Ultrastructure of the Midgut of the Early Third Larval Instar of Chrysomya megacephala (Diptera:Calliphoridae)

[1*]Nancy Taha, [2]Afaf Abdel-Meguid, [1]Ahmed El-ebiarie and [1]Amany.A.Tohamy

1Department of Zoology and Entomology, Faculty of Science, Helwan University,2Department of Entomology,Faculty of Science,Cairo University,Cairo, Egypt.

[*]nancyt0000@yahoo.com

Abstract: The midgut of third larval instar of *Chrysomya megacephala* was studied using transmission electron microscope. The epithelium is mostly formed of columnar cell. The cells are characterized by a striated border of microvilli, thick amorphous basal lamina and tightly packed channels of basal labyrinth. Well-developed peritrophic membrane was observed in the gut lumen to protect the mid-gut cells from possible damage by abrasive food particles.Numerous cell organelles were observed throughout the cell. The structure and function of secretion of digestive enzymes and absorption of nutrients in the mid-gut epithelium of this fly were discussed. [Journal of American Science. 2010;6(10):1-6]. (ISSN: 1545-1003).

Key Words*: Chrysomya megacephala*, mid-gut, Ultrastructure, maggot

1. Introduction

Chrysomya megacephala (F.), the Oriental latrine fly, is a common blow fly species of medical importance in many parts of the world, including Eygpt. Adults may feed on food sources including nectar, animal carcasses, garbage, and other filth materials, or even human food. Therefore, it is possible that mechanical transfer of potential disease causing pathogens, such as bacteria, viruses, protozoa, and helminth eggs, to human food may occur (Greenberg 1973; Sukontason et al. 2000). Larvae of this species are known to cause myiasis in several mammal species, including humans (Zumpt 1965; Kumarasinghe et al. 2000). Another facet of medical importance of this blow fly is its association with human corpses and its relevance to forensic entomology, *C. megacephala* were found connected with cases of human death (Lee 1996; Carvalho et al. 2000; Goff 2000; Lee et al. 2004; Sukontason et al. 2005).

The aim of this paper,was to study the fine structure of mid-gut epithelium of third larval of *C.megacephala* by transmission electron microscopy to clarify the morphology of the mid-gut to provide a structural framework for physiological interpretations of the process involved in this part of alimentary canal.

2. Material and Methods

The laboatory colony of *C. megacephala* used in this study was established in the Department of Entomology,Faculty of Science, Helwan University **.***Chrysomya megacephala* was reared according to the method of Gaber *et al.,* (2005).For TEM,approximately 20 specimens of 3-day-old larvae were removed from the rearing box and individually dissected in phosphate buffer pH of 7.4 under a binocular dissecting microscope (Olympus®, Japan). The mid-gut was separated and divided into anterior, middle and posterior mid-guts and only the middle midgut was studied. The dissected mid-gut was transferred from the phosphate buffer and prefixed with 2.5% glutaraldehyde in phosphate buffer solution at a pH of 7.4 at 4°C for 24 h to accomplish primary fixation. Then rinsed twice with phosphate buffer solution at 10-min intervals. Rinsed specimens were treated with 1% osmium tetroxide at room temperature for 30 minutes for postfixation.

Post-fixation was followed by rinsing twice with phosphate buffer solution and dehydrating with alcohol. To replace the water in the specimens with alcohol, they were subjected to ascending series of alcohol.

After that, organ specimens were placed in acetone for 2 h before transferring into ratios of resin to acetone of 1:3 for 24 h, 1:1 for 24 h, and 3:1 for 24 h, sequentially. This was followed by treatment with pure resin twice for 3 h. Each sample was then embedded in Epon resin by placing them into a plastic block and by incubating at 70°C for 24 h. Semithin section (0.5 µm) of each sample was made with a glass knife on an Ultramicrotome (Boeckeler®, USA). This was followed by staining with 1% methylene blue mixed with 1% Azure II (1:1) to view under a light microscope (Olympus®, Japan). The ultrathin sections (90 nm) were stained

with uranyl acetate and lead citrate then examined with the ZEISS EM 10 electron microscope (Germany).

