Japanese Society of Applied Entomology and Zoology

App. Ent. Zool. **12** (2): 134—141 (1977)

Histological and Histochemical Observation of Intracellular Yeastlike Symbiotes in the Fat Body of the Smaller Brown Planthopper, *Laodelphax striatellus* (Homoptera : Delphacidae)

Hiroaki NODA

Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464, Japan

(Received December 17, 1976)

Yeastlike symbiotes were observed in some cells (mycetocytes) of the fat body in the smaller brown planthopper, *Laodelphax striatellus.* The symbiotes were oval-shaped, about 13μ in length and seemed to propagate by budding. They had a FEULGEN positive nucleus, and were positive to RNA and protein reactions and were strongly stained by PAS reaction. They increased in the mycetocytes with nymphal development, and a large number of them was observed in female adults while the male adults did not possess so many of them. The yeastlike symbiotes moved into the primary oocyte from the posterior pole through the epithelial plug of the ovary in female adult. At the late stage of embryonic development of the next generation, the symbiotes entered the fat body of the abdomen. The mycetocytes had no proteinaceous spheres and had less lipid droplets than the fat body cells.

INTRODUCTION

Some insects are associated with an internal flora of microorganisms. The microorganisms which live in the host cells are generally referred to as intracellular symbiotes and these cells are called mycetocytes (RICHARDS and BROOKS, 1958). Investigators are interested in the symbiotes of insects with a view to insect pathology (BROOKS, 1963), insect nutrition(BROOKS and KRINGEN, 1972; PANT and DANG, 1972), and plant pathology (NASU, 1965). Intracellular symbiotes of some hemipterous insects have been fairly well investigated. They are transmitted through the ovary to the next host generation (BUCHNER, 1965). Some hemipterous insect, aphids and leafhoppers, possess mycetomes as the organ for symbiotes; but the rice planthoppers, *Laodelphax striatellus*, *Nilaparvata lugens*, and *Sogatella furcifera*, do not have such a special organ. The rice planthoppers harbor the yeastlike symbiotes in the fat body and transmit them to the next generation through the ovary (NASU, 1963). MITSUHASHI (1975) attempted the culture of the yeastlike symbiotes of *L. striatellus.*

In this paper, the morphological and histochemical natures of yeastlike symbiotes in *L. striatellus* are investigated, and the fat body cells and the mycetocytes for symbiote habitat are examined. The ovarian transmission of yeastlike symbiotes is also described.

NII-Electronic Library Service

Japanese Society of Applied Entomology and Zoology

MATERIALS AND METHODS

Histological preparations. *Laodelphax striatellus* (Fallén), collected at Kitashirakawa in Kyoto 1965, were reared on rice seedlings at 25°C under a 16-hr light, 8-hr dark cycle. Fresh insects or eggs were fixed in Bouin's mixture or Bodian II mixture. Materials were then washed in 90% and 80% ethanol respectively and dehydrated in butanol series. After embedding in paraffin wax, they were sectioned at 5 to 7μ, and stained with Mayer's haematoxylin-eosin, gentian violet, toluidin blue, or Giemsa's solution buffered at pH 5.

Histochemical techniques. The histochemical techniques were mostly employed by reference to Lison (1960) and Sano (1972).

For nucleic acids, materials were fixed in Bodian II mixture. Feulgen reaction was carried out for the demonstration of DNA. Methyl green-pyronin method was used for both DNA and RNA. Sections were also stained with toluidin blue at pH 4.8 for RNA. Ribonuclease test with 0.02% RNase (Type I-A, Sigma Chemical Co., which was heated at 90°C for 20 min before use) at 65°C for 2 hr was used for the detection of RNA. Nucleic acids were also extracted with HClO.

For the demonstration of protein, Millon reaction and ninhydrin-Schiff reaction were employed in Bouin's mixture, Bodian II mixture, of formalin fixed paraffin sections. Chèvremont et Frédéric's test was also used in frozen sections. Materials were fixed in 10% formalin and embedded in a small block of pig liver. This block was frozen in a dry ice-acetone bath and were sectioned at about 10 μ with a cryostat (SLEE, Type H) at −20°C. The control slides were dipped in saturated $HgCl_2$ solution at 25°C for 60 min before staining. Proteins were also stained with fast green at pH 1.2 (King, 1960) and at pH 8.1 (Alfert and Geschwind, 1953).

