THE UNIVERSITY OF MANITOBA

A HISTOLOGICAL STRUCTURE-FUNCTION STUDY OF THE AVIAN OESOPHAGUS

by

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ABSTRACT

The histology of the oesophagus is described in ten different bird species representing six taxonomic orders. Birds were grouped on the basis of significance testing for several histological measurements. Comparative histology of the avian oesophagus cannot be used to support or reject the avian phylogenetic series or avian taxonomic divisions.

Birds that ingest large food pieces require a distensible oesophagus with long mucosal convolutions. Birds whose primary food preferences do not require oesophageal distension lack mucosal convolutions. The oesophageal mucous membrane is thickest in birds lacking mucosal convolutions. The muscle layers of the oesophagus are thickest in birds which ingest large food pieces. Size and shape of food particles are the most relevant aspects of food texture related to oesophageal adaptation.

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STATEMENT OF THE PROBLEM

The principle purpose of this study was to ascertain whether histomorphological structures of the avian oesophagus in several species could be correlated to any of the following bird characteristics: 1) gross size of the species; 2) gross thickness of the oesophageal wall; 3) feeding habits; 4) taxonomic position within the birds.

Studies by others have indicated that, in various species of birds, histomorphological variations would be found in the oesophagus. A search of the relevant literature did not disclose any extensive reports on correlative histology in the avian oesophagus.

In the early preparatory stages for this study it was apparent that histological terminology is not applied uniformly. It was therefore decided to carefully define by name the various tissue layers and their parts for common adoption. The lack of a comprehensive review of the literature suggested that there was a need for such an organized body of knowledge.

LITERATURE REVIEW

GROSS ANATOMY

BILL

The development of a bill in birds results from the modifications of the upper and lower jaws (Farner, 1960; Young, 1962; Orr, 1966; Weichert, 1970). The bones are covered with a hard horny sheath derived from the epidermis. Teeth are lacking in all modern birds (Young, 1962; Weichert, 1970). The bills have been variously adapted to the feeding habit of the species (Farner, 1960; Young, 1962; Orr, 1966; Weichert, 1970).

Functionally the bill is a prehensile organ adapted for the rapid ingestion of food. The bill helps also to limit the size of the food particle. This is observed in flesh-eating carnivores such as hawks and owls which rip their food into smaller pieces before swallowing it (Farner, 1960; Orr, 1966; Weichert, 1970).

BUCCAL CAVITY

Farner (1960) stated that the buccal cavity is "somewhat arbitrarily and ambiguously designated as the cavity between the upper and lower jaws". The posterior limit is the anterior margin of the glottis. This marks the anterior margin of the pharynx.

The roof of the buccal cavity is hard anteriorly and continues posteriorly with an incomplete secondary palate. This secondary palate is represented by a pair of palatal folds which do not fuse medially

(Farner, 1960; Weichert, 1970). The soft palatal folds become continuous with the roof of the pharynx but the buccal cavity is not distinctly marked off from the pharynx (Ivey and Edgar, 1952; Calhoun, 1954; Farner, 1960).

TONGUE

The tongue is a pharyngeal derivative and has a posterior attachment (Calhoun, 1954; Farner, 1960). Farner (1960) reported that "the adaptations of the tongue are as extensive and varied as those of the bill".

SALIVARY GLANDS

Browne (1922) stated that true salivary glands were not present in the domestic fowl, Gallus domesticus. He stated that rudimentary salivary glands are present in some species and a few, such as the woodpecker, have well developed salivary glands. More recently Chodnik (1948) and Calhoun (1954) studied the salivary glands of the domestic fowl. Farner (1960) stated that the avian salivary glands show as much adaptive variation as that of the tongue and bill. He found that the literature contained numerous opinions concerning the homologies and nomenclature of the avian salivary glands. This resulted in the nomenclature being inconsistent and confusing.

PHARYNX

The junction of the buccal cavity with the pharynx is not clearly defined; this is also true of the area between the pharynx and the oesophagus (Ivey and Edgar, 1952; Calhoun, 1954; Farner, 1960). Farner

(1960) stated that the anterior limit of the pharynx is the anterior margin of the glottis. The pharynx was defined as that area behind the buccal cavity which contains the openings of the following: the posterior region of the buccal cavity, oesophagus, eustachian tubes, larynx, and nasal openings (Calhoun, 1954).

OESOPHAGUS

The avian oesophagus is a relatively long tube leading from the pharynx to the proventriculus. The oesophagus is of uniform diameter and usually dilatable (Browne, 1922; Steel and Churchill, 1942; Calhoun, 1954; Farner, 1960).

Functionally the oesophagus provides a passageway for ingested material from the pharynx to the proventriculus and lubricates this material with secretions from mucous glands. It may also function as a storage organ which may be accomplished by simple dilation of the oesophagus or by a specialized diverticulum, the crop (Calhoun, 1954; Farner, 1960).

The function of the crop, when present, is one of storage and this enables a bird to ingest large quantities of food material in a short period of time (Calhoun, 1954; Farner, 1960; Young, 1962; Orr, 1966; Weichert, 1970).

