## CHAPTER XXI

# OBSERVATIONS

OBSERVATIONS :

Amino Acids :

Qualitative analyses of free amino acids from the different regions of the gut (foregut, midgut along with hepatic eaeca and hindgut) the fat body and the haemolymph of the insects understudy were made by employing unidimensional ascending chromatography. The results obtained from the chromatograms and after comparing with the Rf values of the known standard aminoacids (run along with the samples) it is evident that the pattern of free amino acids showed variations in different tissues of both the insects. Analyses of free amino acids were carried out in the normal or untreated and treated cockroaches and grasshoppers.

Periplaneta americana :

Occurrence of free amino acids and their derivatives in different organs/tissues of the normal or untreated cockroaches is presented in the Table-1. The results indicate that free amino acids exhibited variations in their pattern in different tissues understudy . Among the different regions of the alimentary canal i.e. the foregut, midgut and hindgut the number of free amino acids was twelve, thirteen and twelve respectively while that of the fat body and the haemolymph it was twelve and fourteen respectively. Most of the amino acids showed common occurrence in the different organs of the gut as well as in the fat body and the haemolymph. The following amino acids i.e. alanine, aspartate, glutamine, glycine, hydroxyproline, isoleucine, lencine lysine, methionine, phenylalanine proline, serine, threonine and valine were appeared in the foregut. Of these, methionine threonine and valine did not appear in the midgut, but instead of these the other amino acids i.e.histidine, ornithine, tryptophan and

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TABLE NO. 1

Occurrence of free amino acids and their dervatives in different organs and tissues of P. americana.

| Amino acids | Foregut | Midgut | Hindgut | Fat body | Haemolymph |
|---|---|---|---|---|---|
| Alanine | + | + | + | + | + |
| Arginine | - | + | - | + | - |
| Aspartate | + | + | - | + | - |
| Glutamine | - | + | - | - | + |
| Glutamate | - | - | + | + | + |
| Glycine | + | + | - | - | + |
| Histidine | - | + | - | - | + |
| Hydroxyproline | + | - | + | + | - |
| Isoleucine | + | - | + | + | + |
| Leucine | + | - | + | - | - |
| Lysine | - | + | - | + | + |
| Methionine | + | - | + | - | + |
| Ornithine | - | + | + | - | + |
| Phenylalanine | + | + | + | + | + |
| Proline | + | + | + | + | + |
| Serine | + | + | - | + | - |
| Threonine | + | - | + | + | + |
| Tryptophan | - | + | + | + | + |
| Tyrosine | - | + | + | - | + |
| Valine | + | - | - | - | - |

tyrosine were appeared. The hindgut though possessed the same number of free amino acids as that of the foregut, its pattern was, however, different. Aspartate, glycine, serine and valine of the foregut were found to be missing in the hindgut. Glutamate, ornithine, tryptophan and tyrosine were found to be new arrivals in this region. Thus, from the table it seems that alanine and proline were common to the three regions of the gut. The other amino acids i.e. aspartate, glycine, and serine were common to the foregut-midgut while ornithine, tryptophan and tyrosine to the midgut-hindgut.

Of the twelve amino acids appeared in the fat body and fourteen in the haemolymph, alanine, glutamate isoleucine lysine protine, threonine and tryptophan were of common appearance in these tissues. Arginine, aspartate, hydroxyproline and serine of the fat body did not appear in the haemolymph whereas glutamine, glycine, histidine, methionine and tyrosine of the haemolymph were found to be missing in the fat body.

A comparison of free amino acid pattern (Table 1) of the three regions of the alimentary canal, fat body and the haemolymph indicates that alanine, and proline were found in all these organs and tissues. Except valine (foregut), the other aminoacids exhibited comparison in some of the organs/tissues.

The pattern of free amino acids in the different parts of the alimentary canal, fat body and the haemolymph of the insects exposed to test oil i.e. treated insects also exhibited organ and tissuewise variations (Table 2). From the table it is evident that nine amino acids appeared in the foregut, eleven in the midgut and fourteen in the hindgut. The number of amino acids appeared in the fat body and the haemolymph was twelve and ten respectively. Of the several

## TABLE NO. 2

Amino acid pattern of *P.americana* after the treatment with neem oil.

| Amino acids | Foregut | Midgut | Hindgut | Fat body | Haemolymph |
|---|---|---|---|---|---|
| Alanine | - | + | + | + | + |
| Arginine | + | - | - | - | + |
| Aspartate | - | + | + | + | - |
| Glutamine | + | - | + | - | - |
| Glutamate | - | + | + | + | + |
| Glycine | - | - | + | - | - |
| Histidine | - | - | - | + | - |
| Hydroxyproline | - | + | + | - | + |
| Isoleucine | - | + | - | + | - |
| Leucine | + | - | + | - | - |
| Lysine | + | + | - | - | + |
| Methionine | - | - | + | + | + |
| Ornithine | - | - | - | + | - |
| Phenylalanine | + | - | + | - | - |
| Proline | + | + | + | + | + |
| Serine | - | - | + | + | + |
| Threonine | + | + | + | - | - |
| Tryptophan | - | + | + | + | + |
| Tyrosine | + | + | - | + | + |
| Valine | + | + | + | + | - |

amino acids appeared in the alimentary tract, only proline, threonine, and valine were found to be common to the foregut, midgut and hindgut. Amino acids lysine & tyrosine appeared in the foregut-midgut, aspartate, glutamate, hydroxyproline and tryptophan in the midgut-hindgut and glutamine, leucine and phenylalanine in the foregut-hindgut. Arginine of the foregut, glycine, methionine and serine of the hindgut and isoleucine of the midgut did not appear in either of the organs.

Of the twelve and ten amino acids appeared in the fat body and the haemolymph respectively, alanine, methionine, proline, serine, tryptophan and tyrosine were appeared in both the tissues. Table 2 shows that proline appeared in all the regions of alimentary canal, fat body and haemolymph. Most of the other amino acids were common to most of these orans and tissues.

Organ and tissuewise comparison of free amino acids from both the untreated or treated cockroaches is presented in the Table 3. Majority of the amino acids were found to be common to most of the organs/tissues. However, variations in the number of amino acids in the corresponding organs/tissues were evident. In some of the organs and tissues the number of amino acids was decreased or increased. Among the three regions of the alimentary canal of untreated cockroaches the maximum (thirteen) number of amino acids was found in the midgut but after the treatment with neem oil, the number was found to be increased in the hindgut. The haemolymph of untreated had fourteen amino acids, however this number was reduced to ten in the haemolymph of treated insects.

## TABLE NO.3

Organ/tissuewise comparison of free amino acids in normal and treated *P. americana*

| Amino acids | Foregut | | Midgut | | Hindgut | | Fatbody | | Haemolymph | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Normal | Treated | Normal | Treated | Normal | Treated | Normal | Treated | Normal | Treated |
| Alanine | + | - | + | + | + | + | + | + | + | + |
| Arginine | - | + | + | - | - | - | + | - | - | + |
| Aspartate | + | - | + | + | - | + | + | + | - | - |
| Glutamine | - | + | + | - | - | + | - | - | + | - |
| Glutamate | - | - | - | + | + | + | + | + | + | + |
| Glycine | + | - | + | - | - | + | - | - | + | - |
| Histidine | - | - | + | - | - | - | - | + | + | - |
| Hydroxyproline | + | - | - | + | + | + | + | - | - | + |
| Isoleucine | + | - | - | + | + | - | + | + | + | - |
| Leucine | + | + | - | - | + | + | - | - | - | - |
| Lysine | - | + | + | + | - | - | + | - | + | + |
| Methionine | + | - | - | - | + | + | - | + | + | + |
| Ornithine | - | - | + | - | + | - | - | + | + | - |
| Phenylalanine | + | + | + | - | + | + | + | - | + | - |
| Proline | + | + | + | + | + | + | + | + | + | + |
| Serine | + | - | + | - | - | + | + | + | - | + |
| Threonine | + | + | - | + | + | + | + | - | + | - |
| Tryptophan | - | - | + | + | + | + | + | + | + | + |
| Tyrosine | - | + | + | + | + | - | - | + | + | + |
| Valine | + | + | - | + | - | + | - | + | - | - |

Schistocerca gregaria :

Occurrence of free amino acids in different regions of the alimentary tract, fat body and the haemolymph of grasshopper is presented in the Table 4. Similar to the cockroach, variations in the pattern of amino acids also occured in this insect. Among the three regions of the gut, the foregut and midgut possessed the same number of amino acids i.e. twelve each while the hindgut had ten amino acids. Of the several amino acids found in these three regions of the gut, alanine, glutamate & glycine were of common occurrence in them. The three amino acids namely tryptophan, tyrosine and valine were shared by the foregut- midgut, whereas the leucine was shared by the midgut- hindgut. The foregut & the hindgut possessed the amino acids asparatate and phenylalanine in common. Glutamine histidine and ornithine of the foregut did not appear in midgut and hindgut. Similarly, arginine, isoleucine lysine and methionine of the midgut neither appeared in the foregut nor in the hindgut. Hydroxyproline, serine and threonine of the hindgut were not seen in either of the remaining two regions of the gut.