Carbohydrates were shown by PAS reaction. For the demonstration of glycogen salivary test was employed at 37°C for 60 min before PAS reaction. The tissues were fixed in Carnoy's mixture and the sections were covered with a collodion membrane in order to prevent glycogen from dissolving during PAS reaction. Alcian blue also was used at pH 2.6 for acid mucopolysaccharides.

For lipids, materials were fixed in formalin-calcium, and the frozen sections were made as afore-mentioned. Sudan black B was employed for the demonstration of lipids. Neutral fats were demonstrated with 1% Nile blue. Liebermann-Schultz's test was also carried out for cholesterol.

RESULTS

Distribution and histochemistry of yeastlike symbiotes

Microorganisms, which were similar to the yeast in shape, were recognized in the fat body of *L. striatellus* (Fig. 1). These yeastlike symbiotes were oval-shaped and some of them were budding (Fig. 2). Their size varied and was about 13 μ in length on an average. The vegetative reproduction by budding was only observed in the cells in laboratory cultured insects. Insects harbored them only in the fat body of the abdomen except mature female adults which also possessed them in the ovarioles. The yeastlike symbiotes were never observed in the head and thorax.

In the second-instar nymphs, the yeastlike symbiotes were scattered over the fat body, and in the third-instar nymphs they were found in small groups. In the fourth-