3. Results

The larval mid-gut of *Chrysomya megacepha* is the longest portion of the alimentary canal lying convoluted and twisted within the larval body cavity (Worachote et al. 2007).

Ultrastructurally, the midgut consists mainly of columnar cells that possess microvilli (Figs1,14), numerous lipid spheres (Figs1,11,12), different secretory granules (Fig.2,3), ferritin granules (Fig.4), large lysosomal bodies (Fig.13) and vesicles of rough endoplasmic reticulum surrounded by numerous mitochondria (Figs5,7). The nucleus appeared clear and surrounded by numerous vesicles of rough endoplasmic reticulum (Fig.6).A well-developed peritrophic membrane can be observed (Fig.8) and also lateral cell junctions separating two different cells (Figs9,10).The cells rest on a basement membrane and a basal labyrinth where the plasma membrane contains no extracellular spaces with tight channels (Fig.15).

4. Discussion

The midgut of third larval instar of *C.megacephala* is functionally the most important part of the digestive system, responsible for digestion and absorption of nutrients as in other insect larvae (Dow 1986).

The midgut of *C.megacephala* is similar to those of other Diptera.The presence of microvilli in this species provide an enormous surface area for absorbing materials from the lumen as stated by Romoser (1996).The thickness of the basement membrane in *C.megacephala* is due to the good nutrition during the larval stage (Clements,1992) which facilitates the transport of products between the intestine and the haemolymph (Reinhardt and Hecker,1973;Houk et al.,1980). Lehane and Billingsley(1996) stated that the basal labyrinth is common to insects of other Diptera.In addition, the separation of membranes of basal labyrinth appear to function in ion and water transport out of the lumen (Billingsley,1990).The folds of the basal labyrinth in the middle midgut of *C.megacephala* labyrinth are tightly apposed to one another such that no exracellular spaces are present. This indicates that this region may have no role in transport mechanism.

The lipid spheres are so numerous in *C.megacephala* which may be a state prior to pupation as indicated by Claudia et al. (2001).

The cytoplasm of mid-gut cells of *C.megacephala* possesses numerous vesicles of rough endoplasmic reticulum. Most authors interpret the phenomenon of vesicles of rough endoplasmic reticulum as a transition of the cell synthetic apparatus into a more active state (Billingsley et al., 1983; Lehane 1976a; Staubli et al., 1966 and Filimonova, 1989). Numerous vesicles of rough endoplasmic reticulum are present around the nucleus in *C. megacephala*. Such organization may indicate high synthesis of proteases as mentioned by Staubli et al., (1966) or a regulating mechanism of RNA transport from the nucleus to the cytoplasm as reported by Richards (1975). In *C.megacephala* the mitochondria are concentrated in the apical part of the cell more than basal part which may account for the transport role by apical part than basal part as indicated by (Hecker 1977and Houk, 1977). Numerous secretory granules with different shapes and sizes can be observed in *C. megacephala*,most of which are concentrated in the apical part and some of them project in the gut lumen.This may indicate that the release of digestive enzymes from midgut in *C. megacephala* may be merocrine. Chun-Nu et al., (2000) reported that the release of digestive enzymes from midgut cells of oriental fruitfly is merocrine due to high concentration of secretory granules in the apical part of the cell. Also the presence of numerous secretory granules and numerous vesicles of rough endoplasmic reticulum in *C megacephala* near the apical part of the cell may account for the production of peritrophic membrane as reported by Filimonova (2005).

