This dissertation has been microfilmed exactly as received

RAO, Balakrishna Raghavendra, 1936–
TRYPSIN ACTIVITY ASSOCIATED WITH REPRODUCTIVE DEVELOPMENT IN THE FEMALE TAMPA COCKROACH, NAUPHOETA CINEREA (OLIVIER).

The Ohio State University, Ph.D., 1964
Zoology

University Microfilms, Inc., Ann Arbor, Michigan
TRYPsin ACTIVITY ASSOCIATED WITH REPRODUCTIVE DEVELOPMENT
IN THE FEMALE TAMPA COCKROACH, NAUPHORIA CINerea (OLIVIER)

Dissertation
Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University.

By

** * * * * *

The Ohio State University
1964

Approved by

Frank M. Sisk
Adviser
Department of Zoology and
Entomology
ACKNOWLEDGMENTS

I take this opportunity to express my thanks to Dr. Frank W. Fisk for his valuable guidance and encouragement throughout the course of this study. I am quite indebted to him, to Dr. Carl Venard, and Dr. John D. Briggs for going through this manuscript painstakingly.

My sincere thanks are also due to Mr. Russell Skavaril and Dr. C. R. Weaver for their indispensable help in the statistical analysis of the data and the use of the IBM 7094 and IBM 1620 computers.

I would like to acknowledge the assistance and valuable suggestions of Mr. Deodatt Wadke during the preparation of this manuscript.

Special mention must be made to Miss Sharon Davis for her tireless work in typing the rough draft of this dissertation.
VITA

September 15, 1936  Born—Udipi, Mysore State, India.

1957  B.Sc. Ag. Banaras Hindu University.


1959-60  Lecturer in Agr. Entomology, Coll. of Agr., Dharwar, Mysore State, India.

1960-64  Graduate Teaching Assistant, Dept. of Zool. and Ent., The Ohio State University, Columbus, Ohio 43210.

PUBLICATIONS


FIELDS OF STUDY

Major Field: Entomology

Agricultural Entomology. Banaras Hindu University and Karnatak University, India.

Insect Physiology. Dr. F. W. Fisk, The Ohio State University.

Insect Toxicology. Dr. G. W. Ware, The Ohio State University.
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Trypsin Activity</td>
<td>2</td>
</tr>
<tr>
<td>Trypsin substrate specificity</td>
<td>3</td>
</tr>
<tr>
<td>Proteolytic Enzyme Activity in Insects</td>
<td>5</td>
</tr>
<tr>
<td>Integration of oocyte development, protein synthesis, and protein hydrolysis (digestive trypsin)</td>
<td>12</td>
</tr>
<tr>
<td>Biology of the Cockroach, <em>Nauphoeta cinerea</em> (Olivier)</td>
<td>17</td>
</tr>
<tr>
<td>MATERIALS AND TECHNIQUES</td>
<td>20</td>
</tr>
<tr>
<td>RESULTS</td>
<td>28</td>
</tr>
<tr>
<td>Tables 1 through 7</td>
<td>33</td>
</tr>
<tr>
<td>Figures 1 through 14</td>
<td>39</td>
</tr>
<tr>
<td>DISCUSSION AND CONCLUSIONS</td>
<td>50</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>55</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>57</td>
</tr>
</tbody>
</table>
INTRODUCTION

Protein synthesis is the most essential metabolic activity for growth and reproduction. Ingested food is the primary source of protein. In the process of digestion this protein breaks down enzymatically to amino acids. These amino acids are utilized for the further synthesis of protein. Since the ovarian development is the major event of the adult stage of female insects, most of the protein synthesis is directed toward this process.

Proteolytic enzymes attack the complex food protein at the initial stages, showing the importance of such enzymes in the whole picture of protein synthesis. Trypsin is regarded as the most important of the several proteolytic enzymes. Present-day knowledge of trypsin has revealed more of its specific nature, such as its site of action on the protein molecule.

The attempt of the present work is to find a correlation of trypsin activity to the reproductive development in female Tampa cockroaches (*Nauphoeta cinerea*). Since in this species the food ingested by the adult is the principal source of substrate for enzyme action it is also interesting to know the relation of the type of food eaten to the above correlation. In the discussion an attempt is also made to give an explanation for these correlation.
Trypsin Activity

Emil Fischer (1905) was the first to investigate the specificity of proteolytic enzymes by studying their action upon several peptides. Since his classical work, the generally accepted classification of proteolytic enzymes up until 1935 was on the basis of substrate. Thus they were classified into proteinases and peptidases. Proteinases were supposed to split proteins, proteoses, and peptones into polypeptides, and these polypeptides were assumed to be hydrolyzed by peptidases, finally yielding a mixture of amino acids. Again, proteinases have been supposed to act on substrates of high molecular weight but not on the lower polypeptides and dipeptides. The opposite was assumed for the peptidases. Further, trypsin was characterized by its specificity of attacking anions as compared with pepsin acting on cations and chymotrypsin on zwitterions. Later Bergmann (1942) gave a tentative classification of proteolytic enzymes on the basis of the location of their action in the protein molecule. Hence trypsin, like pepsin and chymotrypsin, was found to attack the centrally located peptide bonds as well as the terminal peptide bonds, and thus it was grouped under the endopeptidases.

Another attempt was made by Hartley (1960) to classify the proteolytic enzymes, based on the mechanism of action, rather than on the origin, specificity, or physiological action. Thus he grouped the following enzymes: (1) serine proteinases, which included chymotrypsin, trypsin, elastase, thrombin, subtilisin,
etc., the active centers of which contained a serine residue that reacted uniquely with organophosphorous compounds; (2) thiol proteinases, like the papain, ficin, bromelain, etc.; (3) acid proteinases, which included the pepsin and renin having their low optimum pH for reaction; and (4) metal proteinases comprising the remainder, such as the carboxypeptidases, aminopeptidases, and dipeptidases.

According to the 'Report on the Commission on Enzymes' (1961) trypsin has been classified as follows:

Hydrolases—Peptide hydrolases—peptide peptidohydrolases or iminopeptide hydrolases—trypsin. Trypsin is said to hydrolyze the peptides, amides, esters, etc., specifically, at bonds involving the carboxyl groups of L-arginine or L-lysine.

**Trypsin substrate specificity**

In order to establish the point of scission in the peptide links, several synthesized peptide derivatives were developed as substrates. The substrates were simple, having one peptide bond to facilitate the identification of the product of hydrolysis. Earliest of the substrates were mostly amides of lysine and arginine derivatives, hydrolyzing enzymatically into ammonia and corresponding amino acid derivatives. It was later discovered that trypsin was a powerful catalyst for the hydrolysis of certain amino acid esters. Both the esterase and the amidase activities were apparently mediated by the same active surface configurations of the enzyme (Schwert et al., 1948). Schwert et al. found that BAME (α-Benzoyl-L-arginine methyl ester) and TSAME (α-p-
toluenesulfonyl-L-arginine methyl ester) were most active and were hydrolyzed about 60 times as fast as their corresponding amides. While comparing BAME and TSAME, they came to the conclusion that TSAME was more subject to hydrolysis by trypsin than was BAME. Trypsin hydrolyzed TSAME about six times as rapidly at pH 8.0. Moreover, BAME was also hydrolyzed by chymotrypsin, whereas TSAME was not.

Later Schwert and Takenaka (1955) used BAEE (α-Benzoyl-L-arginine ethyl ester) as a substrate for trypsin together with BA (α-Benzoyl-L-arginine) as a blank and ATEE (N-acetyl-L-arginine) as a substrate for chymotrypsin, together with tyrosine as the blank for the ultraviolet absorption spectrophotometric analysis of these two enzymes. But it was also found that BAEE was slowly hydrolyzed by chymotrypsin, thus rendering this substrate unsuitable for the assay of traces of trypsin in the presence of a relatively large amount of chymotrypsin. Greater sensitivity was achieved with TSAME (hereafter called TAME), replacing the BAEE for trypsin activity as compared with that of chymotrypsin. Similarly the substrate BTEE (Benzoyl-L-tyrosine ethyl ester), replacing the ATEE, was employed because of its greater sensitivity for chymotrypsin.

Schwert (1948) had further demonstrated the superiority of TAME over BAA (Benzoyl-L-arginine amide) in that TAME did not undergo spontaneous hydrolysis over a wide range of pH; it was hydrolyzed 900 times as rapidly; it was readily soluble in water and the products were not competitively inhibitory.
Considering the above mentioned advantages Hummel (1959) used TAME successfully for the spectrophotometric determination of trypsin. Siegelman et al. (1962) developed, on the other hand, a sensitive colorimetric method for quantitative demonstration of trypsin activity using TAME (originally designated as TSAME). In this method, methanol, one of the hydrolytic products of TAME, was oxidized to formaldehyde, which in turn was determined colorimetrically after the reaction with chromotropic acid. Kinetics of the enzyme reaction and the subsequent chemical reaction with the chromogen have shown a linear function of duration and concentration of enzyme. Also the method has been proved to be quite suitable for the routine assay of a large number of specimens by virtue of the simplicity of technical operations and the rapidity of enzymic hydrolysis.