NII-Electronic Library Service

Japanese Society of Applied Entomology and Zoology

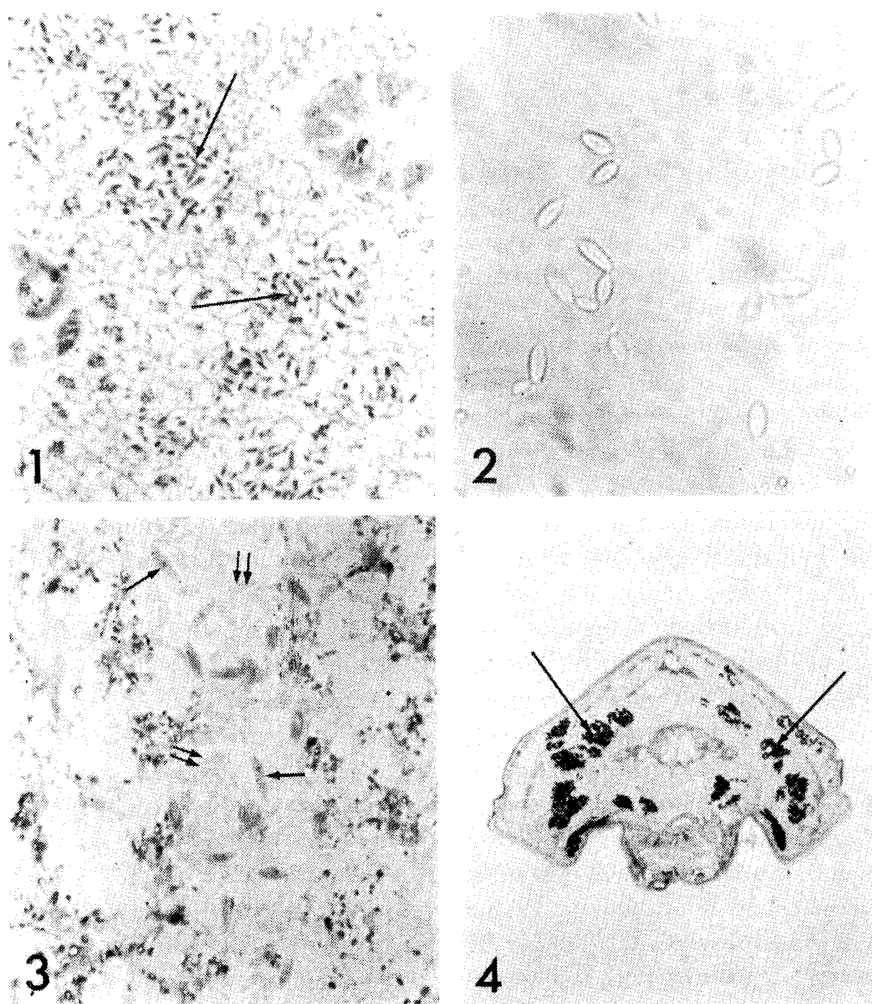

Fig. 1. Yeastlike symbiotes (arrow) in the fat body of nymph (×270)
Fig. 2. Yeastlike symbiotes (×400)
Fig. 3. Methyl green-pyronin test after 1-hr RNase digestion; arrow, stained yeastlike symbiote; double arrow, non-stained yeastlike symbiote (nymph, ×600)
Fig. 4. Distribution of yeastlike symbiotes (arrow) stained by PAS reaction in the cross section of nymph (×60)

and fifth-instar nymphs, they were gathered locally in large groups and increased in number. The female adults harbored a large number of symbiotes in the fat body (Fig. 6). On the other hand, the male adults did not possess so many symbiotes, though just after emergence they possessed a certain number of them. The above observations are in good accord with the previous results in the population change of yeastlike symbiotes (NODA, 1974).

A part of the yeastlike symbiotes, as well as the nuclei of the host cells, was stained pink by the FEULGEN test. This FEULGEN positive part appeared to be the nucleus of the symbiotes, indicating the presence of DNA. SCHIFF's reagent did not color the non-hydrolyzed sections and the reaction following more than 20-min hydrolysis showed weak staining. In pyronin and methyl green the cytoplasm of the symbiotes stained

NII-Electronic Library Service

Japanese Society of Applied Entomology and Zoology

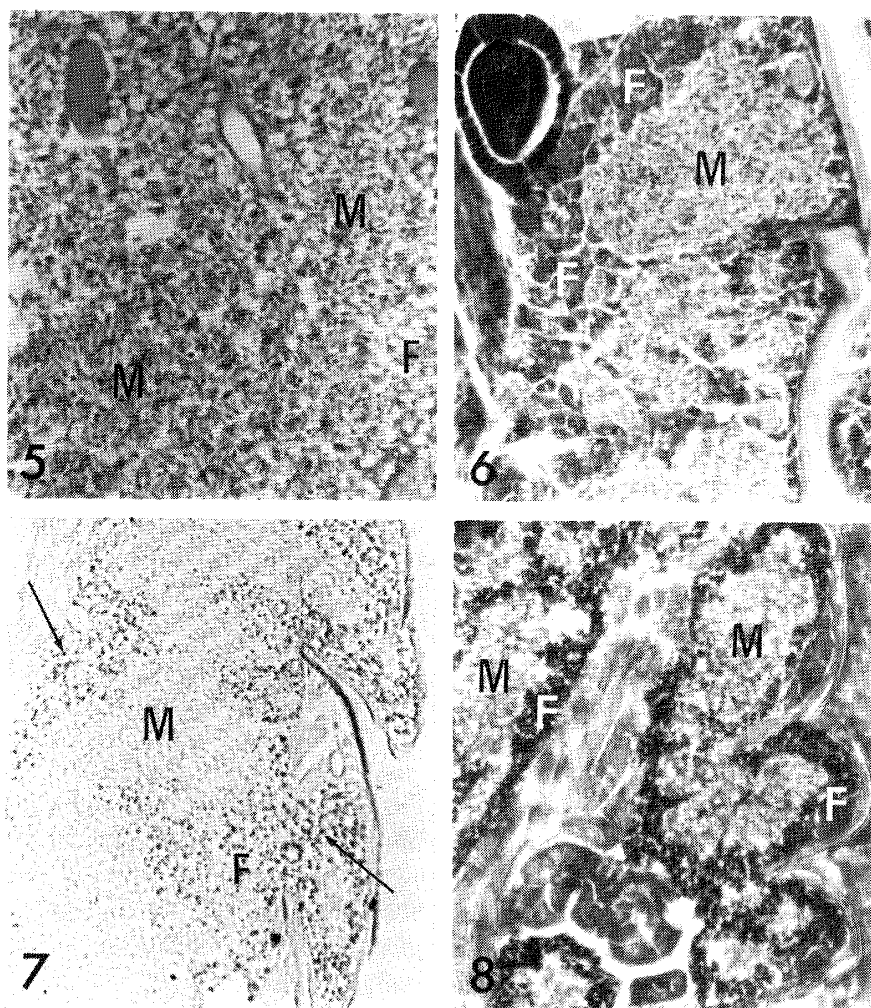

Fig. 5. Fat body cells (F) and mycetocytes (M) of 0-day-old brachypterous female adult (×220)

