Although the histological features of the oropharyngeal cavity of many avian species have been described, often only certain structures or aspects of the oropharynx were studied, for example the tongue (see chapter 5). Owing to their commercial importance, domestic poultry and more specifically the fowl, have received the most attention (Calhoun, 1954; Koch, 1973; Hodges, 1974; McLelland, 1975, 1979, 1990; King and McLelland, 1984; Banks, 1993; Bacha and Bacha, 2000). Although the macroscopic features of the oropharynx or parts thereof have been described in ratites (Meckel, 1829; Cuvier, 1836; Gadow, 1879; Owen, 1879; Pycraft, 1900; Duerden, 1912; Faraggiana, 1933; McCann, 1973; Cho et al., 1984; Bonga Tomlinson, 2000) and other birds (see McLelland, 1979 for a review of earlier literature), the omission of histological data has severely restricted the value of these reports. Gardner (1926) has emphasised the importance of providing histological data, together with macroscopic descriptions, for a more in-depth understanding of structures and their function.

There have been very few histological studies of the ratite oropharynx and none detailing the histological or scanning electron microscopical features of the emu oropharynx. Histological studies of this region in ratites have been limited to the greater rhea (Feder, 1972) and ostrich (Porchescu, 2007; Tivane, 2008), whereas the surface morphology of the entire oropharynx has only been described in the ostrich (Tivane, 2008).

The histological structure of the avian oesophagus displays a remarkable uniformity (Pernkopf and Lehner, 1937; Calhoun, 1954; Warner et al., 1967; Ziswiler and Farner, 1972; Koch, 1973; Hodges, 1974; McLelland, 1975, 1979, 1990; Nickel et al., 1977; King and McLelland, 1984; Banks, 1993; Bacha and Bacha, 2000; Gussekloo, 2006) with the greatest variation appearing to be the presence of either a keratinised or non-keratinised stratified squamous epithelium lining
the organ. An additional variation (at the gross anatomical level) is the absence or presence of a crop.

Histological features of the ratite oesophagus have been documented for the greater rhea (Feder, 1972), ostrich (Porchescu, 2007; Tivane, 2008) and emu (Herd, 1985). These studies all show a similarity between the histology of the ratite oesophagus and that of birds in general, namely a non-keratinised stratified squamous epithelium, oesophageal glands in the distal portion of the lamina propria and the presence of a well-developed muscularis mucosae. The only histological study of the oesophagus of the emu is that of Herd (1985) in which only two specimens were used. The surface morphology of the ratite oesophagus has only been described in the ostrich (Tivane, 2008).

The emu is a commercially important bird and its nutrition and health are paramount to the success of any commercial operation. The emu enjoys a varied diet (Davies, 1978); however, nothing is known of the microstructure of the oropharynx which could affect food selection and intake. For example, it is not known if the emu has a sense of taste. It is therefore necessary to investigate the microstructures of the emu oropharynx to identify structural features that could influence nutrition, food intake and subsequent ingestion, as well as providing a foundation for the recognition of pathology in this region. This chapter will also provide comparative information for future studies of the ratite oropharynx.

3.2 MATERIALS AND METHODS

The heads of 23 sub-adult (14-15 months) emus of either sex were obtained from a local abattoir (Oryx Abattoir, Krugersdorp, Gauteng Province, South Africa) immediately after slaughter of the birds. The heads were rinsed in running tap water to remove traces of blood and then immersed in plastic buckets containing 10% buffered formalin. The heads were allowed to fix for approximately four hours while being transported to the laboratory, after which they were immersed in fresh fixative for a minimum period of 48 hours. Care was taken to exclude air from the oropharynx by wedging a small block of wood in the beak.

After rinsing five of the heads in running tap water, the right commisure of the beak was incised and the mandible reflected laterally by disarticulating the quadratomandibular joint to openly
display the roof and floor of the oropharynx and the proximal oesophagus (Fig. 3.1). Appropriate longitudinal and transverse sections representing areas of interest were excised from the oropharynx and proximal oesophagus (Fig. 3.1). The samples were dehydrated through 70, 80, 96, and 2X 100% ethanol and further processed through 50:50 ethanol: xylol, 2X xylol and 2X paraffin wax (60-120 minutes per step) using a Shandon model 2LE Automatic Tissue Processor (Shandon, Pittsburgh, PA, USA). Tissue samples were then imbedded manually into paraffin wax in plastic moulds. Sections were cut at 4-6 μm, stained with Haematoxylin and Eosin (H&E) and Periodic Acid Schiff Stain (PAS) (McManus, 1946) and viewed and micrographed using an Olympus BX50 equipped with the analySIS CC12 Soft Imaging System (Olympus, Japan).

An additional three heads were collected from birds (5, 15 months & 5 year-old birds) specifically for scanning electron microscopy. The heads were fixed in 10% buffered formalin overnight. Appropriate samples of the oropharynx were removed (Fig. 3.47) after the heads had been rinsed in running water for several hours to remove all traces of phosphate buffer. The samples were dehydrated through an ascending ethanol series (50, 70, 80, 90, 96 and 3X 100%). Due to the size of the tissue blocks, each dehydration step took 60 minutes. The blocks were then critical point dried from 100% ethanol through liquid carbon dioxide in a Polaron E300 Critical Point Drier (Polaron, Watford, England). After critical point drying the samples were mounted on round or rectangular (depending on sample size) aluminium viewing stubs with a conductive paste, Silver Dag (Dag 580 in alcohol), and sputter coated with a thin layer of palladium using a Polaron SEM E5100 coating unit. Areas of interest were viewed using a Jeol NeoScope JCM-5000 SEM operated at 10kV and a Jeol JSM-840 SEM operated at 5kV. Images were digitally captured using Start JCM-5000 and Orion 6.60.4 software, respectively, and described.

The terminology used in this study was that of Nomina Anatomica Avium (Baumel et al., 1993).
3.3 RESULTS

3.3.1 Light microscopic observations

3.3.1.1 Intra-oral mandibular bill skin (Figs. 3.2 – 3.4)

The mandibular bill skin (seen macroscopically as the mandibular rhamphotheca) consisted of a heavily keratinized, pigmented, stratified squamous epithelium, overlying a layer of dense irregular connective tissue (corium). The epithelium formed up to half the thickness of the bill skin. The Str. basale consisted of a tightly packed layer of columnar cells that were interspersed with melanocytes, which were also found in the connective tissue immediately beneath the Str. basale. This layer was followed by a thin Str. spinosum, with the rest of the epithelium composed of an extensive Str. corneum (rhamphotheca). The Str. spinosum appeared in places to be absent and in other areas it was two-three cell layers thick. There was no obvious Str. granulosum. The Str. corneum, which was by far the greatest component of the epithelium, consisted of a narrower, deeper, darker area and a wider, more superficial, lighter area. The Str. basale rested on a fine layer of connective tissue which merged with the dense irregular connective tissue forming the corium. The corium displayed localised areas of loose connective tissue resting on the periosteum of the mandible, and which contained numerous blood vessels, nerves and Herbst corpuscles. The Herbst corpuscles were of varying sizes with the majority being large in size. They were found singly or stacked, grouped or in longitudinal chains and were evenly distributed throughout the corium.

3.3.1.2 Oropharyngeal floor

Based on macroscopic observations (see Chapter 2) the oropharyngeal floor could be divided into the interramal region (Regio interramalis) composed of a rostral pigmented part and a caudal non-pigmented part, and the tongue (see Chapter 5) and laryngeal mound which were situated within the caudal interramal region.
3.3.1.2.1 Interramal region – Rostral pigmented part (Figs. 3.5 – 3.7)

The pigmented area directly caudal to the mandibular rostrum consisted of a keratinized stratified squamous epithelium overlying loosely arranged dense irregular connective tissue, up to six times the width of the epithelium. The intraoral tissues were separated from the underlying integument by a layer of skeletal muscle fibres. The epithelium was undulating, representing the longitudinal mucosal folds and alternating grooves seen macroscopically. Melanocytes were concentrated in the *Str. basale* of the folds, with some cells extending into the *Str. spinosum*. In contrast, the density of melanocytes was greatly reduced in the grooves and where present these cells were scattered in the underlying connective tissue immediately below the *Str. basale* (Figs. 3.5, 3.6). The connective tissue housed numerous capillaries, small nerves and Herbst corpuscles. Connective tissue papillae were absent in this region. The Herbst corpuscles were mainly situated in the connective tissue beneath the mucosal folds (Fig. 3.7). Large blood vessels and nerves were located directly above the skeletal muscle layer.

3.3.1.2.2 The area of transition (Fig. 3.8)

The transitional region was characterised by a loss of mucosal folds and a marked thickening of the epithelium. Melanocytes gradually decreased in density, initially disappearing from the connective tissue and then also from the *Str. basale*. In addition to thickening, the epithelium changed to a non-keratinised stratified squamous epithelium, up to three times the thickness of the rostral keratinized epithelium. A short distance after the loss of the keratinised layer large, simple branched tubular glands (in the large lateral folds) or simple tubular glands (in the region medial to the large lateral folds) appeared in the underlying connective tissue. The epithelium was penetrated by connective tissue papillae carrying capillaries at their tips.