Acknowledgements

I am grateful for my supervisor, Dr. Afaf Abdel-Meguid for her support cooperation and revising this manuscript. Deep thanks for my parents and my husband for supporting me both psychological and financial

Corresponding author
Nancy Taha,
Department of Zoology and Entomology, Faculty ofScience, Helwan University, Cairo, Egypt.
nancyt0000@yahoo.com

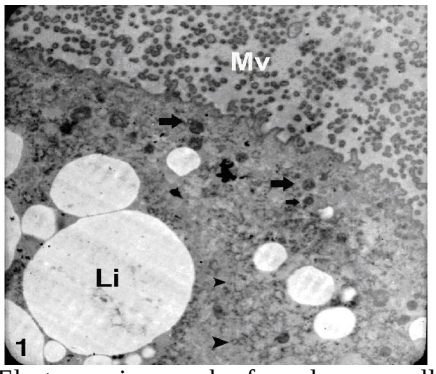

Fig (1): Electron micrograph of a columnar cell in middle mid-gut showing microvilli (Mv), lipid spheres (Li), small dense secretory granules (arrow) and small vesicles of rough endoplasmic reticulum (head arrows). Magnification X 3,000

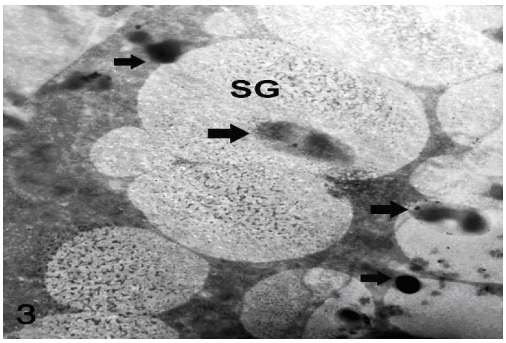

Fig (2): Electron micrograph of a columnar cell in middle mid-gut showing semi-transparent secretory granules may contain ferritin granules (SG), small dense secretory granules extruded by exocytosis (arrows). Magnification X 4,000

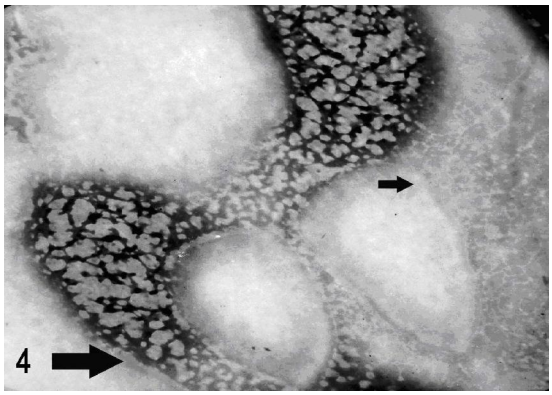

Fig (3): Electron micrograph of a columnar cell in middle mid-gut showing secretory granules, part of it electron-opaque protein and the other part is fibrous mucopolysaccharide (arrows). Magnification X 5,000

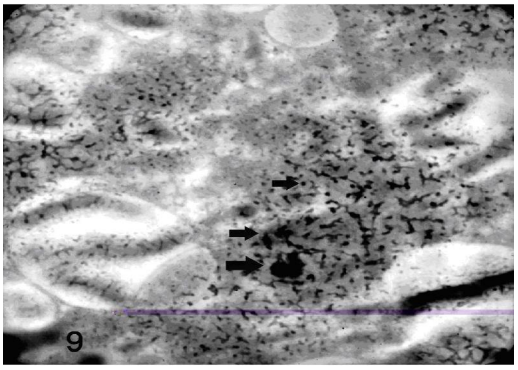

Fig (4): Electron micrograph of a columnar cell in middle mid-gut showing ferritin granules (arrows). Magnification X 5,000

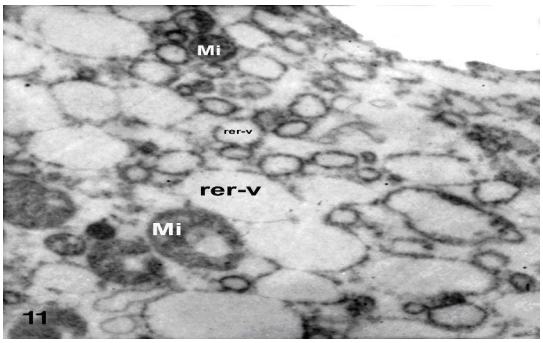

Fig (5): Electron micrograph of a columnar cell in middle mid-gut showing mitochondria (Mi) and vesicles of rough endoplasmic reticulum (rer-v). Magnification X 5,000