Fig. 6. Fat body cells (F) and mycetocytes (M) of 2-day-old brachypterous female adult (×220)

Fig. 7. Granules (arrow) in the fat body cells (F) stained by MILLON reaction (0-day-old female adult, ×170)

Fig. 8. Lipid droplets in the frozen section stained with Sudan black B (female adult, ×100)

red and the nucleus colored green or dark purple. Toluidin blue also stained the symbiote red purple at pH 4.8 while most host tissues were blue or purple. When the tissues were digested for 2 hr at 65°C in RNase before staining, the symbiotes did not stain in pyronin nor toluidin blue. Some symbiotes were, however, stained in the tissues digested with RNase within 1 hr (Fig. 3). RNA of the other symbiotes and host cells easily disappeared by 1-hr RNase digestion. HClO treatment of the tissues prevented the coloration by the above nucleic acid reactions.

The symbiotes were stained pink by ninhydrin-SCHIFF reaction, but did not stain without oxidative deamination. The symbiotes were stained slightly by MILLON reaction. By CHÈVREMONT et FRÉDÉRIC's test, the symbiotes were stained blue, but did

NII-Electronic Library Service

Japanese Society of Applied Entomology and Zoology

not stain after $HgCl_2$ treatment. Fast green also colored them at pH 1.2 and faintly at pH 8.1.

With PAS technique, the yeastlike symbiotes were stained strongly brownish red, the cell wall seeming to stain. PAS reaction showed clearly the distribution of the symbiotes in the insect (Fig. 4). The symbiotes stained by PAS reaction even after salivary digestion. Alcian blue could not stain the symbiotes.

Sudan black B stained the small granules, which seem to be lipid droplets, in the cytoplasm of some symbiotes. The symbiotes failed to be stained by LIEBERMANN-SCHULTZ's test.

Morphology and histochemistry of mycetocytes and fat body cells

The yeastlike symbiotes were observed in some cells of the fat body. In this paper, the cells in which the symbiotes live are called mycetocytes and the other cells of the fat body which do not possess the symbiotes are called fat body cells.

In the nymphal stages, there were few significant morphological differences between mycetocytes and fat body cells except that the fat body cells did not harbor the yeastlike symbiotes and sometimes had small granules. The figures of the fat body of both sexes just after emergence were mostly similar to those of nymphs, but there were numerous ganules in the fat body cells while the mycetocytes had no granules (Fig. 5). The granules in adults were larger than those in nymphs. In the 2-day-old brachypterous male adults, the granules were no longer found in the fat body cells of abdomen. The whole fat body became smaller instead because of the development of gonad and muscles, but the symbiotes were still found in a plentiful number. In the 4-day-old brachypterous male adults, the fat body diminished and the mycetocytes were observed in only a part of it; the number of symbiotes apparently decreased. The fat body cells of the 2-day-old brachypterous female adults did not contain the granules and the cell boundaries became clearer. The cytoplasm of the fat body cells of this stage stained more intensely than those of nymphs and male adults. The mycetocytes were observed in large groups surrounded by the fat body cells, harboring a great number of symbiotes (Fig. 6). Although the ovary of the 4-day-old brachypterous female adults was extremely well developed, the fat body remained; the mycetocytes did not diminish.

The granules which were found in the fat body cells were positive to protein reactions except ninhydrin-SCHIFF reaction. They were stained pale brown by MILLON reaction (Fig. 7) and blue by CHÈVREMONT et FRÉDÉRIC's test. Fast green also stained them green at pH 1.2. The granules were not stained with tests for carbohydrates. These granule, however, colored in pyronin even after RNase digestion (Fig. 3).