STOMACH - GASTRIC APPARATUS

The avian alimentary tract is typically marked by the division of the stomach into an anterior glandular stomach or proventriculus, and a posterior muscular stomach, the gizzard or ventriculus (Browne, 1922; Elias, 1945; Matthews, 1949; Calhoun, 1954; Farner, 1960; Young,

1962; Goin and Goin, 1965; Orr, 1966; Weichert, 1970). Farner (1960) referred to the proventriculus and ventriculus together, as the "gastric apparatus".

Proventriculus. The proventriculus is generally described as a fusiform tubular structure which leads from the oesophagus to the ventriculus and secretes the gastric digestive juices (Matthews, 1949; Calhoun, 1954; Farner, 1960; Goin and Goin, 1965; Orr, 1966; Weichert, 1970). Matthews (1949) described the proventriculus as typically having thicker walls than the oesophagus and being smaller than the ventriculus. He also reported that the thickness of the proventricular walls was primarily due to the deep proventricular glands located in the wall of the organ.

Farner (1960) outlined the functions of the proventriculus. It serves as a passageway for food between the oesophagus and ventriculus and is responsible for the secretion of gastric juice. The proventriculus in some species also assumes the function of a storage organ.

Ventriculus. The ventriculus is located posterior to the proventriculus. Food from the proventriculus moves into the ventriculus which typically grinds food material finely enough for it to be passed into the duodenum. In this way the ventriculus is considered the functional analog of teeth (Dilger, 1957; Farner, 1960).

In addition to the muscular development of the ventriculus there is also the development of a horny layer, the koilin lining, on the grinding surface. Farner (1960) observed that the koilin lining was the most striking histological feature of the ventriculus. It is

produced by the secretory activity of the mucous membrane and is colored in shades of green, yellow or brown by regurgitated bile pigments (Farner, 1960).

Farner (1960) outlined the functions of the ventriculus. It may serve as a storage organ, as a site of "preliminary acid proteolytic digestion" and as the site of the mechanical phase of digestion. The ventriculus is responsible for the movement of food into the duodenum.

Farner (1960) and Young (1962) observed that the anterior chambers of the avian alimentary tract show the most pronounced morphological variations from the general vertebrate pattern. The intestinal apparatus shows less striking morphological variations.

INTESTINE

Small intestine. The small intestine is divided into two anatomical regions, a duodenum and an ileum (Farner, 1960). This contrasts with Batt's (1925) earlier classification of a duodenum and a jejunum. Calhoun (1954) recognized three regions: duodenum, jejunum and ileum. She stated that no distinct line separates the jejunum and ileum and gave no explanation as to what the differences were. Farner's (1960) classification scheme is most satisfactory since it applied to birds in general whereas Batt's (1925) and Calhoun's (1954) scheme was specific for the domestic fowl, Gallus domesticus.

The small intestine, as defined in several authoritative texts, is that portion of the alimentary tract which extends from the ventriculus to the large intestine. The junction of the small intestine and the large intestine is usually marked by a pair of intestinal caeca.

The intestinal caeca are discussed in this section.

The duodenum, which arises from the posterio-medial aspect of the ventriculus, loops around the largest lobe of the trilobed pancreas (Batt, 1925; Rosenberg, 1941; Calhoun, 1954; Farner, 1960). The ascending portion of the loop returns to the level of the ventriculus from where it continues as the ileum. The junction is typically marked by the hepatic and pancreatic ducts which open into the terminal part of the duodenum (Batt, 1925; Calhoun, 1954; Farner, 1960).

The ileum which is arranged in coils, is supported by mesenteries (Calhoun, 1954; Farner, 1960).

Farner (1960) outlined several important functions of the small intestine. It is the principle site of chemical digestion and absorption of nutrients. It is responsible also for moving the contents of its lumen. Farner stated also that the cells of the mucous membrane have a secretory function but little is known about the biochemical nature of such secretions.

Large intestine. The large intestine is continuous with the small intestine. The large intestine begins at the level of the intestinal caeca and continues for a short distance and terminates at the cloaca (Calhoun, 1954; Farner, 1960).

Farner reported that little is known about the functions of the large intestine. He thought that the large intestine had little importance as a digestive organ other than the movement and storage of intestinal material. It may be the site of water absorption because the moisture content of the intestinal material is usually reduced in

the large intestine (Farner, 1960).

Intestinal caeca. The intestinal caeca are often located at the junction of the small and large intestines. They are symmetrically placed on either side of the intestine. Their open ends arise from the intestine in the region approximating the junction of the small intestine with the large intestine. The caecal tubes project anteriorly and terminate blindly as closed tapered ends (Browne, 1922; Calhoun, 1954; Farner, 1960; Goin and Goin, 1965; Weichert, 1970).

In some species of birds the caeca are large while in others they are small. A variety of intermediate conditions also exist. Parrots, woodpeckers and other species were reported to lack caeca (Farner, 1960; Goin and Goin, 1965; Weichert, 1970).

