

A COMPARATIVE STUDY OF THE ANATOMY AND  
HISTOLOGY OF THE ORAL CAVITY AND ALIMENTARY  
CANAL OF TWO SEA TURTLES: THE HERBIVOROUS  
GREEN TURTLE *CHELONIA MYDAS* AND THE  
CARNIVOROUS LOGGERHEAD TURTLE *CARETTA CARETTA*  
(Includes discussion of diet and digestive physiology)

THESIS SUBMITTED BY  
SUSAN MARIE THOMPSON, B.App.Sc. (RCAE)  
IN NOVEMBER, 1980

In partial fulfilment of the requirements for  
the research degree of Master of Science in the Faculty  
of Science of James Cook University of North Queensland.

## THE OESOPHAGUS

5.1 RESULTS5.1.1 Anatomy5.1.1.1 *Chelonia mydas*

The oesophagus is a long, slightly tapering tube connecting the pharynx and the crop. On average it represents 3% of the total length of the alimentary canal (Table 10.3). It is 17.5 to 51 cm long in adult turtles and, when empty, tapers from an internal circumference of 6.0 cm at the anterior end to one of 5.0 cm at the posterior end (Table 10.6).

5.1.1.2 *Caretta caretta*

As in *Chelonia mydas*, the oesophagus is a long slightly tapering tube. While it is shorter in *Caretta caretta* (15 to 19 cm in length in the two individuals examined in this study), it also represents approximately 3% of the total length of the alimentary canal (Table 10.3). The oesophagus is wider in *C. caretta* than in *C. mydas*, tapering from a circumference of 10.3 cm at the anterior end to a circumference of 8.0 cm at the posterior end (Table 10.6).

5.1.2 Internal relief of the mucosal surface5.1.2.1 *Chelonia mydas*

In hatchlings, subadult and adult turtles of this species the oesophagus is completely lined with closely packed, keratinized, conical papillae. These papillae project posteriorly and are orientated parallel to the long axis of the oesophageal tube (Figure 5.1). The dimensions of the papillae change along the length of the oesophagus. At the

proximal end, the papillae are short with small basal diameters and are very tapered. Over the central two quarters of the oesophagus, the papillae become longer and also broader at their bases. In the anterior one quarter of the oesophagus they become even shorter and are blunter or more rounded at their tips, although they remain broad at their bases (Table 5.1). In the transitional area between the oesophagus and the crop, only a few scattered, short, broad, blunt papillae occur (Figure 5.2).

The oesophageal papillae fall into two fairly distinct groups based on their sizes, that is, in a given area a number of large papillae are found between the bases of which also occur numerous smaller papillae (Figure 5.1). In adult turtles of this species the larger papillae are up to 17 mm in length. The smaller papillae are as short as 1.0 mm in length. There is a gradation in size between these two extremes so that the division is arbitrary, however the majority of the papillae are clearly small or large.

The epithelium lying between the bases of the papillae is keratinized and has a crinkled appearance. This crinkled effect is produced by a fine system of folds, which individually extend only short distances along the surface and have no distinct pattern of orientation. It is unlikely that the internal relief of the oesophagus alters greatly during feeding. The conical keratinized papillae are permanent structures. The crinkled epithelium between these papillae, probably allows for the necessary lateral expansion during feeding.

#### 5.1.2.2 *Caretta caretta*

The internal relief of the oesophagus in hatchling, subadult and adult *C. caretta* (Figure 5.3) is like that in *C. mydas*. The oesophageal papillae follow a similar pattern of dimension change along the oesophagus (Table 5.1), but in overall size they are more massive in

Figure 5.1

5

*Chelonia mydas*: Conical spinose papillae lining the oesophagus. The papillae are covered with keratinized stratified squamous epithelium. The majority of the papillae project posteriorly.

Figure 5.2

*Chelonia mydas*: mucosal surface at the transition zone from the oesophagus to the crop. Note the oesophageal papillae become shorter, blunter and less frequent towards the crop. The crop epithelium is folded and creased but not spinose.

Figure 5.3

*Caretta caretta*: conical spinose papillae lining the oesophagus. The papillae are covered with keratinized stratified squamous epithelium. The majority of the papillae project posteriorly. (x 1.2)

Note: lines across tissue segments were caused during preparation and are not natural features.

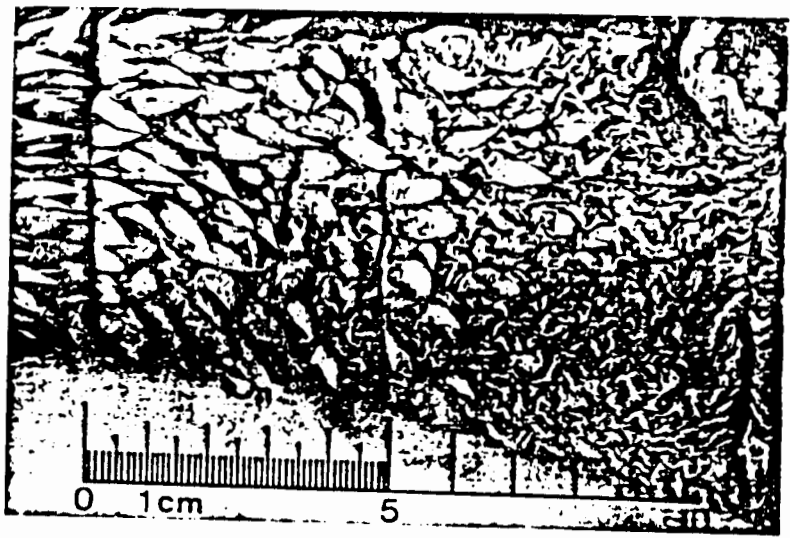
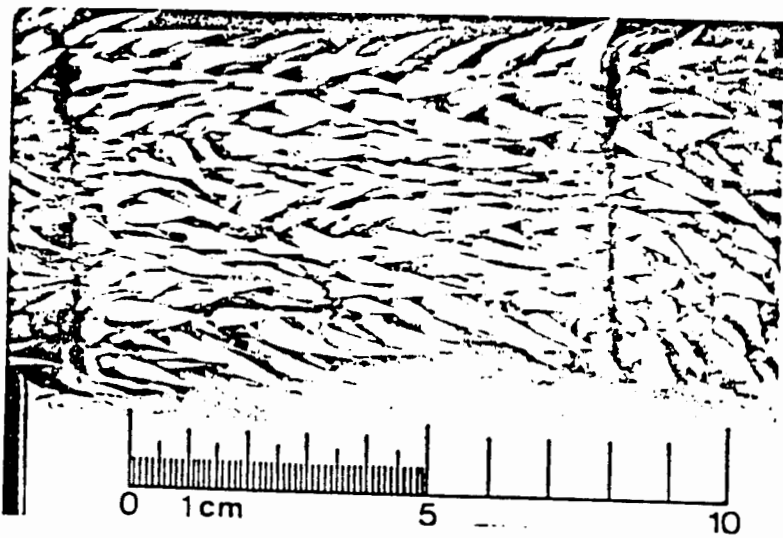


TABLE 5.1

Number of papillae and changes in the dimensions of the papillae, along the length of the oesophagus.  
(One individual of each species examined in detail)

Species	Papillae	Distance from top of oesophagus														
		0 - 5 cm			5 - 10 cm			10 - 15 cm			15 - 20 cm			20 - 25 cm		
		l	d	n	l	d	n	l	d	n	l	d	n	l	d	n
<i>Chelonia mydas</i>	Small	4.4	1.0	7	6.3	1.6	8	5.9	1.9	7	3.2	1.8	3	7.6	2.6	6
	Large	12.9	2.9	10	14.5	4.8	4	13.6	4.6	7	10.9	4.6	5	-	-	-
<i>Caretta caretta</i>	Small	7.5	2.7	6	9.3	3.1	12	8.0	3.5	8	7.3	4.7	9	-	-	-
	Large	17.0	4.6	5	18.7	5.0	9	19.6	7.1	9	18.3	7.5	3	-	-	-

l mean lengths of papillae from tips to bases

d mean diameters of papillae at their bases

n number of papillae measured

Note: a single row of papillae was measured from the top of the oesophagus to the oesophagus crop transition zone.

*C. caretta* than in *C. mydas*. In adult turtles the papillae range in length from 2.7 mm to 23 mm. The majority of the papillae fall towards either end of this range, to form two fairly distinct groups. The epithelium between the papillae is keratinized and crinkled. These crinkles probably smooth out when the oesophagus distends laterally during feeding.

### 5.1.3 Surface epithelium

#### 5.1.3.1 *Chelonia mydas*

The keratinized epithelial tissue of the oesophagus is composed of square or pentagonal, flat, closely adjacent and sometimes overlapping, scale-like cells (Figure 5.4). Each cell has a rough, pitted and bumpy surface. Cells appear to be shed singularly or in small groups and can be seen to be lifting from the surface in places (Figure 5.4). These keratinized cells extend to and over the tips of the oesophageal papillae (Figure 5.5).

In transverse section, the keratinized layer of the epithelium can be seen to stain red with Gordon and Sweets stain (Figure 5.6) and pink with haematoxylin and eosin (Figure 5.7). As seen in these two figures, the stratified, squamous epithelium lining the oesophagus is composed of the usual three distinct layers, an outer Stratum Corneum, a central Stratum Spinosum and an inner Stratum Germinativum. Each stratum is very uniform in appearance and thickness throughout the oesophagus, except at the tip of each papilla where all three strata are slightly thickened. The epithelial layer ranges from 60 to 90  $\mu\text{m}$  in thickness.

#### The Stratum Corneum:

The keratinized Stratum Corneum stains vividly with eosin in haematoxylin and eosin preparations (Figure 5.7). It is composed of dry, devitalized, scale-like, squamous cells. These cells have lost their nuclei and their cytoplasm has largely been replaced by the

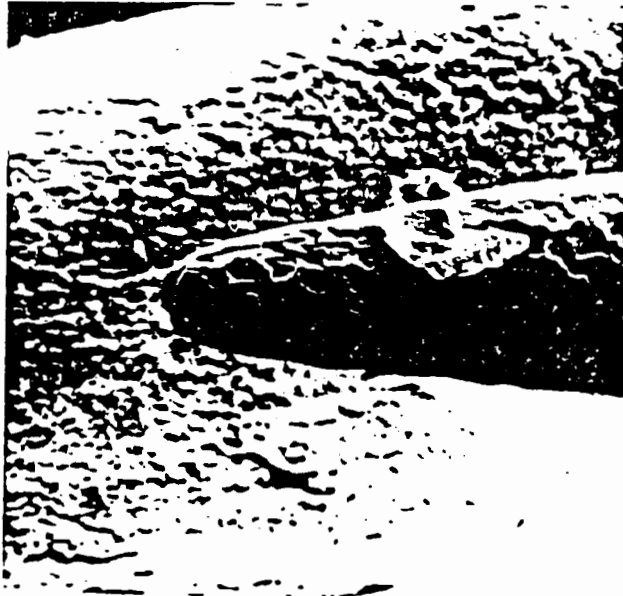
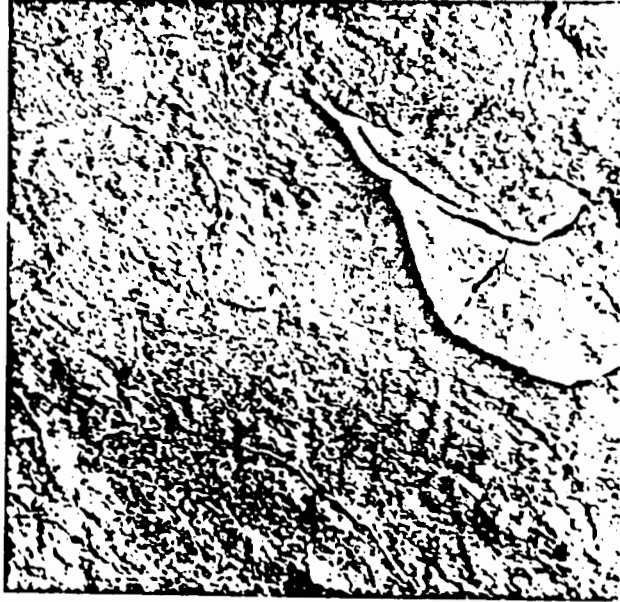
Figure 5.4

*Caretta caretta*: electron micrograph showing the keratinized squamous scale-like cells covering the surface of the oesophageal papillae. Note cells lifting away from surface. (x 935)

Figure 5.5

*Chelonia mydas*: electron micrograph showing the keratinized squamous scale-like cells covering the surface of the oesophageal papillae. Note the keratinized epithelium extends to tip of each papilla. (x 165)





scleroprotein keratin. Each laterally compressed cell is approximately 1.5  $\mu\text{m}$  in width. The entire Stratum Corneum ranges from 25 to 50  $\mu\text{m}$  in thickness.