The fat body of the normal or untreated grasshoppers possessed a maximum of fifteen free amino acids. In the haemolymph, however, only ten amino acids were appeared. Of these, eight amino acids namely glutamine, glutamate lysine, methionine, phenylalanine, proline and tryptophan occurred in both tissues. The remaining seven amino acids of the fat body did not appear in the haemolymph whereas the amino acid glycine of the haemolymph was not seen in the fat body.

Insects treated with neem oil exhibited variations in their free amino acids the pattern of the same being presented in the Table 5. The number of amino acids in the three regions of the alimentary tract

TABLE NO. 4

Occurrence of amino acids in different organs and tissues of *Schistocerca gregaria*.

| Amino acids | Foregut | Midgut | Hindgut | fat body | Haemolymph |
|---|---|---|---|---|---|
| Alanine | + | + | + | + | - |
| Arginine | - | + | - | - | - |
| Aspartate | + | - | + | - | - |
| Glutamine | + | - | - | + | + |
| Glutamate | + | + | + | + | + |
| Glycine | + | + | + | - | + |
| Histidine | + | - | - | + | - |
| Hydroxyproline | - | - | + | + | - |
| Isoleucine | - | + | - | - | + |
| Leucine | - | + | + | + | - |
| Lysine | - | + | - | + | + |
| Methionine | - | + | - | + | + |
| Ornithine | + | - | - | + | - |
| Phenylalanine | + | - | + | + | + |
| Proline | + | + | + | + | + |
| Serine | - | - | + | - | - |
| Threonine | - | - | + | + | - |
| Tryptophan | + | + | - | + | + |
| Tyrosine | + | + | - | + | - |
| Valine | + | + | - | + | + |

is found to be comparatively less than those of the normal grasshoppers. The number obtained for the foregut, midgut and the hindgut was eleven, eight and nine respectively. The amino acids of the foregut were alanine , aspartate, glutamine, glycine, isoleucine, lysine, methionine proline, tyrosine and valine. Of these, alanine and proline were appeared in both midgut and hindgut while isoleucine and methionine in the foregut-midgut. Tyrosine was shared by foregut-hindgut. Arginine, leucine and threonine were common to the midgut-hindgut. The remaining amino acids i.e. aspartate, glutamine, lysine and valine of the foregut, tryptophan of the midgut and glutamate of the hindgut did not share any comparison.

The fat body possessed eleven amino acids and the same number of amino acids was found in the haemolymph but the pattern was different (Table 5). Out of eleven, only three amino acids from each of these tissues did not appear in either of them. Arginine, glutamate and glycine of the fat body did not appear in the haemolymph while alanine hydroxyproline and lysine of the haemolymph were not seen in the fat body. The eight common acids were, aspartate, glutamine, leucine, methionine, phenylalanine, proline, serine and tryptophan.

Organ and tissuewise comparison of amino acids from both untreated, treated grasshoppers is presented in Table 6.

Proteins :

Electrophoretic :

Distinct and reproducible fractions of proteins were observed in different parts of the alimentary canal, fat body and the haemolymph of both the insects. Protein fractions have been numbered in Roman letters in the sequence of their occurrence in the gel, beginnning at the cathode end where electrofractionation was initiated. The rate of

## TABLE NO. 5

Amino acids in different organs/tissues of S.gregaria after exposure to neem oil.

| Amino acids | Foregut | Midgut | Hindgut | Fat body | Haemolymph |
|---|---|---|---|---|---|
| Alanine | + | + | + | - | + |
| Arginine | - | + | + | + | - |
| Aspartate | + | - | - | + | + |
| Glutamine | + | - | - | + | + |
| Glutamate | - | - | + | + | - |
| Glycine | + | - | + | + | - |
| Histidine | - | - | + | - | - |
| Hydroxyproline | - | - | - | - | + |
| Isoleucine | + | + | - | - | - |
| Leucine | - | + | + | + | + |
| Lysine | + | - | - | - | + |
| Methionine | + | + | - | + | + |
| Ornithine | - | - | - | - | - |
| Phenylalanine | + | - | - | + | + |
| Proline | + | + | + | + | + |
| Serine | - | - | - | + | + |
| Threonine | - | + | + | - | - |
| Tryptohan | - | + | - | + | + |
| Tyrosine | + | - | + | - | - |
| Valine | + | - | - | - | - |

TABLE NO.6

Free amino acid pattern in different organs/tissues of untreated and treated S.gregaria.

| Amino acids | Foregut | | Midgut | | Hindgut | | Fat body | | Haemolymph | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Normal | Treated | Normal | Treated | Normal | Treated | Normal | Treated | Normal | Treated |
| Alanine | + | + | + | + | + | + | + | - | - | + |
| Arginine | - | - | + | + | - | + | - | + | - | - |
| Asparatate | + | + | - | - | + | - | - | + | - | + |
| Glutamine | + | + | - | - | - | - | + | + | + | + |
| Glutamate | + | - | + | - | + | + | + | + | + | - |
| Glycine | + | + | + | - | + | + | - | + | + | - |
| Histidine | + | - | - | - | - | + | + | - | - | - |
| Hydroxyproline | - | - | - | - | + | - | + | - | - | + |
| Isoleucine | - | + | + | + | - | - | - | - | + | - |
| Leucine | - | - | + | + | + | + | + | + | - | + |
| Lysine | - | + | + | - | - | - | + | - | + | + |
| Methionine | - | + | + | + | - | - | + | + | + | + |
| Ornithine | + | - | - | - | - | - | + | - | - | - |
| Phenylalanine | + | - | - | - | + | - | + | + | + | + |
| Proline | + | + | + | + | + | + | + | + | + | + |
| Serine | - | - | - | - | + | - | - | + | - | + |
| Threonine | - | - | - | + | + | + | + | - | - | - |
| Tryptophan | + | - | + | + | - | - | + | + | + | + |
| Tyrosine | + | + | + | - | - | + | + | - | - | - |
| Valine | + | + | + | - | - | - | + | - | + | - |

mobility of the fractions was measured in centimeters as the distance from the cathodic end of the gel to the leading end i.e. towards the anode. Differences in the size and staining intensity of fractions were observed among the different organs and tissues employed for the study. The relative thickness of the fractions as well as their staining reaction is diagramatically represented in the electrophoregrams. The differences in the staining intensities of the fractions presumably reflecting in the concentrations of the proteins were assessed by visual comparison.

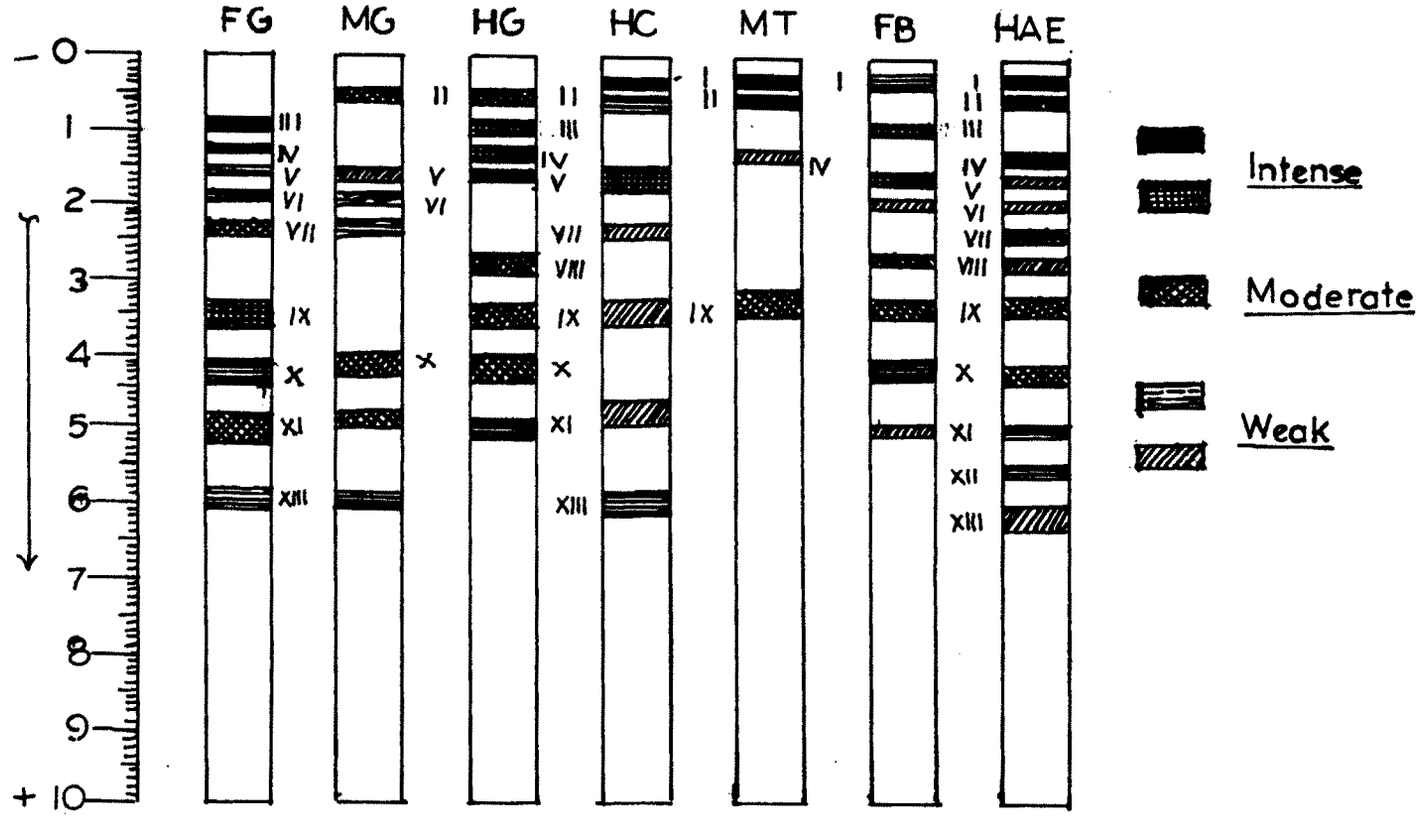
Periplaneta americana :

