CHAPTER IV

DISCUSSION

The anatomy of the alimentary canal of *C. megacephala*, the medically important fly species in Thailand, was clearly investigation in this study using LM, SEM and TEM. This work showed that the alimentary canal was relatively simple, compartmentalized into differently shaped organs. Before this work, the gut anatomical studies of blow fly, *Calliphora vicina*, was shown in gross anatomy by light micrograph (Greenberg 1973). This present work added further information revealed by higher resolution images as followed.

Foregut

The foregut consists of a single tube, beginning in the anterior end being the mouth, esophagus, and anterior portion of the cardia, with the salivary glands and crop emanating from the main digestive tube. Simple tube of esophagus was observed in the third instar and adult. The cuticle (epi- and endocuticle) was still present during metamorphosis of flies, from the third instar until adulthood.

In Diptera, the crop is an alimentary structure that functions in the storage and flow of the ingested food (Stoffolano, Jr. et al. 1995). Micrographs of the crop clearly showed that crop of the third instar was very thin large single sac (see Figure 3), but; appearing as smaller bi-lobed sac in both males (see Figure 8) and females (see Figure 15). This difference may be because of the more importance of the crop using in the third instar to storage food during pupation (metamorphosis to be adult within puparium). Greenberg (1973) reported that in the prepupa, two days after feeding had ceased, the crop was collapsed in to inconspicuous sac. On the other hand, storage of food in the crop of adult was less necessity, based on the small size in adult. The probable reasons may be that adult could search for their food easily and as frequently as they need. The cuticle was clearly found in the crop in the third instar and adult *C. megacephala*, which was relatively similar in structure to that described in the adult fruit fly, *B. dorsalis* (Lee et al. 1998).

Investigation of the salivary glands of the *C. megacephala* has shown that the morphology of this organ was different in the third instar and adult. For the third instar, the simple tube gland observed in this study (see Figure 3) closely resemble those depicted by Evangelista and Leite (2005) for the first instar larva of the bot fly, *D. hominis*. The similarity consists of having simple, tubular glands opening into narrow efferent ducts on each side that converge to form a single median deferent duct leading to the oral cavity. At the ultrastructural level, the composition of the salivary gland of *C. megacephala* larvae appears to be comparable to that of the secretory region of the lateral duct of the larval salivary gland of the ant, *Pachycondyla villosa* (Hymenoptera: Formicidae) (Zara and Caetano 2003). It consists primarily of simple cuboidal epithelium cells and contains fibrous material that is most likely salivary secretion within its lumen.

No significant difference of the morphology of salivary gland was observed between males (see Figures 8, 9A) and females (see Figure 15), in having coil shape at the apex. The coiled salivary gland of *C. megacephala* was parallel to that of other blow fly, *Calliphora vicina* (Rotte et al. 2008). In the highly coiled portion, it was considered secretory, while the big, short straight unpaired duct proximally suggested the re-absorptive (Oschman and Berridge 1970). Moreover, the concentration of mitochondria and the packed cristae within them observed in the cells of salivary gland of adult, shown by males, indicates heightened metabolic activity. This was in agreement with the presence of numerous secretory materials and vesicles inside the cells.