#### The Stratum Spinosum:

In contrast to the Stratum Corneum, the Stratum Spinosum is composed of complete, nucleated cells. These cells are irregularly polyhedral in shape and the nearer to the free surface they occur, the more they are flattened.

Because the cytoplasm of these cells stains lightly with haematoxylin, the boundary between the Stratum Corneum and the Stratum Spinosum is distinct (Figure 5.7). The cell boundaries are clearly visible as fine pink staining (eosinophilic) lines around the cell nuclei. Keratin is first laid down in the last two or three layers of plate-like cells, directly beneath the Stratum Corneum (Figure 5.6). Some of these cells have lost their nuclei. Close to the Stratum Corneum the nuclei are fewer and elongate (6 to 8  $\mu\text{m}$  in width by 13 to 22  $\mu\text{m}$  in length). The nuclei are more numerous and are spherical (less than or equal to 13  $\mu\text{m}$  in diameter) close to the Stratum Germinativum. Each nucleus contains chromatin granules which stain darkly with haematoxylin and one or sometimes two eosinophilic nucleoli (Fig 5.7). In contrast, the remainder of the nuclear material stains lightly with haematoxylin. The spherical nuclei tend to stain more darkly than the ovoid nuclei. The Stratum Spinosum averages 30  $\mu\text{m}$  in depth.

#### Stratum Germinativum:

The Stratum Germinativum consists of a single row of cuboidal to low columnar cells (Figures 5.6 and 5.7) with rounded or bevelled upper edges. The nuclei almost fill the cells and tend to be the same shape as the cells. These cells average 10  $\mu\text{m}$  in height and the nuclei are smaller and more darkly staining than those in the cells of the Stratum Spinosum. Like the nuclei of the Stratum Spinosum, the Stratum

Figure 5.9

Photomicrograph showing the connective tissue matrix in the core of an oesophageal papilla. (Appearance representative of both species). (Stain: Verhoeff-Van Gieson; Culling, 1974). (x 590)

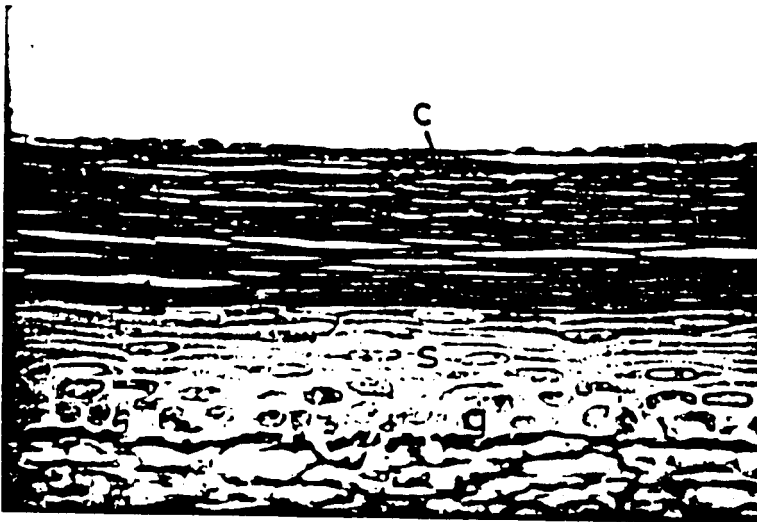
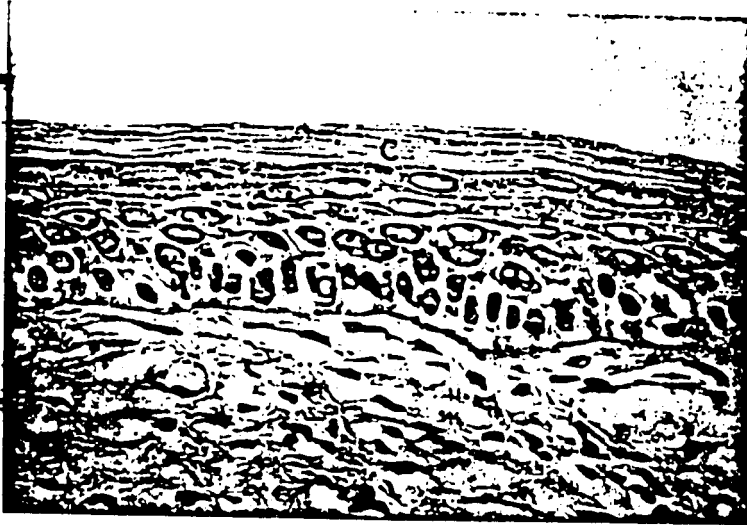
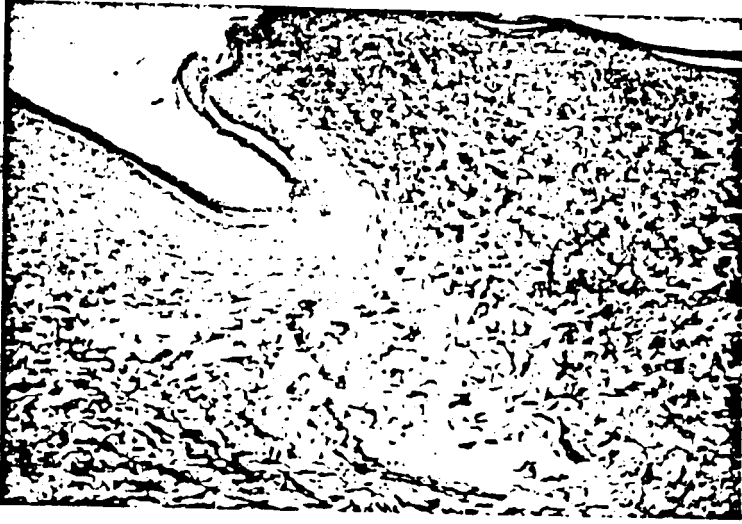
Figure 5.10

Photomicrograph showing the connective tissue matrix adjacent to the muscularis externa in the oesophagus. Note the large mature fibres and fine immature fibres. (Appearance representative of both species). (Stain: Verhoeff-Van Gieson; Culling, 1974). (x 590)

Figure 5.11

Photomicrograph of the elastic fibres immediately adjacent to the muscularis externa which tend to lie parallel to the long axis of the oesophagus. (Appearance representative of both species). (Stain: Verhoeff-Van Gieson; Culling, 1974). (x 230)

Note: Collagenous fibres, red  
Elastic fibres, black  
Smooth muscle fibres, yellow



Germinativum nuclei contain granular chromatin which stains darkly with haematoxylin and one or two prominent eosinophilic nucleoli (Fig 5.7).

Basement membrane:

A fine basement membrane composed of collagen and reticulin fibres (Figures 5.6 and 5.9) borders the Stratum Germinativum. It averages 5  $\mu$ m in thickness and stains slightly with eosin in haematoxylin and eosin preparations.

Thus the epithelium as a whole exhibits cytoplasmic baseophilia, with the lowest stratum staining more darkly because the basal cells are smaller and have a high nuclear to cytoplasmic ratio. The reticular stain (Gordon and Sweet, 1936) showed deposits of reticulin in the keratinized cells of the epithelium. These deposits appear to be first laid down in the cells of the Stratum Spinosum and can be seen in the cells of the Stratum Corneum (Figure 5.6).

5.1.3.2 *Caretta caretta*

The oesophageal epithelium in *Caretta caretta* can not be distinguished histologically from that found in *Chelonia mydas*.

5.1.4 Connective tissue

5.1.4.1 *Chelonia mydas*

An extensive layer of connective tissue lies beneath the epithelium. There is no muscularis mucosae dividing the connective tissue into a lamina propria and a submucosal layer. The connective tissue layer varies in thickness from 0.55mm to 2.85 mm and tends to be thicker in regions where the stratified muscle layer of the muscularis externa is reduced, and locally beneath the larger epidermal papillae.

There are three types of connective tissue fibres present in the oesophageal connective tissue matrix: collagenous, elastic and reticular

Figure 5.8

Photomicrograph showing the connective tissue matrix immediately beneath the epithelium of the oesophagus. Note the band of immature collagenous fibres (red) adjacent to the epithelium and the band containing mature collagenous and elastic fibres (black) adjacent to the collagenous band. (Appearance representative of both species). (Stain: Verhoeff-Van Gieson; Culling, 1974). (x 230)

Figure 5.7

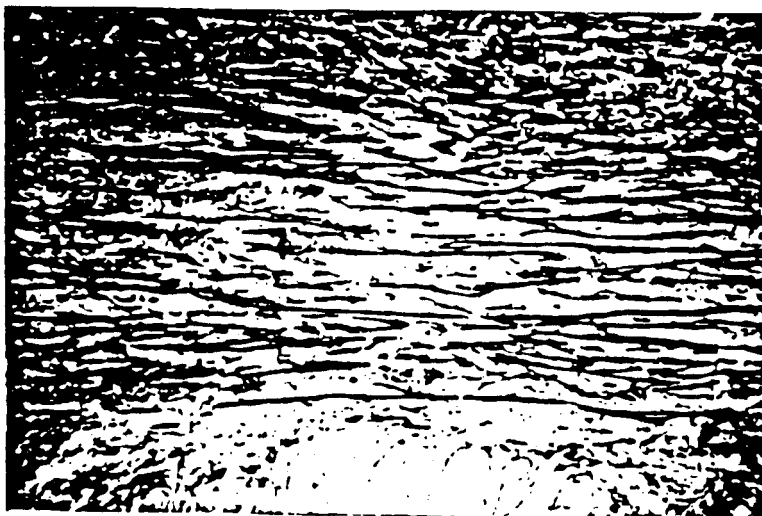
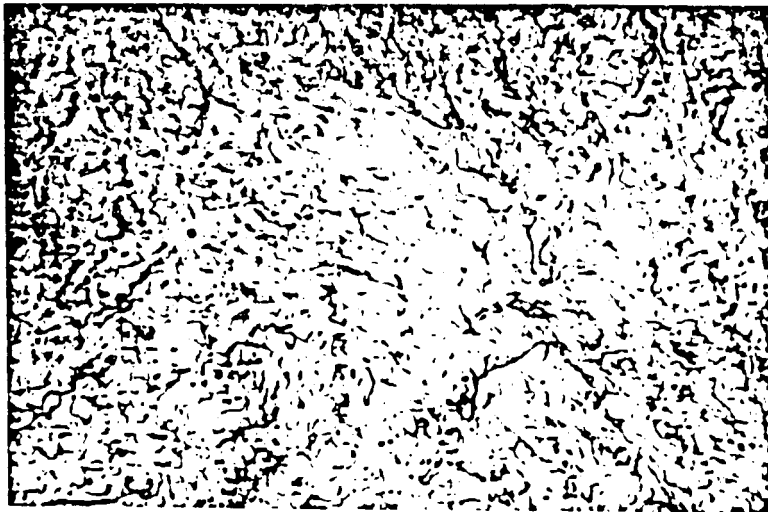
Photomicrograph showing the keratinized squamous epithelium of the oesophagus. Note the three strata of cells as in figure 5.6. Although it is not apparent in this photograph, the nucleoli in the epithelial cells are acidophilic. This is not unusual for cells actively involved in protein production, in this case keratin production. (Appearance representative of both species). (Stain: haematoxylin and eosin; Lillie, 1965). (x 1470)

c    Stratum Corneum;            s    Stratum Spinosum;

g    Stratum Germinativum

Figure 5.6

Photomicrograph showing the keratinized squamous epithelium of the oesophagus. Note that the epithelium is composed of three strata of cells and that the uppermost stratum contains keratin (red) and reticulin (black). (Appearance representative of both species). Stain: Gordon and Sweet; Cook, 1974). (x 1830)



fibres. Both the collagenous and elastic fibres are present as mature (thick, darkly staining fibres, usually in bundles) and immature (fine, threadlike, pale fibres, usually present as single strands) fibres in various combinations.