Protein fractions obtained for each organ/tissue of untreated cockroaches are shown in Fig.1 while the average range of mobility of the fractions is given in the table-7. It is evident from the table that there were variations both in the number as well as in the mobility rates of the fractions obtained from the different parts of the alimenta -ry tract, fat body and the haemolymph. The staining intensity also varied in different fractions. According to the rate of migration, a total of thirteen fractions appeared among the organs/tissues under-study. Both slow and fast migrating fractions (bands) were evident.

Foregut :

Nine fractions were appeared in the foregut. Both slow migrating (III, IV, V, VI, VII) and fast migrating (IX, X, XI, XIII) fractions were evident. There were, however, variations in the size as well as in the staining intensity among both slow and fast migrating fractions. The slow migrating fractions were comparatively thin than those of the anodically migrating ones. The first three fractions III (0.08-0.09), IV (0.11-0.12) and V (0.14-0.16) showed intense staining reaction.

Fig. 1 COCKROACH - NORMAL




FG - Foregut MG - Midgut HG - Hindgut

HC - Hepatic caeca MT - Malpighian tubules

FB - Fat body HAE - Haemolymp

Fraction IX (0.32-0.36) was comparatively less intense. Moderate staining reaction was exhibited by VII (0.22-0.24) and XI (0.48-0.53) fraction. A rather weak staining reaction was seen in the remaining fractions i.e. fraction VI (0.17-0.2) X (0.39-0.44) and XIII (0.59-0.63).

Midgut :

Midgut produced seven fractions. Of these, fraction II (0.05-0.07) was found towards the cathode, fractions V, VI, and VII were slightly fast moving and the remaining fractions i.e. X, XI and XIII were fast migrating ones. The staining intensity of the fractions II, X and XI was moderate. Fraction VI was comparatively intense than these fractions. A very weak staining reaction was observed in the fractions V, VII and XIII. Fractions III, IV and IX of the foregut did not appear in the midgut.

Hindgut :

Protein pattern of the hindgut was more or less similar to that of the foregut with respect to the distribution of the fractions in that it showed two distinct zones. One with slow migrating fractions (II, III, IV, V) and the other with the same number of fractions (VIII, IX, X, XI) but having faster rate of migration. All the fractions of the first zone were comparatively thin than those of the anodical zone. An interesting staining feature was noticed in the fractions of both the zones in that the fraction II at the cathodic end exhibited moderate reaction while the fraction XI at the anodic end was very weak

in its intensity. The remaining fractions, (III, IV, V) and (VIII, IX, X), in each zone showed intense and moderate staining respectively.

Fraction III and IV of the foregut were missing in the midgut but appeared again in the hindgut, with the appearence of fraction VIII(0.25-0.29). However, the anodically migrating fraction XIII of the foregut-midgut was missing in the hindgut.

Hepatic caeca :

Protein pattern was also analysed from the hepatic caeca. Similar to the midgut, the hepatic caeca also produced seven fractions. but they differed in their rate of mobility as well as in their staining feature. Fractions I & II were cathodic & exhibited intense and week staining recation respectively. Fraction III was thick and intense while the remaining fractions (VII, IX, XI, XIII) through broad, were they poorly stained. Fraction I which was absent in the three regions of the alimentary canel, now appeared in this organ. The other fractions i.e. III, IV, VIII & X were disappeared in the hepatic caeca.

Malpighian tubules :

Only four protein fractions appeared in the M. tubules. I, II, IV being sharp with weak staining reaction except the fraction II which showed intense staining. The anodically migrating IX band was thick and exhibited moderate staining.

TABLE NO. 7

Electromobility range of protein fractions from different tissues/organs of normal *P. americana*

| Band No. | Mobility range | Foregut | Midgut | Hindgut | Hepatic Caeca | Malpighian tubules | Fat body | Haemolymph |
|---|---|---|---|---|---|---|---|---|
| I | 0.02 - 0.04 | - | - | - | + | + | + | + |
| II | 0.05 - 0.07 | - | + | + | + | + | - | + |
| III | 0.08 - 0.09 | + | - | + | - | - | + | - |
| IV | 0.11 - 0.12 | + | - | + | - | + | - | + |
| V | 0.14 - 0.16 | + | + | + | + | - | + | + |
| VI | 0.17 - 0.20 | + | + | - | - | - | + | + |
| VII | 0.22 - 0.24 | + | + | - | + | - | - | + |
| VIII | 0.25 - 0.29 | - | - | + | - | - | + | + |
| IX | 0.32 - 0.36 | + | - | + | + | + | + | + |
| X | 0.39 - 0.44 | + | + | + | - | - | + | + |
| XI | 0.48 - 0.53 | + | + | + | + | - | + | + |
| XII | 0.55 - 0.56 | - | - | - | - | - | - | + |
| XIII | 0.59 - 0.63 | + | + | - | + | - | + | - |

The remaining fractions observed in the different regions of the alimentary canal were found to be absent in the M.tubles.

Fat body :

The distributional pattern of proteins in the fat body indicates that it possessed eight fractions. Most of the fractions were comparatively thin except the fraction IX and X. The staining intensity showed variation in different fractions in that fractions I, III, IV were intense, VIII & IX were moderate while VI, X, & XI were weak in their staining reaction. Some of the fractions of the alimentary tract did not appear in the fat body. Fractions II, IV, VII and XIII were absent in this tissue.

Haemolymph :

Among the organs/tissues studied prsently, the haemolymph produced maximum number of protein fractions. Twelve fractions appeared in it. The distributional pattern and the mobility range of different fractions is presented in the Fig.1 and Table 7 respectively. Variation in the staining reaction was also evident. Fractions I, IV stained intensely, VIII, IX and X were moderate while the remaining fractions exhibited weak staining reaction. Fraction III was not seen in the haemolymph.

Thus, the observations made on the distributional pattern and staining intensity of the protein fractions obtained from different parts of the alimentary canal, fat body and