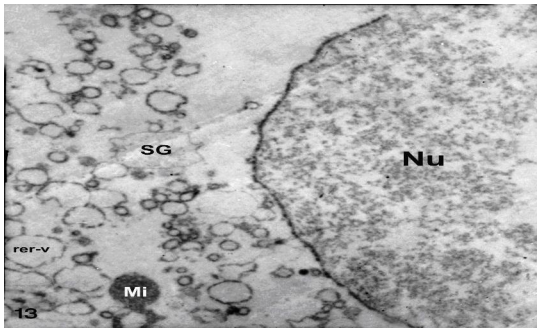

Fig (6): Electron micrograph of a columnar cell in middle mid-gut showing nucleus (Nu) surrounded by numerous vesicles of rough endoplasmic reticulum (rer-v) and secretory granule (SG). Magnification X 4,000

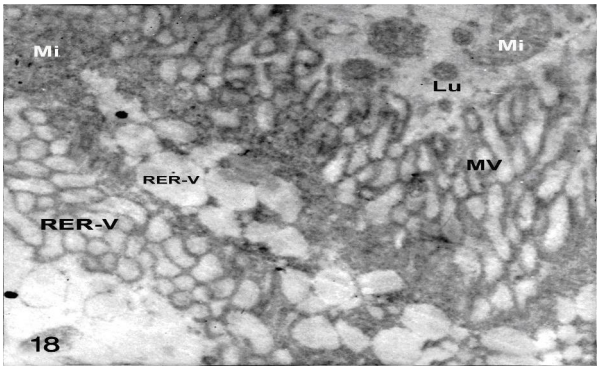

Fig (7): Electron micrograph of a columnar cell in middle mid-gut showing microvilli (Mv), mitochondria (Mi), vesicles of rough endoplasmic reticulum (rer-v) and lumen (Lu).Magnification X 6,000

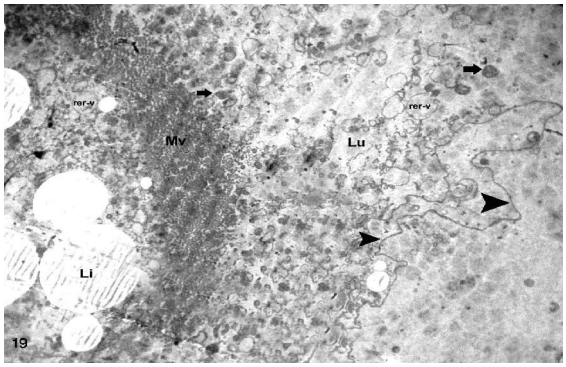

Fig (8): Electron micrograph of a columnar cell in middle mid-gut showing microvilli (Mv),mitochondria (Mi), vesicles of rough endoplasmic reticulum (rer-v) , lumen (Lu), lipid spheres (Li),dense secretory granules (arrow) and peritrophic membrane (head arrow). Magnification X 3,000

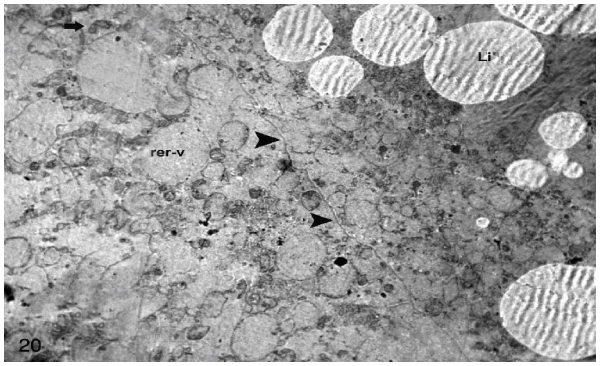

Fig (9): Electron micrograph of a columnar cell in middle mid-gut showing cell junction (head arrow), mitochondria (Mi), vesicles of rough endoplasmic reticulum (rer-v), lumen (Lu), lipid spheres (Li) and dense secretory granules (arrow). Magnification X 4,0000