Collagenous fibres form a dense layer directly beneath the surface epithelium and completely fill the smaller papillae (Figure 5.8). Beneath the smaller papillae this dense layer of mature collagen extends to the muscularis externa, but in the larger papillae it forms only a thin band just beneath the epithelium.

A much finer (though dense and fibrous) connective tissue matrix occupies the core of the papillae. This matrix is composed almost entirely of immature collagenous fibres (Figure 5.9). Some immature elastic fibres are also present. Their numbers increase towards the muscularis externa but decrease peripherally, the smallest number being present in the matrices filling the tips of the papillae. The fine fibrous matrices which fill the large papillae are peculiar to them and occur nowhere else in the gastro-intestinal tract. These matrices do not extend all the way to the muscularis externa beneath the large papillae. The intervening tissue layer contains both mature and immature collagenous and elastic fibres, the mature fibres being particularly numerous (Figure 5.10).

Reticular fibres occurring in association with mature elastic fibres, lie alongside the elastic fibres, and, in some cases, twine around them. These reticular fibres were not seen independently of the elastic fibres in the connective tissue matrices. Reticular fibres also occur in the elastic walls of the blood vessels supplying the connective tissue.

In the connective tissue adjacent to the muscularis externa, elastic fibres are particularly numerous (Figure 5.11). These fibres tend to be orientated parallel to the length of the oesophagus.



Except in this layer, the various connective tissue fibres do not have any set pattern of orientation.

The mature collagenous fibres of the connective tissue matrices are thick and wavy forming interlacing bundles, whereas the finer mature elastic fibres tend to be straighter and although they branch, do not form into bundles. Together both types of fibres form a superimposed meshwork through which are scattered a small number of cells, including wandering lymphocytes, fibroblasts and fibrocytes. No mast, paneth, argyrophil or argentaffin cells were demonstrated by the staining techniques used. (For techniques see table 2.1).

#### Connective tissue mucins:

The presence of neutral mucin was demonstrated in the matrices dominated by mature collagenous and elastic fibres. Those matrices dominated by immature fibres, especially by immature collagen, were shown to contain sulphated acid mucins. These mucins also appeared to be associated with the ground substance between the fibres. The central region or core of the larger papillae is conspicuous in that it stains distinctly blue in sections treated with alcian blue - PAS.

#### 5.1.4.2 *Caretta caretta*

The connective tissue matrices in the oesophagus of *Caretta caretta* are alike in arrangement and composition with those found in *Chelonia mydas*.

#### 5.1.5 Muscularis externa

##### 5.1.5.1 *Chelonia mydas*

The muscularis externa, which contains both striated and smooth muscle bundles, lies directly beneath the connective tissue layer. The oesophagus can be divided into three regions on the basis of the presence and distribution of smooth and striated muscle fibres.

At the pharyngoesophageal boundary there is a prominent ring-shaped band of smooth circular muscle fibres referred to as the pharyngoesophageal sphincter. Except for this small band of smooth muscle, the muscularis externa of the anterior region of the oesophagus is composed entirely of striated muscle fibres. This anterior striated muscle region occupies 27% of the entire length of the oesophagus. The muscularis externa of the next, central region of the oesophagus, contains both an inner striated and an outer smooth muscle layer and occupies 41% of the total length of the oesophagus. Altogether the anterior striated muscle band extends over 68% of the total length of the oesophagus, including the central region of overlap with the smooth muscle band. The muscularis externa in the remaining posterior-most region of the oesophagus is composed entirely of smooth muscle fibres. Altogether the posterior smooth muscle band extends over 72% of the total length of the oesophagus, including the central region of overlap with the striated muscle band.

Striated muscle fibres are longitudinally orientated and always occur closer to the lumen of the oesophagus than the smooth muscle fibres. The smooth muscle layer has a characteristic thick inner circular band and a discontinuous outer longitudinal band. Although the longitudinal band is always thinner than the circular band, it varies considerably in thickness. The longitudinal smooth muscle layer first appears near, or just posterior to, the point at which the striated muscle layer ceases. From the anterior end of the oesophagus, the striated muscle layer gradually decreases in thickness. At its posterior limit, the striated muscle layer is one third or less as thick as at the anterior end of the oesophagus. The smooth muscle layer gradually increases in thickness from anterior to posterior, so that the overall thickness of the muscularis externa along the region of the striated and smooth muscle overlap is approximately the same

(Figure 5.12).

Muscle fibre bundles within the muscularis externa are surrounded by thin layers of fibrous connective tissue. The main component of this connective tissue is collagen, however a few elastic fibres are also present. A connective tissue layer containing a mixture of immature and mature collagenous and elastic fibres, occurs between the smooth circular and smooth longitudinal muscle bands. The entire muscularis layer also contains reticular fibres, which form fine networks around individual muscle fibres and bundles.

#### 5.1.5.2 *Caretta caretta*

The arrangement and composition of the muscularis externa is very similar in *Caretta caretta* and *Chelonia mydas*. As in *Chelonia mydas*, the oesophagus can be divided into three regions on the basis of the presence and distribution of smooth and striated muscle fibres in the muscularis externa. The anterior region of striated muscle occupies 28% of the total length of the oesophagus, the central mixed striated and smooth muscle region 43% and the posterior smooth muscle region the remaining 29%. The most obvious difference in the muscularis externa of this species is the absence of an outer longitudinal smooth muscle band, the smooth muscle layer being composed entirely of circular muscle bundles. Elastic fibres also appear to be more numerous (in *Caretta caretta*) in the connective tissue between the muscle bundles and muscle layers.

#### 5.1.6 Subserosa and serosa

##### 5.1.6.1 *Chelonia mydas*

In most places, a layer of subserosal tissue of variable thickness and composition lies external to the muscularis externa. The usual components of this layer are, collagenous, elastic and reticular fibres,

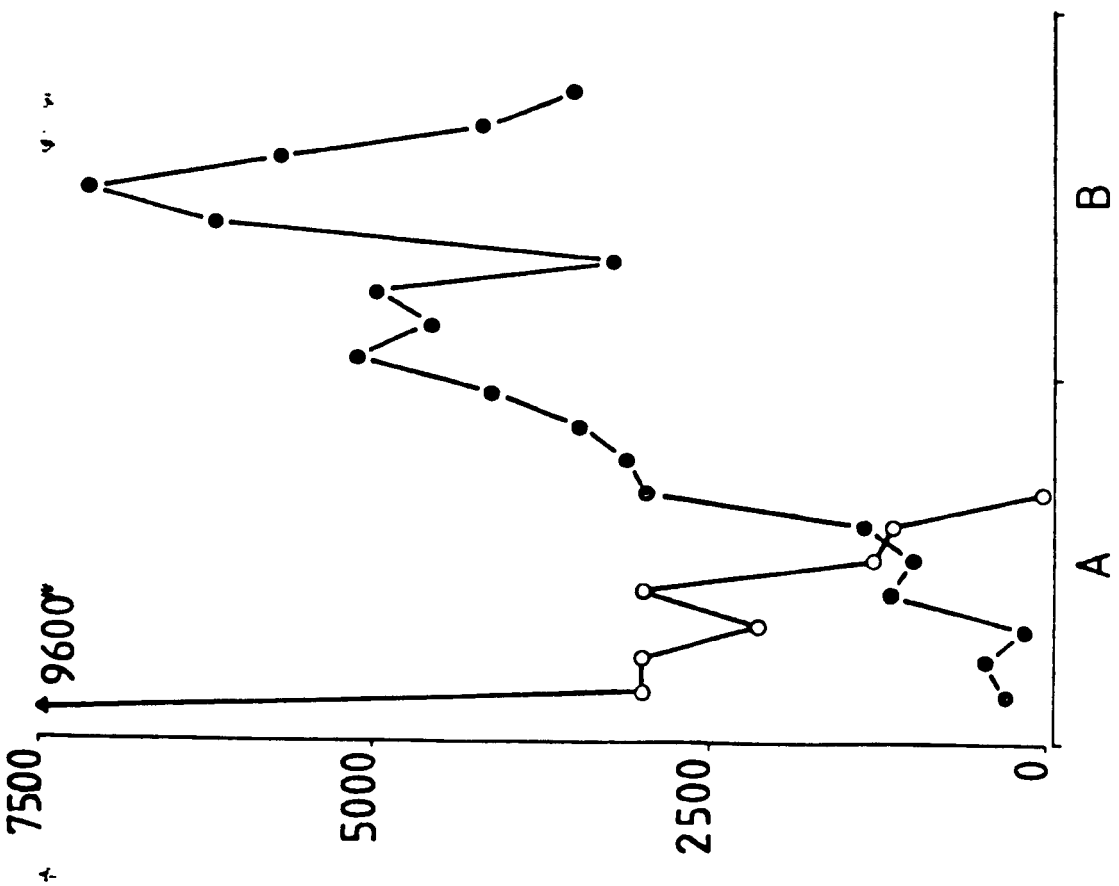
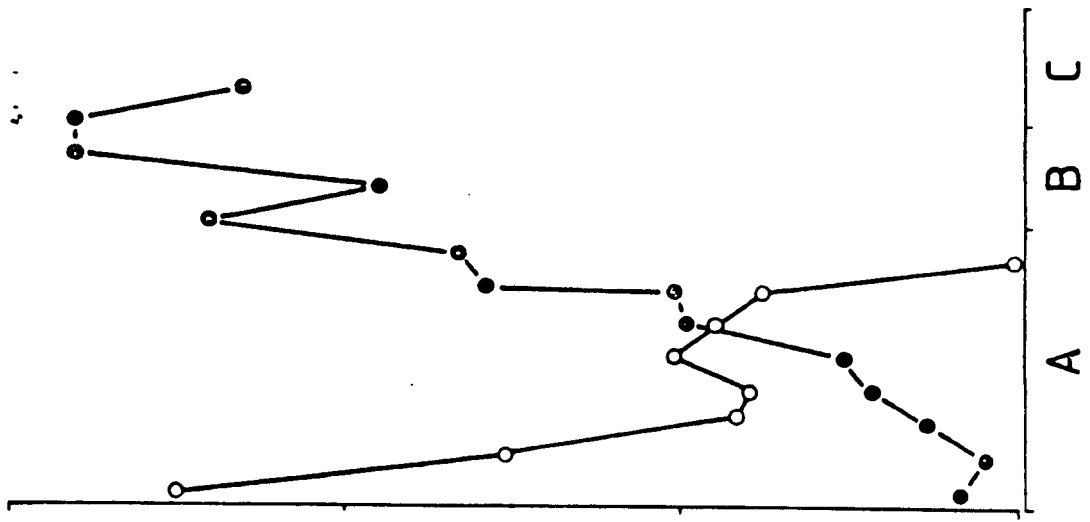
Figure 5.12

Smooth and striated muscle layers of the muscularis externa in the oesophagus. The striated muscle layer decreases in thickness posteriorly while the smooth muscle layer increases in thickness.

*Chelonia mydas*

*Caretta caretta*

0 Striated muscle; ● Smooth muscle; A Oesophagus; B Crop; C Stomach



veins, arteries, and nerves, but it often also contains short bands of longitudinal smooth muscle. This makes it difficult to recognize the boundary between the muscularis externa and subserosa. Elastic fibres are particularly numerous adjacent to the longitudinal muscle band. However the bulk of the subserosal connective tissue lies external to this and is composed largely of mature collagenous fibres. Reticular fibres are particularly numerous in the walls of the veins and arteries, and within the smooth muscle bundles.

