HISTOLOGY

INTRODUCTION

Histological studies are carried out to study the tissues and the organization of the tissues in detail. The histological details reveal the minor cell make up and their influence in the building up of the body tissues. An attempt is made to discuss certain body tissues namely buccal mass, anterior salivary gland, proximal efferent canal, digestive gland (liver), stomach, arm, gills, spiral caecum, testis, ovary and the hatched paralarva.

MATERIAL AND METHODS

Fresh animal samples were collected, the different parts dissected out and stored in 10% formalin solution. Later they were taken for histological sectioning and staining at Vaishnavi labs, Chennai. The slides were studied to bring out the salient characters of different tissues and photographed with the help of a NIKON F10 camera attached to a light microscope.

OBSERVATIONS

Buccal mass

The buccal mass is made of dense muscle fibres. It possesses the radula, beaks and the lips as its parts. The biting of the prey and the scraping of the flesh take place in this region before it is sent to the oesophagus. A section of the buccal mass (2.5 X) (Fig. 1), explains the presence of heavy musculature and the presence of lobes. The radula (R) is seen like a cone – like tooth pattern on one margin. It is found on the entire length of the ribbon. The teeth at the posterior part of the ribbon are
short, stumpy and strong when compared to their counterparts in the anterior end. The salivary papilla (SP) is seen along the inner margin of the buccal opening. Patches of secretory glandular area are seen producing black colour pigments. Fig. 2 shows a section through buccal mass region showing the lobes of the buccal mass, musculature and secretory activity (S). Muscle fibres are closely organized. A magnified portion of the buccal mass (Fig. 3) shows the circular muscles (C) with secretory activity. In between the muscle fibres, secretory zones are present. The secretory glands have secretory cells and loaded with black pigments that are supported by columnar cells (C).

Fig. 1. Buccal Mass with radula at 2.5 X

Fig. 2. Buccal mass at 2.5 X

Fig. 3. Buccal Mass at 40 X
Salivary glands

A section through the anterior salivary glands at 2.5 X magnification reveals secretory activity (Fig. 4). The salivary epithelium is narrow and the salivary glands are made up of hexagonal parenchymatous cells (P). Several lacunae are seen inside the gland (L). Secretions are poured into the middle of the gland (S). At a higher magnification of 40 X (Fig. 5), the Blood cells (BC), Nucleus (N) and Lobules (LO) are clearly visible.

Fig. 4. Posterior salivary gland at 2.5 X
Fig. 5. Posterior salivary glands at 40 X

Proximal efferent canal

A magnified (40 X) section of the proximal efferent canal shows that the canal is gorged with blood cells. The cells may be of haemopoeitic nature (Fig. 6).
Digestive gland (Liver)

The liver contains hepatocyte lobules clearly seen at 2.5 X magnification. These are formed of parenchymatous cells that in turn form a homogenous mass. The Hepatocytes (HC) is polygonal (Fig. 7) and isodeametric. Each hepatocyte contains a distinct centrally placed nucleus. Blood lacunae and narrow long bile canals are also seen. At a higher magnification of 40 X, the hepatocytes (HC) and the lacunae (L) are very clearly seen (Fig. 8).
**Stomach**

The musculature is seen on the outer layers of the stomach. At 2.5 X magnification the digestive caecum and gastric glands are very prominent. Just above the musculature, submucosal coverings are visible with outer muscles (Fig. 9). Connective tissues are seen placed between the muscles near the submucosal layer. Secretory activity is also seen. Rugose - like projections (R) are seen inside the stomach. A closer view at 40 X magnification shows the stomach wall with heavy musculature because of the carnivorous feeding habits (Fig. 10).

**Fig. 9. Section of the stomach at 2.5 X**

- **Fig. 10. Section of stomach at 40 X**

(A: Acetabulum, M: Musculature, GG: Gastric gland, CT: Connective tissue)

**Arm**

A section of the arm at 2.5 X magnification explains the distribution of suckers and arm musculature (Fig. 11). Suckers are more or less globular in nature with a wide acetabulum (A) or chamber. The suckers are surrounded by the infundibular rings (i). The ring is composed of teeth of different dimensions, some are round while others are found to be rectangular (Fig. 12). Some of the suckers are small with (the outer margin) reduced infundibular ring.
Gills

A section through the ctenidia at 2.5 X magnification shows continuous architecture of the gill filaments. The frontal rachis and the gill filaments are composed of rich blood vessels (Fig. 13). Rachis (RA), is composed of soft tissues and blood lacuna (BL). Fig. 14 explains a portion of the gill filament magnified. The filamentous cells are filled with blood cells. Mucus secretions are also seen as mass. Branched blood vessels are also evident in 40 X magnification. The Fig. 15 shows a section of the gill having rich capillary bed (2.5 X).
Spiral caecum

A section through the spiral caecum clearly shows rich secretory activity (S) at 2.5 X magnification. Secretions are abundant by the secretory cells (Fig. 16). Columnar cells are seen in the caecal layer of spiral caecum which is composed of several finger-shaped, diverticular structures with rich secretory functions. Secretions are poured into the central canal. At higher magnification, the secretory columnar cells are very clearly seen (Fig. 17). The caecum is composed of several lobules of different size.
Testis

At lower magnification, the section of testis shows seminiferous tubules with different stages of spermatogenesis. Each seminiferous tubule is a cross section showing distinct central lumen and an outer germinal epithelial layer. Below the germinal epithelial cells, lobules are distinctly seen (L). Inside the seminiferous tubules, zones showing different stages of spermatogenesis are clearly seen (Fig. 18). In certain zones, the lobules show earlier stages of spermatogenesis and in some zones, spermatids and spermatogonia are in developed condition. Spermatozoa are not seen inside the seminiferous tubules. Another section showing a magnified view of germinal epithelium (GE), seminiferous tubules (ST) and somatic cells (SC), which are large and columnar (Fig. 19). Germinal cells are (GC) smaller and narrow. Presence of lobules (LO), lobular walls (LW) are seen along with spermatogonia (SG).