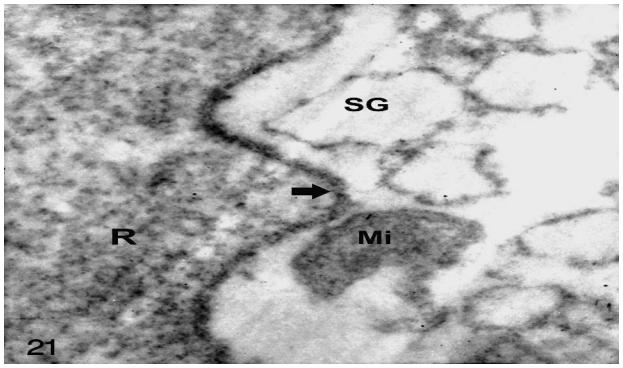

Fig (10): Electron micrograph of a columnar cell in middle mid-gut showing septate junction (arrow), mitochondria (Mi), vesicles of rough endoplasmic reticulum (rer-v) and ribosomes (R). Magnification X 12,000

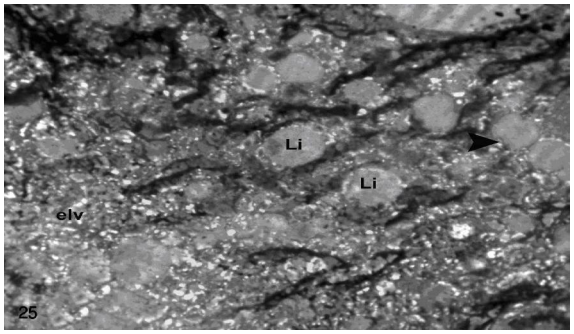

Fig (11): Electron micrograph of a columnar cell in posterior mid-gut showing electron-lucent vacuole (elv) among numerous lipid inclusions (Li). Magnification X 10,000

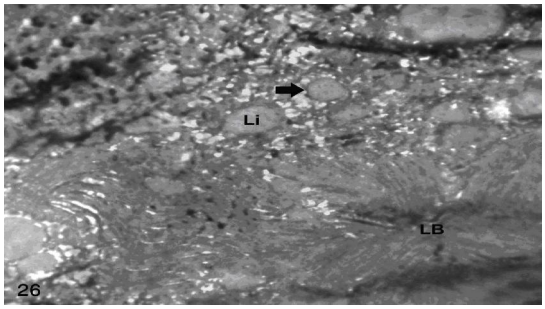

Fig (12): Electron micrograph of a columnar cell in posterior mid-gut showing lamellar bodies (LB), electron-lucent vacuole (elv) and lipid inclusions (Li). Magnification X 15,000

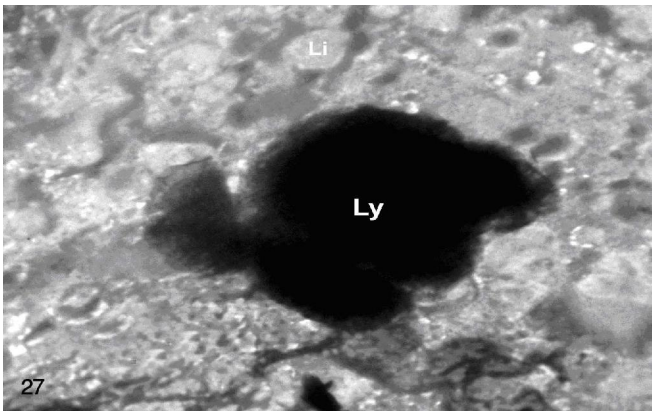

Fig (13): Electron micrograph of a columnar cell in posterior mid-gut showing large lysosomal body (Ly) among lipid inclusions (Li). Magnification X 12,000