The functions of the intestinal caeca are still a matter of speculation (Browne, 1922; Farner, 1960). Browne (1922) and Young (1962) suggested the caeca are responsible for water absorption. This and other proposed functions are listed by Farner (1960).

Cloaca. The cloaca is a posterior continuation of the large intestine. It is a common receptacle for discharged contents from the large intestine, urinary ducts and reproductive canals (Farner, 1960; Young, 1962; Weichert, 1970). The cloaca of birds consists of three chambers. The first is the corprodaeum into which the large intestine passes its contents. Next is the urodaeum into which the ureters and reproductive ducts empty. The proctodaeum continues from the urodaeum and opens to the outside at the anus (Farner, 1960; Young, 1962). Both Young (1962) and Weichert (1970) reported that water absorption takes place

through the walls of the cloaca.

LIVER

The liver is divided into right and left lobes with the right lobe being larger (Farner, 1960). The gall bladder, when present, is adjacent to, and on occasion is found to be partially implanted in, the right lobe of the liver (Batt, 1926; Steel and Churchill, 1942; Calhoun, 1954; Goin and Goin, 1965; Weichert, 1970). Two hepatic ducts are present, one draining each lobe of the liver (Calhoun, 1954; Farner, 1960). Calhoun (1954) reported that in the domestic fowl the left lobe of the liver is drained by the "ductus hepaticus" which empties directly into the duodenum. Calhoun (1954) implied that the gall bladder received bile from the right lobe and stated that the gall bladder drained into the duodenum by the "ductus cysticus". Farner (1960) agreed that the left lobe of the liver is drained by the "left hepatic duct" which communicates directly into the duodenum. Also, the right hepatic duct may have a branch into the gall bladder, or the gall bladder may be a local enlargement of the right hepatic duct. In both cases the duct leading from the gall bladder to the duodenum is the "cystic duct". No information was found to describe how bile was transferred from the right lobe of the liver to the gall bladder. There is apparently some variation.

PANCREAS

The pancreas is situated in the duodenal loop and usually consists of three lobes: dorsal; ventral; splenic (Steel and Churchill, 1942; Calhoun, 1954; Farner, 1960). The dorsal and ventral lobes are the largest while the splenic is smaller. There are usually three pan-

creatic ducts, one from each lobe, which empty into the terminal region of the duodenum (Calhoun, 1954; Farner, 1960).

TERMINOLOGY

Authors use several terms to indentify the tissue layers of the wall of the alimentary tract of vertebrates. While most terminology is acceptable, the following was accepted because of its general use in human histology text books and its common use in the literature pertaining to the avian alimentary tract.

Four main tissue layers compose the gut wall. These layers, arranged in order from the lumen towards the circumference of the organ, were named the following: a) mucosa, b) submucosa, c) muscularis externa, d) adventitia or serosa.

The term mucosa was used by: Andrew (1959); Farner, (1960); Garven (1965); Deane and Padykula (1966); Patt and Patt (1969). Mucous membrane was used synonymously by Ham (1965); Deane and Padykula (1966); Leeson and Leeson (1967).

The mucosa comprises three sublayers. The mucous membrane consists of a sheet of epithelium which lines the gut lumen; the intermediate layer, the lamina propria is a connective tissue layer; the basal layer of the mucosa is the muscularis mucosae which is a smooth muscle layer.

Other names which were given to the mucous membrane were: surface epithelium (Garven, 1965); epithelial lining (Ham, 1965); epithelial membrane (Patt and Patt, 1969); epithelium (Rosenberg, 1941; Calhoun, 1954); mucosal epithelium (Ivey and Edgar, 1952). The term lamina propria was used by Garven (1965); Ham (1965); Bloom and Fawcett

(1968); Patt and Patt (1969). The term tunica propria was used synonymously by: Batt (1925); Ivey and Edgar (1952); Calhoun (1954); Andrew (1959); Patt and Patt (1969).

Most authors referred to the muscle layer of the mucosa as the muscularis mucosae (Batt, 1925; Rosenberg, 1941; Steel and Churchill, 1942; Ivey and Edgar, 1952; Calhoun, 1954; Andrew, 1959; Garven, 1965; Ham, 1965; Bloom and Fawcett, 1968; Patt and Patt, 1969).

The submucosa is the second major tissue layer. It surrounds the mucosa (Rosenberg, 1941; Ivey and Edgar, 1952; Calhoun, 1954; Andrew, 1959; Garven, 1965; Ham, 1965; Patt and Patt, 1969). It is also called the tela submucosa (Bloom and Fawcett, 1968). The basal area of the submucosa is surrounded by a thick muscularis externa (Ivey and Edgar, 1952; Garven, 1965; Ham, 1965; Bloom and Fawcett, 1968; Patt and Patt, 1969). Other terms used synonymously to designate this thick muscle layer are: muscularis (Elias, 1945); lamina muscularis (Calhoun, 1954); muscular coat (Andrew, 1959); tunica muscularis (Leeson and Leeson, 1967).