3.3.1.2.3 Interramal region – Caudal non-pigmented part (Figs. 3.9, 3.10)

This region consisted of a non-keratinised stratified squamous epithelium which overlay a gland-rich connective tissue layer. The epithelium was obliterated in certain areas by large
accumulations of diffuse lymphoid tissue, and which formed round masses emanating from the underlying connective tissue. Between the gland ducts traversing the epithelium were connective tissue papillae which carried capillaries at their tips. Structures resembling taste buds (Fig. 3.17, 3.19) occurred in the epithelium of this region and were very sparse and not associated with gland openings. They were clearly demarcated from the surrounding epithelium which encapsulated them and were composed of elongated elements which could not be clearly distinguished as sensory or supporting cells.

The dense irregular connective tissue beneath the epithelium contained mainly simple tubular mucus-secreting glands (PAS positive). These glands were confined to the more superficial zone of the connective tissue and were densely packed (Fig. 3.10). Large simple branched tubular mucus-secreting glands occurred on the dorsal aspect of the large lateral fold of tissue running parallel to the mandibular rami (Fig. 3.9). However, the blind ending groove or recess enclosed by the large fold and the area medial to it, contained only simple tubular mucus-secreting glands. A rich capillary plexus surrounded the larger glands and the ducts penetrated the full length of the epithelium, opening into the oropharyngeal cavity. The glands present in this area were structurally similar to those described for the tongue (see Chapter 5). The connective tissue beneath the mucosal folds in this region formed a thick core that supported each fold. Situated at the base of the fold was a large artery while nerve and vascular plexuses were situated near the base of the glands (Figs. 3.9, 3.10). Due to the thinning of the connective tissue in the grooves between the folds, the base of the simple tubular glands lay in close proximity to the underlying layer of skeletal muscle which demarcated the intra-oral tissue and integument.

3.3.1.2.4 Mandibular rictus (Figs. 3.11 – 3.13)

The intra-oral mandibular portion of the angle of the mouth (mandibular rictus) was a non-pigmented longitudinally folded tract of tissue. The mandibular rhamphotheca formed its lateral border and it was continuous with the caudal non-pigmented interramal space medially. At the point where the rhamphotheca merged with the non-pigmented tissue, the epithelium was seen to change from a lightly keratinized stratified squamous type with melanocytes to a non-
keratinised stratified squamous type devoid of melanocytes. The epithelium rested on a layer of dense irregular connective tissue, rich in nerves and blood vessels. This connective tissue also housed numerous simple tubular mucus-secreting glands (Fig. 3.12) which opened onto the surface via a duct lined by similar cells to those composing the secretory part of the gland. These glands were situated superficially, directly below the stratum basale of the epithelium and appeared partly intraepithelial in location. Larger simple branched tubular mucus-secreting glands (Figs. 3.11, 3.13) were situated deeper within the connective tissue than the smaller glands. The ducts of the larger glands which opened through the epithelium were lined by invaginated squamous cells from the epithelium which were vertically oriented. The tissue in this area was folded and in some of the folds large aggregations of lymphoid tissue were observed. Some of the aggregations contained a well circumscribed lymphatic nodule, surrounded by a thin layer of connective tissue (Fig. 3.12). The connective tissue papillae penetrated deep into the epithelium and carried capillaries in their tips (Fig. 3.11). Herbst corpuscles occurred in the connective tissue and were mostly associated with the capsule of the large glands (Fig. 3.13), although isolated corpuscles also appeared in the connective tissue (Fig. 3.12). Below the layer of dense irregular connective tissue was a layer of more loosely arranged dense irregular connective tissue, housing nerves and larger blood vessels (Fig. 3.11). This connective tissue rested on skeletal muscle.

3.3.1.3 Laryngeal mound (Mons laryngealis) (Figs. 3.14 – 3.15)

The tissue covering the laryngeal mound was smooth and non-pigmented and consisted of a non-keratinised stratified squamous epithelium which was thinner in the glandular region and thicker in the aglandular region (see below). Beneath the epithelium was a dense irregular connective tissue layer which formed widely spaced papillae that extended a short distance into the epithelium. The connective tissue layer housed mucus-secreting glands, Herbst corpuscles, lymphoid tissue, blood vessels and nerves and rested on skeletal muscle. The laryngeal mound displayed both glandular and aglandular regions. Simple branched tubular mucus-secreting glands (similar to those found elsewhere in the oropharynx) were situated on the dorso-lateral surface of the arytenoid cartilages (Figs. 3.15, 3.38) while the rest of the laryngeal mound was free of glands (Figs. 3.14, 3.38). At intervals, there were small aggregations of diffuse lymphoid tissue, which partially invaded the epithelium. The lymphoid aggregations consisted of scattered lymphocytes separated by connective tissue strands. Herbst corpuscles were present in low
numbers, and were either associated with the glands (Fig. 3.15) or lay isolated in the connective tissue (Fig. 3.14).

3.3.1.4 Laryngo-oesophageal junction (Fig. 3.16)

Macroscopically, the smooth non-pigmented caudal aspect of the laryngeal mound, changed abruptly to the longitudinally folded mucosa of the proximal oesophagus. The histological structure of the caudal aspect of the laryngeal mound was similar to that of the aglandular region of the mound (Fig. 3.14). At the point where the underlying skeletal muscle dissipated, simple tubular glands appeared in the lamina propria, marking the transition to the proximal oesophagus, the structure of which is described below. A structure resembling a taste bud was located in the epithelium of this area (Fig. 3.18). It consisted of a small group of vertically oriented cells lying within a depression in the epithelium and from which small cilia-like structures projected to the surface.

3.3.1.5 Oropharyngeal roof

The oropharyngeal roof was divided into the areas identified macroscopically (see Chapter 2), namely, the rostral pigmented region, the caudal non-pigmented region (housing the choana) and the two pharyngeal folds (Fig. 3.1).

3.3.1.5.1 Pigmented region (Figs. 3.20, 3.21)

The surface lining consisted of a keratinized, pigmented, stratified squamous epithelium (Fig. 3.20), overlying a dense irregular connective tissue layer. The Str. corneum formed up to half the thickness of the epithelium. The melanocytes (Fig. 3.20) were mainly confined to the Str. basale, but extruded pigment granules were also observed in the more superficial layers of the epithelium, particularly the Str. spinosum. The connective tissue abutted the periosteum of the underlying bone and was in places up to four times the thickness of the epithelium. It housed an extensive collection of large nerves and blood vessels as well as numerous Herbst corpuscles (Fig. 3.21). The Herbst corpuscles ranged in position from immediately below the epithelium, to just above the periosteum, although most of these structures were situated centrally in the connective tissue. They varied in size, occurred both
singly, in groups or in chains, and were more or less evenly distributed throughout the tissue. They were comparable in structure, and similarly arranged, to those in the mandibular bill skin (Figs. 3.2, 3.4). In the region forming the median palatine ridge, the connective tissue greatly increased in thickness (Fig. 3.21). At the base of the ridge was a large artery which was a consistent feature in all the specimens studied. In places, the connective tissue formed small, regular papillae which penetrated the epithelium. However, the epithelium and connective tissue generally showed a smooth interface.

3.3.1.5.2 Transitional area (Figs. 3.22, 3.23)

The transition from the pigmented oropharyngeal region to the non-pigmented region was marked by a gradual disappearance of melanocytes from the stratum basale of the epithelium; a gradual increase in thickness of the epithelium as it became non-keratinised; the appearance of connective tissue papillae; and the presence of a small aggregation of diffuse lymphoid tissue within the underlying connective tissue (Fig. 3.23). The deep zone of the connective tissue merged with the supporting connective tissue of the respiratory epithelium lining the nasal cavity (Fig. 3.23).

3.3.1.5.3 Non-pigmented region (Figs. 3.24, 3.25)

The surface was covered by a non-keratinised, non-pigmented stratified squamous epithelium supported by an underlying layer of dense irregular connective tissue. Connective tissue papillae carrying a rich capillary plexus penetrated the epithelium, up to half its depth, at regular intervals. The connective tissue contained large simple branched tubular mucus-secreting glands PAS positive (Figs. 3.24, 3.25) which changed in shape (in a rostral to caudal direction) from dorso-ventrally flattened to more dorso-ventrally elongated. Herbst corpuscles, located in the connective tissue, were most often associated with the glands, as seen elsewhere in the oropharynx, or occurred isolated in the connective tissue (Fig. 3.24). Each gland was surrounded by a capsule of dense irregular connective tissue and was similar in structure to those described in the tongue (see Chapter 5). Rostrally, the tissues of the non-pigmented region were separated from the deeper lying respiratory tissue by an abrupt transition from dense to loose irregular connective tissue. At this junction, large blood vessels and nerves
Chapter 3: Histological Features & Surface Morphology of the Oropharyngeal Cavity & Proximal Oesophagus

were observed. Caudally, a skeletal muscle layer originated which separated the respiratory and oral components from each other. Aggregations of diffuse lymphoid tissue (Fig. 3.25) associated with the ducts of the glands, were occasionally observed. Caudally, occasional simple tubular mucus-secreting glands were interspersed between the larger, simple branched tubular glands.