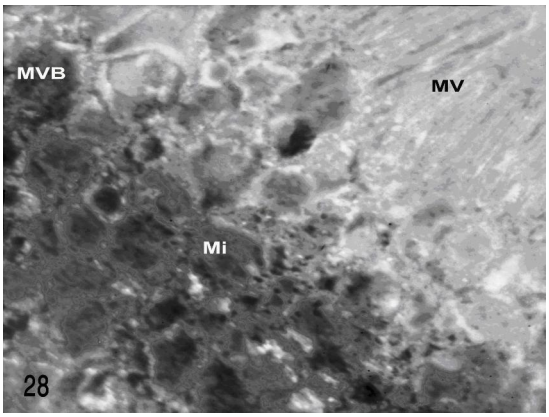

Fig (14): Electron micrograph of a columnar cell in posterior mid-gut showing long compact microvilli (Mv) that are parallel to each other and numerous mitochondria (Mi) that aggregates forming bodies (MVB). Magnification X 10,000

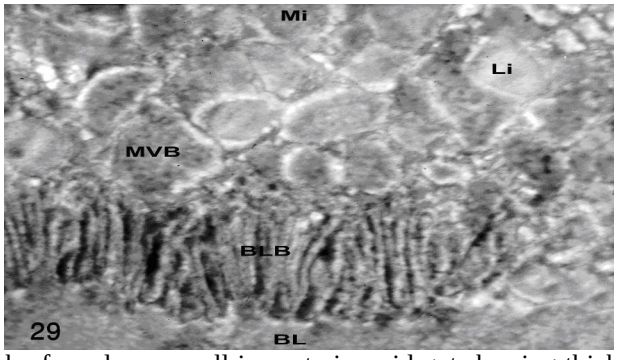

Fig (15): Electron micrograph of a columnar cell in posterior mid-gut showing thick granular basal lamina (BL), long tightly packed basal labyrinth (BLB) and numerous lipid inclusions (Li) that aggregates forming bodies (MVB). Magnification X 10,000

5. References

1. Billingsley PF (1990). The midgut ultrastructure of hematophagous insects. *Ann Rev Entomol 35*: 219-248.
2. Billingsley, P.F. and Downe, A.E.R., (1983). Ultrastructural Changes in Posterior Midgut Cells Associated with Blood Feeding in Adult Female *Phodnius prolixus* Stal (Hemiptera: Reduviidae), *Can. J. Zool.*, vol. 61, pp. 2574.2586.
3. Carvalho LML, Thyssen PJ, Linhares AX, Palhares FAB (2000) A checklist of arthropods associated with pig carrion and human corpses in Southeastern Brazil. Mem Inst Oswaldo Cruz 95:135–138
4. Chun-Nu Hung, Tai-Lung Lin and Wen-Yung Lee (2000).Morphology and ultrastructure of the alimentary canal of the oriental fruitfly,*Bactrocera dorsalis* (Hendel) (Diptera:Tephritidae)(2):The structure of the midgut.*Zoological Studies* 39(4):387-394
5. Cláudia A Andrade-Coêlho/+, Jacenir Santos-Mallet, Nataly A Souza,Ulisses Lins*, Maria Nazareth L Meirelles**, Elizabeth F Rangel, (2001). Ultrastructural features of the Midgut Epithelium of Females *Lutzomyia intermedia* (Lutz & Neiva, 1912) (Diptera: Psychodidae: Phlebotominae), *Mem Inst Oswaldo Cruz, Rio de Janeiro*, Vol. 96(8): 1141-1151.
6. Clements, A. N. (1992). Development, Nutrition and Reproduction *In: The Biology of*