**Proteolytic Enzyme Activity in Insects**

Several insects of economic importance have been studied for their protease activity and many a time the activity has been referred specifically to trypsin. The terms 'protease,' 'proteinase,' and 'proteolytic enzyme' seem to have been used quite synonymously in the following literature.

Wigglesworth (1928), one of the earliest workers of the proteolytic enzymes in insects, reported that the protease of the American cockroach was closely similar to mammalian trypsin. It resembled the pancreatic trypsin in (a) the products of digestion, (b) the relative production of "free acidity" and "formaldehyde
acidity" during digestion, (c) the effect of salts, and (d) in acting upon proteins only on the alkaline side of their isoelectric point. The two enzymes differed in that with a given protein the range of activity of the cockroach protease extends further in the acid direction, as evidenced by the pH activity experiments with different substrates, such as serum albumin, gelatin, caseinogen, and edestin. The theoretical significance of this fact has been attributed to the nature of the substrates used.

Schlottke (1937a, b) was one of the earliest investigators who reported on the stimulated secretion of proteolytic enzymes in response to feeding in insects. He worked with Carabus auratus, a predacious ground beetle, and Tettigonia cantans, an omnivorous pygmy grasshopper. He observed the sharpest increase in trypsin within the first hour after feeding in both these insects. He also reported on the high proteinase activity in the crop and the midgut appendages and mild activity in the midgut proper.

Day and Powning (1949) reported that the pH of the gut contents, except that of the crop, did not vary with the starch and protein diets. They showed that the digestive enzymes were still present in Blatella germanica midgut contents after three days' starvation, but the enzymes studied increased in concentration when the insect was fed, irrespective of the diet. There was, however, a reduction in the activity of proteinase on starvation which was fairly slow in returning to its former level when fed.
Fisk (1950), working with the proteolytic enzyme of *Aedes aegypti*, found that the enzyme activity of mature, unfed females was insignificant, while the activity of midguts from females, permitted a partial blood meal one or two hours prior to dissection, was significant. It seemed apparent that the presence of blood in the stomach stimulated the proteolytic activity but that neither the feeding syndrome in itself, nor the replete, distended abdomen, nor a mixing of precursors and activators from different regions of the digestive system, nor an enzyme origin other than the midgut, was responsible for this stimulation. While determining the pH of the *Aedes* protease activity he found a fairly sharp optimum pH of 7.8 which was the optimum for the trypsin activity.

Fisk and Shambaugh (1952) further investigated the protease activity in relation to feeding. The protease activity of the midgut of *Aedes aegypti* was found to drop rapidly in five minutes to below the residual level characteristic for unfed mosquitoes. This drop was followed by an equally rapid increase for the first few hours which gradually increased till 48 hours, but did not reach the "residual value." The amount of protease activity shown by the midguts of unfed mosquitoes is referred to as the residual value. On feeding sugar solution there was an initial twofold rise in the protease activity during the first hour, but by two hours the activity had dropped to the residual value. Shambaugh (1954), in his studies of protease stimulation, found that the effect of the cellular proteins in a relatively
inert medium is somewhat greater than the residual value. On testing the dialyzable and non-dialyzable portions of the blood for the stimulation of protease, he found that the more important stimulatory factors were in the non-dialyzable plasma protein; namely, fibrinogen, albumin, and gamma globulin of the blood at a concentration of 0.6 per cent. However, the response to the mixture of these three plasma proteins was greater than to any one fraction, but was not greater than the summed effect of the separate fractions. He developed a possible positive correlation between the amount of blood ingested by female mosquitoes and the subsequent protease activity of their midguts which led to the conclusion that an increase in the ingested blood increases the amount of the protein for the midgut proteases.

Dadd (1956) described the work on the proteolytic enzymes of two beetles, Tenebrio molitor and Dytiscus marginalis, having widely different food habits. He reported the spontaneous occurrence of protease in Tenebrio after molt and adult emergence in response to feeding in the active larva and mature adult. Even water or damp cellulose was effective in increasing the secretion in the adult. He showed that the protease was accumulated in the midgut tissue of starved Dytiscus. Within an hour it was largely discharged into the crop. Protease recurred in the midgut tissue in a few hours after feeding, but remained low as long as the crop contained the undigested material. The process of discharge appeared to be independent of synthesis.
Champlain and Fisk (1956) studied the protease activity in the stable fly, *Stomoxys calcitrans*, and they found the optimum pH for the hydrolysis of two substrates for the trypsin action to be 7.8 and 7.9. Protease activity followed by a meal of sucrose solution showed a slight increase for about an hour which quickly declined and remained close to the residual value. Maximum activity was obtained at about 13 hours after the blood meal.

Further work on stable fly trypsin-like protease activity by Patterson and Fisk (1958) showed the zero order type of reaction at the concentration of substrate and homogenate used. It was reported that the midgut homogenate showed at least three trypsin-like substances by paper electrophoresis, each of which seemed to possess three different electromobilities. There was no difference in the trypsin-like activation of midgut homogenate prepared from male and female flies taken from the same population.

From the studies of egg homogenates of *Locusta migratoria migratorioides* Shulov et al. (1957) concluded that the eggs contain at least two kinds of endopeptidases. They tried egg homogenates on casein and on leucylglycylglycine substrates and arrived at an optimum of pH 7.8 and pH 5.6 for these two enzymes.

Rockstein and Kamal (1954) reported the distribution of digestive enzymes in the alimentary canal of larvae of flies of medical and veterinary importance, whereas Kamal (1959) made comparative digestive studies of the digestive enzymes of 13 species of family Calliphoridae and Sarcophagidae (Diptera). Both have given considerable importance to the trypsin distribution.
Waterhouse (1957) summarized most of the above observations made by several workers on the subject of the control of enzyme secretion.

Remarkably enough Greenberg and Paretsky (1955) reported the presence of pepsin, trypsin, and cathepsin from various stages of the housefly on the basis of pH optimum of the enzymatic reaction. All stages, except the egg, had trypsin with a pH optimum between 8 and 9 on casein. A high trypsin activity was shown in the early first instar larvae which decreased gradually showing a minimum in the third instar and prepupal stage. However, the activity was slightly higher in the black stage of the pupa and decreased in the adult stage. Lin and Richards (1956) compared the proteinase activity in the housefly and the American cockroach. Temperature kinetics for both insects showed a similar slope. Patel and Richards (1960) showed that the three trypsinlike components separated from the midguts of adult houseflies had different electrophoretic mobility from the mammalian pepsin and trypsin.

Partial purification was carried on from the homogenates of the larvae of the clothes moth, *Tineola bisselliella*, by Powning and Irzykiewicz (1962a) to test the digestibility of the wool in vitro. The purification resulted in a 400-fold increase of specific activity over the initial water extract. But when compared with trypsin this proteinase was found to possess a distinct 'keratinase' activity (Powning and Irzykiewicz, 1962b).
It had a higher specific activity on casein than the sample of crystalline trypsin used in their experiments.

Thomson and Møller (1963) studied the influence of corpus allatum hormone on the development of the ovaries and on the growth of accessory glands in *Calliphora erythrocephala*. Then they studied a possible effect of this hormone on the protease activity in the blow flies. They found that the enzyme development was controlled by neurosecretory cells. From the dietary experiments they found that the meat-fed flies had a higher protease activity than sugar-fed flies.

Birk et al. (1962) made observations on the factors affecting the growth, by way of studying the inhibition of proteolysis by soybean trypsin inhibitors and haemoglobin as a substrate, subjected to the proteolytic activity of the midgut homogenates of *Tribolium castaneum*, *T. confusum*, and *Tenebrio molitor* in vitro. Their further work was concerned with relation between proteolytic and amylolytic activity in the growing larva. They found that with a relative decrease in proteolytic activity during larval development there was a steady relative increase in amylolytic activity until both activities reached constant levels in the last instars.

"Tenebrio trypsin" and two exopeptidases were differentiated on the basis of the selective inhibition by specific trypsin inhibitors from *Tenebrio molitor* larval midgut homogenate by Applebaum et al. (1964). The Tenebrio trypsin was further purified
by eluting from a column of ECTEOLA-cellulose and further purified by adsorption on a column of CM-cellulose and subsequent elution.

Integration of oocyte development, protein synthesis, and protein hydrolysis (digestive trypsin)

Fraenkel (1940) showed that in blow flies an adequate diet of meat, sugar, and water was necessary for the development of the ovaries. When blow flies were deprived of their medial neurosecretory cells and fed meat their ovaries, corpora allata, accessory glands, and oenocytes were of nearly the same small size as those fed on sugar (Thomsen, 1952). A hypothesis was thus developed that a neurohormone from medial neurosecretory cells (m.n.c.) had an influence on protein metabolism and especially the protein synthesis. Numerous evidences have been accumulated confirming this concept of hormonal control of protein synthesis. Wigglesworth (1954) suggested that the failure of the ovarian development in Rhodnius after allatectomy might have been due to a failure of protein synthesis. Thomsen (1952) showed the relationship of m.n.c., corpora allatum, and corpus cardiacum to the ovarian development in female Calliphora erythrocephala. She found that the flies were able to develop eggs without the corpus cardiacum. The ovaries of the allatectomized flies generally developed only to the stage at which the deposition of yolk began. From later experiments she concluded that in Calliphora the m.n.c. must be regarded as the overall controlling center of the endocrine system that involves protein metabolism. Several
possibilities had to be considered in these experiments. Either the m.n.c. deprived flies ate less meat than the normal ones, or were unable to digest the ingested meat, or, if the meat were digested, they were unable to absorb the amino-acids or resynthesize the absorbed amino-acids. These considerations led to studies of the influence of neurosecretory cells and/or corpus allatum on intestinal protease activity in the Calliphora erythrocephala by Thomsen and Møller (1959, 1963). They found that the stimulation of protease activity was chiefly controlled by the m.n.c., while the corpus allatum had a minor role in the same.