The entire subserosal tissue layer is bounded by a simple squamous mesothelial or serosal membrane.

#### 5.1.6.2 *Caretta caretta*

The subserosal tissue layer is basically the same in *Caretta caretta* and *Chelonia mydas*. There are however no longitudinal smooth muscle bundles in this tissue layer in *Caretta caretta*.

## 5.2 DISCUSSION

### 5.2.1 Epithelium

In many reptiles the oesophageal epithelium contains variably distributed ciliated, microvillous, calciferous, goblet and columnar mucous cells (Table 5.2). According to Dandrifosse (1974), the oesophagus of fresh water turtles is lined with ciliated columnar epithelium interspersed with goblet cells. The oesophageal epithelium in the turtle *Chrysemys picta*, contains both ciliated and goblet cells (Thiruvathukal, 1965). In *Chrysemys picta*, the epithelium in the region where the pharynx joins the oesophagus is composed of stratified squamous cells. This epithelium gradates posteriorly into pseudo-stratified ciliated epithelium. Numerous funnel shaped goblet cells are scattered throughout the mucosa (Thiruvathukal, 1965).

TABLE 5.2

Epithelial cell types present in the oesophagus of reptiles

Group or Species	Cell types present in the epithelium	Reference
Snakes	ciliated, goblet	Luppa (1977)
<i>Xenodon merremii</i>	ciliated, goblet, columnar	Ferri and Medeiros (1975)
Alligator	ciliated, goblet, columnar	Luppa (1977); Reese (1913)
<i>Gehyra mutilata</i>	columnar	Chou (1977)
<i>Tiliqua scincoides</i>	ciliated, goblet, microvillous	Giraud <i>et al.</i> (1978)
<i>Uromastix aegyptia</i>	ciliated, goblet, calciferous, columnar	El-Toubi & Bishai (1955)
<i>Varanus griseus daud</i>	ciliated, goblet, calciferous	Bishai (1961)
<i>Chelonia mydas</i>	keratinized stratified squamous epithelium	This study
<i>Caretta caretta</i>	keratinized stratified squamous epithelium	This study

Neither ciliated, goblet or calciferous cells are present in the oesophageal epithelium of *C. mydas* or *C. caretta*. It seems that this is the only group to have keratinized papillae lining the oesophageal tube and a keratinized stratified squamous oesophageal epithelium. The presence of keratin in the epithelium of the buccal cavity (see Chapter 4) and oesophagus (also the crop, see Chapter 6), could provide a protective barrier against excess water loss from the tissues of the pharynx and oesophagus to the sea water ingested with food. Although much of this water is expelled from the food before it is swallowed (see discussion in Chapter 4), the oesophagus also appears to be adapted to this function. Contraction of the oesophageal musculature could function to squeeze water out of food and expell it anteriorly. This problem would not be encountered by reptiles feeding in fresh water or on land. It would be interesting to know whether the mucosa of the pharynx and oesophagus of sea snakes (family Hydrophiidae) and the marine Iguana, *Amblyrychus cristatus*, are similar to that of *Chelonia mydas* and *Caretta caretta*.

Some fresh water turtles have oesophageal papillae, although these are not as prominent as those in sea turtles. Ballmer (1949) found papillae in *Amyda spinifera* and *Graptemys geographica*. In the latter species, the papillae do not extend to the stomach but become less abundant posteriorly and are absent in the lower half of the oesophagus. In *Amyda*, the papillae almost reach the stomach. There is no "crop" between the oesophagus and the stomach in either of these species. In both species, the entire mucosal surface of the oesophagus, including the papillae, is lined with a ciliated columnar epithelium containing numerous goblet cells. The ciliated cells become more numerous anteriorly and disappear towards the stomach (Ballmer, 1949). In Testudininae (*Testudo graeca*, *Testudo horsfieldii*), the epithelium is stratified



(Luppa, 1977) but is not apparently keratinized nor raised into papillae.

Yoshie and Honma (1976), describe in detail the large keratinized papillae in the oesophagus of *Lepidochelys olivacea*. Identical, although smaller, papillae occur in the oesophagus of *Chelonia mydas*, *Caretta caretta* and *Eretmochelys imbricata* (personal observations). Yoshie and Honma (1976) hypothesized that these "spines" may contribute to the breaking of various foods into smaller pieces. My observations of food samples from the anterior and posterior oesophagus in *Caretta caretta* and *Chelonia mydas* suggest that this is not the case. In addition, my observations on the feeding behaviour of a *Chelonia mydas* turtle kept in captivity for five days, indicate that the papillae probably function to trap and hold ingested food while water is squeezed out of the ingesta and then pumped cranially through the oesophagus and out through the mouth cavity. This action would eliminate most of the water from the ingesta which then becomes compacted into a bolus in the lower oesophagus, or crop, to be passed into the stomach.

### 5.2.2 Oesophageal glands

There was a diversity of opinion concerning the presence and structure of oesophageal glands in reptilian species, among early workers (Nussbaum, 1882; Teschler, 1885; Beguin, 1904; Greschik, 1917-1918; Eisler, 1889; Hoffman, 1890; and Oppel, 1897: in El-Toubi and Bishai, 1959). Folds in the epithelium of the oesophagus, lined by mucous cells, were taken by some early workers to represent simple mucous glands (Nussbaum, 1882; Teschler, 1885: in El-Toubi and Bishai, 1959). Later workers did not consider these folds to be glands (Beguin, 1904; Greschik, 1917-1918). This and more recent work on the oesophageal glands of reptiles is reviewed by Luppa (1977).

Many reptiles lack oesophageal glands altogether (Table 5.3).

There seems to be no correlation between the systematic position of a reptile and the occurrence of oesophageal glands (Luppa, 1977). Ballmer (1949) also notes that there appears to be no correlation between food habits, environmental factors and the presence of glands.

In many turtles, the subepithelial tunica propria contains tubulo-alveolar glands (Table 5.3). These glands vary in size, distribution and number, between species. In all of the species in which tubulo-alveolar glands occur, the glands are lined in their ducts and terminal portions by the same type of cell, that is, by prismatic cells with flattened basal nuclei.

Luppa (1977) gives details of the histochemistry of the main cell types in reptilian oesophageal epithelium. He states that the ciliated cells produce small quantities of PAS-positive mucoproteins and have high enzyme activity. Goblet cells react positively with PAS and tests for acid mucopolysaccharides but have a low enzyme activity (Luppa, 1977). The mucous cells in the "crop" glands of *Chelonia mydas* and *Caretta caretta*, react histochemically like those described by Luppa (1977) from the oesophagus as goblet cells (personal observations).

### 5.2.3 Connective tissue layers

In most reptiles, lymph nodes penetrate either individually or in aggregates from the tunica propria into the epithelium (Luppa, 1977). Many capillaries and larger blood vessels are also present in the fibrous tunica propria. Few lymph cells are found in the tunica propria of the oesophagus in *Chelonia mydas* and *Caretta caretta*, although this tissue does contain large numbers of lymphocytes in other regions of the alimentary tract (personal observations).

4 A muscularis mucosae is normally found only in the posterior section

TABLE 5.3

The presence of oesophageal glands in Reptilia

Group or species	Presence and type of gland	Reference
Snakes	none	Luppa (1977)
<i>Xenodon merremi</i>	none	Ferri and Medeiros (1975)
Alligators	none	Luppa (1977); Reese (1913)
<i>Gehyra mutilata</i>	none	Chou (1977)
<i>Tiliqua scincoides</i>	none	Giraud <i>et al.</i> (1978)
<i>Uromastix acanthinurus</i>	tubulo-alveolar mucous	Luppa (1977)
<i>Uromastix aegyptia</i>	mucous, oxyntic, mucous-oxyntic	El-Toubi & Bishai (1955)
<i>Varanus griseus daud</i>	none	Bashai (1961)
<i>Chrysemys picta</i>	mucous	Thiruvathukal (1965)
<i>Trionyx chinensis</i>	none	Ballmer (1949)
<i>Amyda spinifera</i>	none	"
<i>Emys europaea</i>	no	"
<i>Sternotherus odoratus</i>	none	"

Continued over

TABLE 5.3 (Continued)

Group or species	Presence and type of gland	Reference
<i>Clemmys insculpta</i>	none	Ballmer (1949)
<i>Chrysemys picta</i>	none	"
<i>Emys blandingii</i>	beginnings of glandular formation	"
<i>Terrapene carolina</i>	rudiments of glandular formation	"
<i>Graptenys geographica</i>	rudiments of glandular formation	"
<i>Chelondina longicollis</i>	compound tubular glands	"
<i>Chelys fimbriata</i>	low simple tubular	"
<i>Clemmys caspica</i>	large tubular	"
<i>Testudo graeca</i>	simple deep tubular	"
<i>Chelemys victoria</i>	branched and unbranched, tubular	"
<i>Chrysemys picta</i>	compound tubular, (zymogen)	"
<i>Chelonia imbricata</i>	(probably lower oesophagus = crop)	El-Toubi and Bishai (1955)
<i>Caretta caretta</i>	compound coiled tubuloalveolar (mucous) (in lower oesophagus = crop)	This study
<i>Chelonia mydas</i>	compound coiled tubuloalveolar (mucous) (in crop at posterior end of oesophagus)	This study

of the reptilian oesophagus, although this is by no means the case in all reptiles, and it is usually absent altogether in turtles (Ballmer, 1949; Luppa, 1977). This study shows that *Chelonia mydas* and *Caretta caretta* are similar to other turtles in that they lack a muscularis mucosae in the oesophageal region.

The submucosa, where present, is composed of a fairly dense mass of connective tissue. As in the lamina propria, the types of fibres present vary from species to species. Blood vessels and connective tissue cells lie scattered through the fibrous matrix (Luppa, 1977). The connective tissue adjacent to the muscularis externa in *C. mydas* and *C. caretta* is similar to the submucosal tissue described by Luppa (1977).

#### 5.2.4 Muscularis externa

The oesophageal muscularis externa (or tunica muscularis) in reptiles, follows a basic pattern as described by Luppa (1977). The inner layer is composed of circular smooth muscle and the outer layer, where present, of longitudinal smooth muscle fibres. The outer longitudinal layer is generally missing in the anterior portion of the oesophagus, or is present as small scattered bundles of muscle fibres, which consolidate in the posterior oesophagus into a well defined band. This outer band may reach the same thickness as the inner circular band but it is generally thinner.

In the turtle species examined by Ballmer (1949), the muscularis externa is typical of most reptiles. The longitudinal muscle band is less prominent at the anterior end, but becomes as prominent as the circular band in the posterior oesophagus. The presence of striated muscle is mentioned only by Thiruvathukal (1965), for the fresh water turtle *Chrysemys picta*. Thiruvathukal records that there is a prominent

arrangement of striated muscle in the anterior part of the oesophagus. Otherwise the distribution of muscle fibres in the oesophagus of *Chrysemys picta* follows a pattern typical of reptiles in general (Luppa, 1977).

In *Chelonia mydas* and in *Caretta caretta* the muscularis externa is peculiar in a number of respects. A well developed band of striated muscle is present in the anterior 68 to 71% of the length of the oesophagus, and in the anterior 27 to 28% of the oesophagus there is no smooth muscle band at all, although it is well developed over the remainder of the oesophagus. The outer longitudinal smooth muscle band is absent altogether in *Caretta caretta*, but is present as a thin band in the posterior region of the oesophagus in *Chelonia mydas* (personal observations).

#### 5.2.5 Subserosa and serosa

The serosal band is variable in thickness and in composition within and between species. Where a subserosa is present in turtles, it is composed mainly of connective tissue fibres, connective tissue cells, major blood vessels and some nerves (Ballmer, 1949). The serosa is usually a simple squamous membrane and surrounds the entire oesophagus. The subserosa and serosa in both *Chelonia mydas* and *Caretta caretta* was like that described by Ballmer (1949) and Luppa (1977).