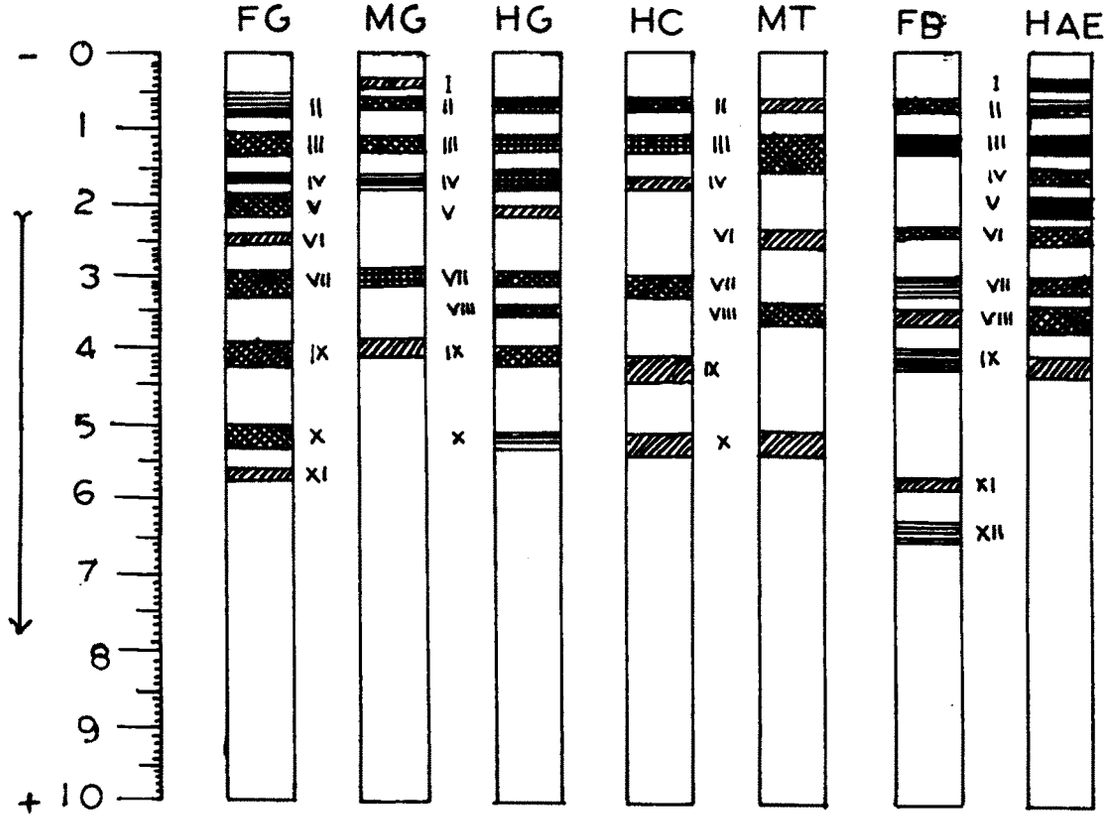
the haemolymph indicate that in general, the anodically migrating fractions were comparatively broad and showed weak staining reaction. Further, most of the fractions exhibited similarities in their mobility range while some of them appeared and disappeared in some organs / tissues understudy.

Cockroches treated with neem oil exhibited an interesting distributional pattern of protein fractions. In general, the thickness of the bands increased in most of the organs and tissues understudy. In a similar way most of the fractions showed an increased staining reaction. The rate of mobility also differed in different organs/tissues. In all, twelve fractions appeared but organ and tissuewise variations were evident. The electrophoregram indicated in Fig. 2, shows the distributional pattern of its fractions while their mobility range is presented in the Table 8.

Foregut :

The foregut of the treated cockroach possessed the same number (nine) of fractions as that of the foregut of untreated ones. Most of the fractions i.e. band II (0.06-0.08), III (0.11-0.12), V (0.18-0.20), VII (0.29-0.33), IX (0.40-0.44) and X (0.51-0.52) were thick while remaining bands IV (0.13-0.15), VI (0.23-0.25) and XI (0.57-0.59) were comparatively thin. Among the thin bands, fractions IV was intense while the remaining two were very weak. Rest of the bands exhibited moderate staining reaction.

Fig. 2 COCKROACH – TREATED




FG – Foregut MG – Midgut HG – Hindgut

HC – Hepatic caeca MT – Malpighian tubules

F B – Fat body HAE – Haemolymph

Midgut :

The midgut produced six fractions of which I (0.03-0.05), II (0.06-0.08), III (0.11-0.12) and IV (0.13-0.15) were slow migrating while the band VII (0.29-0.33) and IX (0.44-0.46) were comparatively fast moving. Intense staining was observed in band VII, moderate in II & III while weak in the remaining ones. Band I was not seen in the foregut whereas fraction V, VI & anodically migrating X & XI of the foregut were missing in the midgut.

Hindgut :

The number and distributional pattern of the fractions was more or less the same as that of the hindgut of untreated insects. The fast migrating bands (V & X) of the cathodic & anodic zones stained poorly while fractions II, III, IV and VII, VIII, IX of the respective zones exhibited intense and moderate staining reactions. Bands XI (foregut) and I (midgut) did not appear in the hindgut but the others were retained with the appearance of a new additional band(VIII).

Hepatic caeca :

It produced six bands of which three were slow migrating and thin and three with fast migrating but slightly thick. Staining patterns differed in them in that II & VII showed moderate reaction, III was intense while IV, IX & X were faint. Bands V and VIII of the hindgut did not appear in the hepatic caeca.

TABLE NO.8

Distributional pattern of protein fractions of treated *P.americana*.

| Band No. | Mobility range | Foregut | Midgut | Hindgut | Hepatic caeca | Malpighian tubules | Fat body | Haemolymph |
|---|---|---|---|---|---|---|---|---|
| I | 0.03 - 0.05 | - | + | - | - | - | - | + |
| II | 0.06 - 0.08 | + | + | + | + | + | + | + |
| III | 0.11 - 0.12 | + | + | + | + | + | + | + |
| IV | 0.13 - 0.15 | + | + | + | + | - | - | + |
| V | 0.18 - 0.20 | + | - | + | - | - | - | + |
| VI | 0.23 - 0.25 | + | - | - | - | + | + | + |
| VII | 0.29 - 0.33 | + | + | + | + | - | + | + |
| VIII | 0.34 - 0.37 | - | - | + | - | + | + | + |
| IX | 0.4 - 0.44 | + | + | + | + | - | + | + |
| X | 0.51 - 0.52 | + | - | + | + | + | - | - |
| XI | 0.57 - 0.59 | - | - | - | - | - | + | - |
| XII | 0.63 - 0.65 | - | - | - | - | - | + | - |

Malpighian tubules :

Of the five factions appeared, fraction III was very thick while the others were comparatively less thick. It has moderate staining reaction similar to that of fraction VII Remaining fractions (II, VI, V) were poorly stained.

Fat body :

Fat body produced eight fractions buttheir distributional pattern indicates that they appeared in three zones: the slow migrating zone with two fractions (II & III) middle zone with four fractions (VI, VII, VIII & IX) and an anodically migrating zone having XI & XII fractions (Fig.2). Except fractions III (intense), II & VI (moderate) the remaining bands showed weak staining reaction.

Haemolymph :

Nine fractions appeared in the treated insects. The size of the fractions increased gradually in fast migrating bands. Fractions I, III & V were intense while fractions IV, VI, VII & VIII were moderately stained. A weak staining reaction was exhibited by II & IX fractions.

The distributional pattern of the protein fractions of the organs/tissues studied from the treated insects (Table 8) indicates that most of the fractions exhibited comparison while others did not. Fraction I, for example, appeared in the midgut.

8249
A.

and haemolymph only whereas XI in the foregut and fat body. Fraction XII was found only in the fat body. Some fractions found in one organ were found to be missing in the others.

Organ/tissuewise comparison of the protein fractions of both untreated & treated insects is presented in the figures 3 a to fig. 3 e and in fig. 4 a and 4 b. In case of foregut (fig. 3 a) most of the fractions exhibited similarity while others differed in their mobility range. Fractions IV-III, V-IV, VI-V, VII-VI and IX-X of untreated and treated foregut showed more or less the same range of mobility while fractions III, IX, X and XIII of untreated and fraction II, VII, IX and XI of the treated foregut did not show any comparison. The distributional pattern of fractions varied greatly in the midgut of untreated and treated insects. Only few bands (II-I, V-IV, X-IX) showed comparison. Fractions VI, VII, XI and XII of the normal midgut did not appear in the treated one. In the hindgut (Fig. 3 c) most of the fractions, except V of treated, exhibited similarities in their mobility range. In the normal hepatic cacea (Fig.3 d) there were seven fractions but in treated six bands were seen, but only II & V of the former exhibited comparison with the II & IV of the latter. The distributional pattern in the malpighian tubules, (Fig.3 e) differed greatly. There were variations in the size of the fractions as well as in the staining intensity. Fractions II-II, IV-III & IX-VIII exhibited comparison.