Highnam (1962) reported that a hormone from m.n.c. regulated the ovarian development in Schistocerca gregaria. Further Highnam and Lusis showed (1962) that the blood protein concentration, which normally increased just before and during yolk deposition and decreased as the oocytes reached their maximum length, remained at a low level if the m.n.c. of the young adult females were cauterized. Further evidence was given by Hill (1962) who showed that in Schistocerca gregaria whenever the m.n.c. were active the protein concentration of the haemolymph was high and when they were inactive the concentration remained low. A high haemolymph protein concentration was found to be correlated with the developing ovaries. This was further supported by the fact that one of three haemolymph protein fractions obtained by electrophoresis was found to increase greatly after ovariectomy and during yolk deposition and thus
seemed to be concerned with the ovarian development. Later, Highnam et al. (1963) proved that the corpus allatum did not affect the protein synthesis in the desert locust. He found that in the allatectomized females the oocytes did not develop though the blood protein reached the concentration at which yolk deposition normally occurred. Thus they concluded that protein synthesis was controlled by the neurosecretory system during the oocyte development, while the corpora allata secrete a gonadotropic hormone which facilitates protein uptake by the growing oocytes.

Khan (1963a) studied the distribution of proteinase, invertase amylase activity in various regions of the alimentary canal of Locusta migratoria L. and found that caeca showed a higher proteinase (4 times) activity than the midgut, both in male and female. The proteinase activity in female, however, was higher than that of male. In her further studies Khan (1963b) showed the variation in the fed and unfed locusts of both male and female. (Proteinase activity was reduced to half in starved females.) She found that the amount of enzyme in the tissue was very small as compared with that in the lumen. Thus she concluded that when enzyme was produced in the tissue it was rapidly discharged into the lumen.

Highnam et al. (1963) showed a correlation between the oocyte resorption and the neurosecretory system which, in turn, controlled the haemolymph protein concentration, that is, the protein 'availability' to the oocytes. Competition by individual
oocytes for the available protein played an important part in the oocyte resorption. They found that partial ovariectomy in the desert locust resulted in the reduction of the oocyte resorption.

Thomsen (1964) pointed out that though the m.n.c. hormone has a profound influence on the development of the ovaries it cannot be considered as the "gonadotropic" hormone as such (as per the usage by Fraenkel and Heiao, 1963) because it also regulates the secretion of proteolytic enzymes by the midgut cells.

A few questions remain to be investigated. What changes in the blood protein concentration reflect changes in the gut protease activity? How does the latter correlate with the ovarian development? Having established this correlation, how is the correlation affected by different diets and the mated or virgin conditions? What is the relation of midgut total protein content to the oocyte development? (This could be a key point to ascertain the amino acid sources for the protein metabolism and the changes in the blood protein content.)

Hence the present line of work was mainly concerned with investigating, for the cockroach, *Nauphoeta cinerea*, the correlation of protease activity to oocyte development under several different conditions. This information, with the additional contribution of the blood protein variation, should answer most, though not all of the linking mechanisms in insectan protein synthesis; namely, the protein source, enzymic hydrolysis to synthesis, hormonal control of the protein metabolism, transport and final utilization of the synthesized protein for the development of eggs.
In the present investigation a correlation between the oocyte development and the protease activity, specifically the trypsin activity, was developed. It is thought that the trypsin activity is the prime step to the protein metabolism to provide an ultimate amino acid pool for the further protein synthesis in the insect body. Egg development being one of the major events in the reproductive stage of the adult female the metabolic activity is directed toward the protein synthesis at this site. The principal source of the raw material for this metabolic activity is the food ingested by the insect. Since the trypsin activity is mostly concentrated in the midgut it could well be understood that there might be a direct relationship between the trypsin activity and the oocyte development. Some of the other problems which subsequently arise following this work would be: the control mechanism of the appropriate stimulating hormone; intermediary stages or storage of proteins and amino acids either in the blood or elsewhere; and the possibility of other organs concerned with the protein metabolism in addition to the midgut.

Two other important factors can be mentioned at this stage which influenced the present study. First, a more specific and reliable method of establishing trypsin activity, the importance of which has been mentioned in detail elsewhere and second, the work of Roth and Stay (1962) on *Nauphoeta cinerea* which clearly showed a strong correlation between mating and oocyte development in this particular species of cockroach.
Thus the following investigations were carried out in the present work:

1. To establish a correlation between the midgut trypsin activity and the oocyte development in adult female Nauphoeta cinerea cockroaches.
2. To compare the correlations noted with mated versus virgin females.
3. To study the influence of a high-carbohydrate diet, high-protein diet, or starvation on the initial correlation.
4. To study the relationship of the total protein of the midgut with trypsin activity in all the above situations.

Biology of the Cockroach, Nauphoeta cinerea (Olivier)

Nauphoeta cinerea (Olivier), commonly known as the Tampa roach, was first discovered in Mauritius in 1780 (Illingworth, 1942). Though distinctively a circumtropical species it spread to the new world through commercial shipping. Illingworth (1942) made some observations on these cockroaches as early as 1914 from Hawaii and he reported an outbreak of the cockroaches in 1942 in Honolulu. The first report of their establishment in the United States was in Tampa, Florida, in 1952 by Gresham.

According to Rehn's classification (1951), Nauphoeta cinerea (Olivier) belongs to family Blattidae and subfamily Epilamprinae. Roth and Willis (1954) grouped these cockroaches under the 'false ovoviviparous' type, modifying Hagan's (1951) definition of viviparity. The ootheca is extruded out from the
uterus and then retracted completely into the brood sac, where the eggs inside the ootheca remain till hatching. During the gestation period the embryos of Nauphoeta absorb water, but derive no nutritive material from the mother (Roth and Willis, 1954). Recently Roth and Hahn (1964) worked out the variation in the size of new born nymphs in Nauphoeta and Diploptera.

The adult male measures about 28 mm and the adult female about 40 mm. Both are grey in color with two longitudinal black lines bordering the prothorax. Males are identified by the anal style in addition to the cerci and by the narrow terminal abdominal sternite. Willis et al. (1958) made detailed observations on the reproduction and development of Nauphoeta cinerea.

Observations made in this laboratory revealed that copulation was initiated by a short duration courtship by the male. The male raised his wings in front of the female so as to expose the tergal gland. The responsive female climbed over the male from the rear and started nibbling this scent gland. As she was engaged in this act, the male extended his abdomen from underneath the female and shot out the phallomere to reach the female's genitalia and subsequently hooked on to it. At this time the female, being disturbed, tried to move away from the male, but the male having twisted his abdomen by this time, came to an end-to-end position with the female. They remained in this position from 14 to 21 minutes with an average of 16 minutes. Roth and Willis (1954) described the mating of this and other species of cockroaches.
The transfer of the sperms from male to female is accomplished by means of a spermatophore. The sperms migrate from the deposited spermatophore to the spermatheca of the female. The eggs, as they pass out of the oviduct into the genital chamber, are fertilized (Roth and Willis, 1954).

Roth and Lewis (1957) conducted the longevity experiments with this species.
MATERIALS AND TECHNIQUES

Rearing technique

The Tampa roach was mass reared in polystyrene containers ("Crisper Chest") of 30.5 cm x 25.5 cm x 10 cm, covered with a partially screened lid. The inner wall of the crisper was smeared with the roach barrier (thin slurry prepared by mixing vaseline and mineral oil) about 5-8 cm from the top. The roaches had a constant supply of water and dog biscuits. The advanced immature stages were removed from the cultures and transferred to clean crispers to obtain the freshly molted adults. Every day as they emerged these adults were transferred to 15 cm x 7.5 cm circular crystalline dishes, sexed, and identified with a number and treatment. These crystalline dishes were also smeared with the roach barrier and kept covered with the screen lid or cheese cloth with a rubber band. All were watered but fed differently according to the desired diet; namely, normal or balanced diet (dog biscuits), high protein diet, carbohydrate diet, and starved.

Since the air-conditioned rearing room did not receive any daylight, it was artificially simulated with the diurnal rhythm by

1. Austin dog biscuits—Sunshine Biscuits, Inc., Long Island City, N. Y. Crude protein (Min.)--22.0%; Crude fat (Min.)--2.5%; Crude fiber (Max.)--4.5%; Ash (Max.)--9.0%; Moisture (Max.)--10.0%.