The adventitia lies adjacent to the muscularis externa and forms the outer investing layer of the oesophagus (Ivey and Edgar, 1952; Andrew, 1959; Garven, 1965; Ham, 1965; Bloom and Fawcett, 1968; Patt and Patt, 1969). The term 'adventitia' is used where the outer connective tissue of the gut wall unites with connective tissues in surrounding areas. In the body cavity the organs are suspended by mesenteries which are extensions of the parietal peritoneum. The term serosa is used to indicate the peripheral layer of the oesophagus which is located in the body cavity. The serosa is formed by a contin-

uation of the parietal peritoneum by way of dorsal mesenteries around the gut wall (Batt, 1925; Ivey and Edgar, 1952; Andrew, 1959; Garven, 1965; Ham, 1965; Patt and Patt, 1969).

HISTOLOGY OF THE OESOPHAGUS

Mucosa

Mucous membrane. The mucous membrane consists of stratified squamous epithelium (Batt, 1925; Steel and Churchill, 1942; Ivey and Edgar, 1952; Calhoun, 1954; Andrew, 1959; Farner, 1960). Andrew (1959) reported that in some species of birds, the superficial cells showed pronounced flattening. Cornification of the surface epithelium occurred in doves and ducks (Ivey and Edgar, 1952; Andrew, 1959; Farner, 1960). The surface epithelium tends to slough off in the domestic fowl (Calhoun, 1954) and the ring-necked pheasant (Steel and Churchill, 1942). In the domestic fowl the junction between the mucous membrane and the lamina propria is marked by papillae of the lamina propria (Calhoun, 1954). The thickness of the mucous membrane varies from species to species (Farner, 1960).

Lamina propria. The lamina propria is a relatively thin layer of areolar connective tissue situated between the mucous membrane and the muscularis mucosae (Farner, 1960). The lamina propria, as described in several authoritative texts, consists of a fibrous, loose connective tissue layer with blood and lymph vessels, nerves, and varying amounts of lymphatic tissues. The mucous glands, located in the mucosa, usually project deeply into the lamina propria (Batt, 1925; Steel and Chur-

chill, 1942; Ivey and Edgar, 1952; Calhoun, 1954; Andrew, 1959; Farner, 1960). Batt (1925) observed smooth muscle fibres scattered in the oesophageal lamina propria of the domestic fowl. The lamina propria has accumulations of diffuse and/or nodular lymphatic tissue (Calhoun, 1954; Andrew, 1959; Farner, 1960). The lymphatic tissue intermingled anatomically with mucous glands and mucous ducts (Andrew, 1959). Batt (1925) observed many lymphatic wandering cells in the lamina propria of the domestic fowl. Andrew (1959) reported that the boundary between the mucous membrane and the lamina propria was often obliterated by accumulations of lymphatic tissue.

Calhoun (1954) found that in the domestic fowl the amount of elastic tissue in the lamina propria increased as a consequence of age.

Muscularis mucosae. The muscularis mucosae was situated between the lamina propria and the submucosa (Batt, 1925; Steel and Churchill, 1942; Ivey and Edgar, 1952; Calhoun, 1954; Farner, 1960). Andrew (1959) discussed a few conflicting opinions on whether homologies existed between the various oesophageal muscle layers. He reported that in the domestic fowl and taxonomically related birds a definite longitudinally disposed muscularis mucosae was present. He then stated that "in a great many birds the layer which should correspond to the muscularis mucosae consists of inner longitudinal and outer circular bundles, the reverse of that seen where two layers are present in other vertebrates." The difficulty in recognizing the different oesophageal muscle layers was the result of inadequate development of both the submucosa and the outer longitudinal layer of the

muscularis externa. It was reported also that on occasion the outer muscle layer was lacking (Farner, 1960).

Several authors described an anatomical relationship of the muscle layers which was the correct version. They stated that the longitudinally oriented muscularis mucosae was well developed in birds and that an outer longitudinal layer of the muscularis externa was inadequately developed and often absent (Batt, 1925; Steel and Churchill, 1942; Ivey and Edgar, 1952; Calhoun, 1954; Farner, 1960).

Submucosa

The submucosa in the bird consists of a connective tissue layer which lies between the basal border of the muscularis mucosae and the muscularis externa (Ivey and Edgar, 1952; Andrew, 1959). The submucosa is poorly developed in birds (Batt, 1925; Steel and Churchill, 1942; Calhoun, 1954; Farner, 1960). Calhoun (1954) observed that the submucosa of the domestic fowl was barely discernable in one area while in other areas of the same oesophagus it widened out to contain blood vessels and nerves.

Muscularis externa

In the preceding description of the muscularis mucosae reference was made to the muscularis externa. It consisted of a well developed inner circular layer and a poorly developed outer longitudinal layer of smooth muscle. It lies adjacent to the basal border of the submucosa (Batt, 1925; Ivey and Edgar, 1952; Calhoun, 1954; Andrew, 1959; Farner, 1960).

Adventitia

The adventitia consists of a peripheral investing layer of fibrous connective tissue. (Ivey and Edgar, 1952; Andrew, 1959; Garven, 1965; Ham, 1965; Bloom and Fawcett, 1968; Patt and Patt, 1969).