The cardia is a distinctive organ in Diptera that located at the posterior end of the foregut and anterior end of the midgut. Longitudinal sections of the organ in the third instar (see Figure 6B), males (see Figure 12B) and females *C. megacephala* (see Figure 19D) clearly showed two major compartments: the anterior foregut tissue and posterior midgut tissue. This corresponded with those seen in the adult blow fly, *Lucilia cuprina* (Binnington et al. 1998), stable fly, *Stomoxys calcitrans* (Lehane 1997) or the fruit fly, *B. dorsalis* (Lee et al. 1998). According to Binnington et al. (1998), the cardia of adult *L. cuprina* composed of interlocking formation zones, anterior tissue and posterior tissue. The peritrophic membrane was secreted from the three formation zones. In the present study, semi-thin and ultra-thin investigations reveal that the peritrophic membrane first appears in the posterior midgut tissue of the cardia of both third instar and adult, indicating that it is a type II peritrophic membrane that actually forms in the cardia (Lehane 1997). This agrees with Tellam et al. (2000) who found that intrinsic peritrophic matrix protein, peritrophin-95, was synthesized in the cardia of third instar larvae of the blow fly, *Lucilia cuprina*. Similarly, Hao et al. (2003) documented the synthesis of peritrophic membrane constituents in the cardia of the adult tsetse fly, *Glossina morsitans morsitans*. In this same study, the cardia was also found to play a crucial role in immunity in this tsetse fly species. The results of *C. megacephala* larvae evidently showed that the peritrophic membrane started at the posterior midgut tissue of cardia (see Figure 6B) and ran along the canal until the anus. Two major functions of the midgut peritropic membrane have been reports: to providing a barrier against microorganisms and protecing the midgut cells from abrasion by food particles (Chapman 1998). In blow fly, *Calliphora vicina*, the peritropic membrane was readily permerable to amino acids, disaccharides, and smaller molucules, but not to starch or protein (e.g., albumin, casein or gelatin), suggesting the semi-selective ultrafilter (Greenberg 1973). In addition, this study has clearly shown that the peritropic membrane was well developed in the midgut of third instar. However, the precise time the peritropic membrane is formed in the first or the second instar is unknown, and this subject is merit for further investigation.

Large area of muscle was observed beneath the anterior foregut tissue of adult, as demonstrated in females (see Figures 19D, 19E). This finding confirmed information that the cardia was thought to act as sphincter, controlling the passage of food and preventing regurgitation (Greenberg 1973).

Midgut

The midgut of adult *C. megacephala* arised in the middle of the thorax and proceed as a straight tube into the abdomen, where it coiled and convoluted. The midgut occupied the longest tube of the alimentary canal of flies, both the third instar and adult. The midgut length of third instar was longer about two times than that of males or females (see Table 2).

In this study, four large blind end tubes of the gastric caeca were observed in the third instar *C. megacephala* by attaching to the end of cardia (see Figures 3, 4B). This resembled those seen in the third instar of blow fly *C. vicina* (Greenberg 1973) and the flesh fly, *Liosarcophaga dux* (Sukontason et al., unpublished data). Nevertheless, this was in contrast with the absence of gastric caeca reported in the third instar of the bot fly, *Dermatobia hominis* (Evangelista and Leite 2003). The presence of short microvilli at the free surface of the gastric caeca cells is evidence of increased surface area for more efficient nutrient absorption (Chapman 1998). Evidently, no gastric caecae was observed in the adult *C. megacephala,* both males (see Figure 8) and females (Figure 15). It is possible that this organ was not necessary for functioning in the adult; but, in turn, essential in the larval stage.

The presence of microvilli along the midgut epithelial cells was observed in the third instar (see Figure 6F) and adult male *C. megacephala* (see Figure 13B). This corresponds with similar findings reported in other insects such as the sand flies, *Lutzomyia intermedia* (Andrade-Coelho et al. 2001) and *L. longipalpis* (Leite and Evangelista 2001, Secundino et al. 2005); fruit fly, *Drosophila auraria* (Dimitradis 1991); mosquitoes, *Aedes (Stegomyia) aegypti*

(Zieler et al. 2000, Moncayo et al. 2005) , and *Anopheles darlingi* (Okuda et al. 2005); tick, *Haemaphysalis longicornis* (Matsuo et al. 2003); beetle, *Dendroctonus valens* (Silva-Olivares et al. 2003); bee, *Melipona quadrifasciata anthidioides* (Neves et al. 2003); or the wingless firebrat, *Thermobia domestica* (Rost et al. 2005). In addition, long microvilli located on the midgut cells of male *C. megacephala* observed in this study using TEM (see Figure 13B) was parallel with that found in the third instar of fly, *Dermatobia hominis* (Evangelista and Leite 2003). Occurrence of microvilli on cells typically indicates regions where large amounts of absorption and/or secretion take place.

A large number of secretory vesicles were evident in the midgut cells of *C. megacephala*, both in the third instar and adult. Darkly staining vesicles were not only observed inside the cells, but on many occasions were also seen outside the cells often bound to the apical cell membrane, suggesting a secretory role of these cells in the midgut. This corresponded with previous reported that the darkly staining, granule vesicles were associated with a secretory function (termed secretory vesicles); whereas, the large, lightly staining vesicles (termed lipid vesicles) were associated with absorption (Lehane 1998). Secretory granules are most likely released from their vesicles by a process known as exocytosis in which the secretory vesicles move to the inner apical surface of the cell, fuse with the cell membrane and release the secretory granules into the gut lumen (Chapman 1998).