3.3.1.5.3.1 Maxillary rictus (Figs. 3.26-3.29)

The tissue in this region was similar to that described above for the non-pigmented region. It differed slightly in that the dense connective tissue layer was relatively wider with a greater amount of loose connective tissue, carrying blood vessels and nerves, present below the dense connective tissue layer (Fig. 3.26). A higher frequency of diffuse lymphoid tissue aggregations (Fig. 3.27), associated with both the glands (predominantly large, simple branched tubular) and isolated in the connective tissue, as well as a higher frequency of deep penetrating connective tissue papillae, (Fig. 3.26) were observed. Herbst corpuscles were present and were mostly arranged in groups and not associated with the glands (Fig. 3.28 – inset). The corpuscles were structurally similar to those found elsewhere in the oropharynx (see Chapter 5). The fibrocytic lamellae forming the outer core of the corpuscles demonstrated a faint PAS-positive reaction (Fig. 3.29).

3.3.1.5.3.2 Non-pigmented region – fold caudo-lateral to the choana (Figs. 3.30, 3.31)

The ventral aspect of the small fold of tissue observed at the caudo-lateral edge of the choana displayed similar features to that of the non-pigmented region (Fig. 3.30) with which it was continuous. The epithelium lining the dorsum of the fold (effectively forming the ventrum of the pocket) changed from a stratified squamous type to a ciliated columnar epithelium, which only occurred within the confines of the pocket. This transition was characterised by the appearance of large aggregations of diffuse and nodular lymphoid tissue which occupied the connective tissue enclosed within the blind-ending pocket (Figs. 3.30, 3.31). The presence of lymphoid tissue in this region was a consistent feature in all the specimens examined. The supporting connective tissue housed glands, lymphoid tissue, Herbst corpuscles, blood vessels and nerves. In the connective tissue adjacent to the blind-ending pocket was a large muscular artery (Figs. 3.30, 3.31). The glands within the pocket were simple tubular mucus-secreting
glands (PAS-positive, Fig. 3.31), although the lateral aspects of the pocket contained both the above mentioned glands as well as large, simple branched tubular mucus-secreting glands (Fig. 3.31).

3.3.1.6 Pharyngeal folds (Figs. 3.32 – 3.37)

The rostral fixed part of the pharyngeal folds was continuous with the oropharyngeal roof caudal to the choana. The epithelium and connective tissue elements were similar to those described for the non-pigmented region of the roof. There was a low frequency of simple tubular mucus-secreting glands scattered amongst the evenly spaced large simple branched tubular mucus-secreting glands (PAS positive, Fig. 3.33), which formed the bulk of the glandular tissue. The lumen of some of the glands was lined by a pseudostratified ciliated columnar epithelium (Fig. 3.35). Most of the glands were associated with variably sized aggregations of diffuse lymphoid tissue. Caudally (seen macroscopically as large pitted openings), the glands increased in size as did the associated aggregations of lymphoid tissue. Randomly distributed units of nodular lymphoid tissue occurred within the diffuse lymphoid aggregations. More caudally the epithelium was penetrated by a lower frequency and regularity of connective tissue papillae. Below the dense irregular connective tissue was a thin layer of loose irregular connective tissue containing blood vessels and nerves as well as adipose tissue. Below this layer was a layer of skeletal muscle.

The free part of the pharyngeal folds displayed the largest glands and the greatest amount of associated lymphoid tissue. At the most caudal extremity, the pharyngeal fold was separated by a crypt that was only present in the free part of the fold (Fig. 3.34). It varied in depth between the specimens. The tissue flap forming the ventral boundary of the crypt (continuous with the ventral surface of the pharyngeal fold) was generally free of lymphoid tissue, the connective tissue layer was thinner, and large, simple branched tubular glands (more typical of the rest of the oropharynx) opened into the oropharynx. The glands on the opposite side of the fold and which opened into the crypt were both large, simple branched tubular and, more rostrally, simple branched tubular glands (Fig. 3.34). The part of the pharyngeal fold forming the dorsal boundary of the crypt contained a few simple branched tubular glands and a large mass of diffuse lymphoid tissue. Dorsal to the crypt, a pocket or recess was formed between the dorsum
of the pharyngeal fold and a caudo-lateral projection of tissue (see Chapter 2). The part of the tissue projection protruding beyond the pharyngeal fold contained both types of glands and scant lymphoid tissue (Fig. 3.34). However, the pocket or recess was lined almost entirely by a dense mass of diffuse lymphoid tissue, with only occasional simple tubular glands being seen (Figs. 3.34, 3.37). Variable amounts of nodular lymphoid tissue were also present in the tissue lining the recess (Figs. 3.36, 3.37). The dorsal surface of the pharyngeal fold consistently displayed a small nodule of lymphoid tissue which protruded into the pocket or recess, suspended by a stalk of connective tissue (Fig. 3.36). The dorsal aspect of the projection merged rostrally with the dorsal aspect of the pharyngeal fold, which rostrally reflected on itself and marked the beginning of the proximal oesophagus, forming the depth of the retropharyngeal recess (Fig. 3.37). This portion of the pharyngeal fold showed fewer and smaller amounts of lymphoid tissue and contained simple tubular glands only. The oesophagus in the retropharyngeal recess displayed features similar to the rest of the proximal oesophagus (see below).

3.3.1.7 Proximal cervical oesophagus (Oesophagus Pars cervicalis) (Figs. 3.39 – 3.46)

The oesophagus was composed from within outwards of four main layers, namely, the mucosa, submucosa, muscular layer (Tunica muscularis) and adventitia (Figs. 3.39, 3.40).

The mucosa was formed by a non-keratinised stratified squamous epithelium, a dense irregular connective tissue (lamina propria) and a thick longitudinally oriented smooth muscle layer, the muscularis mucosae. The epithelium was penetrated by the long necks of glands emanating from the lamina propria, and contained sparse taste buds. The taste buds were elongated structures, which were clearly discernable from the surrounding epithelium and displayed a definite taste pore (Fig. 3.46). They were not directly associated with the glandular tissue. The taste buds were composed of vertically oriented cellular elements that displayed both round vesicular nuclei and denser, more elongated nuclei. However, it was not possible with the staining technique used to discern sensory and supporting cells from one another or the presence of nerve processes. The lamina propria housed numerous mucus-secreting glands (PAS positive), which were of the simple tubular type, some of which were branched. In the mucosal folds the glands did not appeared to penetrate far into the lamina propria (in the mucosal folds the lamina propria was thick due to the absence of the muscularis mucosae) (Figs. 3.40-3.43). In contrast, in the mucosal grooves, the glands appear to occupy the
full width of the lamina propria (in the mucosal grooves the lamina propria was thin due to the presence of the muscularis mucosae) (Figs. 3.40, 3.43). The glands displayed features typical of the mucus-secreting glands (Figs. 3.44, 3.45) described in the oropharynx. Unlike in the oropharynx, no large, simple branched tubular glands were observed. Diffuse lymphoid tissue was also present in the lamina propria and was situated between the numerous glands which were often excluded (Fig. 3.43). The muscularis mucosae was the most prominent layer in the oesophagus but did not extend into the folds of the mucosa (Figs. 3.39-41, 3.43). It was composed of longitudinally arranged smooth muscle cells and was similar in thickness to the tunica muscularis. The longitudinal folds of the proximal oesophagus were formed by the epithelium and lamina propria only.

The submucosa was a very thin connective tissue layer which in places was hardly discernable (Figs. 3.39, 3.40). It carried large blood vessels and nerves as well as the submucosal plexus.

The tunica muscularis (Figs. 3.39, 3.40, 3.43) was composed of a thicker inner circular and thinner outer longitudinal layer of smooth muscle. Between the two layers was a nerve plexus and associated neurons, the myenteric plexus.

The tunica adventitia (Fig. 3.39), composed of loose connective tissue, formed the outermost layer of this region and contained large blood vessels, nerves and adipose tissue.

3.3.2 Scanning electron microscopic observations

Samples for SEM (Fig. 3.47) were taken from the interramal region (including the rostral pigmented and caudal non-pigmented parts, and large lateral fold), the pigmented and non-pigmented parts of the roof (including the median palatine ridge), the ventrum of the pharyngeal fold and tissue projection, and the proximal oesophagus.

3.3.2.1 Oropharyngeal floor

At low magnification two distinct parts were visible, a region displaying many crevices and folds (representing the rostral longitudinally folded keratinised oropharyngeal floor) (Figs. 3.48, 3.49) and a smoother region consisting of a few larger, well-defined folds (representing the caudal
non-keratinised glandular oropharyngeal floor) (Fig. 3.51). High magnification of the longitudinal folds of the rostral region revealed numerous oblique, transverse and longitudinal fissures on the surface (Figs. 3.49, 3.50). Few individual desquamating cells (Fig. 3.50) were visible on the surface. The transition between the keratinised and non-keratinised regions (Fig. 3.48) was broad and bordered rostrally by the abrupt ending of the longitudinal folds and caudally by the flaky appearance of the non-keratinised region. The transitional zone was composed of a broad sheet of desquamating cells (Fig. 3.48), similar to those observed in the keratinised region of the oropharyngeal roof (see below). The large lateral fold of the non-keratinised region displayed individually desquamating cells, giving it a flaky appearance, and large, evenly dispersed openings, often obscured by mucus-secretion from the underlying glands (Fig. 3.56). In the younger bird, the fold medial to the large lateral fold displayed a similar surface but with fewer openings (Figs. 3.51, 3.52). The surface of the more medial folds (Fig. 3.55) was uneven and undulating. High magnification of these folds revealed surface cells covered with dense microvilli (Fig. 3.57) which were compacted at the cell boundaries, thus clearly demarcating the individual cells (Figs. 3.55, 3.57). Numerous small openings were also present on this surface and were also surrounded by cells densely packed with microvilli (Figs. 3.55, 3.57). In the older birds, the region medial to the large lateral fold displayed numerous evenly spaced small openings. In all the specimens studied, the grooves between the folds displayed a lumpy, uneven surface with large and small openings (Figs. 3.51, 3.52). This surface was covered by cells densely packed with microvilli, and which were concentrically arranged around the gland openings (Figs. 3.54, 3.57). The large openings were situated in the walls of the grooves (Figs. 3.51, 3.52) whereas the smaller openings occupied the depths of the grooves. The large openings were lined by a concentric pattern of cells giving them a ridged appearance (Figs. 3.52, 3.53).