DEFINITION OF GLAND STRUCTURE

Several types of exocrine glands are found in the oesophagus of birds. They do not conform structurally to the distinct types found in mammals but rather show intermediate forms or combinations of different types so that there is no universal agreement regarding the descriptive terminology in general use. Their secretory components are organized in different ways. A description follows of the terminology used to decribe these glands.

Exocrine glands may be either simple or compound. A simple exocrine gland was described as a secretory unit connected directly to the surface epithelium (Ham, 1965; Bloom and Fawcett, 1968). A branching duct system was termed a compound gland (Ham, 1965; Bloom and Fawcett, 1968). The anatomical category of glands are: simple tubular; simple alveolar; simple branched alveolar; simple tubuloalveolar. The term tubuloalveolar was used to designate those glands which have both tubular and alveolar secretory units. Intermediate forms also exist (Ham, 1965).

OESOPHAGEAL GLANDS

The oesophagus contains only mucous glands (Browne, 1922; Batt, 1925; Steel and Churchill, 1942; Calhoun, 1954; Andrew, 1959; (Kaden, 1936; Schreiner, 1900; Swenander, 1899, 1902 - as summarized by Farner,

1960). These glands occur in the lamina propria in most species but never in deeper tissue layers (Andrew, 1959; Farner, 1960). In a number of birds the major portion of each gland lies within the epithelial mucous membrane, that is, intraepi-

thelial in position. This results in a small portion of each gland being situated in the lamina propria (Andrew, 1959).

Kaden (1936), as cited by Farner in 1960, classified mucous glands in various bird groups according to the position and morphology of the glands. The classification scheme indicates also morphological variations:

"(1) Simple tubular glands in epithelial layer with unspecialized ducts; e.g. Asio otus
(2) Simple tubular glands extending about two-thirds into the lamina propria, with unspecialized ducts; e.g. Coloeus monedula
(3) Simple tubular glands, partly in the lamina propria, with ducts differentiated in form; e.g. Larus
(4) Simple tubuloalveolar glands, partly in the lamina propria, with long ducts differentiated in form; Parus
(5) Folded tubular glands, partly in the lamina propria, with ducts undifferentiated; e.g. Dryobates major
(6) Simple tubuloalveolar glands, entirely in lamina propria, with slight degree of inner folding; ducts differentiated in form and epithelium; e.g. Turdus
(7) Folded tubuloalveolar glands, entirely in lamina propria but with epithelial capsule; ducts differentiated in form and epithelium; e.g. Garrulus glandarius
(8) Folded tubuloalveolar glands, entirely in lamina propria; ducts differentiated in form and epithelium; e.g. Falco tinunculus
(9) Deeply folded tubular glands, entirely in lamina propria; ducts with specialized epithelium; e.g. Columba livia."

Farner (1960) reported that no correlations existed between the following:

a) glandular morphology and bird feeding habit
b) glandular morphology and bird phylogeny
c) glandular position and bird feeding habit
d) glandular position and bird phylogeny

Swenander (1899, 1902), as cited by Farner in 1960, stated that birds feeding on animal foods often had simple tubular glands. Andrew (1959) noted that the mucosal glands in most birds were alveolar in shape while in some birds the glands were tubular in shape.

GENERAL REMARKS

The avian oesophagus conforms histologically to the general vertebrate pattern (Browne, 1922; Farner, 1960). The gross and microscopic structure is similar throughout its length, though the number of glands may vary (Steel and Churchill, 1942; Calhoun, 1954). The oesophagus was described as a thin elastic distensible tube from the pharynx to the proventriculus (Browne, 1922; Bradley and Grahame, 1951; Calhoun, 1954; Farner, 1960).

Birds do not masticate their food. The oesophagus, by its capability for considerable dilation, displays a structure-function relationship by being able to accommodate a large particle of food. To this end the mucosa has longitudinal folds, termed "mucosal convolutions", which permit such expansion (Matthews, 1949). Welty (1962) reported that the diameter of the fully dilated oesophagus is related to the size of the food particle swallowed. Insect eaters and those species which break up their food before swallowing have narrow tubes while birds which swallow larger items tend to have a larger oesophagus. Farner (1960) concurred stating that birds which eat bulky food items and those which use the oesophagus to store ingested materials, tended to have an oesophagus with a large diameter and much folding of the mucosa. The species he lists as those with large oesophageal diameters are; grebes, loons, auks, puffins, petrels, gulls, cormorants, storks, herons, coots, gullinules, hawks, owls, and piscivorous kingfishers. The species which Farner listed as having extensive mucosal convolutions are; penguins, auks, petrels, gulls, gannets, pelicans, ducks, geese, grouse, hawks, owls, goatsuckers, crows and waxwings. Farner (1960)

also mentioned that species which ate small food items and those which reduced their food into smaller pieces before these could be swallowed tended to have a small oesophagus. These include many insectivores and graminivores.