Fig. 18. Male reproductive system at 2.5 X

![Image of testis showing seminiferous tubules and lobules](image18)

(GE: Germinal epithelium, ST: Seminiferous tubules, SG: Spermatogonia, LO: Lobules)

Fig. 19. Male reproductive system at 40 X

![Image of testis showing germinal epithelium and somatic cells](image19)

(GC: Germinal cells, SM: Somatic cells, LW: Lobular walls, SG: Spermatogonia, SPT: Spermatids, SP: Spermatocytes)

Ovary

A section taken through the ovary showing ovarian follicles and oviduct (OV) explains ovarian follicles (FC), which are many in number and are in different
shapes and size (Fig. 20). Some of the ovarian follicles are ruptured releasing a mass of oocytes (O) into the duct (FM). Some of the follicles are abnormal in shape (FA). Follicular wall is found to be ingressed into the middle of the follicle as a fold (Fl). The infolds are of different dimensions. In a magnified view of 40 X the portion of the ovary shows follicular wall or oocytes (Fig. 21). The gonad gland oviduct (Fig. 22) shows circular central mass with differential shells.

Fig. 20. Ovary at 2.5 X

Fig. 21. Gonad gland of oviduct at 40 X

(Fig. 20: Ovarian follicles, OV: Oviduct, OO: Oocytes, FA: Abnormal follicles, Fl: Follicular fold)

(Fig. 21: Ovarian follicles, OO: Oocytes)

Fig. 22. Gonad gland of oviduct at 2.5 X

(Fig. 22: Ovarian follicles)
Paralarva

The higher magnification (40 X) clearly shows the cephalic region and arms of the paralarvae having large suckers and infundibulum as seen in Fig. 23. The stomach region of the paralarvae is visible containing the ink sac (Fig. 24).

Fig. 23. Section of cephalic region at 2.5 X

Fig. 24. Section of stomach region at 2.5 X

(A: Arm, SU: Sucker, BM: Buccal mass) (IS: Ink sac, GL: Gills)

DISCUSSION

A detailed study on the fine structures of *Sepia pharaonis* has been carried out. The buccal mass area is surrounded by dense muscle fibres that may be useful to the animal while feeding by contraction and relaxation. Most cephalopods have two pairs of salivary glands, which are the anterior and posterior salivary glands (Meglitsch and Schram, 1991). This is true in the case of *Sepia pharaonis*. The anterior salivary gland in the present study has many hexagonal parenchymatous cells. The salivary papilla inside the buccal mass region possessing secretory cells suggests the release of secretions at the time of feeding.

As explained earlier, the digestive organ is the biggest organ in the body of cuttlefishes. The digestive gland or hepatopancreas is paired in *Sepia* consisting of a
brownish liver and a whitish pancreatic region (Meglitsch and Schram, 1991). The brownish liver region is clearly visible in the case of *Sepia pharaonis* but the whitish region of the pancreas is much paler in colouration. Here the cells are made of a homogenous mass suggesting the presence of Hepatocytes arranged as lobules. The secretory cells in the digestive glands are triangular and occur in groups for *Sepia pharaonis* while the digestive gland is coated with this connective tissue membrane for the gastropod *Hemifusus pugilinus* (Benny, 1996). The stomach is lined with chitin and is presumably a derivative of the stomodeum (Meglitsch and Schram, 1991).

In the gills of the gastropod *Hemifusus pugilinus*, the epithelium is provided with a cuticle, ciliated to a varying extent and the mucocyte cells are sparingly present among the epithelial cells (Benny, 1996), while the mucus secretion for *Sepia pharaonis* is seen as a mass with rich blood vessels. The ctenidia have architecture of gill filaments with rich blood vessels.

For *Sepia pharaonis*, the presence of higher number of spermatocytes in the center of the tubule suggests that the animal is in the maturing stage as reported earlier by Gabr et al., (1998) for *Sepia pharaonis*. The spermatozoa were not seen in the tubules of the testis. This may be due to the maturation of spermatids into sperms that takes place in the seminferous tubules of the testis. Rahim and Chandran (1984) have reported that the transportation of spermatids on the anterior vas deferens in the cephalopod *Loligo duvauceli*. Gabr et al., (1998) have reported that in the pre spawning stage of *Sepia dolfusi*, follicular epithelium begins to invade the oocytes as follicles of tissue with higher mitotic rate, making the end of their maximum penetration into the oocytes in the formation of the scyncitium. The folds of the follicular syncytium are active in the vitellogenesis and formation of the chorion. In the follicles with heavy
infolding, the number of oocytes is low. From ruptured follicles, oocytes enter as a long mass of cells into the oviduct (OE).

The results from the present study will be useful to minutely look into different species of the same genus for micro-taxonomical characters in the future. This will also form a basis for further research on the different organs during development of the cuttlefish *Sepia pharaonis*. 