Mosquitoes. London, Chapman & Hall, p. 509.
7. Dow (1986).Insect midgut function.Adv.Insect Physiol.19:187-328.
8. Filimonova, S.A., (1989).Morphological Analysis of Digestion in the Flea *Leptopsylla segnis* (Siphonaptera: Leptopsyllidae), *Parazitologiya*, vol. 23, pp. 480.488.
9. 3-Goff ML (2000) A fly for the prosecution: how insect evidence helps solve crimes. Harvard University Press, MA
10. Filimonova (2005).Morphological study of digestive cycle in bloodsucking biting midges of Genus Culicoides.Journal of Evolutionary Biochemistry and Physiology,Vol.41,No.2,2005,pp.176-185.
11. Greenberg B (1973) Flies and disease, Vol. II: biology and disease transmission. Princeton University Press, NJ
12. Hecker, H. (1977) Structure and function of midgut epithelial cells in culicidae mosquitoes (Insecta,Diptera). Cell Tissue Res 184:321-341.
13. Houk, E.J., (1977). Midgut Ultrastructure of *Culex tarsalis* (Diptera: Culicidae) before and after Bloodmeal, *Tiss.Cell*, vol. 9, pp. 103.118.
14. Houk, E. J.; Chiles, R. E. & Hardy, J. L. (1980). Unique midgut basal lamina in the mosquito *Aedes dorsalis* (MEIGEN) (Insecta, diptera). *Int. J. Insect. Morphol. Embryol 9*:161-4,
15. 5-Kumarasinghe SPW, Karunaweera ND, Ihalamulla RL (2000) A study of cutaneous myiasis in Sri Lanka. *Int J Dermatol* 39:689–694
16. Lee HL (1996) Recovery of forensically important insect larvae from human cadavers in Malaysia (1993–1996). *Malays J Pathol* 18:125–127
17. Lee HL, Krishnasamy M, Abdullah AG, Jeffery J (2004) Review of forensically important entomological specimens in the period of 1972–2002. Trop Biomed 21:69–75
18. Lehane, M.J., (1976a).Digestive Enzyme Secretion in *Stomoxys calcitrans* (Diptera: Muscidae), *Cell Tiss. Res.*,vol. 170, pp. 275.287.
19. Lehane, M. J. & Billingsley, P. F. (1996). Structure and ultrastructure of the insect midgut. I*n: Biology of the Insect Midgut*. Chapman & Hall, p. 486.
20. Reinhardt, C. & Hecker, H. (1973).Structure and function of the basal lamina and of the cell junctions in the midgut epithelium (stomach) of female *Aedes aegypti* L. (insecta: diptera). *Acta Trop., 30 (3)*:213-35
21. .
22. Richards GA (1975). The ultrastructure of the midgut of hematophagous insects. 481 *Acta Trop 32*: 83-95.474 482
23. Romoser WS (1996). *The Biology of Disease Vectors*, University Press of Colorado, 606
24. Stäubli, W., Freyvogel, T.A., and Suter, J., (1966). Structural Modification of the Endoplasmic Reticulum of Midgut Epithelial Cells of Mosquitoes in Relation to Blood Intake. *J. Microscopie*, vol. 5, pp. 189.
25. Sukontason K, Bunchoo M, Khantawa B, Piangjai S, Sukontason K, Methanitikorn R, Rongsriyam Y (2000) Mechanical carrier of bacterial enteric pathogens by *Chrysomya megacephala* (Diptera: Calliphoridae) in Chiang Mai, Thailand. Southeast *Asian J Trop Med Public Health* 31(Suppl 1):157–161.
26. Sukontason KL, Narongchai P, Sukontason K, Methanitikorn R, Piangjai S (2005) Forensically important fly maggots in a floating corpse: the first case report in Thailand. *J Med Assoc Thai* 88:1458–1461.
27. Worachote Boonsriwong & Kom Sukontason & Jimmy K. Olson & Roy C. Vogtsberger & Udom Chaithong & Budsabong Kuntalue & Radchadawan Ngern-klun & Surasak Upakut & Kabkaew L. Sukontason (2007). Fine structure of the alimentary canal of the larval blow fly *Chrysomya megacephala* (Diptera: Calliphoridae). *Parasitol Res* 100:561–574.
28. Zumpt F (1965) Myiasis in man and animals in the old world.Butterworths, London Res., 22:149-154.

4/1/2010