Nucleic acid reactions showed that the nuclei of mycetocytes and fat body cells had DNA and the cytoplasm of these cells was rich in RNA. The cytoplasm of these cells also stained slightly by protein reactions. There was also PAS positive substance in the cytoplasm of mycetocytes and fat body cells in the sections covered with a collodion membrane. This PAS positive substance was digested by saliva, which demonstrates that it is glycogen. In the fat body cells there were many lipid droplets which stained in Sudan black B. These lipid droplets also colored red in Nile blue, indicating that the lipid droplets consisted of neutral fats. On the other hand, in the mycetocytes there were fewer and smaller lipid droplets than in the fat body cells (Fig. 8). LIEBERMANN-SCHULTZ's test did not show typical coloration of the lipid droplets.

NII-Electronic Library Service

Japanese Society of Applied Entomology and Zoology

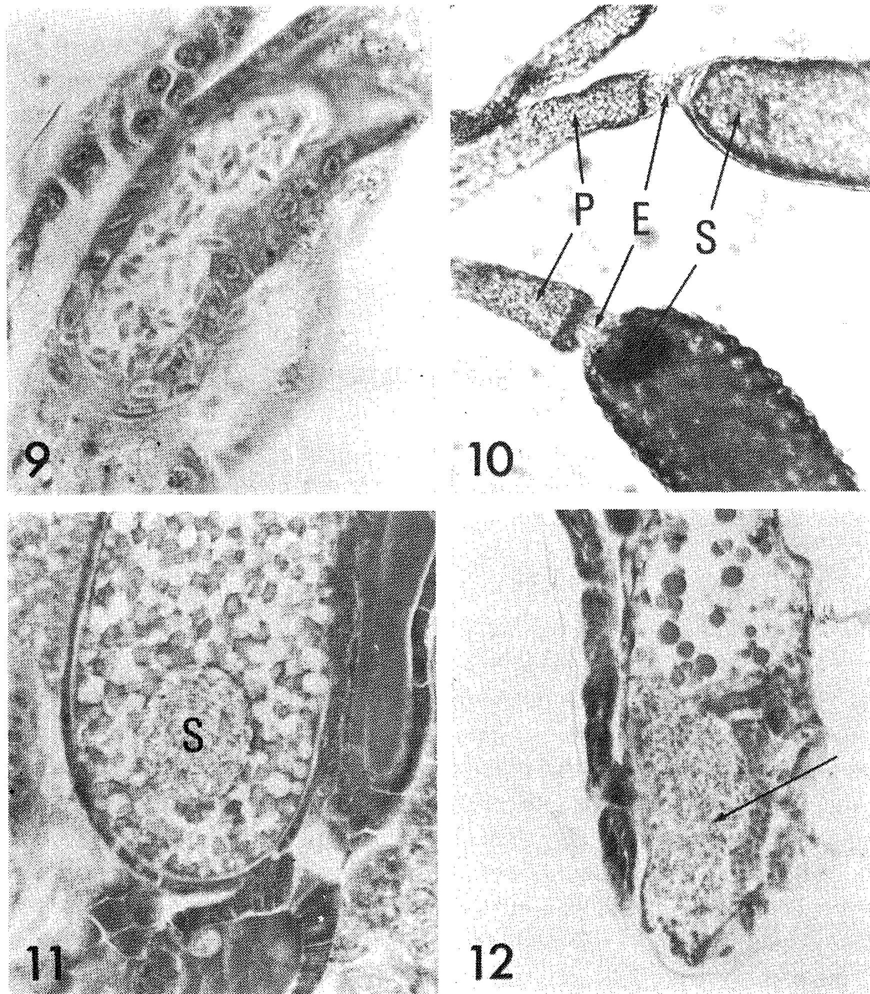

Fig. 9. Yeastlike symbiotes entering the primary oocyte (×500)
Fig. 10. Epithelial plug (E) between pedicel (P) and primary oocyte; S, symbiote ball (×160)
Fig. 11. Symbiote ball (S) in the posterior part of the egg in the female (×250)
Fig. 12. Yeastlike symbiotes (arrow) entering the fat body of embryo (×240)