TAXONOMY AND FEEDING HABITS OF SPECIES INVESTIGATED

CLASSIFICATION OF BIRDS SELECTED

The reported taxonomic positions of the birds were found to be in agreement with several authorities; namely, Mayr and Amadon (1951), Wetmore (1951), Storer (1960). These authors agreed that Pelecaniformes were the most promitive group and Passeriformes the most highly advanced.

Little information was available concerning the inter-relationships of the avian orders. Mayr and Amadon (1951) reported that some taxonomists considered the Falconiformes and Pelecaniformes to be distantly related. They also felt that any similarities of the pelicans to the gulls is the result of convergent evolution. The resemblance of hawks and owls is also a result of convergence (Young, 1962). Friedmann (1955) reported that "within such complex groups as the passerine birds there is still no agreement as to the 'best' or most 'natural' sequence in which to list the included families".

Recent studies of avian taxonomy are based on reconstructing evolutionary history. Much palaeontological evidence is now available though bird fossils are rarer than those of mammals (Storer, 1960). The problem of avian phylogeny is so complex that the development of a scheme of classification which expresses the whole phylogeny is impossible (Mayr and Amadon, 1951; Storer, 1960).

The birds are listed according to the "Check-list of North American Birds, of the American Ornithologists Union" (1957).

ORDER	FAMILY	SPECIES
Pelecaniformes	Pelecanidae	Pelecanus erythrorhynchos Gmelin 1789 (white pelican)
Falconiformes	Accipitridae	Buteo jamaicensis (Gmelin) 1788 (red-tailed hawk)
Gruiformes	Rallidae	Fulica americana Gmelin 1789 (American coot; mud hen)
Charadriiformes	Laridae	Larus delawarensis Ord 1815 (ring-billed gull)
		Larus pipixcan Wagler 1831 (Franklin's gull)
Strigiformes	Strigidae	Bubo virginianus (Gmelin) 1788 (great horned owl)
Passeriformes	Tyrannidae	Tyrannus tyrannus (Linnaeus 1758) (eastern kingbird)
	Hirundinidae	Iridoprocne bicolor (Vieillot) 1807 (tree swallow)
	Corvidae	Corvus brachyrhynchos Brehm 1822 (common crow)
	Ploceidae	Passer domesticus (Linnaeus) 1758 (house sparrow)

FEEDING HABITS OF BIRDS SELECTED

It was difficult to classify the feeding habits of the bird species in this study. This section will outline briefly the feeding habits of the selected species.

BIRD	FEEDING CLASSIFICATION	FOOD PREFERENCE
White pelican	Carnivorous	Piscivorous
Red-tailed hawk	Carnivorous	Small mammals & birds
American coot	Herbivorous	Graminivorous
Ring-billed gull	Carnivorous	Invertebrates & fish
Franklin's gull	Carnivorous	Invertebrates
Great horned owl	Carnivorous	Small mammals & birds
Eastern kingbird	Carnivorous	Insectivorous
Tree swallow	Carnivorous	Insectivorous
Common crow	Omnivorous	
House sparrow	Herbivorous	Granivorous

Pelecanus erythrorhynchos (white pelican)

The white pelican is almost entirely a fish eater (Taverner, 1926; Martin *et al.*,1961). Typically it eats large numbers of small, sluggish, easily caught fish found in shallow water (Bent, 1922; Taverner, 1926; Martin *et al.*,1961). The white pelican is not a diver but catches its food near the water surface while swimming or wading. Besides fish, pelicans eat frogs and salamanders (Godfrey, 1966). Pelicans are carnivorous.

Buteo jamaicensis (red-tailed hawk)

This hawk prefers small rodents, gophers, ground squirrels and meadow mice. Rabbits, small birds, snakes and lizzards are also eaten but this hawk seems to prefer furred animals which it usually swallows whole (Taverner, 1926; Bent, 1937; Martin et al., 1961; Godfrey, 1966). This is a carnivore.

Fulica americana (American coot)

The American coot (mud hen) is primarily a vegetative feeder concentrating on submerged aquatic vegetation and seeds (Taverner, 1926; Martin et al., 1961). Animal foods are of secondary importance. These consist of aquatic beetles, bugs, dragonflies and damsel flies and a considerable number of mollusks as well as crustaceans (Martin et al., 1961). This species is primarily herbivorous.

Larus delawarensis (ring-billed gull)

Taverner (1926) reported that gulls eat all forms of animal matter; fish, crustaceans, mollusks, insects, offal, and sometimes young birds and mice. The ring-billed gull is inclined to eat fish, beetles, grasshoppers and crickets, true bugs, amphibians, and mollusks (Martin et al., 1961; Godfrey, 1966). Gulls are scavengers that may be classified as carnivorous with preferences for invertebrates.

Larus pipixican (Franklin's gull)

This bird has similar feeding habits to other gulls but consumes a larger variety of invertebrates. Its diet consists chiefly of cutworms, wireworms, grasshoppers and crickets, bees, dragonflies and

damsel flies, spiders and fish (Martin et al., 1961; Godfrey, 1966). Franklin's gulls are carnivores with preferences for invertebrates.