3.3.2.2 Oropharyngeal roof

Two different regions of the roof were apparent at low magnification, a smooth rostral area representing the keratinised region and a ‘flaky’ caudal area representing the non-keratinised region (Fig. 3.60). The transition between the two regions was abrupt (Fig. 3.60). The smooth area typically displayed sheets of desquaming cells (Fig. 3.58). Individual cells were polygonal in shape and displayed microridges on their free surface (Fig. 3.59). The non-keratinised region of the oropharyngeal roof displayed individual desquamating cells or rows of cells giving it a more flaky appearance (Fig. 3.60, 3.61). The surface cells in this region were also polygonal
shaped. Large gland openings (Figs. 3.61, 3.62) as well as numerous small gland openings (Figs. 3.63, 3.64) were visible in this region. The smaller gland openings (found in the more caudal aspect of the non-keratinised oropharyngeal roof) were surrounded by concentrically arranged cells covered by a dense mass of microvilli (Fig. 3.64).

3.3.2.3 Pharyngeal folds

At low magnification the surface of the pharyngeal folds displayed similar features to that of the non-keratinised roof, exhibiting a flaky appearance due to the desquamation of individual cells (Fig. 3.65, 3.69). Higher magnification of the surface cells revealed a complex pattern of branching and anastomosing microplicae (Fig. 3.66). The complexity of this pattern varied between individual cells. However, in the immediate vicinity of gland openings the surface cells displayed dense masses of microvilli (Figs. 3.67, 3.68). The gland openings in this region (Figs. 3.65, 3.67, 3.69) were more numerous and larger than those of the oropharyngeal roof. Both large and small gland openings were present, with the former (presumably representing the openings of the underlying large, simple branched tubular glands) being more numerous and apparent. The cells forming the ducts of the large glands were vertically aligned, in some instances appearing to form folds (Fig. 3.67). The duct lining cells also displayed masses of microvilli (Fig. 3.67) and were continuous with the zone of similarly adorned cells surrounding the duct openings. The gland openings were often filled with a plug of mucous (Fig. 3.67) and patches of cilia were apparent (Figs. 3.67, 3.68). Small, randomly distributed globular structures were also present (Fig. 3.68).

In the younger bird the caudo-lateral tissue projection of the pharyngeal fold displayed a more irregular surface than the pharyngeal fold (Fig. 3.69, 3.70). The large gland openings (Figs. 3.69, 3.70) were bigger than those of the pharyngeal fold and appeared raised or crater-like (Figs. 3.69, 3.70). Small gland openings surrounded by circumferentially oriented cells (Fig. 3.71) were also present. The surface cells adopted a variety of shapes, their cells boundaries were not clearly defined and they were covered with masses of densely-packed microvilli (Figs. 3.71, 3.72). Occasional ciliated cells were interspersed between the microvilli-rich cells (Fig. 3.72). Numerous small raised nodules (presumably rounded cells) (Figs. 3.70, 3.72) were situated on the surface of this tissue and displayed a pattern of microplicae (Fig. 3.71, 3.72). Numerous cell projections were apparent in this region and occurred in the form of long slender rods or club-shaped structures (Figs. 3.71, 3.72). Numerous globular structures lay scattered on or between
3.3.2.4 Proximal oesophagus

On low magnification, the mucosal folds of the proximal oesophagus appeared as smooth, gently rounded, longitudinally oriented structures exhibiting a convoluted or wavy pattern. A degree of branching and anastomosing was observed while strands of mucus were visible between and adhering to the folds (Fig. 3.73). The surface of the folds was pitted by the openings of underlying glands (see light microscopy). Both large and small openings were apparent, with the small openings being more numerous and generally scattered around the larger openings (Figs. 3.73-3.75). Strands of mucus representing the secretions from the underlying glands were visible in most of the openings and occasionally on the cell surfaces (Figs. 3.75, 3.76, 3.78). In the young bird, desquamating surface cells, unlike the rest of the oropharynx, were not a feature of this region. The surface cells were polygonal and characterised by clearly demarcated cell boundaries accentuated by an accumulation of microvilli (Figs. 3.75, 3.77, 3.78). All cell surfaces in the proximal oesophagus, including the cells lining the gland duct openings, displayed densely arranged microvilli (Figs. 3.76-3.78). Scattered, raised nodules (Fig. 3.74) lay on the surface between the gland openings. In the older birds, gland openings were more crater-like in appearance and the surface cells with microvilli were restricted to the regions immediately surrounding and lining the gland openings. Thus the predominant cell surface pattern in the older birds for this region was microplicae.

3.4 DISCUSSION

3.4.1 The oropharynx

3.4.1.1 Epithelium

The entire oropharyngeal cavity of the emu was lined by a stratified squamous epithelium. This is the same finding for the ostrich (Tivane, 2008) and for other birds in general (Fahrenholz, 1937; Calhoun, 1954; Warner et al., 1967; McLelland, 1975, 1979; Nickel et al., 1977; King and McLelland, 1984). The stratum corneum of the epithelium covering the bill is termed the rhamphotheca (Hodges, 1974). The stratum granulosum is not very apparent in the emu, a
feature also noted in the chicken (Hodges, 1974). The epithelium of the rostral oropharynx in the emu contains melanocytes and is keratinised, and manifests macroscopically as the rostral pigmented parts of the floor and roof (see Chapter 2). Although the greater rhea (Feder, 1972) and ostrich (Tivane, 2008) do not have a rostral pigmented oropharynx, the epithelium of the rostral part of the roof is also keratinised in both species, as well as the rostral floor in the ostrich (Tivane, 2008). Feder (1972) makes no mention of the histology of the oropharyngeal floor in the greater rhea. The transition between the rostral keratinised stratified squamous epithelium and the caudal non-keratinised stratified squamous epithelium in the emu is abrupt, a feature also noted in the ostrich (Tivane, 2008). Thus the epithelia lining the emu, greater rhea and ostrich oropharyngeal cavities are similar. Keratinisation of the oropharyngeal epithelium also occurs in other birds to varying degrees (Fahrenholz, 1937; Nickel et al., 1977; McLelland, 1979; King and McLelland, 1984).

In areas subject to abrasion the epithelium is keratinised (McLelland, 1979; King and McLelland, 1984) and the degree of keratinisation varies according to the amount of mechanical stress involved (Nickel et al., 1977). In the emu, the rostral pigmented parts of the floor and roof of the oropharynx are keratinised. In the cranioinertial feeding method employed by ratites (Bonga Tomlinson, 2000; Gussekloo and Bout, 2005), the food is handled by the bill tips only and held in the most rostral portions of the oropharynx prior to being transported to the proximal oesophagus. Therefore the keratinisation of these areas in the emu as well as in other ratites such as the ostrich (Tivane, 2008) that employ the same feeding strategy, protects the parts of the rostral oropharynx involved in the handling of food and thus subject to the most abrasion.

3.4.1.1.2 Taste buds (Caliculi gustatorii)

In birds, the presence or absence of taste buds in the oropharynx has been heavily debated due to the different eating habits and diet of birds (Moore and Elliott, 1946). In the emu, structures resembling taste buds are located in the oropharyngeal epithelium in the caudal interramal region, the tongue root (see Chapter 5) and at the laryngo-oesophageal junction. This is the first report of taste buds in the oropharynx of a ratite. No taste buds were identified in the greater rhea (Feder, 1972) or ostrich (Tivane, 2008), although, the former author notes that the possibility of their existence could not be ruled out.
Birds possess a very low number of taste buds in comparison to other vertebrates (Berkhoudt, 1985). This would be true for the emu as putative taste buds were very sparse and only observed in a few sections. As the emu swallows its food whole, employing the ‘catch and throw’ (Gussekloo and Bout, 2005) or cranioinertial feeding method (Bonga Tomlinson, 2000), in which the food lands near or into the oesophageal entrance before swallowing, there would be a limited need or opportunity for taste during the intra-oral transport of food. It would thus seem appropriate that any taste receptors found in the emu oropharynx would be sparse and located in the most caudal regions.

A reason for the difficulty in locating taste buds, as noted by Moore and Elliott (1946), is the fact that they are obscured by the connective tissue papillae and the ducts of glands traversing the epithelium. Submucosal papillae and salivary ducts can also easily be mistaken for taste buds, depending on the plane of sectioning (Lindenmaier and Kare, 1959). Moreover, taste buds are most often associated with glands (Gentle, 1971b; Bacha and Bacha, 2000). The presence of many deep connective tissue papillae and gland openings in the emu oropharynx would certainly complicate and mask the identification of taste buds in this species. Taste buds can either occur free in the mucosa or be associated with salivary glands (Botezat, 1910; Nickel et al., 1977; Berkhoudt 1985). The structures found in the emu oropharynx were not associated with gland openings and were distinct entities within the epithelium. A definite taste pore as well as vertically oriented elongated cells were identified, although it was not possible to discern supporting from sensory cells as described by Berkhoudt (1985).