Transovarial passage and embryo infection of yeastlike symbiotes

The observations of the ovary disclosed that the symbiotes infect the epithelial plug which is located between the vitellarium and pedicel (BONHAG, 1958). The yeastlike symbiotes in the epithelial plug were morphologically identical with those in the mycetocytes. These symbiotes appear to move from the mycetocytes to the epithelial plug and enter the ovariole through the epithelial plug (Fig. 9). Then, they infect the primary oocyte from the posterior pole (Fig. 9, 10). The entrance of the symbiotes in the ovariole was observed only at the posterior pole of the primary oocyte, and the yeastlike symbiotes could not be found to infect from the other parts of an ovariole. The symbiotes entered the first oocyte before yolk deposition was finished, and then the symbiote ball was formed. The symbiote ball surrounded by a membrane was buried in the yolk in the posterior part of the egg (Fig. 11). The symbiote ball consisted

NII-Electronic Library Service

Japanese Society of Applied Entomology and Zoology

of cell-like structures and symbiotes were enclosed in the egg and were transmitted to the next host generation. From the point of view of egg maturation of *L. striatellus*, it is remarkable that maturation occurred only in the primary oocyte in an ovariole.

Just after oviposition the symbiote ball was located at the posterior part of the egg, and 2 days after oviposition it was found at the anterior part. Then it moved again to the posterior part according to the embryonic development(NASU and SUENAGA, 1958). The symbiotes then entered the fat body of the abdomen from the posterior part (Fig. 12). Thus, newly hatched nymphs harbor the yeastlike symbiotes in the fat body of the abdomen.

DISCUSSION

Many kinds of yeastlike symbiotes have been reported in hemipterous insects (BUCHNER, 1965). The manner of endosymbiosis between *L. striatellus* and its yeastlike symbiotes, the symbiotes living in the fat body, seem to be one of the popular ones in hemipterous insects. The yeastlike symbiotes in *L. striatellus* appear to propagate by budding in the mycetocytes. The RNA of some symbiotes were digested within 1 hr with RNase but that of the others was not. In this respect, there may be a possibility that the types or forms of RNA are not the same among the developmental stages of the symbiotes. Another possibility is that the different types of yeastlike symbiotes co-exist in the mycetocytes.

BUCHNER (1965) stated that there are various modes of ovarian transmission of symbiotes but infection limited to the posterior pole is by far the most common method and it is constantly being varied. *L. striatellus* has telotrophic type ovary, and oocyte maturation occurs only in the primary oocyte in an ovariole. The yeastlike symbiotes in *L. striatellus* enter only through the epithelial plug and infect only the primary oocyte. Then the first oocyte finishes yolk deposition and forms the egg shell. After the primary oocyte has moved to the pedicel, the secondary oocyte contacts the epithelial plug. The symbiotes infect the next oocyte in the same manner. However, there are still problems as to how they move or are transferred to the epithelial plug from the mycetocytes and why this transmission occurs in company with ovary development. After oviposition, the yeastlike symbiotes enter only the fat body of the abdomen from the posterior part during embryonic development. This seems to be at least one reason why the yeastlike symbiotes live only in the fat body of the abdomen.

Histochemical characteristics of the fat body cells are mostly similar to those shown in other insects (NAIR and GEORGE, 1964; BUTTERWORTH et al., 1965; WAKU and SUMIMOTO, 1969). The granules in the fat body cells were stained by protein reactions and stained in pyronin even after RNase digestion. They seem to consist of protein and some other substances. In this respect, these granules should be called proteinaceous spheres, as pointed out by PRICE (1973). These proteinaceous spheres were not found in the mycetocytes. There were further differences between fat body cells and mycetocytes; the lipid droplets were richer in the fat body cells than in the mycetocytes, and the fat body cells showed morphological differences between mature female adults and nymphs though the mycetocytes did not show significant changes through a host's life. In view of the present observations, it is reasonable to say that the mycetocytes are different from the fat body cells in their function or physiology and the mycetocytes are suitable for symbiote habitat, although it is still obscure what caused

NII-Electronic Library Service

Japanese Society of Applied Entomology and Zoology

these differences between mycetocytes and fat body cells in *L. striatellus*.