Bubo virginianus (great horned owl)

The great horned owl feeds on a wide variety of animal life. Its primary food preference is small mammals such as rabbits, rats and weasels, but also it eats birds, mice, frogs, large insects and crayfish (Taverner, 1926; Bent, 1937; Martin et al., 1961; Godfrey, 1966). The indigestible materials such as bones, fur and feathers are regurgitated as pellets. It is classified as a flesh-eating carnivore.

Tyrannus tyrannus (eastern kingbird)

The eastern kingbird eats mainly invertebrate animal material which is made up largely of honeybees, ants, grasshoppers, various beetles, bugs and flies. Plant food, including wild fruit and berries, makes up a small part of the eastern kingbird's diet (Taverner, 1926; Martin et al., 1961).

Iridoprocne bicolor (tree swallow)

The food of the tree swallow consists mainly of invertebrates. The main food source includes flies, beetles, ants, bees, wasps and other flying insects. Foods of lesser importance include moths, grasshoppers, dragonflies, other insects, and spiders (Taverner, 1926; Martin et al., 1961; Godfrey, 1966). Plant foods, which include a few fruits, make up a small part of their diet (Martin et al., 1961).

Corvus brachyrhynchos (common crow)

The crow is an omnivore. Its diet is balanced between plant and

animal foods without a predilection for any specific type (Bent, 1946; Taverner, 1926; Martin et al., 1961; Welty, 1962; Godfrey, 1966). Animal foods are mainly insects and the eggs and the young of birds. Plant foods consist of such grains as corn and wheat (Martin et al., 1961; Godfrey, 1966).

Passer domesticus (house sparrow)

The house sparrow is mainly a grain-eating bird and a variety of seeds such as oats, wheat and corn make up the bulk of the diet (Taverner, 1926; Martin et al., 1961). Insects are consumed also as food (Martin et al., 1961).

METHODS AND MATERIALS

COLLECTION OF SPECIMENS AND REMOVAL OF ORGANS

Most specimens were taken in south-western Manitoba and a few were collected in Netley Marsh near the south end of Lake Winnipeg. All birds collected were mature and healthy adults. No data were taken on weight, size, plumage and sex of the specimens. The birds were killed by gunshot which did not destroy the alimentary tract. The organs of the alimentary tract were removed by dissection. Tissues not exceeding 10 mm^2 were cut from these organs, rinsed in 0.85% saline solution, and placed in fixative. Tissues from the midregion of the oesophageal length were examined for this study.

HISTOLOGICAL TECHNIQUES

Fixation

Initially four fixatives were used. These were: 10% formalin solution (Armed Forces Laboratory Manual, 1960); Zenker's fixative (Humason, 1962); Bouin's fixative (Humason, 1962); FAA (formaldehyde; ethyl alcohol; glacial acetic acid) (Galigher and Kozloff, 1964) (see Appendix).

When 10% formalin solution was used the tissues were placed in the fixative for 18 to 24 hours. This was followed by washing the tissues in running tap water for 8 hours.

Tissues fixed with Bouin's fixative were treated for 18 to 24 hours. They were washed in several changes of 50% ethyl alcohol in order to remove the picric acid which was deposited by the fixative.

Picric acid which remained was later removed in the alcohols of higher concentration during dehydration.

Tissues fixed with Zenker's fixative were treated for 18 to 24 hours and then washed in running tap water for 8 hours. If excess hardening occurred the tissues were chromated for 12 to 24 hours in 3% potassium dichromate before being washed. After tissues were washed they were placed in 30% and then 50% ethyl alcohol each for 1 hour. Tissues were transferred from the 50% ethyl alcohol solution to a saturated solution of iodine in 70% ethyl alcohol for 7 hours to remove any mercuric bichloride precipitate left in the tissues after fixation. Tissues were then taken through an alcohol series for complete dehydration.

When FAA was used the tissues were fixed for 18 to 24 hours. This was followed by washing them in running tap water for 8 hours. Tissues fixed with FAA could be sectioned without difficulty and this fixative was selected for use in this study.

Dehydration; Clearing; Infiltration; Embedding

When the 10% formalin solution, Zenker's fixative, and FAA were used the tissues were washed in running tap water for 8 hours. Tissues fixed with Zenker's fixative were treated in a saturated solution of iodine and 70% ethyl alcohol to remove any excess mercuric bichloride. Tissues fixed with Bouin's fixative were washed in 5 to 7 changes of 50% ethyl alcohol before being completely dehydrated in the solutions of increasing alcohol concentrations.

Dehydration. After the tissues were washed in running tap water they were transferred to an ethyl alcohol series of increasing concen-

trations for complete dehydration. Before the tissues were transferred to the pure clearing solution of toluene they were treated for a period of one hour in a solution of 50% absolute ethyl alcohol and 50% toluene.

Clearing. Two changes of toluene were used for 1.5 hours each before the tissue was transferred to melted paraffin for infiltration.

Infiltration. Tissues cleared in toluene were transferred to two changes of paraffin and remained in each solution for 1.5 hours. The embedding medium was Tissuemat which had a melting point of 61°C.