## CHAPTER VI

## THE CROP

6.1 RESULTS6.1.1 Anatomy6.1.1.1 *Chelonia mydas*

In *Chelonia mydas* the crop is a muscular "U" shaped tube connecting the oesophagus and the stomach. In adult turtles it has an internal circumference of 5.0 cm to 9.7 cm (Table 10.6) and is usually wider posteriorly than it is anteriorly. There is no external indication of the junction between the oesophagus and crop, but there is a noticeable constriction at the junction of the crop and the stomach. The crop represents, on average, 2.4% of the total length of the alimentary canal in adult and subadult turtles (Table 10.3).

6.1.1.2 *Caretta caretta*

In *Caretta caretta* the crop is a short, straight, narrow tube connecting the oesophagus to the stomach. In the two adult turtles (Cc2, Cc3) of this species examined in detail the internal circumference of the crop was 6.3 to 7.0 cm and 6.5 to 7.0 cm (Table 10.6). The length of the crop was measured in five adult turtles and ranged from 6.0 to 11.0 cm (Table 10.3). The crop represents, on average, 0.9% of the total length of the alimentary canal in this species (Table 10.3).

Externally, there is no indication of the junction between the oesophagus and crop. The junction of the crop and the stomach is indicated by the sudden increase in circumference of the alimentary canal at the anterior end of the stomach.

## 6.1.2 Internal relief of the mucosal surface

### 6.1.2.1 *Chelonia mydas*

Internally the transition from the oesophageal mucosa to the crop mucosa is gradual. The oesophageal papillae become less numerous and the area covered by wrinkled leaf-like folds (as found between the papillae in the oesophagus), more extensive, until the entire surface is covered with this highly wrinkled crop epithelium (Figure 6.1). The non-keratinized glandular epithelium of the lower crop is hidden between the keratinized folds of the mucosal surface. At the junction of the crop and the stomach the keratinized epithelium of the crop borders the mucus cell epithelium of the cardiac glands (Figure 6.2).

### 6.1.2.2 *Caretta caretta*

Internally, the mucosa of the oesophagus gives way abruptly to the mucosa of the crop. The oesophageal papillae cease some 6.0 to 11.0 cm anterior to the junction of the crop and the stomach (Figure 6.3). The keratinized epithelium in the nonglandular region of the crop is highly folded (Figure 6.3). The mucus glands at the posterior end of the crop are much more numerous and more extensive in *C. caretta* than in *C. mydas*. As a consequence the epithelial mucus cells surrounding the gland pits form a continuous layer in most places and the gland pits are not recessed between keratinized folds, as they are in *Chelonia mydas*. Only a few small folds of keratinized epithelium occur in the cardiac band (Figure 6.3). The glandular mucosa has a lobular appearance and is coated with mucus. It forms a narrow band, approximately 2.0 cm wide, at the posterior end of the crop, adjacent to the gastric mucosa. In fresh, unfixed material the glandular mucosa of the crop is a yellowish-orange colour while the gastric mucosa is a reddish-brown colour (Figure 6.4).



Figure 6.3

↑ #  
*Caretta caretta*: mucosal surface at the oesophagus, crop and stomach junction. There is a short transition zone at each junction. (x 0.85)

o oesophagus  
 c crop  
 s stomach  
 g crop glands

Figure 6.2

↑ #  
*Chelonia mydas*: mucosal surface at the junction of the crop and stomach. The crop epithelium gives way to gastric epithelium along a clearly defined line. (x 1.13)

c crop  
 s stomach

Figure 6.1

↑ #  
*Chelonia mydas*: mucosal surface at the junction of the oesophagus and crop. The Spinose oesophageal epithelium gives way to non spinose crop epithelium over a short transition zone. (x 1.1)

o oesophagus  
 c crop

Note: transverse furrows across segments are an artifact of preparation.



Figure 6.4

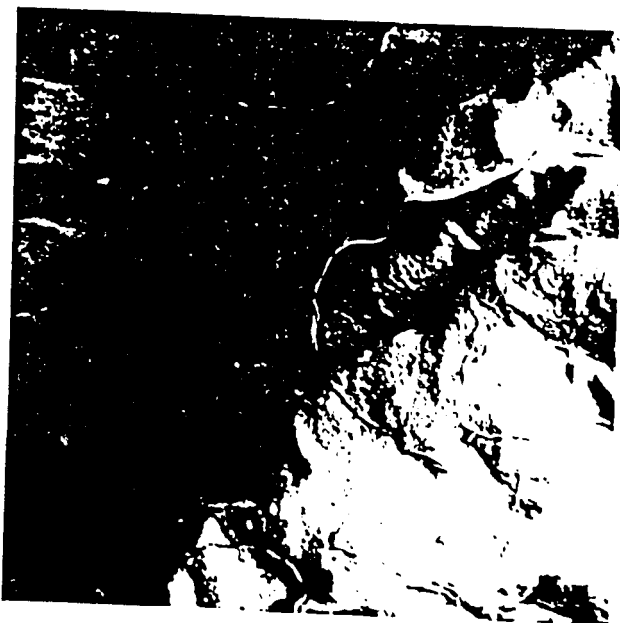
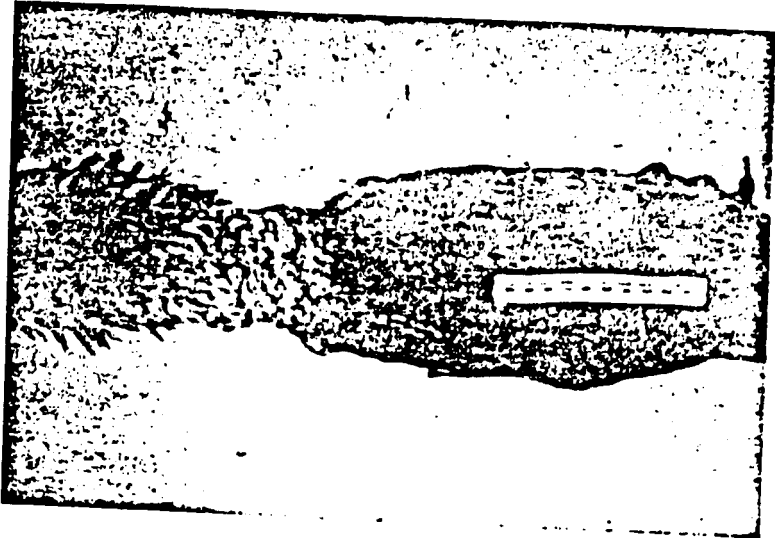
*Caretta caretta*: junction of oesophagus and crop, and the crop and stomach. Nonglandular crop (white); glandular crop (orange); stomach (red-brown). (x 0.3)

Figure 6.5

*Chelonia mydas*: electronmicrograph showing the keratinized, squamous epithelium of the crop. Note the crinkled surface and the squamous cells lifting away from surface. (x 60)

Figure 6.6

*Chelonia mydas*: electron micrograph showing the keratinized, squamous cells of the epithelial surface in the crop. (x 740)



### 6.1.3 Surface epithelium: non glandular

#### 6.1.3.1 *Chelonia mydas*

Under the scanning electron microscope, the keratinized epithelial surface of the crop is very similar to that of the oesophagus, but the surface has a much more crinkled appearance (Figure 6.5). The keratinized scale-like epithelial surface cells are similar to those of the oesophagus (Figure 6.6).

In section, the keratinized stratified squamous epithelium of the crop is almost identical with that found in the oesophagus and it is unnecessary to describe it again.

#### 6.1.3.2 *Caretta caretta*

Under the scanning electron microscope, and in section, there appears to be no difference between the keratinized epithelium in the crop of this species and that found in the crop of *C. mydas*.

### 6.1.4 Glands

#### 6.1.4.1 *Chelonia mydas*

In the posterior crop the mucous cells which form the epithelium adjacent to the gland pit, can not be seen with the scanning electron microscope as the glands lie at the bases of deep keratinized crypts (Figure 6.7).

When a longitudinal section was made through the glandular region of the crop in turtles cm3 and cm4, the glands were seen to occupy a band of 3.5 cm in extent. Within this band, small groups of simple, coiled, tubular mucous glands were seen to open to the mucosal surface via short secretory ducts (Figure 6.8). One or two of these gland groups open into a broad pit at the base of each deep keratinized crypt. In the gland pits, the epithelium surrounding the openings of the secretory ducts is composed of simple low columnar mucous cells (Figure

Figure 6.7

*Chelonia mydas*: electron micrograph of a crypt opening into a crop gland. Glands are recessed below the keratinized surface at the base of crypts which are lined in their distal portions by keratinized epithelium. (x 90)

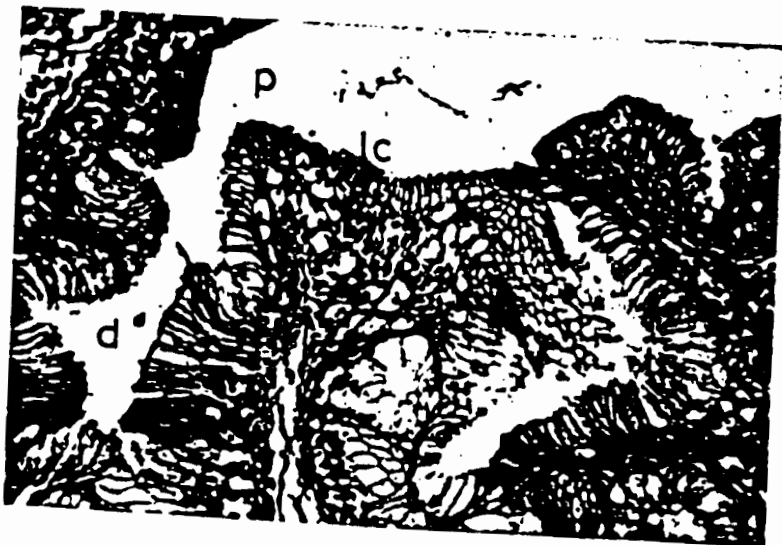
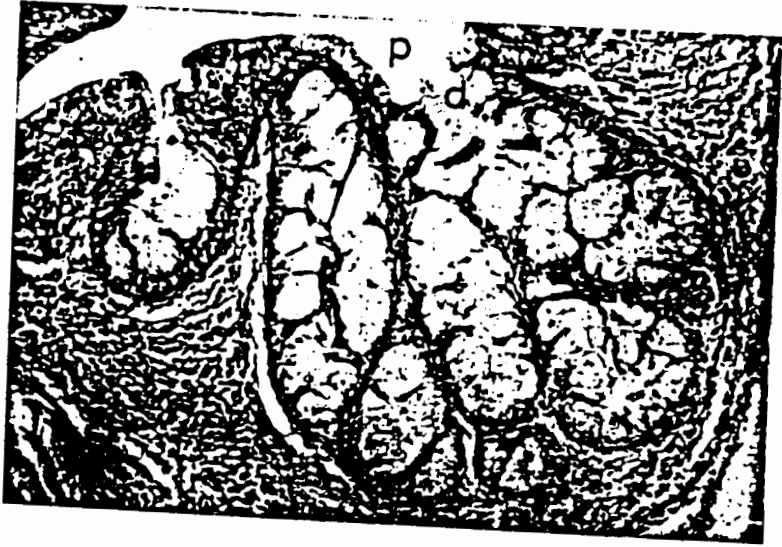
Figure 6.8

*Chelonia mydas*: photomicrograph showing a cluster of crop glands at the base of a gland crypt. (Stain: haematoxylin and eosin; Lillie, 1965). (x 300)

p	gland pit	;	lc	low columnar cells
d	gland duct	;	tc	tall columnar cells

Figure 6.9

*Chelonia mydas*: photomicrograph showing the mucous cell epithelium lining the gland pits and ducts in the crop glands. Low columnar epithelium gives way to tall columnar epithelium. Large numbers of lymphocytes occur in the epithelial tissues. (Stain: haematoxylin and eosin; Lillie, 1965). (x 670)



6.9). Each small group of glands forms an ovoid-shaped mass which is recessed into the subepithelial tunica propria beneath the gland pit. There is no muscularis mucosae in the crop and the tunica propria occupies the entire space between the epithelium and the muscularis externa. Each ovoid-shaped gland mass is located within the inner two thirds of the subepithelial tunica propria layer and is approximately 0.94 mm in depth from the epithelial surface of the gland pit. In any one longitudinal section through the glandular band of the posterior crop, only a few groups of glands can be seen interspersed with connective tissue.