2. Fish meal—Menhaden. Protein--60%; Fat (Max.)--9%; Salt (Max.)--4%.

3. Mixture—(Lab preparation). Starch--80%; Sugar--10%; Cellulose--10%.
using three 100-watt lamps kept at a suitable height. The light went on at 8:30 A.M. and off at 8:30 P.M. every day. The temperature was maintained at an average of 24°C with a relative humidity of 40-60 per cent.

**Mating**

Matings were induced on the fifth, sixth, and seventh day to determine the best day for assuring ready mating. Though the males displayed some of the typical mating behavior by raising their wings in front of the females, the majority of females on the fifth day did not pay any attention to them. On the sixth day some of the females responded readily, some with a little delay and hesitation, yet some did not show any signs of sexual behavior. On the seventh day the females readily received the displaying males in a matter of a few seconds after being placed with them in the mating containers. Hence the seventh day was fixed as the day for effecting mating where mated females were wanted.

**Dissection**

A minimum of three roaches was used for each day's observation. Each roach was anaesthetised separately with carbon dioxide and weighed in the torsion balance. Then it was dissected in cold roach saline to expose the alimentary canal from

\[ \text{The roach physiological saline was prepared by dissolving} \]
\[ \text{NaCl--10.93 g; KCl--1.57 g; CaCl}_2--0.85 \text{ g; MgCl}_2--0.17 \text{ g; NaHCO}_3--0.17 \text{ g in 1,000 ml of water and pH adjusted to 7.85.} \]
which the midgut was severed and transferred to a labelled centrifuge tube which was quickly placed in deep freeze. The eggs were removed from the body, placed in the cavity slide with a little saline, the oocytes were separated and their length was measured by means of an ocular micrometer. The ocular micrometer had been calibrated for the different objectives. An average of measurements of five oocytes from each egg mass was taken for the final reading. In case of the oothecal formation of the mated series the oothecae were weighed to assess the rate of development in addition to the length of the ovules.

Homogenate preparation

The midguts were homogenized individually. The frozen midgut from the centrifuge tube was transferred to the tissue grinder and homogenized with 0.25 ml of cold 7.8 pH Tris-citrate buffer, rinsed with 0.35 ml and then with 0.5 ml of more buffer, each time emptying the liquid homogenate into the centrifuge tube. The tubes were then centrifuged again at approximately 2,420 rpm for 5 minutes. The supernatent liquid was filtered through glass wool to obtain 1 ml of the sample which was then stored in the deep freeze in an acid-cleaned, labelled tube of 75 mm x 10 mm till needed for the tests.

Protein test

Total protein was quantitatively estimated by the Folin-Ciocalteau-Phenol method of Lowry et al. (1951). The procedure
was slightly modified for convenience. The following reagents were necessary for the test:

Solution A: 2% anhydrous sodium carbonate prepared in 0.1N NaOH.

Solution B: 0.5% copper sulfate prepared in 1% Na-K tartrate.

Solution C: 1 ml of solution B made up to 50 ml with solution A and diluted to 250 ml with distilled water.

Solution D: 50% of phenol reagent.

Solutions C and D were prepared just before the experiment.

Standard: Standard solution was prepared by dissolving Armour crystalline bovine plasma in water (200 ug/ml).

Blank: Water has been used for the blank reading.

A 50 ul portion of the thawed sample of the homogenate was diluted to 1 ml with water in a test tube; 1 ml of water served as the blank. All tubes were made in duplicate. To each of the tubes 5 ml of solution C was added at a regular interval of ½ minute and stirred. After exactly 10 minutes of reaction 0.1 ml of solution D was added by forcing it into the solution, stirring quickly with the help of rotary vibrator. The color was allowed to develop for about 30 minutes and reading was taken in Klett-Summerson photoelectric colorimeter with a red filter (660 mu).
During each test a standard of 200 ug/ml (1 ml) was used to check the aging various dilutions of the Bovine plasma albumin. The blank reading was subtracted from the average readings of the duplicates. This was read against the standard curve to obtain the bovine plasma albumin equivalents (ug/ml). This value has been used in the data to represent the total protein of the midgut content (0.05 ml sample).

**Trypsin test**

Having discussed the merits of TAME as a substrate it was decided to use it as the standard substrate for the trypsin test. The procedure adopted for the testing was mainly from the method of Siegelman et al. (1962) for the quantitative estimation of the trypsin activity in human blood serum with some suitable modifications. The following reagents were prepared for the test:

- **Buffer**: Boric acid-Borax buffer 0.05M aqueous with pH 8.0. The boric acid solution was prepared first to which the borax solution was added to raise the pH 8.0.
- **Substrate**: TAME 0.25M prepared by dissolving 950 mg in 10 ml of water. Remaining solution was refrigerated to be used for the next day test. Thus the possibility of autohydrolysis was avoided.
- **Calcium chloride buffer**: 0.05M, aqueous adjusted to pH 2.5 with HCl. This has been used as a diluent for the trypsin.

---

5 Standard: Crystallized bovine plasma albumin (Armour Laboratories).

6 p-Toluenesulfonyl-L-arginie methyl ester HCl (N. B. Co., Cleveland, Ohio).
Trypsin: For standard—2X, Crystalline, salt free. The stock solution of 100 ug/ml was frozen.

Trichloroacetic acid (TCA): 10% for terminating the enzyme reaction and 5% TCA for blank and diluent of methanol "key" standard.

Potassium permanganate—2% aqueous, as an oxidizing agent.

Sodium sulfite—10% aqueous, as a reducing agent.

Chromotropic acid—the working reagent was prepared by mixing 100 ml of 2% aqueous chromotropic acid solution with 200 ml of cold water in a liter flask. To this 600 ml of chilled concentrated sulfuric acid was added little by little over a period of about two hours and the flask was kept in cold water. Finally the volume was made to the liter mark with water. The solution was refrigerated.

Methanol "key" standard: $3 \times 10^{-3}$M methanol prepared in 5% TCA. All the reagents were stored in the refrigerator.

Two sets of centrifuge tubes were marked as A and B and each test of an homogenate was duplicated. In all tubes 0.8 ml of borate buffer was placed and to each of the A tubes 0.05 ml thawed homogenate was added and stirred, starting with B tubes and ending with A tubes. The tubes were then incubated at 37°C in a magniwhirl waterbath for an hour. During this time the tubes were covered to retard the possible slow evaporation of methanol.

---

7 Crystalline, 2X, Salt free (N. B. Co., Cleveland, Ohio).
8 4,5-Dihydroxy-2, 7-naphthalenedisulfonic acid, disodium salt. Eastman Organic Chemicals, Rochester, N. Y.).
which is one of the end products. At the end of the incubation period the tubes were placed in cold water for one minute. One ml of 10% TCA was added to each tube and stirred, starting from A tubes and ending with B tubes. To the B tubes 0.05 ml of corresponding homogenates were added and stirred again. All the tubes were then centrifuged at 2,620 rpm approximately for 5 minutes. One ml sample of the supernatent liquid from each tube was placed in a similarly labelled test tube and 0.2 ml of KMnO₄ was added and mixed. After about one minute 0.2 ml of Na₂SO₃ was added and mixed till the solution decolorized. Then 8.6 ml of chromotropic reagent were added forcibly to get a rapid mixing of the materials. All the tubes were then placed in the boiling water bath for 15 minutes. During this period the tubes were covered with marbles to retard the excess evaporation. The tubes were cooled in cold running water for 3 minutes. The solution attained a deep purple color which was read in the Klett-Summerson photoelectric colorimeter with a 600 mu, amber colored filter. The B tubes served as the blanks for the respective A tubes. Apart from these blanks a TCA blank (1 ml of 5% TCA) and a methanol "key" standard (1 ml of the standard) were introduced along with the test homogenate tubes just before the oxidation process.

A set of 8 tubes was (4As and 4Bs) worked at a time for the sake of loading convenience of the centrifuge machine. Klett
readings of B tubes were deducted from the A tubes and the readings were averaged from the duplicates. These readings were read against the trypsin standard curve to obtain the trypsin activity in terms of pure crystalline trypsin.
RESULTS

Preliminary experiments

To determine the active region of the trypsin activity in the alimentary canal the following experiment was performed. The cockroach was dissected under saline. The salivary glands were removed and the alimentary canal was dissected out and separated into foregut, midgut, and hindgut. The salivary glands and the three gut portions were homogenized separately and were tested for trypsin activity. As shown in Table 1 and Figure 1, the salivary glands did not show any trypsin activity, whereas the foregut and the hindgut showed slight activity and the midgut, including the ceaca, showed the highest activity. Khan (1963b) showed that the proteinase activity was highest in ceaca as compared to the midgut in Locusta migratoria.

Another experiment was set up to determine a suitable incubation period for the homogenate and the substrate system. The tubes were incubated at 37°C for 15, 30, 45, 60, 75, 90, 105, and 120 minutes along with respective blanks for each set. Following incubation they were suitably treated for the development of the color. The Klett-Summerson colorimeter readings along with the blank readings are listed in Table 2 and graphically represented in Figure 2. From the above results an incubation period
of 60 minutes was selected as most suitable for further trypsin activity estimations.