The structures resembling taste buds found in the emu oropharynx were similar to the isolated receptors depicted by Botezat (1910) and appeared similar in shape to those described and depicted for birds in general (Botezat, 1910; Moore and Elliott, 1946; Gentle, 1971b; Nickel et al., 1977; Lindenmaier and Kare, 1959; Warner et al., 1967). Taste buds in birds also appear similar to those found in other vertebrates (Moore and Elliott, 1946; Gentle, 1971b). However, a more detailed comparative study will be needed to ascertain whether the taste buds in the ratite oropharynx are comparable to those found in other birds. Further studies will also be needed, employing alternative staining techniques, to fully describe the structure of the emu taste buds.

The most obvious function of taste buds in the emu would be the discrimination of food. The sense of taste is an important motivator for feeding as well as for initial food selection in birds (Gentle, 1971a). Taste encourages nutrient intake as well as helping to discriminate against
possible harmful foods (Kare and Rogers, 1976) by screening the intake of food and water (Berkhoudt, 1985). Initial food selection, however, may not be an important function of taste in the emu, as noted above, due to the particular feeding method of this bird where food is most likely only tasted after ingestion. In birds, food selection is also based on size, shape, colour and texture as well as taste and olfaction (Berkhoudt, 1985). It would seem plausible that all these factors would also influence food intake in the emu.

3.4.1.3 Connective tissue

The layer of connective tissue supporting the epithelium of the oropharynx in the emu could not be clearly divided into a lamina propria and a submucosa, a feature also noted in the greater rhea (Feder, 1972) and chicken (Calhoun, 1954). This is due to the absence of a muscularis mucosae (Calhoun, 1954). Thus for the purposes of this study this tissue was termed the underlying connective tissue. The connective tissue in the emu formed capsules around the glands, and housed blood vessels, nerves, Herbst corpuscles, melanocytes, lymphoid tissue and glandular tissue, in similar fashion (except for the melanocytes) to that described in the ostrich (Tivane, 2008).

3.4.1.3.1 Glands (Glandulae oris, Glandulae pharyngis)

Glandular tissue was a major feature of the non-pigmented regions of the emu oropharynx and was located in the connective tissue of the non-pigmented floor, tongue (see Chapter 5), lips of the glottis, the non-pigmented roof, rictus and pharyngeal folds. The environment and condition of the animal is reported to influence both the size and number of glands present in the oral and pharyngeal cavities (Tucker, 1958) and glands are best developed in birds with a dry diet, such as seed or insect eaters (King and McLelland, 1984). The emu has a varied diet also consuming seeds and insects (Davies, 1978), thus there is a high gland density in the emu oropharynx. The glands in the greater rhea (Feder, 1972) and ostrich (Porchescu, 2007; Tivane, 2008) oropharynx are also abundant in comparable regions to those in the emu (see Chapter 2).

The nomenclature used to describe the grouping of avian salivary glands has been found in the past to be both inconsistent and confusing (Ziswiler and Farner, 1972). This is partly due to the fact that in birds the regions of glandular tissue tend to merge with one another (Tucker, 1958). Fahrenholz (1937) grouped the oropharyngeal glands of birds into: mandibular, lingual and
crico-arytenoid glands in the floor, and palatine and sphenopterygoid glands in the roof. Tucker (1958), alternatively, distinguishes the oral angular glands (often rudimentary), the palatine group consisting of palatine, median, lateral, anterior, posterior, internal and external glands, the intermandibular group consisting of anterior, posterior, external posterior and inferior posterior glands and the pharyngo-oesophageal group consisting of pterygoidal palatine, crico-arytenoidal and oesophageal glands. Thus the glandular regions in the emu oropharynx were grouped and named according to their location (Fig. 3.38). The following groups were recognised, namely, caudal intermandibular, lingual (see Chapter 5), crico-arytenoid, oral angular (buccal), caudal palatine and pharyngeal glands. The groups of glands identified in the greater rhea and ostrich were not named (Feder, 1972; Tivane, 2008). The two types of glands (large, simple branched tubular and small, simple tubular glands, see below) observed in the emu oropharynx differed in distribution. The caudal intermandibular glands were formed by both types of glands. The crico-arytenoid glands were composed of the large simple branched tubular type and the oral angular glands consisted of both types. The caudal palatine and pharyngeal glands consisted predominantly of the large simple branched tubular units with only a few simple tubular glands being present.

Two types of salivary glands were evident in the emu, namely, small simple tubular mucus-secreting glands (single and branched) and large simple branched tubular mucus-secreting glands, similar to those noted in the ostrich (Tivane, 2008). The glands in the greater rhea (Feder, 1972) were described as being tubulo-alveolar with typical mucus-secretory features. No further mention of size or details of their structure were provided (Feder, 1972). Hodges (1974) and McLelland (1979) state that the salivary glands of the oral and pharyngeal cavity in birds are compound tubular structures. Although large, the branched tubular glands seen in the emu did not reveal a complex duct system and were therefore not compound in nature. Tubular glands are the most common type found in birds with the alveolar type being the exception (Fahrenholz, 1937). The large glands manifested as the doughnut-shaped structures observed macroscopically with their openings to the surface the small central spot or depression (also noted by Gardner (1927) in other birds studied). The openings of the salivary glands of the chicken (King and McLelland, 1984) and ostrich (Tivane, 2008) are also seen as small openings macroscopically.

In birds, definitive salivary glands do not occur; instead they are replaced by collections of large numbers of simple and branched tubular mucus-secreting glands lined by large mucus cells (Banks, 1993). Thus the salivary glands of birds are mostly a collection of individual glands
combined in a glandular field forming a polystomatic gland (Fahrenholz, 1937). This situation is evident in the emu as well as in the greater rhea (Feder, 1972) and ostrich (Tivane, 2008). Monostomatic salivary glands are, however, found in poultry (Saito, 1965). Collections of glands with a single opening form a continuous layer in the connective tissue of the fowl (McLelland, 1975, 1979). In all the ratite species studied (emu, ostrich and greater rhea) all the glands were mucus-secreting only. The salivary glands in birds are most often tubular with the serous elements normally absent (Ziswiler and Farner, 1972), a feature also apparent in the ratites. The glands of the emu oropharynx compare to the similar simple branched tubular, tubulo-alveolar and alveolar mucus-secreting glands found in many birds (Calhoun, 1954; Warner et al., 1967; Hodges, 1974; McLelland, 1975, 1979; Samar et al., 1999).

The lumen of some of the large, simple branched glands of the pharyngeal folds in the emu displayed a pseudostratified ciliated columnar epithelium, presumably to assist in extrusion of mucus from the glands. The mucus secretions of the oropharyngeal glands apparently accumulate in the large lumen below the epithelium and moves to the surface through short ducts. Thus extrusion of viscid secretion may be due to the action of cilia, where present, as well as through pressure build-up of accumulated secretions. The large openings would offer little resistance to the passage of the secretions. Hodges (1974), notes that the presence of smooth muscle fibres around glands is disputed in birds. The large glands in the emu are surrounded by a clear connective tissue capsule with no evidence of smooth muscle (with the staining techniques used), a finding similar to that in the ostrich (Tivane, 2008). Connective tissue capsules around glands in other birds have also been noted (Warner et al., 1967; Hodges, 1974). However, in the quail (Warner et al., 1967) smooth muscle fibres were identified surrounding the glands in the oropharynx.

The main function of the salivary glands in birds is mucogenesis to form saliva (Ziswiler and Farner, 1972) which provides moisture and lubrication for food bolii (Ziswiler and Farner, 1972; Nickel et al., 1977; King and McLelland, 1984; Gargiulo et al., 1991; Liman et al., 2001). Mucins are visco-elastic organic components of mucus formed by high molecular weight glycoproteins and coat all mucosal surfaces (Tabak et al., 1982). They provide protection from desiccation and mechanical damage, help maintain cellular water balance, provide lubrication and are antimicrobial in action (Tabak et al., 1982). Sticky saliva also assists in the backward propulsion of food and prevents regurgitation (McLelland, 1990). All these functions would be fulfilled by the mucus-secreting glands in the emu oropharynx.
3.4.1.3.2 Herbst corpuscles

Herbst corpuscles were located throughout the oropharynx of the emu, except for the pharyngeal folds, and in the glandular regions were mostly associated with the large glands. Sensory corpuscles occur in the roof of the greater rhea (Feder, 1972) and the oropharynx (excepting the tongue) of the ostrich (Tivane, 2008). Herbst corpuscles occur in the beak of ducks and geese, the oral cavity, tongue, subcutaneous connective tissue, muscles and adjacent to joints. In the deep dermis they are found in the legs, beak and feathered skin (Gottschaldt, 1985). Their presence in the oropharynx of many birds has also been confirmed (Wight et al., 1970; Ziswiler and Farner, 1972; Hodges, 1974; Berkhoudt, 1979).