ACKNOWLEDGEMENTS

The author is grateful to Prof. T. Saito of this laboratory, Prof. S. Kawase of Sericultural Laboratory of this Faculty, Dr. S. Nasu of National Institute of Agricultural Sciences, Dr. K. Sōgawa of Tropical Agriculture Research Center, and Dr. J. Kitoh of Nagoya University School of Medicine for their helpful suggestions and encouragements. Thanks are due to Mr. H. Honda for insect rearing. Acknowledgement is also made to Prof. M. A. Brooks of Department of Entomology, Fisheries and Wildlife, University of Minnesota for critical reading of this manuscript.

REFERENCES

Alfert, M. and I. I. Geschwind (1953) A selective staining method for the basic proteins of cell nuclei. *Proc. Nat. Aca. Sci.* **39**: 991-999.

Bonhag, P. F. (1958) Ovarian structure and vitellogenesis in insects. *Ann. Rev. Entomol.* **3**: 137-160.

Brooks, M. A. (1963) The microorganisms of healthy insects. In *"Insect Pathology, An Advanced Treatise* I" (ed. E. A. Steinhaus) pp. 215-250. Academic Press, New York.

Brooks, M. A. and W. B. Kringen (1972) Polypeptides and proteins as growth factors for aposymbiotic *Blattella germanica* (L.). In *"Insect and Mite Nutrition"* (ed. J. G. Rodriguez) pp. 353-364. North-Holland Publ. Co., Amsterdam.

Buchner, P. (1965) *Endosymbiosis of Animals with Plant Microorganisms.* Interscience, New York.

Butterworth, F. M., D. Bodenstein, and R. C. King (1965) Adipose tissue of *Drosophila melanogaster*. I. An experimental study of larval fat body. *J. exp. Zool.* **158**: 141-154.

King, R. C. (1960) Oogenesis in adult *Drosophila melanogaster*. IX. Studies on the cytochemistry and ultrastructure of developing oocytes. *Growth* **24**: 265-323.

Lison, L. (1960) *Histochimie et Cytochimie Animales, Principes et Methodes.* Gauthier-Villars & Co., Paris.

Mitsuhashi, J. (1975) Cultivation of intracellular yeast-like organisms in the smaller brown planthopper, *Laodelphax striatellus* Fallén (Hemiptera, Delphacidae) *Appl. Ent. Zool.* **10**: 243-245.

Nair, K. S. S. and J. C. George (1964) A histological and histochemical study of the larval fat body of *Anthrenus vorax* Waterhoue (Dermestidae: Coleoptera). *J. Insect Physiol.* **10**: 509-517.

Nasu, S. (1963) Studies on some leafhoppers and planthoppers which transmit virus disease of rice plant in Japan. *Bull. Kyushu Agr. Expt. Stn* **8**: 153-349. (In Japanese with English summary).

Nasu, S. (1965) Electron microscopic studies on transovarial passage of rice dwarf virus. *Jap. J. appl. Ent. Zool.* **9**: 225-237.

Nasu, S. and H. Suenaga (1958) On the embryonic development of planthoppers. *Bull. Kyushu Agr. expt. Stn* **5**: 71-84. (In Japanese with English summary).

Noda, H. (1974) Preliminary histological observation and population dynamics of intracellular yeast-like symbiotes in the smaller brown planthopper, *Laodelphax striatellus* (Homoptera : Delphacidae). *Appl. Ent. Zool.* **9**: 275-277.

Pant, N. C. and K. Dang (1972) Physiology and elimination of intracellular symbiotes in some stored product beetles. In *"Insect and Mite Nutrition"* (ed. J. G. Rodriguez) pp. 331-322. North-Holland Publ. Co., Amsterdam.

Price, G. M. (1973) Protein and nucleic acid metabolism in insect fat body. *Biol. Rev.* **48**: 333-375.

Richards, A. G. and M. A. Brooks (1958) Internal symbiosis in insects. *Ann. Rev. Entomol.* **3**: 37-56.

Sano, Y. (1972) *Histological Technics, Theoretical and Applied.* Nanzando Company Tokyo. (In Japanese).

Waku, Y. and K. Sumimoto (1969) Light- and electron-microscopical study of the fat cells in the metamorphosing silkworm. *Bull. Fac. Textile Fibres, Kyoto Univ. of Ind. Arts Textile Fibres* **5**: 256-287. (In Japanese with English summary).

NII-Electronic Library Service