There is a gradual transition from the keratinized, stratified, squamous epithelium lining the crop and the gland crypts, to the simple columnar mucous epithelium lining the secretory ducts of the crop glands. The Stratum Corneum gradually thins until it disappears. Scattered cuboidal mucous cells, measuring approximately 12 by 10  $\mu\text{m}$ , begin to appear among the cells of the thickened Stratum Spinosum. These cuboidal cells have rounded basal nuclei with diameters averaging 6  $\mu\text{m}$ , a small amount of eosinophilic cytoplasm and a small apical mucous cap that is up to 10  $\mu\text{m}$  in height (Figure 6.9). Gradually the mucous cells become more numerous and taller until they form a continuous layer of columnar mucous cells 37 to 50  $\mu\text{m}$  high adjacent to and lining the secretory ducts leading from the individual glands (Figure 6.9). These latter mucous cells closely resemble those found in the gastric mucosa. Each has a large mucous cap that is variable in size and is located distal to the rounded basal nucleus. The nuclei average 10  $\mu\text{m}$  in diameter. The mucous cap and the nucleus of each cell are separated by a narrow band of eosinophilic cytoplasm. In haematoxylin and eosin preparations each nucleus is seen to contain darkly staining chromatin granules. The remainder of the nuclear material stains lightly with haematoxylin.



The prismoid mucous cells lining the tubules of each gland are all of one type. A large mucous cap of variable size occupies almost the entire cell and presses against the flattened basal nucleus which measures 3 by 10  $\mu\text{m}$ . These mucous cell nuclei stain more darkly with haematoxylin than those of the surface epithelium. The cells of the gland tubules are shorter than those in the secretory duct and average 25  $\mu\text{m}$  in height by 2  $\mu\text{m}$  in diameter (Figures 6.9 and 6.10).

The mucus material in the cells of the gland pit duct and tubules does not stain in haematoxylin and eosin preparations but reacts positively to stains for acid and neutral mucopolysaccharides (Table 6.1). In all the mucous cells the mucus product is dominated by acid mucopolysaccharides which include sialomucin, strongly sulphated acid mucin and weakly sulphated acid mucin (Figure 6.11). No pepsin is produced by any of these cells (Table 6.1).

#### 6.1.4.2 *Caretta caretta*

Under the scanning electron microscope, the epithelium on the basal sides of the small keratinized folds which occur between the crop glands, is unlike the epithelium found in any other region of the alimentary canal. Each cell in this epithelium has a clearly defined, pentagonal-shaped, collar or border surrounding and separating it from the adjacent cells (Figure 6.12). Although most of these cells are roughly pentagonal in outline, they are irregular in size and shape. The apical surface of each cell is covered with what appear to be short microvilli (Figure 6.13). Where the above described epithelium gives way to the surface cells of the glandular epithelium, the collars bordering the cells disappear and the cells have more prominent microvilli covering their apical surfaces (Figure 6.14). The secretory ducts of the crop glands open to the surface between these latter cells (Figure 6.15). The cells above the crop glands closely resemble those of the gastric mucosa

Figure 6.10

Photomicrograph of cells lining the crop gland tubules. Epithelium lining the gland tubules is simple, tall and columnar. Each cell contains a large mucus filled theca. (Appearance representative of both species). (Stain: haematoxylin and eosin; Lillie, 1965).  
(x 940)

Figure 6.12

*Caretta caretta*: electron micrograph showing the cells of the epithelium which occurs between the keratinized squamous epithelium and the glandular epithelium of the crop. Note "collar" like border around each cell. (x 460)

Figure 6.13

*Caretta caretta*: electron micrograph showing microvilli on the surface of the "collar" cells adjacent to the crop glands.  
(x 2310)

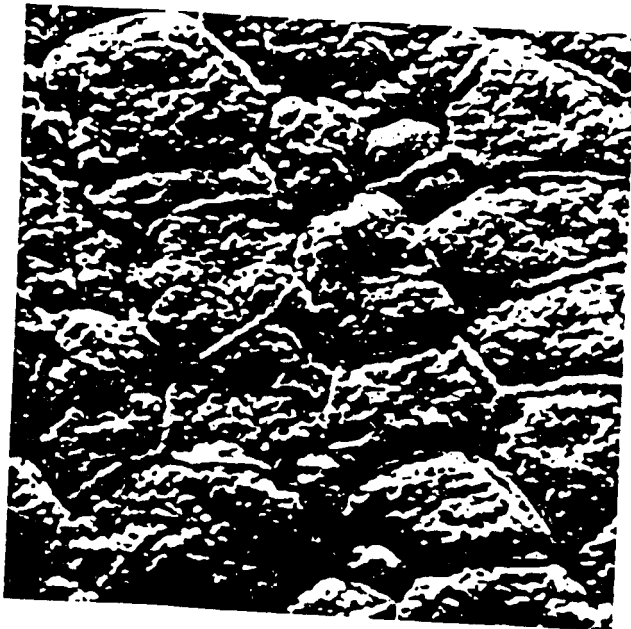
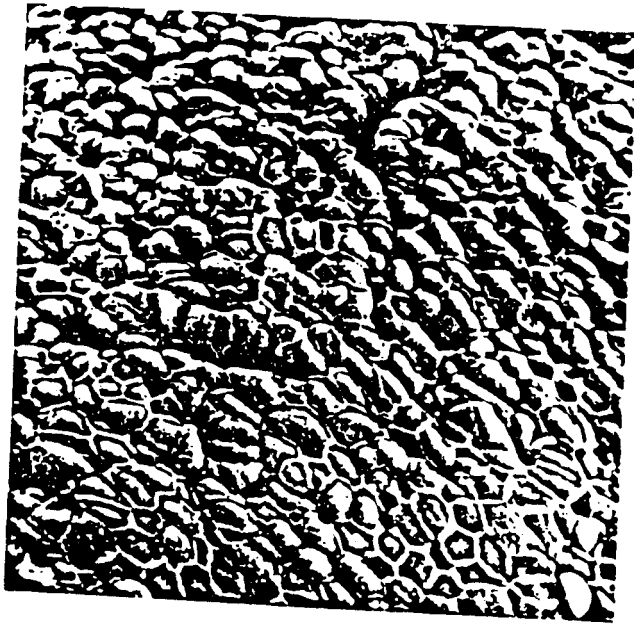





Figure 6.11

Mucins produced in the crop gland cells of

*Chelonia mydas*

*Caretta caretta*

- s surface epithelium; P gland pit; i gland isthmus; n gland neck;
- f gland fundus; N neutral mucin; ss strongly sulphated acid mucin;
- ws weakly sulphated acid mucins;  less than;  greater than;
-  greater than or equal to; + from anterior to posterior crop glands.

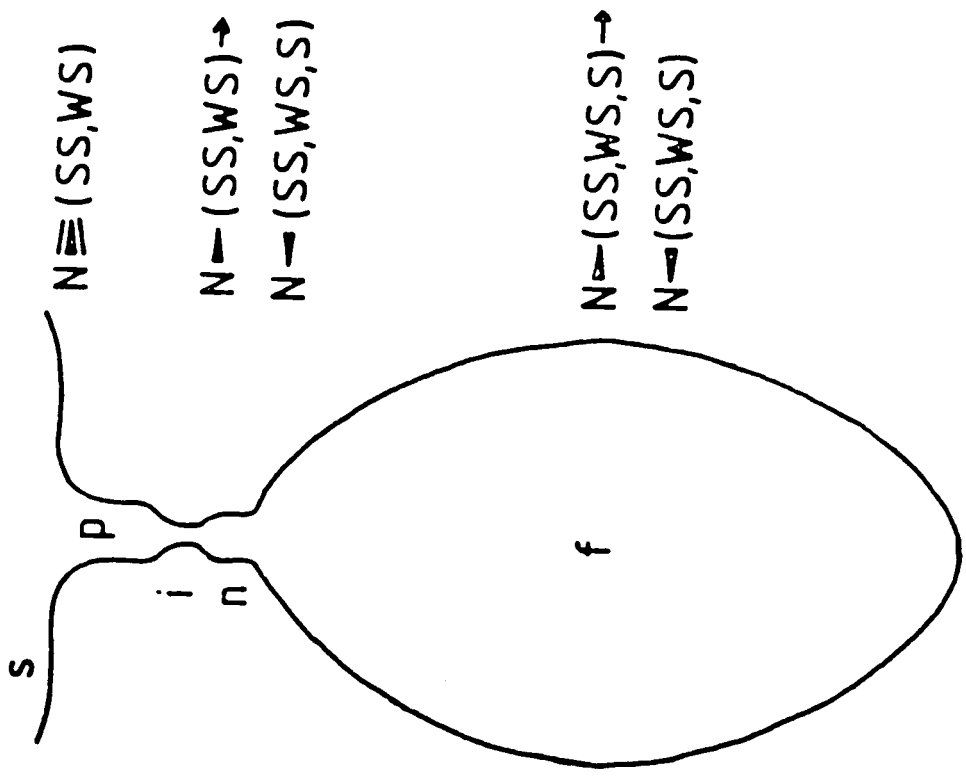
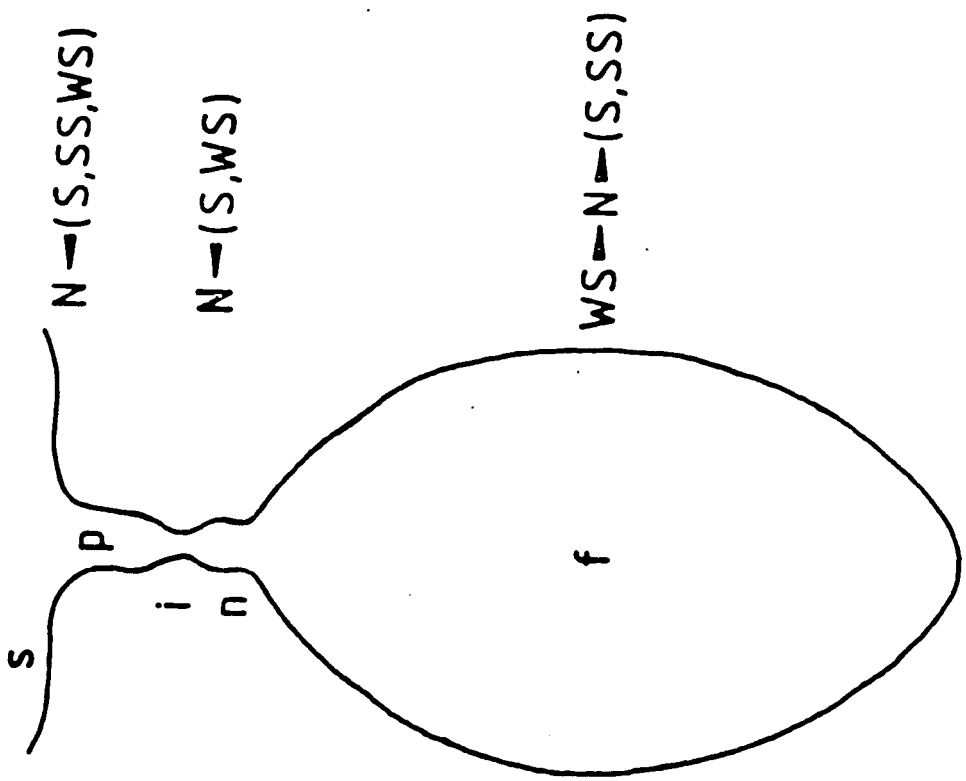