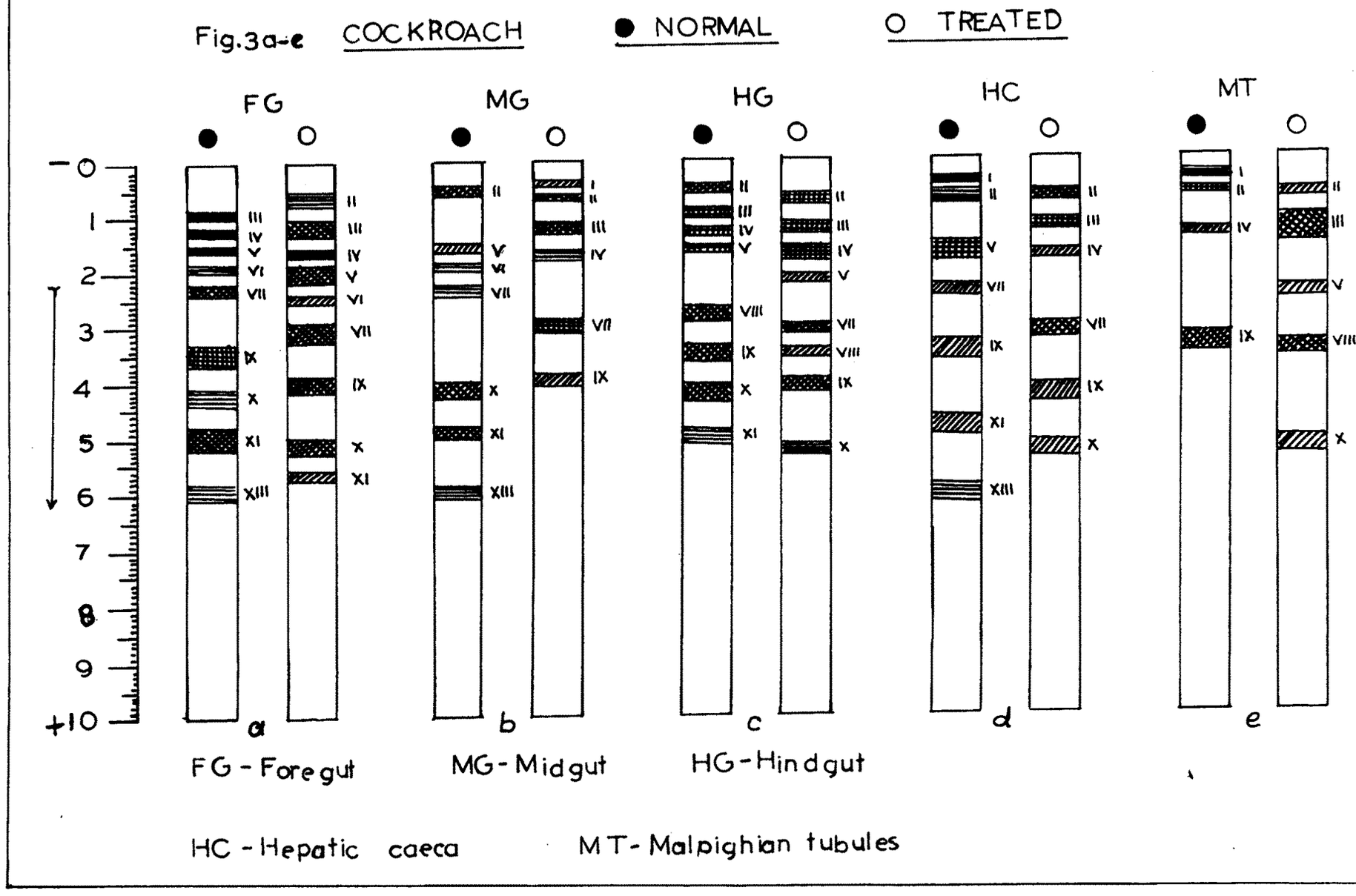


Fig.3a-e COCKROACH
● NORMAL
○ TREATED
FG
MG
HG
HC
MT
a
b
c
d
e
FG - Foregut
MG - Midgut
HG - Hindgut
HC - Hepatic caeca
MT - Malpighian tubules

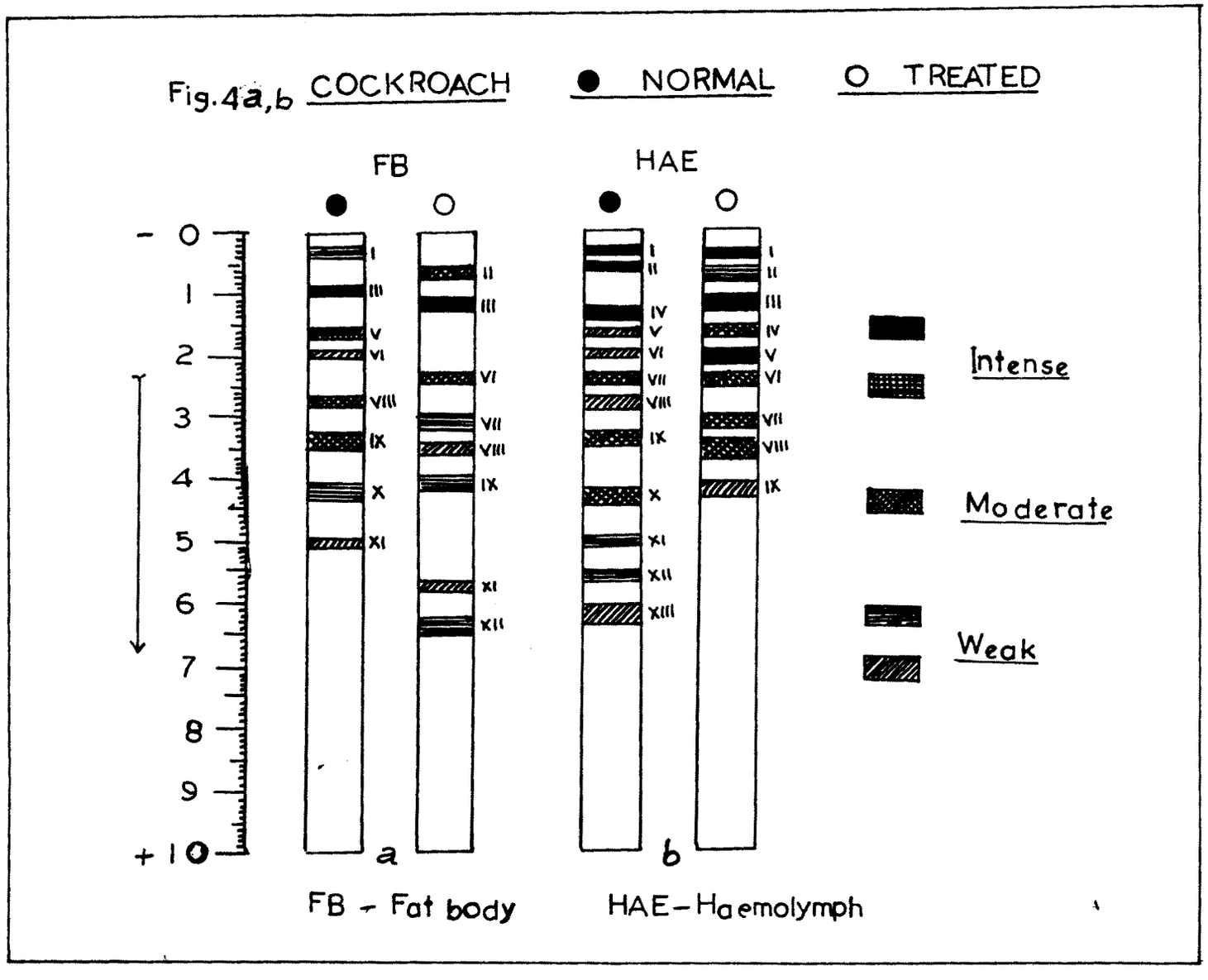


Fig. 4a,b COCKROACH
NORMAL
TREATED
FB
HAE
- 0
1
2
3
4
5
6
7
8
9
+ 10
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
a
b
Intense
Moderate
Weak
FB - Fat body
HAE - Haemolymph