**Standard curves**

A standard curve was drawn from the Klett readings plotted against trypsin concentration, using a series of dilutions of pure trypsin. The trypsin used was twice crystallized and salt free. These solutions were prepared in 0.05M CaCl₂ buffer at pH 2.5. The resulting standard curve for trypsin activity is shown in Figure 3.

Similarly a standard curve was drawn for the total protein determination from the Klett readings plotted against the concentration of a series of dilutions of bovine plasma albumin. Figure 4 represents the standard curve of total protein. These standard curves have been used to convert the Klett readings into trypsin equivalents and bovine plasma concentration equivalents respectively.

**Graphical representation of results of trypsin activity**

Data on individual cockroaches are shown in the tables 5 to 9. The information shown in these tables includes the age from the last molt, weight, average oocyte length, trypsin activity represented by trypsin equivalent and total protein in terms of bovine plasma albumin concentration. In case of the mated females the measurements of the eggs were continued even after the ovulation. The weight of the ootheca was also taken into consideration.
as an additional criterion for the egg development. The eggs were removed from the ootheca in order to measure their length.

Data on trypsin activity, as related to the age since emergence, were analyzed by means of the least square method to determine if there was any linear relationship between them. The results were analyzed by an IBM 7094 computer (The Ohio State University). Since a linear function could not be fitted to the above results they were further assorted for the fitting of polynomial curves of second and third degree. The polynomial expressions were obtained from an IBM 1620 computer (The Ohio Agricultural Experiment Station, Wooster) and evaluated to obtain the theoretical values of Y for the corresponding values on the X axis. Second and third degree polynomial curves were drawn by joining these points. Mean values of the average oocyte length were plotted to represent the oocyte development against the age of the roach since emergence. Linear regression lines were fitted for the total protein values against the age by means of the least square method from the IBM 7094 computer.

The graphs were drawn in two sets. The first set of figures represents the trypsin activity values with the polynomial curve and the oocyte length versus age. The second set shows the distribution of total protein values with the regression line and the polynomial curve of the trypsin activity versus age.

In the case of mated females on normal diet the trypsin activity increased with the development of the oocytes until ovulation and then started decreasing gradually as shown in Figure 5.
This increase coincided with the period of active deposition of yolk in the oocytes. The oocytes shortened in length and increased during the ovulation period and then continued to grow by absorbing water. Hence there is a slight dip in the oocyte growth curve as it is based on length measurements. Another feature of the mated condition was that there was a rapid growth of oocytes following mating.

The oocyte development in unmated females on normal diet was slow and gradual as seen in Figure 6. There was no high initial growth rate as was seen in the mated ones. Trypsin activity increased as the oocytes developed until the commencement of ovulation. But thereafter, unlike the mated condition, the activity further increased until it reached a peak and then dropped off rapidly.

The growth rate of the oocytes of starved, unmated females was still lower and the oocytes never reached their full length before ovulation as shown in Figure 7. Carbohydrate-fed unmated cockroaches (Fig. 10) also showed an initial lowering of trypsin activity like that of starved ones. But after about 20 days it started rising gradually. The oocyte growth was gradual and they reached their maximum length before ovulation. In the protein-fed unmated females (Fig. 9) there was a pronounced high initial trypsin activity which decreased to its lowest value by about the 25th day and then started rising. However, the oocytes reached their full length before ovulation.
In all the above categories of unmated females the oocytes started dehydrating when they reached the uterus; they turned yellow and translucent and were finally aborted. As for the trypsin activity in all these cases there was a general trend of increase even before the oocytes reached maturity.

**Graphical representation of the results of total protein content**

The total protein content of the midgut of the mated females remained nearly the same throughout this period though the trypsin activity showed a rise and then a fall as seen in Figure 11. In the unmated females there was a slight rise in the total protein content whereas in the starved ones there was a gradual fall (Fig. 8 and 12). In all the above cases initial amount of protein was almost the same. But in carbohydrate-fed and protein-fed females there was a high initial content of total protein which decreased in a rather steep slope. However, one can see from Figures 13 and 14 that there is no direct relationship between total protein content and trypsin activity in the protein-fed and the carbohydrate-fed cockroaches.
### TABLE 1
**TRYPSIN ACTIVITY OF VARIOUS REGIONS OF ALIMENTARY CANAL**

<table>
<thead>
<tr>
<th>Regions Tested</th>
<th>Salivary Gland</th>
<th>Foregut</th>
<th>Midgut</th>
<th>Hindgut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin Eq. ug/ml</td>
<td>0</td>
<td>0.6</td>
<td>2.9</td>
<td>0.55</td>
</tr>
</tbody>
</table>

### TABLE 2
**EFFECT OF INCUBATION TIME ON THE TRYPSIN ACTIVITY OF HOMOGENATE**

<table>
<thead>
<tr>
<th>Time of Incubation (minutes)</th>
<th>Klett Readings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homogenate</td>
</tr>
<tr>
<td>15</td>
<td>48</td>
</tr>
<tr>
<td>30</td>
<td>87</td>
</tr>
<tr>
<td>45</td>
<td>120</td>
</tr>
<tr>
<td>60</td>
<td>135</td>
</tr>
<tr>
<td>75</td>
<td>150</td>
</tr>
<tr>
<td>90</td>
<td>155</td>
</tr>
<tr>
<td>105</td>
<td>160</td>
</tr>
<tr>
<td>120</td>
<td>162</td>
</tr>
</tbody>
</table>
### TABLE 3
**DATA ON MATED FEMALES FED ON NORMAL DIET**