TABLE 6.1  
 REACTIONS OF THE CROP GLAND CELLS TO TESTS FOR MUCINS AND PEPSIN

Stain Series	Epithelial pit surface cells		Secretory duct cells		Tubule or fundus cells	
	<i>Chelonia mydas</i>	<i>Caretta caretta</i>	<i>Chelonia mydas</i>	<i>Caretta caretta</i>	<i>Chelonia mydas</i>	<i>Caretta caretta</i>
PAS	**	**	**	**	**	*
AB-PAS pH2.5	+/+*	+/+*/++	+/+*	+/+* →	+/+*	**/+++/+*
AB pH2.5	+++	++ → +++	+++	++ → +++	++	++
AB-PAS pH1.0	+/+*/++	+/+*/++ → **/++*/++	**/++*/++	+/+*/++ → **/++*	+/+*	+/+*/++ → **/++*
AB pH1.0	+	+ ; ++	+	+ ; ++	+++ → 0	+ ; ++
AB-PAS pH0.2	**/++	**/++	**/++	**/++	** ; **/++	**/++
AB pH0.2 (iii)	++	+	++ + 0	+	0 ; ++	+
H <sub>2</sub> SO <sub>4</sub> digest + AB pH2.5	++	++ → +++	++	++	+	+
Bowie (I)	0	0	0	0	0	0
Bowie (II)	0	0	0	0	0	0
Liisberg	0	0	0	0	0	0

Key: 0 clear + Pale blue ++ blue +++ dark blue \* Pale magenta \*\* magenta \*\*\*\* dark magenta  
 ++ Purple + Apex to base of gland → Anterior to posterior of gland region (I) Orange G-crystal violet (II) Ethyl violet-Biebrich scarlet (iii) Sallomucin digest AB Alcian blue PAS periodic acid Schiff —→ order of abundance, most dominant to least dominant

Figure 6.14

*Caretta caretta*: electron micrograph showing microvillous cells lining the epithelial surface surrounding the pits leading into the crop glands and lining the gland pit themselves. (x 2310)

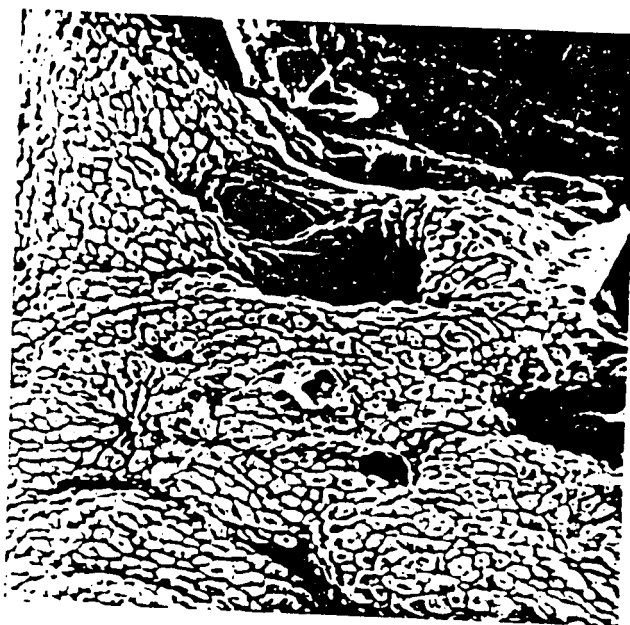
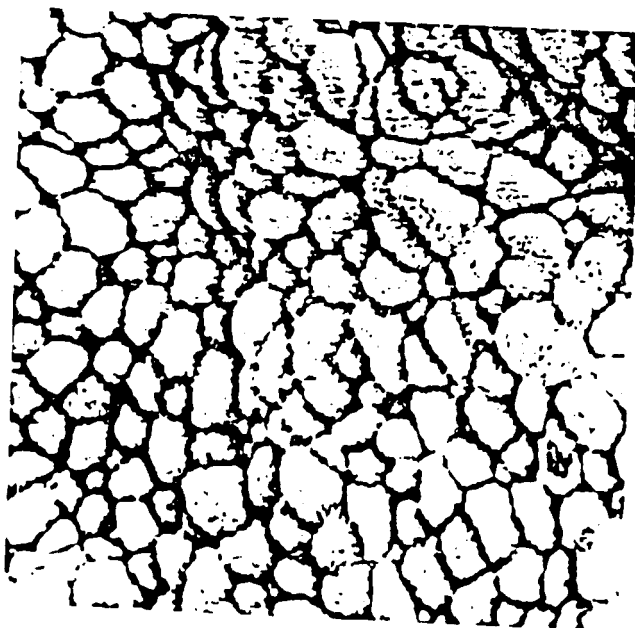
Figure 6.15

*Caretta caretta*: electron micrograph showing secretory ducts opening to the surface from the crop glands. (x 1870)

Figure 6.16

*Caretta caretta*: photomicrograph of a longitudinal section through the junction of the crop and stomach showing the crop gland tubules, lymph node and cardiac gland tubules. (Stain: haematoxylin and eosin; Lille, 1965). (x 250)

- c crop gland tubules
- l lymph node
- g cardiac gland tubules





in their appearance when viewed under the scanning electron microscope.

When a section through the crop region is viewed under the light microscope, the keratinized stratified, squamous epithelium, the "cellar" cell epithelium and the simple columnar mucous cell epithelium, can not be distinguished in appearance from the epithelia surrounding and making up the gland pits in *C. mydas*. It seems reasonable to assume that the epithelium seen under the scanning electron microscope (in *C. caretta*) to be composed of cells bordered by "collars", is equivalent to the simple cuboidal mucous cell epithelium (seen under the light microscope) which occurs between the keratinized epithelium and the tall columnar mucous cell epithelium of the gland pits in both species.

When a longitudinal section was taken through the glandular region of the crop in turtles cc2 and cc3 the glands were seen to occupy the posterior 3.1 cm of the crop. The glands themselves are not formed into small groups, as they are in *C. mydas*, but instead occupy almost the entire tunica propria tissue layer of this region, and are up to 6.40 mm in depth. The simple columnar mucous cell epithelium which lies above the glands is only occasionally interrupted by keratinized stratified squamous and simple cuboidal mucous cell epithelium. Adjacent gland tubules are so closely packed together that there is only a small amount of connective tissue separating them (Figure 6.10).

The crop glands lie adjacent to the cardiac gland region of the stomach. There is an abrupt boundary between the crop and cardiac gland regions (Figure 6.16). An area of lymphatic tissue usually occurs beneath the glands at the boundary of the crop and cardiac gland regions (Figure 6.16).

Although the crop glands in *C. caretta* are more extensive in number and in size than they are in *C. mydas*, the cells which they contain can not be distinguished under the light microscope from those found in

*C. mydas* and they need not be described. However, these cells do not react in the same manner, as the cells in *C. mydas*, to stains for acid and neutral mucopolysaccharides (Table 6.1). The overall results suggest that neutral mucopolysaccharide is the dominant product of the surface mucous cells above the glands and of the cells in the ducts and tubules of the more anterior glands, while acid mucopolysaccharides are the dominant products of the cells in the ducts and tubules of the more posterior glands (Figure 6.11). There is no clear boundary between the more neutral anterior and the more acid posterior glands. Instead there is a gradual transition from one to the other. None of the cells in the crop glands produce pepsin (Table 6.1).

#### 6.1.5 Connective tissue

##### 6.1.5.1 *Chelonia mydas*

There is no muscularis mucosae dividing the connective tissue layer of the crop. The entire tissue layer between the epithelium and the muscularis externa can be termed the tunica propria.

The connective tissue fibres of the tunica propria in the non-glandular region of the crop tend to be formed into three bands. Directly beneath the epithelium is an inner band of dense collagenous connective tissue. Mature collagenous fibres are the major component of this band, which also contains immature elastic fibres, small blood capillaries and immature collagenous fibres. Underlying this inner band is a more extensive central band composed of smooth muscle fibres and mature collagenous fibres. It is well supplied with small and large veins and arteries and contains small scattered groups of elastic fibres. Adjacent to the muscularis externa is the third band of connective tissue which is more extensive than the two inner bands. It is composed entirely of mature and immature collagenous and elastic

fibres and scattered bundles of longitudinal smooth muscle. This external band is much less vascular than the central band and is bordered by the large veins of the central band. Elastic fibres are very prominent and are evenly distributed through the collagenous fibre matrix.

The inner and central bands extend into the wrinkled mucosal folds of the crop, suggesting that they are equivalent to the mucosa, while the outer band is completely external to the folded mucosa suggesting that it is equivalent to the submucosa. The few blunt papillae at the anterior end of the crop contain cores of immature connective tissue fibres like those found in the core of the oesophageal papillae (see Section 5.1.4 above). Reticular fibres are distributed as in the oesophageal tunica propria.

In the glandular region of the crop, the arrangement of connective tissue fibres is very different from that described above for the anterior non-glandular region. There are no obvious bands of fibres, the tissue is not as dense, elastic fibres are confined to the connective tissue closely adjacent to the muscularis externa and there is a very extensive invasion of lymph cells into the connective tissue matrix.

Collagenous fibres are the major component of the connective tissue between the glands. Both mature fibres in bundles and threadlike immature fibres make up the connective tissue matrix. Reticular fibres are also numerous in this matrix. They form fine borders around small groups of gland tubules dividing them into islands of mucous gland cells (Figure 6.17). Reticular fibres are particularly numerous in association with the wandering lymph cells in this connective tissue and with the lymph nodes at the junction of the crop and the stomach.

Adjacent tubules, within a single gland, are separated by a few collagenous and reticular fibres or by only reticular fibres and the

Figure 6.17

Photomicrograph showing the reticular fibres surrounding the tubules of a crop gland. (Representative of both species). (Stain: Gordon and Sweet; Cook, 1974). (x 750)

Figure 6.18

Photomicrograph of the connective tissue matrix between the base of the crop gland tubules and the muscularis externa. (Representative of both species). (Stain: Verhoeff-Van Gieson; Culling, 1974). (x 230)



basement membranes around the cells of the tubules (Figure 6.17). The oval shaped groups of glands are separated by bands of connective tissue measuring up to 80  $\mu\text{m}$  in width. Connective tissue fibres surrounding the glands and their tubules tend to be orientated parallel to the basement membranes of the tubules, while elsewhere there is no obvious pattern of orientation in the connective tissue fibres.

Connective tissue underlying the glands is well supplied with capillaries and veins. Adjacent to the muscularis externa the connective tissue contains bundles of smooth muscle fibres arranged in a wide discontinuous band. This band probably represents the beginning of the muscularis mucosa which is present in the stomach (see Section 7.1.5 below). The surrounding collagenous connective tissue contains mature elastic fibres. These fibres are most prominent adjacent to the muscularis externa.

There are no mast, paneth, argyrophil or argentaffin cells in the tissue layers of the crop (see Appendix 5). Small parasitic bodies present in the epithelium and in the connective tissue matrix, stain in a way similar to paneth cells making interpretation of results for this stain difficult (see Appendix 6).

#### 6.1.5.2 *Caretta caretta*

The tunica propria beneath the nonglandular region of the crop is formed into three bands, in a very similar way to the connective tissue in this region of the crop in *C. mydas*.

The connective tissue surrounding the crop glands has the same structure and composition as the connective tissue in the nonglandular crop region. The inner and central bands extend into the epithelial folds between the gland groups, while the central and outer most bands lie beneath the glands, the outer most band adjacent to the muscularis externa.

In places where the glands are closely packed together, each gland is separated from adjacent glands by a band of collagenous and reticular fibres, up to 25  $\mu\text{m}$  in width. Between the gland tubules within each gland, these bands of connective tissue are only a few micrometers in width. Adjacent gland tubules are sometimes separated only by their basement membranes and the associated reticular fibres (Figure 6.17). Some fine elastic fibres occur in the connective tissue matrix between the glands and their tubules.

The connective tissue directly beneath the glands is dense and collagenous. It contains some reticular fibres but very few elastic fibres. Adjacent to the muscularis externa, the connective tissue contains many elastic fibres and bundles of smooth muscle fibres arranged longitudinally (Figure 6.18). Some circular smooth muscle is present. These muscle bundles do not form a continuous band but probably represent the beginning of the muscularis externa which occurs in the stomach (see Section 7.1.5 below).