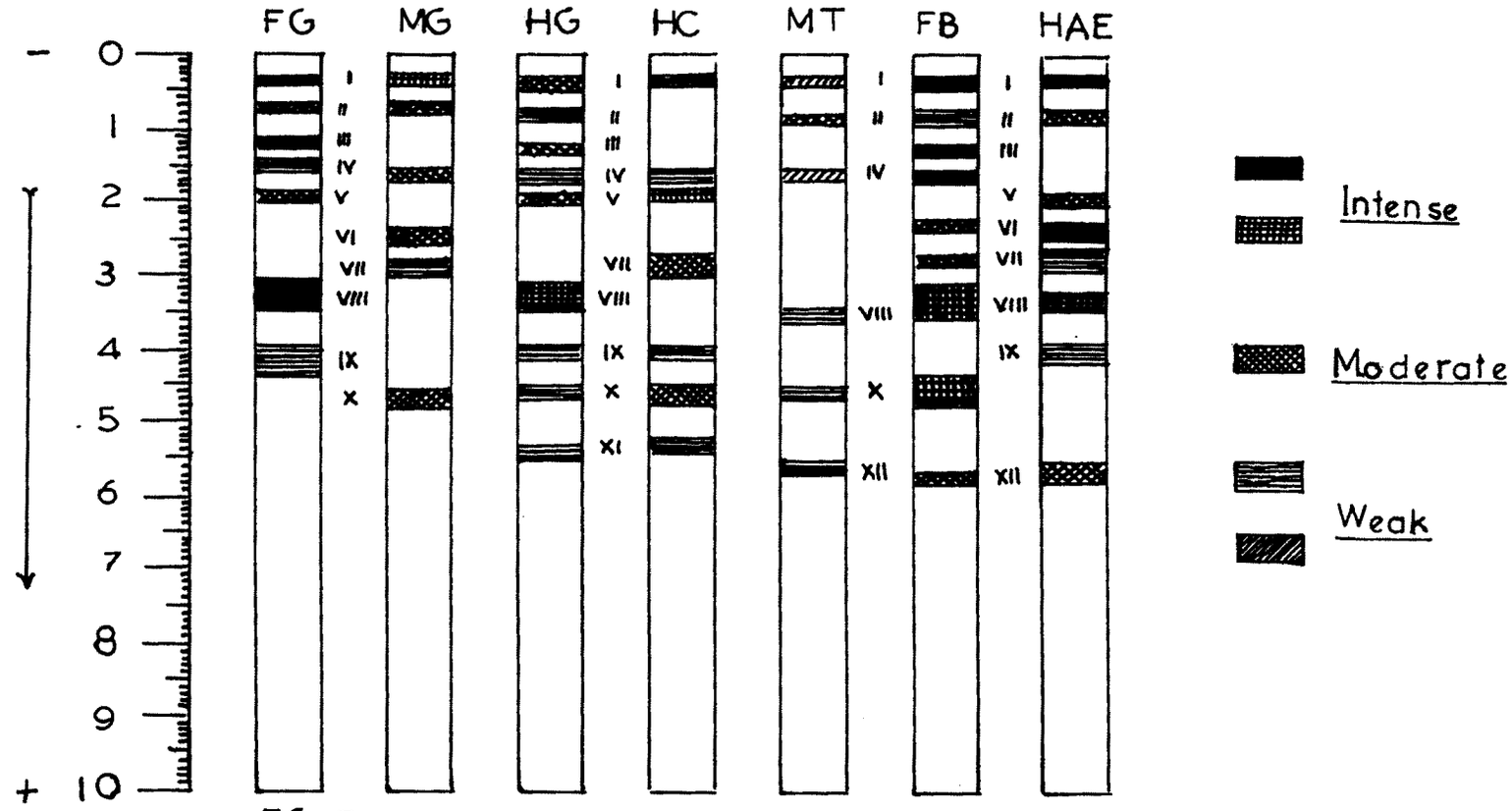
The number of fractions remained the same (Fig. 4 a) in the untreated and treated fat body but exhibited difference in their mobility range. Most of the fractions did not exhibited any comparison in their staining intensity. Fractions I, III, V, VI, VIII, X and XI of the normal did not have their counterparts in the treated fat body. In case of the haemolymph, the number of fractions reduced to nine in the treated insects. The anodically migrating fraction of the normal haemolymph did not appear in the treated one (Fig. 4 b). However, the staining intensity increased in most of fractions in the treated insects.

Schistocerca gregaria :

Protein fractions obtained from the different regions of the alimentary canal, fat body and the hacmolymph of un - treated grasshoppers were also distinct and reproducible. Variations in the number of fractions as well as in the staining intensity were also evident. Distributional pattern of fractions is diagramatically represented in fig. 5 while their range of mobility is given in table 9. A total of twelve fractions appeared among the different organs/tissues understudy.

The number of fractions appeared in each region of the alimentary tract varied. The foregut produced seven bands, the midgut had six while in the hindgut nine bands were appeared.

Fig 5 GRASSHOPPER – NORMAL




FG Fore gut MG – Midgut HG – Hindgut

HC – Hepatic caeca MT – Malpighian tubules

FB – Fat body HAE – Haemolymph

The hepatic caeca and Malpighian tubules which were also employed for the study, showed distinct protein pattern in that they produced seven and six fractions respectively.

Most of the fractions from the three regions of the gut exhibited similarity in their rate of migration. However, they differed in their staining intensity. Both slow and fast migrating bands were evident. Fraction I (0.03-0.05), II(0.07-0.09) and IV (0.14-0.16) were found to be common to the foregut, midgut and hindgut, while fraction III (0.12-0.13), V(0.17-0.19), VIII (0.32-0.36) and IX (0.39-0.42) did not appear in the midgut but were common to both the foregut and hindgut. Fraction VI (0.22-0.25) and VII (0.28-0.3) of the midgut neither appeared in the foregut nor in the hindgut. The anodically migrating band XI(0.53-0.56) of the hindgut was not found in the foregut and midgut. In general the anodically migrating fractions were thick especially the fractions VIII(foregut-hindgut) and IX(foregut). Fractions I, III (foregut) and VIII(foregut-hindgut) showed intense staining reaction while II (foregut-midgut), III (hindgut), IV, VI & X (midgut) and fractions V (foregut-hindgut) had moderate staining. The remaining fractions were weak in their staining intensity.

Most of the fractions from the hepatic caeca and Malpighian tubules did not show comparison in their range of mobility. Only fractions I, IV & X showed identity. Fractions V, VII, IX & XI of the hepatic caeca did not appear in the M.tubules while II, VIII & XII of the M.tubules were not seen in the hepatic caeca. All the bands of M.tubules were thin and exhibited weaker staining reaction except fraction II (moderately stained). The staining reaction of the fractions from hepatic caeca is shown in the fig.5

TABLE NO.9

Mobility range of protein fractions from different organs/tissues of untreated S.gregaria

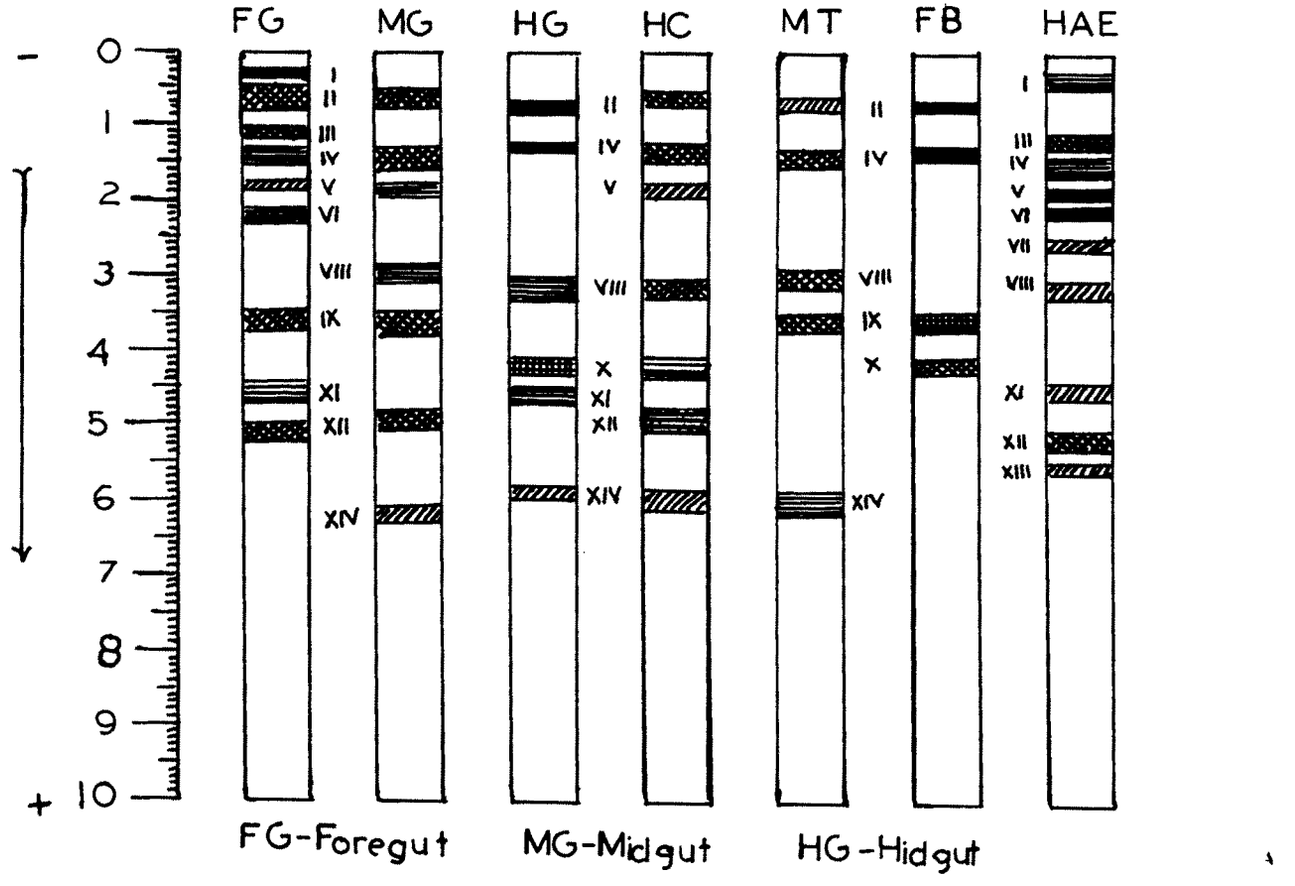
| Band No. | Mobility range | Foregut | Midgut | Hindgut | Hepatic caeca | Malpighian tubules | Fat body | Haemolymph |
|---|---|---|---|---|---|---|---|---|
| I | 0.03-0.05 | + | + | + | + | + | + | + |
| II | 0.07-0.09 | + | + | + | - | + | + | + |
| III | 0.12-0.13 | + | - | + | - | - | + | - |
| IV | 0.14-0.16 | + | + | + | + | + | + | - |
| V | 0.17-0.19 | + | - | + | + | - | - | + |
| VI | 0.22-0.25 | - | + | - | - | - | + | + |
| VII | 0.28-0.3 | - | + | - | + | - | + | + |
| VIII | 0.32- 0.36 | + | - | + | - | + | + | + |
| IX | 0.39-0.42 | + | - | + | + | - | - | + |
| X | 0.46-0.48 | - | + | + | + | + | + | - |
| XI | 0.53-0.56 | - | - | + | + | - | - | - |
| XII | 0.57-0.6 | - | - | - | - | + | + | + |