The dermis (corium) of the bill skin in the emu was aglandular and contained numerous Herbst corpuscles. Herbst corpuscles have also been found in the bill skin of domestic poultry (Calhoun, 1954; Warner et al., 1967; Berkhoudt, 1979) and the bill of the kiwi (Cunningham et al., 2007). In the keratinised, aglandular regions of the emu oropharynx, the corpuscles were situated near the base of the connective tissue layer and were mostly single but sometimes occurred in groups or chains. In comparison to the rest of the structures and regions of the emu oropharynx, the corpuscles were mainly concentrated in the pigmented roof. In the ostrich (Tivane, 2008) the median palatine ridge was a very pronounced structure in comparison to that in the emu (see Chapter 2). Herbst corpuscles were concentrated in this ridge, as well as in the mucosal ridges on the floor of the oropharynx (Tivane, 2008). However, no such concentration of corpuscles was noted in the emu median palatine ridge. In contrast, they were evenly distributed throughout the pigmented roof, and the median ridge/s on the oropharyngeal floor (present in the ostrich [Tivane, 2008]), were absent in the emu. The corpuscles decreased in number in the non-keratinised (non-pigmented glandular) regions of the oropharynx, a finding similar to that in the greater rhea (Feder, 1972) and the ostrich (Tivane, 2008).

The connective tissue encapsulating the avian Herbst corpuscle is reported to be continuous with the perineurium of the nerve fibre supplying it and the lamellae consist of delicate connective tissue (Nickel et al., 1977). The continuity between the Herbst corpuscle capsule and the perineurium of the associated nerve could not be demonstrated in the emu material studied. The structure of the Herbst corpuscles in the oropharynx of the emu was similar to those identified in the tongue (Crole and Soley, 2008; Chapter 5) and in the ostrich oropharynx (Tivane, 2008). The
emu Herbst corpuscle is also similar to those described in the chicken (Cobb and Bennet, 1970; Wight, 1970; Hodges, 1974; Dimitrov, 2003). Gottschaldt (1985) provides a review of the earlier literature, as well as a description of Herbst corpuscles; from this it is apparent that the emu Herbst corpuscle, at the light microscopic level, appears similar to other avian Herbst corpuscles. A more detailed comparative study of these structures, however, will be needed to clarify this situation.

3.4.1.3.3 Lymphoid tissue

In the emu oropharynx, lymphoid tissue was located in the connective tissue of the caudal non-pigmented glandular interramal region, the tongue (see Chapter 5), the rictus, the junction of the pigmented and non-pigmented roof, the mucosal folds lateral to the choana, the infundibular cleft and pharyngeal folds and was mainly associated with the glands present in these regions. The association of lymphoid tissue with glands has been noted in the ostrich (Tivane, 2008) and in other birds (Calhoun, 1954; Warner et al., 1967; Hodges, 1974). Lymphoid tissue is abundant in the oropharynx of birds (Rose, 1981) and is especially concentrated in the pharyngeal region (Barge, 1937; Nickel et al., 1977; McLelland, 1979) where it has been termed the *lymphonoduli pharyngeales* (Rose, 1981; Rautenfeld, 1993). The pharyngeal folds in the emu represented the *lymphonoduli pharyngeales* (pharyngeal tonsils).

Lymphoid tissue occurred in the emu oropharynx as numerous areas or patches of diffuse lymphoid tissue, some of which featured nodular concentrations. The occurrence of both diffuse and nodular lymphoid tissue was noted in the ostrich (Tivane, 2008) as well as in other birds (Ziswiler and Farner, 1972; Nickel et al., 1977). Nodular lymphoid tissue was mainly seen in the rictus, the mucosal folds lateral to the choana and the pharyngeal folds. Each pharyngeal fold in the emu demonstrated a small protrusion of tissue on its caudo-lateral edge. This tissue was almost entirely lymphoid in nature (composed of both diffuse and nodular tissue). This feature of the emu pharyngeal fold is unique amongst the ratites.

Lymphocytes constitute the main component of lymphoid tissue, with the T-lymphocytes being responsible for cell mediated immune responses and the B-lymphocytes, which synthesize and secrete antibodies after transforming to plasma cells, providing humoral immunity (Rose, 1981).
3.4.2 Proximal cervical oesophagus

As previously described (Herd, 1985, and confirmed in the present study), the oesophagus of the emu is composed, in sequence, of a stratified squamous epithelium overlying a loose connective tissue lamina propria containing glands, a longitudinal muscularis mucosae, a thin submucosa and a broad inner circular and thin outer longitudinal external muscle layer. Additional to the description of Herd (1985), it was noted in the present study that the epithelium is non-keratinised, the glandular tissue is composed of tubular and simple branched tubular mucus-secreting glands (PAS-positive staining), lymphoid tissue is present in the lamina propria, the muscle layers are composed of smooth muscle and the outermost layer is the tunica adventitia. It is unclear from the study of Feder (1972) and Herd (1985) which part of the oesophagus was sampled in the greater rhea and emu respectively, however, the results from this study show the proximal oesophagus of the emu to be similar to the results of Herd (1985).

The oesophagus of the greater rhea (Feder, 1972) and ostrich (Tivane, 2008) is also lined by a non-keratinised stratified squamous epithelium, as in the emu (present study). This is a feature common to most birds (Pernkopf and Lehner, 1937; Calhoun, 1954; Warner et al., 1967; Hodges, 1974; McLelland, 1975; Bacha and Bacha, 2000). However, in some birds this epithelium may be partially or completely keratinised (Koch, 1973; King and McLelland, 1984; McLelland, 1990), a feature not seen in the emu (present study). Fowler (1991) states that the ratite oesophagus appears cornified, but as indicated above, the epithelium in the emu remains uncornified. In the hatchling greater rhea, sheets of ciliated columnar epithelium, in the process of sloughing, were observed on the stratified squamous epithelium (Feder, 1972). Although ciliated cells were seen elsewhere in the oropharynx, ciliation was never observed in the emu oesophagus.

Taste buds were found in the proximal oesophagus of the emu. This is the first report of such structures in the ratite oesophagus. They had the typical appearance of those described for birds (Botezat, 1910; Moore and Elliott, 1946; Gentle, 1971b; Nickel et al., 1977; Lindenmaier and Kare, 1959; Warner et al., 1967) and were similar to those identified in the emu oropharynx (see above). The presence of taste buds in this segment of the emu upper digestive tract is probably not unusual as in the eating method employed by this bird (Bonga Tomlinson, 2000; Gussekloo and Bout, 2005) the oesophagus is one of the first areas to receive ingesta. Thus food selection by taste in the emu may most likely occur after swallowing (see above).
Despite the occurrence of large amounts of lymphoid tissue in the avian oesophagus (Pernkopf and Lehner, 1937) it is only mentioned in a few studies (Warner et al., 1967; Banks, 1993). Lymphoid tissue in the oesophagus is termed *lymphonodi oesophageales* (Rose, 1981), however, its actual existence in birds is questioned by Rautenfeld (1993). Although specific aggregations of lymphoid tissue are formed in the oesophagus of the emu, they do not constitute oesophageal tonsils. Both the ostrich (Tivane, 2008) and the emu display lymphoid tissue in the oesophagus. The lymphoid tissue of the emu was mainly composed of diffuse lymphoid tissue and was situated in the *lamina propria* in association with the glands. This tissue would obviously imply an immunological function for the oesophagus, as in the oropharynx.

A prominent feature of the avian oesophagus is the presence of numerous simple tubular mucus-secreting glands, also noted in the ostrich (Tivane, 2008) and greater rhea (Feder, 1972). In the emu, the oesophageal glands are situated in the *lamina propria* (Herd, 1985) (although much of their length is enclosed in the epithelial lining) (present study) into which they extend for only a short distance, a feature similar to that in the ostrich (Porchescu, 2007; Tivane, 2008) and greater rhea (Feder, 1972). This is in contrast to mammals where glands are situated in the submucosa (Ross et al., 2003). In birds the oesophageal glands are noted to lie in the *tunica mucosae* (Ziswiler and Farner, 1972) or more specifically, the *lamina propria* (McLelland, 1975). In the emu the glands are simple tubular, sometimes branched, mucus-secreting (PAS-positive) glands. Oesophageal glands of other birds have been reported to range from tubular to alveolar (Ziswiler and Farner, 1972), mainly alveolar with some branching (Warner et al., 1967) or branched (Koch, 1973). Hodges (1974) notes that in the chicken, the same type of glands found in the oropharynx occur in the oesophagus. The simple tubular glands also occurred in the oropharynx (see above) and tongue (see Chapter 5) in the emu.

The *muscularis mucosae* in the emu represented the thickest layer of the oesophagus and consisted of longitudinally oriented (Herd, 1985; present study) smooth muscle fibres, a feature noted in the greater rhea (Feder, 1972) and ostrich (Tivane, 2008). This appears to be a general feature of the avian oesophagus (Calhoun, 1954; Warner et al., 1967; Ziswiler and Farner, 1972; Hodges, 1974; Gussekloo, 2006). In the greater rhea (Feder, 1972) and ostrich (Tivane, 2008) oesophagus the *muscularis mucosae* was present in the longitudinal folds of the mucosa, a feature not noted in the emu. However, Tivane (2008) reported that the folds of the proximal oesophagus in the ostrich were lower than those situated more distally and that the *muscularis*
mucosae was only present in the larger folds. Thus the presence of the muscularis mucosae in the larger folds of the distal oesophagus of the emu cannot be ruled out.