As in *C. mydas*, the entire crop gland region contains a diffuse infiltration of wandering lymphoid cells. Large aggregations of lymph cells occur between the glands, particularly those glands situated adjacent to the stomach (Figure 6.16).

No mast, paneth, argentaffin or argyrophil cells were observed in the tissue layers of the crop (see Appendix 5). The parasitic bodies which occur in *C. mydas* and stain in a similar way to paneth cells, were also observed in *C. caretta* (see Appendix 6).

#### 6.1.6 Muscularis externa

##### 6.1.6.1 *Chelonia mydas*

An extensive muscularis externa underlies the connective tissue of the crop. It consists of the characteristic inner wide band of circular

smooth muscle and an outer, much narrower, longitudinal smooth muscle band. The distribution of connective tissue fibres around the muscle bundles and between the two bands of smooth muscle is similar to that described for the oesophagus (see Section 5.1.5 above).

#### 6.1.6.2 *Caretta caretta*

In *C. caretta* there is no outer longitudinal smooth muscle band associated with the muscularis externa. In all other respects the muscularis externa is like that in *C. mydas*.

### 6.1.7 Subserosa and serosa

#### 6.1.7.1 *Chelonia mydas*

The subserosal connective tissue on the outer side of the muscularis externa carries many large veins, arteries and some nerves. There are fewer elastic fibres in this tissue than in the subserosal connective tissue of the oesophagus (see Section 5.1.4 above). A simple squamous membrane composed of flattened nucleated cells borders the outer edge of the subserosa.

#### 6.1.7.2 *Caretta caretta*

The serosal tissues are very similar to those described in *C. mydas*.

## 6.2 DISCUSSION

### 6.2.1 Anatomy

A crop or crop-like region has not previously been described for any other member of the phylum Reptilia. In *C. caretta* and in *Eretmochelys imbricata* (the marine 'hawksbill' turtle) this region of



the alimentary canal is a short nonpapillate extension of the oesophagus (personal observations), while in the herbivorous *C. mydas*, it is much larger and is bound into a tight "U" shape by external mesenteries. The wall of the crop in *C. mydas* is very muscular. In this species the crop probably functions to form and to store, for a short time, a large bolus of plant material. Observations of the ingesta suggest that the crop is not involved in the maceration or digestion of food. These possibilities are further discussed in Chapter 10.

#### 6.2.2 Crop glands

Both *C. caretta* and *C. mydas* have well developed simple branched coiled tubular glands in the posterior region of the crop. These glands are very similar to the glands found in the oesophagus of the lizard *Uromastyx acanthinurus* (Luppa, 1977). In these three species the ducts and terminal portions of these glands are lined by prismoid cells with flattened basal nuclei.

The cells in the crop glands of the two sea turtle species examined produce both acid and neutral mucins. No oxyntic or peptic cells were observed in these glands. The gland cells have a structure typical of mucin producing cells and their cytoplasm is non-granular. In view of the histological and histochemical properties of these cells (above), it is unlikely that digestion begins until food enters the stomach.

The mucous glands in the crop are much more extensive in *C. caretta* than in *C. mydas*. This difference could be due to the different nature of the foods ingested by the two species. Molluscs form the major component of the diet for *C. caretta* and the ingesta therefore contains many jagged fragments of shell. While the oral cavity and the oesophagus are protected from abrasion by the keratinized epithelium, the stomach

and the remainder of the alimentary canal are not. A thick layer of mucus could reduce the chances of damage by abrasion while still allowing the passage of digestive enzymes to the ingesta (Heatley, 1959). Algae and sea grasses form the major component of the diet in *C. mydas*. These foods are far less likely to damage the surface epithelium of the alimentary canal and would not need to be as liberally coated with mucus as mollusc fragments.

### 6.2.3 Muscularis mucosae

In the two species of turtle examined, the muscularis mucosae appears as a discontinuous band in the posterior region only of the crop. Although there is no muscularis mucosae dividing the connective tissue layer, the connective tissue matrix adjacent to the muscularis mucosae closely resembles the submucosal tissue in the stomach. The condition in *C. mydas* and *C. caretta* seems to be typical of reptiles in general because Lippa (1977) states that the muscularis mucosae is ordinarily found only in the most posterior region of the oesophagus in reptiles.

## CHAPTER VII

### THE STOMACH

#### 7.1 RESULTS

##### 7.1.1 Introduction and Anatomy

###### 7.1.1.1 *Chelonia mydas* (adult turtles)

In *Chelonia mydas* the stomach is a long sausage shaped organ with the posterior one third of its length doubled back along the side of the lesser curvature (Figure 3.2). The anterior end of the stomach tapers sharply to the cardia where its internal circumference, when empty, is 8 cm. Even when empty the corpus of the stomach has a greater internal circumference (11 cm) than any other region of the gastro-intestinal tract. The posterior (pyloric) portion of the stomach tapers gently to the pylorus where it has an internal circumference of 6.25 cm when empty. These circumference measurements vary slightly from turtle to turtle. The shape of the stomach is entirely constant within the species, even in hatchling turtles.

In the size range of *Chelonia mydas* examined during this study (Table 10.3), the total length of the stomach varied from 35.7 cm in a turtle with a curved carapace length (C.C.L.) of 47.7 cm, to 104 cm in a turtle with a C.C.L. of 98.7 cm. In the former case, the length of the stomach represents 5.7% of the total length of the alimentary canal (oesophagus and crop included) while in the latter specimen, it represents 6.0%. The mean value for the turtles examined was 5.97% (Table 10.3).

###### 7.1.1.2 *Caretta caretta* (adult turtles)

In *Caretta caretta* the stomach is simple, and somewhat flask-shaped (Figure 3.3). At the cardia, the internal circumference of the stomach

the cellulose content of algae is less than that of land plants. He reported values as low as 2.5% and up to 8.2%, considerably lower than records for tender land herbs which contain 10.5% to 19.9% cellulose (% of dry substance).

Herbivorous animals feeding on marine algae may be at a considerable advantage compared with land herbivores. The former include the iguanids and *Chelonia mydas*. In these cases the comments by Skoczylas (1978) that herbivorous reptiles are wasteful consumers and that those reptiles utilizing symbiotic gut bacteria probably make use of only a small part of the calorific value of their food, may not be entirely justified.

#### 10.2.4.2 *Chelonia mydas* as a herbivore

*Chelonia mydas* has a functionally, although not anatomically, defined caecum just posterior to the ileo-colic valve (Bjorndal, 1979; personal observations; see Chapter 9). There is a rich cellulolytic bacterial and protozoan flora in the caecum and colon (Bjorndal, 1979; Fenchel et al., 1979).

According to Bjorndal (1979), *Chelonia mydas* in the Caribbean Sea select young plant material (sea grasses), low in lignin content. The caecum is the initial site of cellulolytic activity (Bjorndal, 1979). Bjorndal (1979) was able to show that 83% of digested organic matter, 82% of digested cellulose and 58% of digested hemicellulose were digested in the caecum and large intestine. Fenchel et al. (1979) reported that organic matter, total carbohydrate and cellulose were broken down with efficiencies of about 78, 92 and 95% respectively. They consider that the high efficiency of cellulose breakdown in the alimentary canal of the green turtle compare with the upper range of efficiencies measured in ruminants (Hungate, 1975) and the dugong (Murray et al., 1977). Bjorndal (1979) estimated that a 50 kg turtle at an average temperature of 30°C digests cellulose and hemicellulose as efficiently as a 250 kg dugong

(Murray *et al.*, 1977) and at least as efficiently as a ruminant (Hungate, 1975), with respect to fibre digestion. The apparent digestibility coefficient values for *C. mydas* were 69 and 64% (Bjorndal, 1979). These values are higher than reported for herbivorous lizards (Skoczylas, 1978). These higher values may be due to fermentation and breakdown of fibre in the caecum and colon of the green turtle (Bjorndal, 1979). Volatile fatty acid and lactate values are low in the oesophagus, stomach and small intestine of *C. mydas* indicating a low level of carbohydrate digestion (Bjorndal, 1979). The levels of volatile fatty acid and lactate are significantly higher in the caecum and colon, indicating an increase in fermentation. A sharp drop in the level of volatile fatty acid and lactate production in the mid colon to cloaca indicates that these end products are probably absorbed in the caecum and large intestine. The proportions of volatile fatty acids produced were in the sequence acetate > butyrate > propionate (Bjorndal, 1979). These results, although different from those for ruminants, are similar to results of comparable analyses for the dugong, *Dugong dugon* (Murray *et al.*, 1977), the quokka *Setonix brachyurus* (Moir, 1968) and the porcupine *Erethizon dorsatum* (Johnson and McBee, 1967).

Bjorndal (1979) estimated that the caecum provided 15.2% of the daily energy budget for *Chelonia mydas*. She considers that the fermentation in the remainder of the large intestine provides even more energy than fermentation in the caecum. This is a reasonable assumption considering that there is an increase in the volatile fatty acid concentration posterior to the caecum and that the colon swells rapidly with gases once it is removed from the turtle, indicating that there is active fermentation going on in the colon. The caecum value is thus likely to be a great underestimate of the total contribution of the gut fermentation to the energy balance of *C. mydas* (Bjorndal, 1979).

Personal observations on *Chelonia mydas* consuming algae show that half of the digesta in the lower ileum is in the form of intact pieces of algae. The remaining components of the digesta are the consistency of a fine "mud" with some very small fragments of algae. A large percentage then of the ingested algae passes through the oesophagus, crop, stomach and small intestine, without being visibly changed. While absorption of nutrients from the "mud" and from the broken ends of the algae fragments would take place along the small intestine, it is obvious that much of the potential food would be lost if no further breakdown of the digesta occurred in the large intestine. Observations show that the algal fragments which appear undigested in the ileum, pass through the caecum and enter the anterior colon. The majority of these fragments are reduced to a "mud-like" paste by the time they reach the mid colon.

In the lower half of the colon the digesta becomes increasingly dry and is consolidated into lumps of "paste-like" material. Closer examination of this "paste" reveals that it contains a large number of translucent sheath-like tubules. These are presumably the outer walls of the original algal fragments. It seems probable that the symbiotic bacteria reported by Bjorndal (1979) and Fenchel et al. (1979) are responsible for the breakdown of the cell wall material in the algae fragments which pass seemingly intact into the colon. Observations which indicate that this process is completed in the anterior half of the large intestine correlate with Bjorndal's (1979) observations on volatile fatty acid levels. The major function of the posterior colon appears to be the absorption of fermentation products and fluids. The great length of the large intestine allows for both the effective breakdown of the plant cell wall material and the thorough absorption of all useful products.

From the work by Fenchel et al. (1979), Bjorndal (1979) and this study, it appears that *Chelonia mydas* is an effective consumer of plant material. The considerable length of the intestine and the presence of symbiotic micro-organisms would seem to be adequate adaptations for efficiently utilizing a diet of marine algae and angiosperms. The construction of the serrated plates in the margin of the beak (Chapter 4) and the large size of the turtle are two additional factors which contribute to the success of this reptilian species as a herbivore.

*Chelonia mydas* has an extremely muscular crop at the anterior end of the stomach which may serve as a storage and consolidating organ for the small pieces of plant material passing down the oesophagus. In this way, once a large bolus of material has been accumulated and coated with mucus by the crop glands, it would be passed into the corpus of the stomach. The mucus coating presumably reduces the abrasive effect of the digesta on the lining of the stomach and supplies an adsorptive surface for gastric enzymes. The oesophagus and crop do not appear to play any role in the actual digestion process as extremely fresh and undigested looking samples of algae have been taken from the crop of *Chelonia mydas* during this study.

Many of the algal fragments in the stomach, especially those near the mucosa, have a "mushy" appearance and seem to be more fragmented than the algae in the crop. This fraction (> 50%) of the algae is further reduced to a mud-like slurry in the small intestine. It appears that a considerable portion of the ingested algae is broken down through digestion by enzymes in the acid stomach and more neutral small intestine, while the remainder is attached by cellulolytic bacteria and protozoa in the large intestine.