Distributional pattern of the fractions obtained from the fat body and the haemolymph (fig.5) indicates that most of the fractions were prominent and exhibited variations in the size as well as in the staining intensity. Nine fractions were evident in the fat body while eight in the haemolymph. Fractions I, II, VI, VII, VIII & XII were common to both the tissues while fractions III, IV and X of the fat body and fractions V and IX of the haemolymph did not have their counterparts.- Fractions VIII & X of the fat body were very thick and exhibited intense staining reaction. The anodically migrating fractions VI, VII, VIII, IX and XII of the haemolymph were of moderate size. The staining reaction of the fraction I (fat body-haemolymph), III, IV (fat body) and VI (haemolymph) was intense whereas those of VI, VII and XII (fat body)& II,V & XII (haemolymph) was moderate. Most fraction of both the fat body and haemolymph showed comparison with those of the different region of the gut.

Fractions obtained from the treated insects exhibited variations in their pattern of distribution, number and staining intensity. Fig.6 presents the distributional pattern of the fractions obtained from the different regions of the alimentary canal, fat body and haemolymph. Mobility range of the fractions is given in the Table 10. From the fig. 6 it appears that fractions from the organs/tissues understudy show cathodic and anodic zones of distribution, each with variable number of fractions.

Among the different parts of the alimentary tract maximum number of the bands was evident in the foregut (nine), while the midgut and hindgut produced seven and six fractions respectively.

Fig. 6 GRASSHOPPER – TREATED



The hepatic caeca produced seven bands while five fractions appeared in the M.tubules. Fractions I(0.03-0.04), III(0.11-0.12) and VI (0.21-022) of the foregut did not appear in the remaining parts of alimentary canal, whereas II(0.06-0.08) and IV (0.13-0.15) were common to all of them. Fraction V(0.18-0.2 )was missing in the hindgut and M.tubules, VIII(0.30-0.35) in the foregut, IX in the hindgut and hepatic caeca and XIV in the foregut. Fraction XII XXXXXXXX (0.49-0.52) disappeared in the hindgut and M.tubules. The remaining fractions were common to foregut-midgut (IX), hindgut-hepatic caeca (X) and foregut-hindgut (XI).

It is interesting to note that the fat body of treated insects produced minimum number (four) of fractions while the heamolymph had ten fractions. Of the four fractions, two fractions (II & IV) had slow migration rate while the remaining two (IX & X) had faster rate of migration. All of them, except X, stained intensely. Fraction II, IX and X of the fat body did not appear in the haemolymph. The distributional pattern and staining intensity of haemolymph fractions is presented in the electrophoregram (fig.6). Some of the fractions of haemolymph exhibited comparison with those of the alimentary tract.

A organ and tissuewise comparison of the fractions from both the normal or xxxx untreated and treated insects is presented in the figures 7a, b, c, d, e and fig. 8a, b.

Fig.7a indicates that the number of fractions increased in the foregut after the treatment of oil. In general, the thickness as well as the staining intensity of most of the fractions increased. Most of the fractions, however, exhibited more or less the same range of migration except the anodically migrating ones.

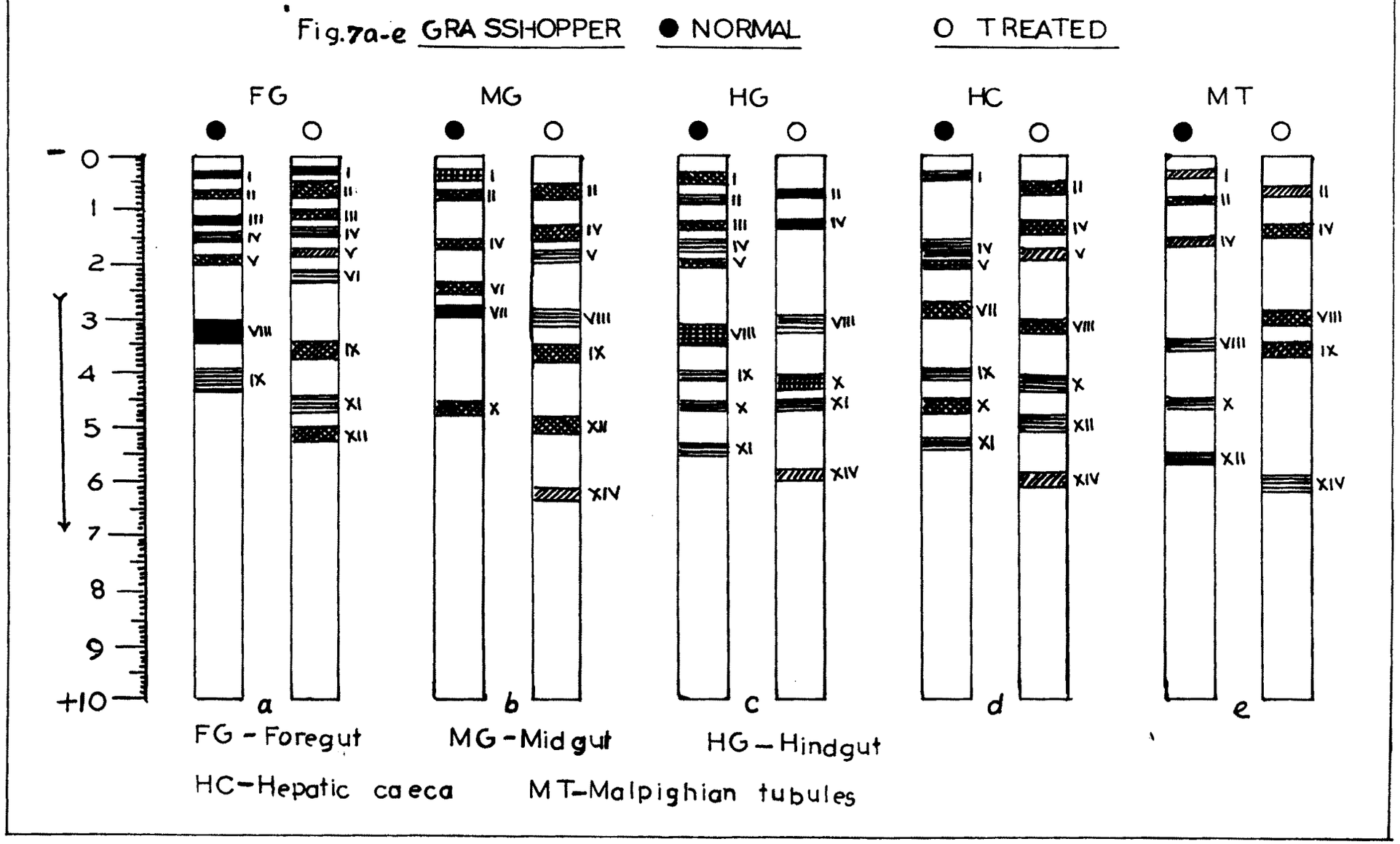


Fig.7a-e GRASSHOPPER ● NORMAL O TREATED
FG
MG
HG
HC
MT
0
1
2
3
4
5
6
7
8
9
+10
a
b
c
d
e
FG - Foregut
MG - Midgut
HG - Hindgut
HC - Hepatic caeca
MT - Malpighian tubules