The submucosa in the emu oesophagus was weakly developed (Herd, 1985; present study) and was situated between the muscularis mucosae and tunica muscularis. It was composed of a loosely arranged irregular dense connective tissue and carried blood vessels and nerves (submucosal plexus). This finding is similar to that in the greater rhea (Feder, 1972) and ostrich (Tivane, 2008) as well as in other birds (Calhoun, 1954; Warner et al., 1967; Ziswiler and Farner, 1972; Hodges, 1974; Gussekloo, 2006).

In the emu, the tunica muscularis was composed of a thicker inner circular and thinner outer longitudinal (Herd, 1985; present study) smooth muscle layer and was surrounded by the loose irregular connective tissue of the adventitia. Both of these layers were similar to those described for the greater rhea (Feder, 1972) and ostrich (Tivane, 2008). The features of the tunica muscularis and adventitia of the emu were also typical for those described in other birds (Pernkopf and Lehner, 1937; Calhoun, 1954; Warner et al., 1967; Ziswiler and Farner, 1972; Hodges, 1974; Banks, 1993; Gussekloo, 2006). Although Owen (1879) reported that the oesophagus of the kiwi contained an outer circular and inner longitudinal layer, the uniformity of the layers of the muscular tunic described in ratites and other birds (see above) would make it seem unlikely that this arrangement would differ in the kiwi.

3.4.3 Scanning electron microscopy

The description of surface features was based mainly on observations of the 5 month-old specimen, although the basic features observed were consistent with those of the older birds. The main difference appeared to be an increase in cell sloughing in the older birds and the replacement of large areas of cell surfaces displaying microvilli (young bird) by surfaces displaying microplicae (older birds).

The SEM findings for the oropharyngeal floor of the emu revealed a difference in appearance of the keratinised and non-keratinised surfaces noted histologically. The keratinised region displayed sheets of desquamating cells whereas the non-keratinised region displayed individual desquamating cells. Individual desquamating cells were also a feature noted in the oropharynx and oesophagus of the ostrich (Tivane, 2008).
In the emu, higher magnification of the surfaces studied in the oropharynx and proximal oesophagus, revealed 4 different cell surface features, namely: microridges, microplicae, microvilli and cilia. Microridges were present on the surface cells of the keratinised areas only. In the non-keratinised regions, microplicae were present on cells free of microvilli and cilia as well as on the raised round cells of the pharyngeal folds. Microvilli were present on cells lining all small gland openings and large gland openings or parts there of. Microvilli also adorned cell surfaces in areas surrounding the gland openings and the luminal surface of the proximal oesophagus. Cilia were present in isolated patches in the ducts of gland openings and in the vicinity of the openings. No specialised features were noted for the ostrich (Tivane, 2008).

The openings seen in all regions of the emu oropharynx represented the underlying glands. Large openings represented those of the large simple branched tubular mucus-secreting glands whereas the small openings represented the simple tubular mucus-secreting glands. Both large and small openings were often filled with cellular debris and mucus-secretions from the underlying glands. In the ostrich (Tivane, 2008) only one type of opening was described (which also represented underlying glands) and showed similar features to that of the large gland openings observed in the emu.

Although only simple tubular (and sometimes branched) glands were identified histologically in the proximal oesophagus, both large and small openings were observed on the luminal surface using SEM. The small openings were more numerous and represented the simple tubular glands seen histologically, a feature also noted in the ostrich (Tivane, 2008). However, it was not possible to ascertain what type of underlying glands the large openings represented. Following the pattern seen in the oropharynx, it may be possible that the large openings represent large, simple branched tubular glands, such as those commonly seen in the oropharynx, or are merely enlarged openings of the simple tubular glands. However, the large, simple branched tubular glands were not observed histologically.

Although taste buds were identified histologically, structures typically representing taste buds were not resolved by SEM. However, due to the relatively small areas sampled for SEM and the scarcity of taste buds in the emu oropharynx, this study does not rule out the possibility of these structures being identified by SEM. Another possibility could be that the taste buds may be difficult to visualise due to their size and morphological characteristics, and may possibly even
be masked by mucus-secretions or desquamating cells. Further studies, incorporating larger tissue samples will be needed to positively identify these structures in the oropharynx and proximal oesophagus of the emu using this technique.

3.5 REFERENCES


Figure 3.1: Emu head opened and the two halves reflected to show the areas sampled for light microscopy.

Floor of the oropharynx: The pigmented mandibular rhamphotheca (A), the pigmented rostral interramal region (B), the area of transition (C), the caudal non-pigmented interramal region (D), the mandibular rictus (E), the laryngeal mound (G) and the transition to the oesophagus (H).

Roof of the oropharynx: The pigmented roof (1), the non-pigmented roof (2), the transitional area (3), the maxillary rictus (4), the mucosal flap lateral to the caudal choana (5), rostral attached pharyngeal fold (6), caudal free pharyngeal fold and the caudo-lateral projection (7), proximal oesophagus (8). Bar = 5mm.
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Figure 3.2: The mandibular bill skin showing the *Stratum corneum* (Sc) (*rhamphotheca*) overlying the *str. spinosum* (Ss) and *Str. basale* (Sb). The corium (Co) houses Herbst corpuscles (*). Melanocytes (M) are concentrated in the *Str. basale*.

Figure 3.3: The *rhamphotheca* of the mandibular bill skin formed by the dark (Scd) and light regions of the *Str. corneum* (Scl). *Str. spinosum* (Ss), *Str. basale* (Sb), melanocytes (M) and corium (Co).

Figure 3.4: The corium of the mandibular bill skin displaying the dense connective tissue (Dct) typical of this layer and an area of loose connective tissue (Lct) housing Herbst corpuscles (*). Epithelium (E), *Str. basale* (Sb) with melanocytes (dark line).
**Figure 3.5:** Two folds and an intervening groove (G) in the rostral pigmented interramal region. *Str. corneum* (Sc), melanocytes (M) in the *Str. basale* (Sb), melanocytes in connective tissue (Mct), connective tissue (Ct), blood vessel (Bv) and nerve (*).

**Figure 3.6:** Two folds and an intervening groove (G) in the rostral pigmented interramal region. Note the concentration of melanocytes (M) in the *Str. basale* (Sb) of the fold (F), their disappearance from this layer in the groove and their presence (Mct) restricted to the underlying connective tissue (Ct). *Str. corneum* (Sc).
Figure 3.7: A Herbst corpuscle (H) in the connective tissue (Ct) of the rostral pigmented interramal region. Note the desquamation of the Str. corneum (Sc). Melanocytes (M).

Figure 3.8: Floor of the oropharynx showing the zone of transition from the keratinised stratified squamous epithelium (Kse) to the thicker non-keratinised stratified squamous epithelium (Sse). Note the attenuation of the keratinised Str. corneum (Sc) and its eventual disappearance (*) as well as the appearance of connective tissue papillae (P) and glands (Gl) in the non-keratinised region. Connective tissue (Ct), skeletal muscle (Sm).
Figure 3.9: The large lateral fold in the caudal interramal region showing the large, simple branched tubular glands (Lg) restricted to the dorsal (D) surface with simple tubular glands (Sg) present on the ventral (V) surface and opening to the medial-facing groove or recess (R). The inset shows the immediate continuation of the floor medial to the large fold. Connective tissue (Ct), small fold (F), simple tubular glands (*).

Figure 3.10: Enlargement of a similar area to that shown in the inset in Fig. 3.9. The fold (F) of the caudal interramal region displays simple tubular mucus-secreting glands (Sg) only. Note the numerous blood vessels (Bv) within the underlying connective tissue (Ct).
Figure 3.11: Mandibular rictus displaying large, simple branched tubular glands (Gl), diffuse lymphoid tissue (*) and large blood vessels (Lbv) in the underlying dense connective tissue (Dct). Note the gland opening (arrow) coursing through the epithelium (E) and the regular, deep connective tissue papillae. Loose connective tissue (Lct).

Figure 3.12: A collection of diffuse lymphoid tissue (Dlt) and nodular lymphoid tissue (Nlt), simple tubular glands (Sg) and a Herbst corpuscle (arrows) in the mandibular rictus. Epithelium (E).

Figure 3.13: A large simple branched tubular gland (Gl) with associated Herbst corpuscles (*) in the connective tissue (Ct) of the mandibular rictus. Note the large lumen (L) filled with pale basophilic material (mucus).
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Figure 3.14: A glandular region of the laryngeal mound displaying a thick stratified squamous epithelium (Sse) and dense irregular connective tissue (Ct) resting on skeletal muscle fibres (Sm). A single Herbst corpuscle is outlined by the arrows.

Figure 3.15: Glandular region of the laryngeal mound displaying a thinner stratified squamous epithelium (Sse) than the aglandular region. The underlying connective tissue (Ct) contains large simple branched tubular glands (Gl). Note the Herbst corpuscle (arrows) associated with the glands. Gland opening (Go).

Figure 3.16: The laryngo-oesophageal junction marked by the appearance of simple tubular glands (Sg). Stratified squamous epithelium (Sse), connective tissue (Ct).
Figure 3.17: A taste bud in the non-pigmented floor, adjacent to the tongue body. The taste bud is demarcated (arrows) from the surrounding stratified squamous epithelium (Sse). Sensory and supporting cells (S) are not clearly defined. Opening to the surface (*). Nerve (N).