<table>
<thead>
<tr>
<th>Number</th>
<th>Insect</th>
<th>Age Days*</th>
<th>Weight (mg)</th>
<th>Average Oocyte Length (mm)</th>
<th>Weight (mg)</th>
<th>Trypsin Eq. (ug/ml)</th>
<th>Bovine Plasma Albumin Eq. (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM1008-7</td>
<td>12</td>
<td>800</td>
<td>3.17</td>
<td>3.75</td>
<td>260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1008-8</td>
<td>12</td>
<td>730</td>
<td>3.28</td>
<td>2.25</td>
<td>220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1008-9</td>
<td>12</td>
<td>600</td>
<td>2.86</td>
<td>2.05</td>
<td>270</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1111-4</td>
<td>17</td>
<td>665</td>
<td>3.30</td>
<td>1.05</td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1111-5</td>
<td>17</td>
<td>630</td>
<td>3.68</td>
<td>0.8</td>
<td>145</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1111-10</td>
<td>17</td>
<td>670</td>
<td>3.45</td>
<td>1.5</td>
<td>345</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1111-11</td>
<td>17</td>
<td>725</td>
<td>3.53</td>
<td>0.85</td>
<td>190</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1023-1</td>
<td>22</td>
<td>790</td>
<td>4.00</td>
<td>128</td>
<td>495</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1023-2</td>
<td>22</td>
<td>775</td>
<td>3.79</td>
<td>125</td>
<td>225</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1023-3</td>
<td>22</td>
<td>800</td>
<td>3.74</td>
<td>123</td>
<td>185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1028-4</td>
<td>27</td>
<td>820</td>
<td>3.95</td>
<td>122</td>
<td>320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1028-5</td>
<td>27</td>
<td>725</td>
<td>3.95</td>
<td>112</td>
<td>300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1028-6</td>
<td>27</td>
<td>735</td>
<td>3.95</td>
<td>102</td>
<td>390</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1229-1</td>
<td>32</td>
<td>790</td>
<td>3.68</td>
<td>130</td>
<td>285</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1229-2</td>
<td>32</td>
<td>990</td>
<td>3.68</td>
<td>152</td>
<td>475</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1229-3</td>
<td>32</td>
<td>860</td>
<td>3.68</td>
<td>128</td>
<td>325</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1119-12</td>
<td>38</td>
<td>635</td>
<td>3.95</td>
<td>138</td>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1119-13</td>
<td>38</td>
<td>765</td>
<td>3.68</td>
<td>148</td>
<td>250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1119-14</td>
<td>38</td>
<td>660</td>
<td>3.95</td>
<td>140</td>
<td>315</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1119-15</td>
<td>38</td>
<td>690</td>
<td>3.66</td>
<td>160</td>
<td>205</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM0120-1</td>
<td>47</td>
<td>675</td>
<td>4.73</td>
<td>172</td>
<td>1.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM0120-2</td>
<td>47</td>
<td>865</td>
<td>4.99</td>
<td>210</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM0120-3</td>
<td>47</td>
<td>705</td>
<td>4.73</td>
<td>190</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1105-1</td>
<td>52</td>
<td>925</td>
<td>5.14</td>
<td>267</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1105-2</td>
<td>52</td>
<td>940</td>
<td>5.14</td>
<td>240</td>
<td>0.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1105-3</td>
<td>52</td>
<td>855</td>
<td>4.74</td>
<td>230</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1105-8</td>
<td>54</td>
<td>740</td>
<td>4.74</td>
<td>203</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mated on the seventh day.*
<table>
<thead>
<tr>
<th>Number Insect</th>
<th>Age Days</th>
<th>Weight (mg)</th>
<th>Average Oocyte Length (mm)</th>
<th>Trypsin Eq. (ug/ml)</th>
<th>Bovine Plasma Albumin Eq. (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1107-1</td>
<td>5</td>
<td>735</td>
<td>1.30</td>
<td>2.30</td>
<td>220</td>
</tr>
<tr>
<td>S1107-2</td>
<td>5</td>
<td>740</td>
<td>1.32</td>
<td>1.25</td>
<td>255</td>
</tr>
<tr>
<td>S1107-3</td>
<td>5</td>
<td>725</td>
<td>1.28</td>
<td>1.05</td>
<td>205</td>
</tr>
<tr>
<td>S1009-4</td>
<td>10</td>
<td>770</td>
<td>2.37</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>S1009-5</td>
<td>10</td>
<td>575</td>
<td>2.26</td>
<td>3.95</td>
<td>150</td>
</tr>
<tr>
<td>S1009-6</td>
<td>10</td>
<td>695</td>
<td>2.42</td>
<td>3.3</td>
<td>200</td>
</tr>
<tr>
<td>S1011-4</td>
<td>15</td>
<td>735</td>
<td>2.69</td>
<td>1.8</td>
<td>310</td>
</tr>
<tr>
<td>S1011-5</td>
<td>15</td>
<td>660</td>
<td>2.97</td>
<td>2.1</td>
<td>350</td>
</tr>
<tr>
<td>S1011-6</td>
<td>15</td>
<td>725</td>
<td>2.79</td>
<td>2.05</td>
<td>350</td>
</tr>
<tr>
<td>S1013-4</td>
<td>20</td>
<td>570</td>
<td>2.42</td>
<td>1.25</td>
<td>185</td>
</tr>
<tr>
<td>S1013-5</td>
<td>20</td>
<td>635</td>
<td>2.93</td>
<td>2.8</td>
<td>215</td>
</tr>
<tr>
<td>S1013-6</td>
<td>20</td>
<td>565</td>
<td>2.48</td>
<td>1.0</td>
<td>210</td>
</tr>
<tr>
<td>S1019-1</td>
<td>25</td>
<td>735</td>
<td>3.05</td>
<td>5.9</td>
<td>210</td>
</tr>
<tr>
<td>S1019-2</td>
<td>25</td>
<td>725</td>
<td>3.16</td>
<td>2.85</td>
<td>260</td>
</tr>
<tr>
<td>S1019-3</td>
<td>25</td>
<td>725</td>
<td>3.42</td>
<td>2.15</td>
<td>230</td>
</tr>
<tr>
<td>S1030-5</td>
<td>30</td>
<td>850</td>
<td>3.42</td>
<td>6.1</td>
<td>260</td>
</tr>
<tr>
<td>S1030-6</td>
<td>30</td>
<td>845</td>
<td>3.16</td>
<td>4.6</td>
<td>125</td>
</tr>
<tr>
<td>S1030-7</td>
<td>30</td>
<td>780</td>
<td>4.21</td>
<td>6.5</td>
<td>170</td>
</tr>
<tr>
<td>S1020-7</td>
<td>35</td>
<td>925</td>
<td>3.26</td>
<td>2.1</td>
<td>385</td>
</tr>
<tr>
<td>S1020-8</td>
<td>35</td>
<td>785</td>
<td>x</td>
<td>4.6</td>
<td>240</td>
</tr>
<tr>
<td>S1020-9</td>
<td>35</td>
<td>730</td>
<td>x</td>
<td>7.1</td>
<td>245</td>
</tr>
<tr>
<td>S1102-4</td>
<td>40</td>
<td>725</td>
<td></td>
<td>4.8</td>
<td>500</td>
</tr>
<tr>
<td>S1102-5</td>
<td>40</td>
<td>840</td>
<td></td>
<td>2.1</td>
<td>730</td>
</tr>
<tr>
<td>S1102-6</td>
<td>40</td>
<td>890</td>
<td></td>
<td>4.6</td>
<td>345</td>
</tr>
<tr>
<td>S1025-1</td>
<td>44</td>
<td>900</td>
<td></td>
<td>2.3</td>
<td>315</td>
</tr>
<tr>
<td>S1025-2</td>
<td>44</td>
<td>665</td>
<td></td>
<td>7.2</td>
<td>150</td>
</tr>
<tr>
<td>S1025-3</td>
<td>44</td>
<td>815</td>
<td></td>
<td>10+</td>
<td>825</td>
</tr>
<tr>
<td>S1121-1</td>
<td>45</td>
<td>785</td>
<td></td>
<td>10+</td>
<td>315</td>
</tr>
<tr>
<td>S1121-2</td>
<td>45</td>
<td>730</td>
<td></td>
<td>10+</td>
<td>175</td>
</tr>
<tr>
<td>S1102-10</td>
<td>50</td>
<td>830</td>
<td></td>
<td>2.15</td>
<td>355</td>
</tr>
<tr>
<td>S1102-11</td>
<td>50</td>
<td>780</td>
<td></td>
<td>3.75</td>
<td>420</td>
</tr>
<tr>
<td>S1102-12</td>
<td>50</td>
<td>700</td>
<td></td>
<td>5.05</td>
<td>315</td>
</tr>
<tr>
<td>S1120-7</td>
<td>55</td>
<td>745</td>
<td></td>
<td>2.1</td>
<td>380</td>
</tr>
<tr>
<td>S1120-8</td>
<td>55</td>
<td>685</td>
<td></td>
<td>1.9</td>
<td>260</td>
</tr>
<tr>
<td>S1120-9</td>
<td>55</td>
<td>620</td>
<td></td>
<td>1.5</td>
<td>285</td>
</tr>
<tr>
<td>S1120-10</td>
<td>55</td>
<td>770</td>
<td></td>
<td>1.1</td>
<td>255</td>
</tr>
<tr>
<td>S1103-10</td>
<td>60</td>
<td>860</td>
<td></td>
<td>1.9</td>
<td>335</td>
</tr>
<tr>
<td>S1103-11</td>
<td>60</td>
<td>815</td>
<td></td>
<td>1.6</td>
<td>460</td>
</tr>
<tr>
<td>S1103-12</td>
<td>60</td>
<td>600</td>
<td></td>
<td>2.45</td>
<td>215</td>
</tr>
<tr>
<td>Number Insect</td>
<td>Age (Days)</td>
<td>Weight (mg)</td>
<td>Average Oocyte Length (mm)</td>
<td>Trypsin Eq. (ug/ml)</td>
<td>Bovine Plasma Albumin Eq. (ug/ml)</td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
<td>-------------</td>
<td>---------------------------</td>
<td>--------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>ST1015-1</td>
<td>5</td>
<td>565</td>
<td>1.26</td>
<td>1.8</td>
<td>290</td>
</tr>
<tr>
<td>ST1015-2</td>
<td>5</td>
<td>730</td>
<td>1.37</td>