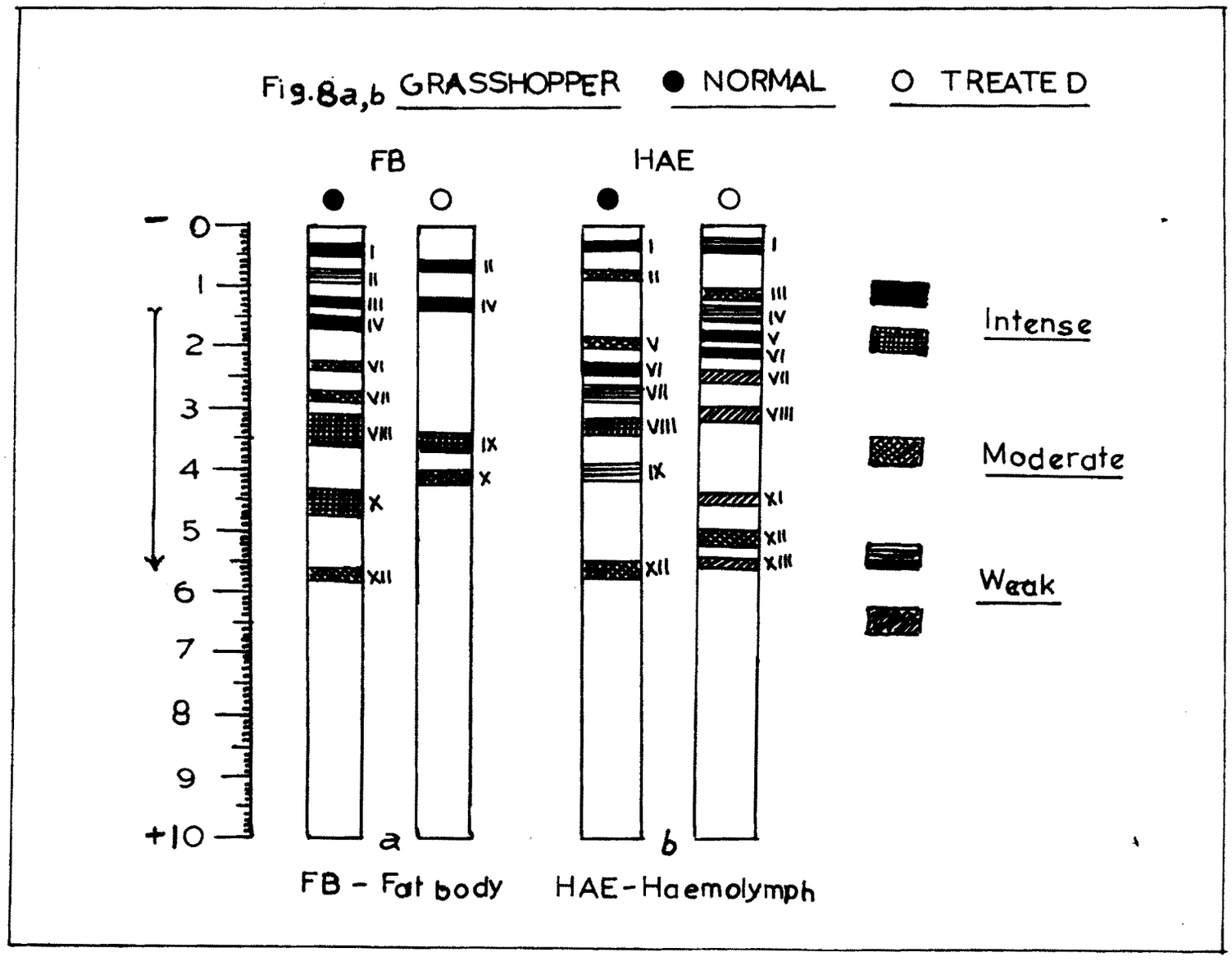


Fig.8a,b GRASSHOPPER ● NORMAL ○ TREATED
FB
HAE
0
1
2
3
4
5
6
7
8
9
+10
I
II
III
IV
VI
VII
VIII
X
XII
II
IV
IX
X
I
II
V
VI
VII
VIII
IX
XII
I
III
IV
V
VI
VII
VIII
XI
XII
XIII
a
b
Intense
Moderate
Weak
FB - Fat body
HAE - Haemolymph

In case of midgut of treated grasshoppers the fraction exhibited comparatively faster rate of migration. They also exhibited an increase in their size. Only few fractions i.e. II-II, VII-VIII & X-XII showed nearly the same range of migration in untreated and treated insects.

The number of fractions was found to be reduced in the treated hindgut (fig.7c). According to the rate of mobility, fraction I, IV, V and XI of the normal hindgut did not appear after the treatment with neem oil. The other fractions (II-II, III-IV, VIII-VIII, IX-X and X-XI) were identical in both the cases.

There was no change in the number of fractions of hepatic caeca after exposure to the test oil. However, they did exhibit change in the rate of migration as well as in the staining reaction (fig.7d). The fast migrating fractions, in general, exhibited poor staining reactions. A similar variation in the rate of migration of the fractions was evident in the M.tubules after the treatment of neem oil (fig.7e), Though the number of the fractions was less (five), but they increased in size & staining intensity as compared to the untreated one.

A marked change in the number of fractions was noticed in the fatbody of the treated insects (fig.8a). Most of the fractions of normal fat body were missing. Only two fractions (III-IV & VIII-IX) exhibited comparison in their range of migration.

A comparison of the fractions of the normal and treated haemolymph is presented in fig.8b. The fractions in both cases exhibited three distinct zones, a)cathodic, b) middle and c)anodic zone. Variations in the number of fractions in each zone were evident. Middle zone contained maximum fractions in both normal and treated insects.

## TABLE NO.10

Electromobility range of protein fractions from treated *S.gregaria*.

| Band No. | Mobility range | Foregut | Midgut | Hindgut | H.caeca | M. tubules | Fat body | Haemolymph |
|---|---|---|---|---|---|---|---|---|
| I | 0.03 - 0.04 | + | - | - | - | - | - | + |
| II | 0.06 - 0.08 | + | + | + | + | + | + | - |
| III | 0.11 - 0.12 | + | - | - | - | - | - | + |
| IV | 0.13 - 0.15 | + | + | + | + | + | + | + |
| V | 0.18 - 0.2 | + | + | - | + | - | - | + |
| VI | 0.21 - 0.22 | + | - | - | - | - | - | + |
| VII | 0.25 - 0.26 | - | - | - | - | - | - | + |
| VIII | 0.3 - 0.33 | - | + | + | + | + | - | + |
| IX | 0.35 - 0.38 | + | + | - | - | + | + | - |
| X | 0.42 - 0.43 | - | - | + | + | - | + | - |
| XI | 0.45 - 0.46 | + | - | + | - | - | - | + |
| XII | 0.49 - 0.52 | + | + | - | - | - | - | + |
| XIII | 0.53 - 0.55 | - | - | - | - | - | - | + |
| XIV | 0.6 - 0.63 | - | + | + | + | + | - | - |

Biochemical :

Quantitative analyses of water soluble proteins were carried out on normal and treated insects of both the species. The same organs i.e. different regions of the alimentary tract (foregut, midgut, hepatic caeca M. tubules), fat body and the haemolymph were employed for the study. The estimations were also performed on these organ/tissues of both males and females of these insects. The average values of the protein contents for the untreated and treated cockroaches are given in the Table 11 while of the grasshopper in Table 12. The results indicate that a sexwise difference in the protein content of both the species was evident. In general, the females of both the insects possessed comparatively higher values than those of the males.

The amount of protein in different organs/tissues ranged between 73.95 to 178.27 mg/gm wet weight of the tissue in the normal males while it was between 88.76 to 205.35 mg/gm in the females. Of the various organs and tissues, the maximum amount of protein was found in the haemolymph of both the sexes. The results obtained in the different organs/tissues of the grasshopper indicate that the amount of protein was found to be more or less the same as those of the cockroaches. In it the values ranged between 97.02 to 195.62 mg/gm, in the males and between 79.6-201 mg/gm in the females. The fat body and the haemolymph had the higher values of the protein content. It is interesting to note that the amount of protein increased in different organs/tissues of both the insects after the treatment with neem oil. The values of protein content after the treatment of oil in males and females of both the insects are presented in same tables (11 & 12).

TABLE NO.11

Values of protein content in different organs/tissues of normal and treated males and females of P.americana.

| | Normal | | Treated | |
|---|---|---|---|---|
| Organ/tissue | Male | Female | Male | Female |
| Foregut | 153.68 | 160.93 | 212.3 | 217.75 |
| Midgut | 125.75 | 138.75 | 158.25 | 170.2 |
| Hindgut | 142.63 | 151.25 | 201.85 | 199.1 |
| H.Caeca | 130.4 | 122.76 | 150.1 | 179.06 |
| M.tubules | 73.95 | 88.76 | 165.5 | 160.95 |
| Fat body | 148.8 | 171.5 | 203.07 | 229.74 |
| Haemolymph | 178.27 | 205.35 | 221.45 | 236.09 |

## TABLE NO.12

Values of protein content in the normal and treated males & females of *S.gregaria*.

| Organ/ tissue | Normal | | Treated | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Foregut | 145.6 | 154.95 | 201.75 | 210.6 |
| Midgut | 128.57 | 142.63 | 181.05 | 203.35 |
| Hindgut | 154.95 | 160.93 | 192.0 | 198.03 |
| H.Caeca | 116.55 | 131.3 | 159.35 | 177.1 |
| M.tubules | 97.02 | 79.6 | 130.06 | 139.45 |
| Fat body | 195.62 | 201.35 | 206.5 | 241.4 |
| Haemolymph | 181.5 | 190.15 | 230.27 | 235.15 |