Figure 3.18: Putative taste bud at the laryngo-oesophageal junction.

Figure 3.19: A circumscribed area in the stratified squamous epithelium (Sse) of the non-pigmented floor showing a collection of vertically oriented cells (*) with features typical of a taste bud.
Figure 3.20: Epithelial lining of the pigmented region of the oropharyngeal roof. Note the columnar cells of the *stratum basale* (Sb) and the interspersed melanocytes (*) which are also obvious in the *stratum spinosum* (Ss). The thickness of the *stratum corneum* (Sc) places it out of the plane of focus. Connective tissue (Ct).

Figure 3.21: Transverse section through the median palatine ridge. The low magnification inset demonstrates the boundaries of the ridge (blue arrowheads) and the large artery (A) typically situated at its base. At higher magnification a single Herbst corpuscle (*) is seen in the less compacted region of the underlying connective tissue (Ct). Desquamation (arrows) of the surface cells of the *str. corneum* (Sc) is obvious. *Str. basale* (Sb).
Figure 3.22: Transition between the pigmented and non-pigmented regions of the oropharyngeal roof. The keratinized stratified squamous epithelium (Kse) of the aglandular pigmented region gradually widens (*) as it changes to the non-keratinised stratified squamous epithelium (Sse) of the glandular non-pigmented region. Gland (Gl), connective tissue (Ct).

Figure 3.23: An area similar to that shown in Fig. 3.22 but demonstrating an aggregation of diffuse lymphoid tissue (Dlt) below the transition from a keratinized stratified squamous epithelium (Kse) to a thicker non-keratinised stratified squamous epithelium (Sse). Melanocytes (arrows), transition area (*), connective tissue (Ct) shared between the oral and nasal (N) portions of the roof.
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Figure 3.24: The non-pigmented, glandular oropharyngeal roof lined by a stratified squamous epithelium (Sse). Note how the dense connective tissue (Dct) houses the glands (Gl) and a Herbst corpuscle (*) whereas the deeper, more loosely arranged connective tissue (Lct) contains large blood vessels (Bv).

Figure 3.25: Glandular region of the oropharyngeal roof showing the PAS-positive staining reaction of the large, simple branched tubular glands (Gl). The diffuse lymphoid tissue (Dlt) obliterates part of the overlying stratified squamous epithelium (Sse), appearing to breach the surface (*).

Figure 3.26 & 3.27: Sections through the mucosa of the maxillary rictus indicating the regularity and depth of the connective tissue papillae (arrows) in the maxillary rictus. Large, simple branched tubular glands (Gl) and aggregations of diffuse lymphoid tissue (Dlt) are present in the dense connective tissue (Dct). Loose connective tissue (Lct), gland opening (*), stratified squamous epithelium (Sse).
Figure 3.28: A group of Herbst corpuscles at the maxillary rictus (inset). Higher magnification of one of the corpuscles in longitudinal section details the central pink axon (A) surrounded by the inner core (Ic) with Schwann cell nuclei (black arrows), the outer core (Oc) with fibroblast nuclei (blue arrows) and the subcapsular space (Scs) below the fibrous outer capsule (Ca).

Figure 3.29: The PAS-positive staining reaction shown by the simple branched tubular glands (Gl) of the maxillary rictus. The fibrocytic lamellae of a Herbst corpuscle (*) also demonstrate a faint PAS-positive reaction. Connective tissue (Ct).
Figure 3.30: Transverse section of the small fold on the glandular region of the oropharyngeal roof lateral to the choana. Note the large, simple branched tubular glands (Lg) on the ventral surface (V) and the simple tubular glands (Sg) lining the recess (R) beneath the fold. Similar glands occur in the deeper lying respiratory mucosa (arrows). The large blood vessel (Lbv) and diffuse (Dlt) and nodular (*) lymphoid tissue situated at the angle of the recess, were consistently present. Stratified squamous epithelium (Sse), tissue of non-pigmented roof (Npr), direction of the choana (blue arrow), connective tissue (Ct).

Figure 3.31: A similar view of the fold to that depicted in Fig. 3.30 but sectioned closer to its edge. The large, simple branched tubular glands (Lg) are confined mainly to the ventral surface of the pharyngeal fold and the lateral edges of the recess. These glands and the simple tubular glands (Sg) display a PAS-positive staining reaction. Large blood vessel (Lbv), connective tissue (Ct), diffuse lymphoid tissue (*).
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Figure 3.32: Ventral surface of the pharyngeal fold displaying numerous, large, simple branched tubular glands (Gl) and associated lymphoid tissue (*) in the connective tissue (Ct). The lymphoid tissue consists of diffuse and nodular accumulations. Note the large opening to the surface of a gland (arrow).

Figure 3.33: Similar region to that shown in Fig. 3.32 illustrating the PAS-positive staining reaction of the glandular tissue (Gl). Note the large accumulations of lymphoid tissue (*) associated with the glands. Connective tissue (Ct), large gland openings (arrows).

Figure 3.34: Caudo-lateral aspect of the pharyngeal fold (black double-headed arrows) depicting the large opening (arrows) to the tonsilar crypt (*). Note the PAS-positive staining reaction of the figure on the right, showing the mucus-secreting properties of the glands. Connective tissue (Ct), lymphoid tissue (Lt), large, simple branched tubular glands (Lg), simple tubular glands (Sg), pocket or recess (yellow star) between the pharyngeal fold and the caudo-lateral tissue projection (white double-headed arrows). Protruding surface of the caudo-lateral projection (dotted bracket) (see Chapter 2 - Fig. 2.17).
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**Figure 3.35:** Psuedostratified ciliated columnar epithelium (bracket) lining the lumen of a large, simple branched mucus-secreting gland in the pharyngeal fold. Mucous cells (Mc).

**Figure 3.36:** Pocket or recess (yellow star) between the dorsal surface of the pharyngeal fold (D) and the ventrum of the caudo-lateral tissue projection (T). Note the large nodule (bracket) of lymphoid tissue (Lt) projecting dorsally into the recess from the pharyngeal fold, held by a connective tissue (Ct) stalk. Nodular lymphoid tissue (*).

**Figure 3.37:** Rostral extent of the pocket or recess (arrows) illustrated in Fig. 3.36. The figure on the right also shows the rostral extent of the retropharyngeal recess (Rr). The caudo-lateral tissue projection (T) formed the ventral border of the retropharyngeal recess. Connective tissue (Ct), diffuse (Dlt) and nodular (*) lymphoid tissue, large simple branched tubular gland (Gl), simple tubular glands (Sg).
Figure 3.38: Schematic representation of the mucus-secreting glandular fields identified in the oropharynx and proximal oesophagus of the emu: Caudal intermandibular (Ci, purple), lingual (L, black), radical (R, turquoise), crico-arytenoid (Ca, blue), oral angular (Oa, red), caudal palatine (Cp, yellow), pharyngeal (P, green), oesophageal (O, white) glands. Bar = 5mm.
Figure 3.39: Transverse section of the proximal oesophagus depicting the lamina propria (Lp) containing simple tubular glands (Gl), the thick longitudinal muscularis mucosae (Mm), the very thin submucosa (Sm) with blood vessels (*), the inner circular (Tmc) and outer longitudinal (Tml) layers of the tunica muscularis and the adventitia (A).

Figure 3.40: Transverse section of the proximal oesophagus depicting the epithelium (**), lamina propria (Lp) containing simple branched glands seen in longitudinal (Gll) and cross section (Glc), the thick longitudinal muscularis mucosae (Mm), the very thin submucosa (Sm), and the inner circular (Tmc) and outer longitudinal (Tml) layers of the tunica muscularis. Note how the mucosa is thrown into folds (F) which fill the lumen (L).

Figure 3.41: A mucosal fold in the proximal oesophagus consisting of a core of connective tissue (lamina propria) (Lp), containing simple tubular glands in longitudinal (Gll) and cross section (Glc). Note the large blood vessel (Bv) carried in the centre of the fold as well as the absence of the muscularis mucosae. Stratified squamous epithelium (Se).
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Figure 3.42: High magnification of the simple tubular glands (Gl) situated in the lamina propria (Lp) of the proximal oesophagus. Note that the base of the glands (Glb) extend only a short distance into the lamina propria. Non-keratinised stratified squamous epithelium (Se). Lumen (L).

Figure 3.43: PAS-positive staining reaction of the simple tubular mucus-secreting glands (Gl) in the proximal oesophagus. Aggregations of lymphoid tissue (Lt) lie between the glands. Lumen (L), mucosal fold (Mf), muscularis mucosae (Mm), submucosa (Sm), tunica muscularis (Tm), stratified squamous epithelium (Se).

Figure 3.44 & 3.45: High magnification of the mucus-secreting cells (Mc) which form the simple tubular oesophageal glands (PAS-positive stain reaction, Fig. 3.45). Note the typical features, basal nuclei (Bn) and basophilic foamy cytoplasm (*), of the mucus-secreting cells. Lumen (L), lamina propria (Lp), cross section of the basal part of the cells (double-headed arrow).
Figure 3.46: Enlargement of a taste bud (circled in inset) located in the non-keratinised stratified squamous epithelium (Se) of the proximal oesophagus. Structures identifiable were the taste pore (Tp), encapsulating epithelium (arrows) and vertically oriented, elongated cells (star). ** indicates another possible taste bud sectioned superficially.