	54.6 ±7.7	43.3 ±5.0	41.4 ±7.2	33.1 ±2.0	30.4 ±2.1	26.9 ±3.1	23.0 ±3.03	22.5 ±1.6	21.0 ±2.0	19.3 ±3.0
Percent sugar/wet weight	1.71	1.69	1.40	1.23	1.13	1.11	1.09	0.91	0.81	0.72	0.72	0.72	0.65
Trehalose as percent/ wet weight	1.22	1.22	0.95	0.84	0.79	0.78	0.83	0.77	0.80	0.66	0.67	0.65	0.65
Trehalose as percent/ total sugar	71.6 ±1.3	72.6 ±6.2	68.1 ±1.9	68.7 ±5.1	69.9 ±1.3	70.0 ±3.7	76.5 ±2.4	84.6 ±5.3	98.7 ±3.5	92.2 ±10.0	93.2 ±6.2	93.9 ±6.2	97.6 ±4.3

Quantitative changes in amino acids during development

(µg/individual)

S. No.	Name of Amino Acid	L_1	L_2	L_3	L_4	L_5	PP	P	Adult Male (24 hr)	Adult female (24 hr)
1	Alanine	0.144	0.476	0.600	0.665	1.200	1.734	1.543	1.457	2.000
2	Arginine	0.302	1.190	1.060	0.900	1.985	1.590	2.000	0.600	0.572
3	Aspartic Acid	0.381	1.082	1.525	1.933	2.145	2.214	2.000	0.904	1.113
4	Cystine	-	-	0.561	0.344	1.409	0.381	0.500	0.400	0.563
5	Glutamic Acid	0.435	1.680	2.250	3.293	3.293	2.667	4.350	5.100	2.360
6	Glutamine	0.200	1.260	1.360	1.575	1.661	2.380	2.470	0.390	1.332
7	Histidine	0.550	0.800	0.970	0.999	1.213	1.000	1.460	0.630	0.800
8	Leucine	0.057	0.099	0.137	0.350	0.525	0.579	0.666	0.225	0.435
9	Lysine	0.083	0.232	0.750	0.760	1.425	1.553	2.000	0.395	0.417
10	Methionine	-	1.090	0.250	0.300	0.475	0.526	0.825	0.400	0.666
11	Phenylalanine	-	-	-	-	0.570	0.410	0.513	0.332	0.407
12	Proline	0.200	0.118	0.145	0.168	0.187	0.092	0.070	0.213	0.238
13	Threonine	0.040	0.132	0.210	0.510	0.475	0.430	0.555	0.163	0.444
14	Tyrosine	0.167	0.556	0.830	0.999	1.206	1.472	1.806	1.670	0.810
15	Unknown	0.013	0.080	0.097	0.174	0.430	0.100	0.220	0.070	0.121
16	Unknown	-	-	-	-	-	0.160	0.150	-	-
17	Valine	0.090	0.303	0.392	0.632	0.900	0.866	1.134	0.470	0.646
	Total	2.662 ±0.201	9.098 ±1.402	11.137 ±1.210	14.202 ±1.700	19.104 ±2.010	18.206 ±1.937	22.762 ±2.367	13.424 ±1.603	13.479 ±1.434

Quantitative changes in amino acids during aging (µg/insect) in males

S. No.	Name of Amino Acid	1 day	2 day	3 day	4 day	6 day	8 day	10 day	12 day
1	Alanine	1.457	1.130	1.053	1.153	0.762	0.670	0.660	0.590
2	Arginine	0.600	0.533	0.315	0.267	0.333	0.333	0.600	0.710
3	Aspartic Acid	0.904	2.235	1.390	1.200	1.130	1.222	1.200	1.190
4	Cystine	0.400	0.235	0.167	0.200	0.254	0.310	0.600	0.715
5	Glutamic acid	5.100	6.140	5.320	3.200	2.700	2.000	1.263	0.829
6	Glutamine	0.390	0.666	1.030	0.923	0.910	1.000	0.793	0.572
7	Histidine	0.630	0.660	0.730	0.800	0.330	0.760	0.347	0.372
8	Leucine	0.225	0.094	0.213	0.450	0.275	0.225	0.184	0.169
9	Lysine	0.395	0.522	0.533	0.333	0.487	0.660	0.852	0.915
10	Methionine	0.400	0.600	0.333	0.132	0.236	0.400	0.193	0.165
11	Phe.Alanine	0.332	0.900	0.333	0.125	0.250	0.250	0.364	0.410
12	Proline	0.213	0.222	0.200	0.167	0.120	0.131	0.086	0.074
13	Threonine	0.163	0.176	0.277	0.200	0.133	0.200	0.133	0.110
14	Tyrosine	1.670	0.720	0.660	0.444	0.330	0.500	0.306	0.976
15	Unknown	0.070	0.127	0.143	0.033	0.058	0.086	0.095	0.101
16	Valine	0.470	0.755	0.693	0.378	0.286	0.237	0.444	0.292
	Total	13.424 ±1.603	15.765 ±1.782	13.395 ±1.013	10.010 ±0.321	8.744 ±0.912	8.934 ±0.634	9.125 ±1.032	8.690 ±0.512

Quantitative changes in amino acids (µg/insect) during aging in females

S. No.	Name of Amino Acid	1 day	2 day	3 day	4 day	6 day	8 day	10 day	12 day
1	Alanine	2.000	1.230	0.810	1.301	1.140	0.998	0.993	0.686
2	Arginine	0.572	0.525	0.330	0.300	0.280	0.240	0.549	0.429
3	Aspartic acid	1.118	1.200	1.480	1.524	2.380	1.571	1.333	1.110
4	Cystine	0.563	0.220	0.150	0.333	0.385	0.400	0.503	0.310
5	Glu. acid	2.860	4.000	4.610	4.490	2.830	2.000	1.230	0.811
6	Glutamine	1.332	0.620	1.170	1.221	1.273	1.260	1.666	1.158
7	Histidine	0.800	0.918	1.082	0.600	1.000	1.066	1.270	0.635
8	Leucine	0.435	0.156	0.240	0.540	0.625	0.250	0.313	0.338
9	Lysine	0.417	0.431	0.670	0.660	0.970	0.950	1.200	0.957
10	Methionine	0.666	1.249	0.333	0.325	0.300	0.263	0.205	0.222
11	Phe. alanine	0.407	0.450	0.188	0.051	0.256	0.400	0.461	0.370
12	Proline	0.238	0.222	0.210	0.184	0.238	0.177	0.122	0.082
13	Threonine	0.444	0.277	0.237	0.090	0.198	0.193	0.275	0.182
14	Tyrosine	0.810	0.750	0.333	0.300	0.439	0.503	0.640	0.555
15	Unknown	0.121	0.140	0.174	0.160	0.116	0.105	0.086	0.032
16	Valine	0.646	1.000	1.354	0.394	0.858	0.680	0.520	0.472
	Total	13.479	13.388	13.371	12.483	13.338	11.056	11.371	8.349
		±1.434	±1.219	±1.279	±0.987	±1.536	±1.054	±0.918	±0.627