Musculature covering the alimentary canal of *C. megacephala* was more distinct in adult than the third instar. As for adult especially males, a striking circular muscle was prominent in the anterior midgut (see Figure 9C) and hindgut, particularly colon (see Figure 10B) and rectum (see Figure 10D), suggesting the high movement of these organs.

Hindgut

It is obvious that microvilli line the entire lumen of the Malpighian tubules of *C. megacephala* larvae. This resembles that described for the fire ant, *Solenopsis saevissima* (Arab and Caetano 2002) and the Malpighian papillae of the dipluran, *Campodea (Monocampa) quilisi* (Pigino et al. 2005).

The presence of a cuticle comprised of a narrow layer of epicuticle and rather thick layer of endocuticle within the foregut and hindgut of *C. megacephala*, both the third instar and adult male. In the foregut, it was apparent in the esophagus; while in the hind gut, it was apparent in the pyrolus, ileum, colon, rectum and anus. The presence of cuticle in this study resembles most other insects such as the fruit fly, *B. dorsalis* (Lee et al. 1998) and the beetle, *D. valens* (Silva-Olivares et al. 2003).

In the hindgut, morphological feature of the rectum was markedly different between the third instar and adult *C. megacephala*, both males and females. Rectum of the third instar appears as long, slender tube, whereas those of males and females were similar in appearance, showing a muscular cone-shaped structure and four protruding muscle-free rectal pads at the anterior end of the gland. In this regard, no sexual dimorphism is observed. The appearance of rectal gland of adult *C. megacephala* is comparable with that of male fruit fly, *Bactocera papayae* (Khoo and Tan 2005). In this study, the histological section of females revealed the presence of rectal pad that extended into the gland as rectal papillae (see Figures 21A, 21C). This observation corresponded with Khoo and Tan (2005) who described the rectal gland of *B. papayae* that the rectal pads extended into the gland as rectal papillae of which having large nuclei in the cell. In this study, TEM section revealed large oval nucleus within cells of rectal papillae (see Figure 14C), that was resemble with the area of rectal papillae previously reported in *B. papayae* (Khoo and Tan 2005). Also, large amount of mitochondria was similar to *B. papayae*. As for the muscle covering the rectum, only circular muscle was detected in both males and females in *C. megacephala*. Contrary to this observation, Khoo and Tan (2005) have demonstrated that rectum of male *B. papayae* showed both circular and faint longitudinal muscles; whereas, that of female was covered only circular muscle. The presence of only circular muscle in rectal gland has been suggested the ability of this organ to contract only in the dorsal-ventral direction (Khoo and Tan 2005).

Rich supplied of tracheae was observed in the rectal pad of male and female *C. megacephala*. This was in accordance with the previous reported in the rectal gland of blow fly, *Calliphora erythrocephala* (Gupta and Berridge 1966); male and female *B. papayae* (Khoo and Tan 2005), strongly suggesting that these glands involved in active aerobic activity (Khoo and Tan 2005). It has been suggested that water, chloride and probably other ions were absorbed at the ractal pad (Greenberg 1973).

The muscular fibers observed within the walls of the alimentary canal were most apparent in the hindgut region of *C. megacephala* both third instar and males. Musculature was apparent beginning in the ileum and became more pronounced moving posteriorly down the hindgut, with the greatest intensity of myo-epithelial cells being found in the anal tube. These observations strongly suggest that intense activity of muscle contractions is performed in the hindgut region of the alimentary canal.

In conclusion, the results of this work obtained from using a combination of light microscopy, SEM, and TEM represented a step toward information on the ultrastructure of the alimentary canal of *C. megacephala*, both in the third instar and adulthood. Understanding the functional morphology of several organs in the alimentary canal was provided. The ultrastructural characteristics of each component within the foregut, midgut and hindgut were found to have differences from both morphological and functional viewpoints which help to clarify and better understand each particular organ in the alimentary canal of this species.