<td>1.05</td>
<td>170</td>
</tr>
<tr>
<td>ST1015-3</td>
<td>5</td>
<td>730</td>
<td>1.32</td>
<td>1.6</td>
<td>200</td>
</tr>
<tr>
<td>ST1020-1</td>
<td>10</td>
<td>535</td>
<td>1.97</td>
<td>1.15</td>
<td>200</td>
</tr>
<tr>
<td>ST1020-2</td>
<td>10</td>
<td>550</td>
<td>1.89</td>
<td>2.0</td>
<td>240</td>
</tr>
<tr>
<td>ST1020-3</td>
<td>10</td>
<td>710</td>
<td>1.44</td>
<td>1.45</td>
<td>220</td>
</tr>
<tr>
<td>ST1025-1</td>
<td>15</td>
<td>530</td>
<td>1.92</td>
<td>1.15</td>
<td>215</td>
</tr>
<tr>
<td>ST1025-2</td>
<td>15</td>
<td>500</td>
<td>1.97</td>
<td>0.9</td>
<td>190</td>
</tr>
<tr>
<td>ST1025-3</td>
<td>15</td>
<td>580</td>
<td>1.97</td>
<td>0.6</td>
<td>125</td>
</tr>
<tr>
<td>ST1103-1</td>
<td>20</td>
<td>645</td>
<td>2.15</td>
<td>1.2</td>
<td>230</td>
</tr>
<tr>
<td>ST1103-2</td>
<td>20</td>
<td>600</td>
<td>2.42</td>
<td>0.8</td>
<td>190</td>
</tr>
<tr>
<td>ST1103-3</td>
<td>20</td>
<td>770</td>
<td>2.24</td>
<td>1.3</td>
<td>170</td>
</tr>
<tr>
<td>ST1127-1</td>
<td>25</td>
<td>505</td>
<td>2.59</td>
<td>0.85</td>
<td>130</td>
</tr>
<tr>
<td>ST1127-2</td>
<td>25</td>
<td>560</td>
<td>2.76</td>
<td>0.85</td>
<td>155</td>
</tr>
<tr>
<td>ST1127-3</td>
<td>25</td>
<td>560</td>
<td>2.59</td>
<td>0.95</td>
<td>195</td>
</tr>
<tr>
<td>ST1230-1</td>
<td>31</td>
<td>470</td>
<td>1.90</td>
<td>0.75</td>
<td>160</td>
</tr>
<tr>
<td>ST1230-2</td>
<td>31</td>
<td>550</td>
<td>2.59</td>
<td>0.4</td>
<td>130</td>
</tr>
<tr>
<td>ST1230-3</td>
<td>31</td>
<td>530</td>
<td>2.42</td>
<td>0.45</td>
<td>195</td>
</tr>
<tr>
<td>Number Insect</td>
<td>Age (Days)</td>
<td>Weight (mg)</td>
<td>Average Oocyte Length (mm)</td>
<td>Trypsin Eq. (ug/ml)</td>
<td>Bovine Plasma Albumin Eq. (ug/ml)</td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
<td>-------------</td>
<td>---------------------------</td>
<td>--------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>C1231-1</td>
<td>2</td>
<td>560</td>
<td>1.05</td>
<td>3.65</td>
<td>345</td>
</tr>
<tr>
<td>C1231-2</td>
<td>2</td>
<td>750</td>
<td>1.05</td>
<td>3.35</td>
<td>435</td>
</tr>
<tr>
<td>C1231-3</td>
<td>2</td>
<td>690</td>
<td>1.05</td>
<td>2.5</td>
<td>200</td>
</tr>
<tr>
<td>C1231-4</td>
<td>2</td>
<td>640</td>
<td>1.05</td>
<td>3.7</td>
<td>485</td>
</tr>
<tr>
<td>C1220-1</td>
<td>3</td>
<td>655</td>
<td>1.05</td>
<td>2.75</td>
<td>335</td>
</tr>
<tr>
<td>C1220-2</td>
<td>3</td>
<td>800</td>
<td>1.05</td>
<td>2.6</td>
<td>415</td>
</tr>
<tr>
<td>C1220-3</td>
<td>3</td>
<td>760</td>
<td>1.05</td>
<td>1.75</td>
<td>190</td>
</tr>
<tr>
<td>C1218-1</td>
<td>5</td>
<td>750</td>
<td>1.05</td>
<td>1.55</td>
<td>290</td>
</tr>
<tr>
<td>C1218-2</td>
<td>5</td>
<td>865</td>
<td>1.05</td>
<td>1.2</td>
<td>455</td>
</tr>
<tr>
<td>C1218-3</td>
<td>5</td>
<td>655</td>
<td>1.05</td>
<td>1.55</td>
<td>380</td>
</tr>
<tr>
<td>C1218-4</td>
<td>5</td>
<td>650</td>
<td>1.05</td>
<td>1.2</td>
<td>340</td>
</tr>
<tr>
<td>C1219-1</td>
<td>10</td>
<td>750</td>
<td>1.90</td>
<td>1.05</td>
<td>295</td>
</tr>
<tr>
<td>C1219-2</td>
<td>10</td>
<td>780</td>
<td>2.02</td>
<td>1.35</td>
<td>365</td>
</tr>
<tr>
<td>C1219-3</td>
<td>10</td>
<td>525</td>
<td>2.00</td>
<td>1.1</td>
<td>315</td>
</tr>
<tr>
<td>C1219-4</td>
<td>10</td>
<td>785</td>
<td>2.07</td>
<td>1.05</td>
<td>405</td>
</tr>
<tr>
<td>C1221-1</td>
<td>15</td>
<td>670</td>
<td>2.31</td>
<td>0.9</td>
<td>255</td>
</tr>
<tr>
<td>C1221-2</td>
<td>15</td>
<td>750</td>
<td>2.48</td>
<td>1.3</td>
<td>310</td>
</tr>
<tr>
<td>C1221-3</td>
<td>15</td>
<td>550</td>
<td>2.17</td>
<td>0.8</td>
<td>260</td>
</tr>
<tr>
<td>C1222-1</td>
<td>20</td>
<td>750</td>
<td>2.76</td>
<td>0.65</td>
<td>215</td>
</tr>
<tr>
<td>C1222-2</td>
<td>20</td>
<td>565</td>
<td>2.42</td>
<td>0.75</td>
<td>285</td>
</tr>
<tr>
<td>C1222-3</td>
<td>20</td>
<td>610</td>
<td>2.42</td>
<td>0.9</td>
<td>305</td>
</tr>
<tr>
<td>C1222-4</td>
<td>20</td>
<td>605</td>
<td>2.93</td>
<td>0.5</td>
<td>170</td>
</tr>
<tr>
<td>C1222-5</td>
<td>25</td>
<td>670</td>
<td>2.76</td>
<td>0.55</td>
<td>250</td>
</tr>
<tr>
<td>C1222-6</td>
<td>25</td>
<td>680</td>
<td>2.84</td>
<td>0.5</td>
<td>195</td>
</tr>
<tr>
<td>C1222-7</td>
<td>25</td>
<td>730</td>
<td>3.21</td>
<td>1.3</td>
<td>310</td>
</tr>
<tr>
<td>C1222-8</td>
<td>25</td>
<td>590</td>
<td>2.76</td>
<td>0.9</td>
<td>103</td>
</tr>
<tr>
<td>C0122-1</td>
<td>30</td>
<td>730</td>
<td>2.93</td>
<td>0.8</td>
<td>190</td>
</tr>
<tr>
<td>C0122-2</td>
<td>30</td>
<td>700</td>
<td>3.11</td>
<td>0.65</td>
<td>110</td>
</tr>
<tr>
<td>C0122-3</td>
<td>30</td>
<td>770</td>
<td>3.11</td>
<td>1.8</td>
<td>110</td>
</tr>
<tr>
<td>C1223-1</td>
<td>35</td>
<td>680</td>
<td>-</td>
<td>1.05</td>
<td>240</td>
</tr>
<tr>
<td>C1223-2</td>
<td>35</td>
<td>830</td>
<td>-</td>
<td>1.8</td>
<td>690</td>
</tr>
<tr>
<td>C1223-3</td>
<td>35</td>
<td>810</td>
<td>-</td>
<td>2.45</td>
<td>585</td>
</tr>
<tr>
<td>Number Insect</td>
<td>Age (Days)</td>
<td>Weight (mg)</td>
<td>Length (mm)</td>
<td>Trypsin Eq. (ug/ml)</td>
<td>Albumin Eq. (ug/ml)</td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
<td>-------------</td>
<td>-------------</td>
<td>---------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>P1214-1</td>
<td>2</td>
<td>790</td>
<td>1.05</td>
<td>4.4</td>
<td>300</td>
</tr>
<tr>
<td>P1214-2</td>
<td>2</td>
<td>740</td>
<td>1.18</td>
<td>2.55</td>
<td>240</td>
</tr>
<tr>
<td>P1214-3</td>
<td>2</td>
<td>635</td>
<td>1.05</td>
<td>5.0</td>
<td>380</td>
</tr>
<tr>
<td>P1213-1</td>
<td>3</td>
<td>855</td>
<td>1.18</td>
<td>3.9</td>
<td>395</td>
</tr>
<tr>
<td>P1213-2</td>
<td>3</td>
<td>760</td>
<td>1.05</td>
<td>3.75</td>
<td>200</td>
</tr>
<tr>
<td>P1213-3</td>
<td>3</td>
<td>605</td>
<td>1.05</td>
<td>5.0</td>
<td>265</td>
</tr>
<tr>
<td>P1211-1</td>
<td>5</td>
<td>880</td>
<td>1.05</td>
<td>4.35</td>
<td>465</td>
</tr>
<tr>
<td>P1211-2</td>
<td>5</td>
<td>810</td>
<td>1.18</td>
<td>2.75</td>
<td>285</td>
</tr>
<tr>
<td>P1211-3</td>
<td>5</td>
<td>740</td>
<td>1.05</td>
<td>5.7</td>
<td>405</td>
</tr>
<tr>
<td>P1213-4</td>
<td>10</td>
<td>515</td>
<td>1.90</td>
<td>3.35</td>
<td>255</td>
</tr>
<tr>
<td>P1213-5</td>
<td>10</td>
<td>630</td>
<td>2.07</td>
<td>3.1</td>
<td>270</td>
</tr>
<tr>
<td>P1213-6</td>
<td>10</td>
<td>670</td>
<td>1.90</td>
<td>3.75</td>
<td>215</td>
</tr>
<tr>
<td>P1224-1</td>
<td>15</td>
<td>680</td>
<td>2.42</td>
<td>2.05</td>
<td>260</td>
</tr>
<tr>
<td>P1224-2</td>
<td>15</td>
<td>590</td>
<td>2.42</td>
<td>2.25</td>
<td>335</td>
</tr>
<tr>
<td>P1224-3</td>
<td>15</td>
<td>690</td>
<td>2.27</td>
<td>2.3</td>
<td>235</td>
</tr>
<tr>
<td>P1224-4</td>
<td>15</td>
<td>780</td>
<td>2.42</td>
<td>2.35</td>
<td>235</td>
</tr>
<tr>
<td>P1214-4</td>
<td>20</td>
<td>580</td>
<td>2.37</td>
<td>2.25</td>
<td>215</td>
</tr>
<tr>
<td>P1214-5</td>
<td>20</td>
<td>640</td>
<td>2.10</td>
<td>1.7</td>
<td>220</td>
</tr>
<tr>
<td>P1224-5</td>
<td>20</td>
<td>630</td>
<td>2.59</td>
<td>1.4</td>
<td>200</td>
</tr>
<tr>
<td>P1224-6</td>
<td>20</td>
<td>710</td>
<td>2.76</td>
<td>1.25</td>
<td>220</td>
</tr>
<tr>
<td>P1224-7</td>
<td>25</td>
<td>640</td>
<td>2.93</td>
<td>0.9</td>
<td>170</td>
</tr>
<tr>
<td>P1224-9</td>
<td>25</td>
<td>575</td>
<td>2.76</td>
<td>1.0</td>
<td>170</td>
</tr>
<tr>
<td>P1224-10</td>
<td>25</td>
<td>500</td>
<td>2.59</td>
<td>1.05</td>
<td>175</td>
</tr>
<tr>
<td>P1211-4</td>
<td>30</td>
<td>860</td>
<td>3.31</td>
<td>2.8</td>
<td>300</td>
</tr>
<tr>
<td>P1211-5</td>
<td>30</td>
<td>510</td>
<td>2.07</td>
<td>2.5</td>
<td>245</td>
</tr>
<tr>
<td>P1211-6</td>
<td>30</td>
<td>570</td>
<td>2.35</td>
<td>2.35</td>
<td>290</td>
</tr>
</tbody>
</table>
Fig. 1.—Distribution of the trypsin activity in the alimentary canal of the cockroach.
Fig. 2.—Effect of incubation time of the homogenate and substrate mixture at 37°C on trypsin activity in midgut of the cockroach.
Trypsin (2X, salt free) µg/ml (prepared in pH 2.5, CaCl$_2$ buffer)

**Fig. 3.**—Standard curve for trypsin activity, incubated for one hour at 37°C with TAME substrate.
Fig. 4.—Standard curve for the total protein determination.
Trypsin activity and the oocyte development in the normal diet, mated female cockroaches from the day of adult emergence.
Fig. 6.—Trypsin activity and the oocyte development in unmated females on normal diet.
Bovine plasma albumin x 100 μg/ml

Fig. 7—Trypsin activity and the oocyte development in the starved, unmated females.

Fig. 8—Total protein and the trypsin activity in starved, unmated females.
Fig. 9.—Trypsin activity and the oocyte development in the protein-fed unmated females.

Fig. 10.—Trypsin activity and the oocyte development in carbohydrate-fed unmated females.
Fig. 11.—Total protein and the trypsin activity in normal diet, mated females.
Fig. 12.—Total protein and the trypsin activity in normal diet, unmated females.
Fig. 13.—Total protein and the trypsin activity in protein-fed, unmated females.

Fig. 14.—Total protein and the trypsin activity in carbohydrate-fed, unmated females.
DISCUSSION AND CONCLUSIONS

Hormones are important in the sequential and quantitative control of various physiological processes in insects. Reproduction is one such major process that involves the hormonal control of protein synthesis. There appears to be a direct correlation between the proteolytic enzyme activity and the reproductive organs. The enzyme activity provides metabolites, e.g., amino acids, dipeptides and polypeptides, via the haemolymph, needed for the protein synthesis in the reproductive organs.

From the mode of trypsin activity found in the mated females it becomes evident that the enzyme titer in the midgut was higher when there was a demand for protein synthesis in the ovaries. During the period of egg development and yolk deposition the proteolytic enzyme showed a higher activity, presumably to supply the metabolites for the protein synthesis. When oocyte maturation was complete the enzyme activity diminished. Further increase in size of the eggs was accounted for by their absorption of water without the need for further protein synthesis.

Several workers observed that when females were kept with males the eggs matured rapidly. Norris (1954) reported that in the desert locust there was an increased maturation in females when kept along with the males. Highnam (1962) has shown that the neurosecretory system releases material into the haemolymph
shortly before ovarian development begins and continues this release throughout the major part of ovarian development. Toward the end of yolk deposition the release of the material ceases. Trypsin activity, as observed in the present work, apparently coincides with the appearance and disappearance of the above described neurosecretory material. Let us assume a trypsin inhibitor factor responsible for the lowering of the trypsin activity in the mated females. Since this factor appears, in the mated females, just at the completion or termination of oocyte maturation, we can associate it with the above neurosecretory material. Highnam (1962) reported that the material was released with a little delay in the females reared without males. Though this observation could not be ascertained by means of trypsin activity in the present study, there is evidence of the absence of the trypsin inhibitory factor in the unmated females. As a result the trypsin activity continued, uncontrolled, until it reached a peak, about 20 days later. So it can be concluded that the trypsin inhibitory factor is controlled by the neurosecretory material of Highnam (1962) and influenced by the mating behavior.

Hill’s (1962) observation on haemolymph protein concentration is in general agreement with the above information. He showed that in the females reared with the mature males, the haemolymph protein concentration rises as neurosecretory material is released into the haemolymph and at the end of yolk deposition it falls. In females reared without males, the protein
concentration rises only slowly and reaches a high level only when neurosecretory material is released. It is obvious that presence of the high protein concentration is due to the activity of proteolytic enzymes like trypsin. Hence the high trypsin activity is evident in the unmated females.

In case of the starved, unmated females there were at least two factors which could account for the observed lower trypsin activity, namely, the unmated condition and the lack of substrate in the form of food. In the protein-fed and the carbohydrate-fed, unmated females the trypsin activity showed a similar decline. The protein-fed ones showed a higher initial trypsin activity (in the first five days after emergence) which decreased abruptly, whereas, in the carbohydrate-fed females there was a gradual decrease of trypsin activity. However, a rise in activity was seen in both types at about the 20th to 25th day period following emergence which coincided with the decline in the trypsin activity in the mated condition. This period also coincided with the proximity of maturation of the eggs. Thus we can conclude that the trypsin inhibitor factor has a timely appearance, that is, a little before the completion of egg maturation. This factor does not appear at this stage in the unmated condition and hence, the increased trypsin activity is accounted for. Because of a change in diet at adult emergence (all nymphs were on the normal diet) there was a decrease in the consumption of the food in case of the protein and the carbohydrate-fed
females and thus a partially starved condition was induced. The initial fall of the trypsin activity could be attributed to this condition as it reduced the amount of substrate for trypsin action.

The total protein of the midgut of mated females on normal diet remained the same during the period of study. The total protein of the midgut of the unmated females fed on normal diet increased gradually. Looking at the trypsin behavior and the ample supply of food available, the observation could be justified, that there was a greater demand for the substrate for trypsin action in the case of the unmated females on normal diet. In the starved condition the initial amount of food being depleted gradually, the total protein content showed a reduction. Similarly, the reduction in the protein-fed and the carbohydrate-fed, unmated females was probably due to the lesser consumption of food.

Thus in the opinion of the author, the most important contribution from the present work is the concept of a trypsin inhibitor factor. This factor is probably of neurosecretory origin and associated with the mating behavior. It appears prior to ovulation and inhibits the trypsin activity soon after maturation, thus bringing about the control of trypsin in the normal, mated condition. Where mating is prevented this factor is not released, regardless of the nature of the food, hence its absence in unmated females. The appearance of this factor is physiologically timed and in the absence of this factor there is a rise
in the trypsin activity. Data given here are suggestive, but do not prove the presence of an inhibitory factor as such. However, this concept serves to explain the rise and fall of trypsin activity in the cockroach.
SUMMARY

1. The trypsin activity of mated female Nauphoeta cinerea cockroaches fed a normal diet increased during maturation of the oocytes and receded after ovulation. The oocyte development was rapid and the total protein content of the midgut remained almost constant during the period of study. Following ovulation and the formation of the ootheca there was no increase in trypsin activity though the eggs continued to increase in size inside the oothecal sac within the vestibule.

2. In the unmated females fed a normal diet the trypsin activity increased even after ovulation. The reason for this is presumed to be the lack of some kind of trypsin inhibitor factor, presumably of neurosecretory origin which, in the normally fed and the mated females, was stimulated by the association with the male. The total protein content of the midgut increased during the period of study and rate of growth of the oocytes was slow, followed by resorption and abortion.

3. Starved unmated females showed a decrease in the trypsin activity. The oocyte development was gradual and did not reach usual size before ovulation. The total protein content of the midgut declined considerably.

4. The protein-fed and the carbohydrate-fed, unmated females showed progressive decreases in the trypsin activity. In
the former there was a high initial increase in activity which
decreased abruptly, whereas, in the latter there was no initial
increase and the decrease was gradual. The protein content of
the midgut, however, decreased considerably in both. The oocytes
attained full length before ovulation. However, the oothecae
were aborted.

5. A very specific, sensitive and convenient colorimetric
micromethod, utilizing TAME (p-toluenesulfonyl-L-arginine methyl
ester HCl) has been adopted from Siegelman et al. (1962) as the
standard method for trypsin estimation.

6. The Folin-Ciocalteau phenol procedure for the
estimation of total protein was a modification of the one
described by Lowry et al. (1951).
LITERATURE CITED


Bergmann, M., and J. S. Fruton (1941). The specificity of proteinases advances in enzymology and related subjects. 1:63.


Gresham, W. B. (1952). Nauphoeta cinerea (Olivier) established in Florida. Florida Ent. 35:77.