Embedding. Tissue-Tec molds and Tissue-Tec universal embedding rings were used to block the tissues. Blocks were stored in envelopes which were easily catalogued.

Pieces of oesophageal tissue were oriented either perpendicular or longitudinal to the cutting surface of the paraffin blocks. Cross and longitudinal sections were then prepared from the paraffin blocks.

Sectioning

Tissue sections, 8 to 10 microns in thickness, were cut on a rotary microtome fitted with an AO microtome knife. The sectioning properties of the blocks were improved by soaking them in cold water for 24 to 48 hours prior to sectioning. Tissue sections were floated onto a Tissue-Tec water bath held at a temperature of approximately 50°C. These sections were then transferred to microscope glass slides.

Standard microscope slides, 75mm by 25mm, and approximately 1 mm thick were used. These had a frosted end which permitted easy

marking. The tissues were then mounted and were left to dry for 72 hours.

Staining Procedures

Standard Hematoxylin-Eosin stain, Mallory's stain, and Verhoeff's Elastin stain were used in this study. When Hematoxylin-Eosin or Mallory's stain were used the tissues were deparaffinized and completely hydrated before staining. Tissues stained with Verhoeff's Elastin stain were deparaffinized and partially hydrated (to 70% ethyl alcohol). Xylene was used to deparaffinize. After two changes of xylene, each of two minutes duration, slides were transferred to absolute ethyl alcohol to remove the xylene. Slides were next hydrated through a series of increasing aquatic dilutions (see Appendix for steps in deparaffinization and hydration).

Hematoxylin-Eosin Stain. The basic procedures for using this stain are well documented (Armed Forces Laboratory Manual, 1960; Humason, 1962; Gray, 1964; Drury et al.,1967) (see Appendix). The hematoxylin stain was differentiated with acid alcohol (see Appendix). Differentiation was complete when the dark blue-to-red colour of the nuclei contrasted sharply with a light blue colour of the cytoplasm. Slides were next placed in running tap water for 15 minutes to be 'blued' and then counterstained for a minute in eosin-Y. Slides were transferred from eosin to a 70% solution of ethyl alcohol and through a series of aqueous-alcohol solutions of increasing alcohol concentrations to absolute alcohol. Colour intensity of the counterstain was controlled by timed immersions in the 70% and 95% ethyl

alcohol solutions. Tissues were completely dehydrated in absolute ethyl alcohol. Alcohol was removed with two changes of xylene and the slides were coverslipped (see Appendix).

Mallory's Stain. The staining procedure followed the method after Pantin (1946) as outlined by Humason (1962). Tissues were placed in a mordant of saturated $HgCl_2$ plus 5% glacial acetic acid after being deparaffinized and hydrated. Mordanting was necessary when the fixative did not contain $HgCl_2$; that is, 10% formalin, Bouin's fixative, and FAA. The excess $HgCl_2$ was then removed by Lugol's solution (see Appendix), and the Lugol's solution removed by a 5% sodium thiosulfate solution (see Appendix) before the slides were washed and stained. Two staining solutions, Mallory I and Mallory II, and a mordant of phosphomolybdic acid were used (see Appendix).

The acid fuchsin of the Mallory I solution was a nuclear stain. This was made possible by the separation of DNA from the chromatin by the fixatives:

> "After most fixatives the DNA will still be present but will not mask the colouring of the protein by an acid dye unless a basic dye is used as well" (Baker, 1958).

The acid fuchsin was rinsed and differentiated in distilled water. Slides were placed in phosphomolybdic acid solution for five minutes, rinsed briefly in distilled water, and placed in Mallory II solution for two minutes. The mordanting action of phosphomolybdic acid increased the selectivity of the aniline blue and orange G of the Mallory II solution. Aniline blue was differentiated in 90% alcohol. The alcohol was removed in two changes of xylene and a cover slip

mounted with DPX was used (see Appendix). Aniline blue stained collagen whereas the orange G, with its high penetrating capabilities, stained the cytoplasm (Baker, 1958).

The reason for separating the mordant and the Mallory II solutions was to control separately the mordanting and staining times. Separating the mordant from the stain prevented the oxidization of the stain by the mordant. After slides were stained in Mallory II they were rinsed in distilled water. This was followed by treatment for five minutes in a 1% acetic acid wash which rendered the tissues more transparent without altering their colour.

Verhoeff's Elastin Stain. Humason (1962) described this staining procedure. This technique was primarily used to identify elastin fibres. It was also valuable for general tissue comparisons in conjunction with other stains (see Appendix).

The staining procedure required deparaffinization and partial hydration to 70% ethyl alcohol before the slides were placed in Verhoeff's stain for 15 minutes.

After staining, the slides were rinsed in distilled water and differentiated in 2% ferric chloride solution. This was described by Humason (1962) as a regressive staining procedure using excess mordant. The excess mordant acted to break up the mordant-dye complex in the tissue. Slides were placed in 5% aqueous sodium thiosulfate solution for a minute and washed in running tap water for two minutes before counterstaining. The counterstain was Ponceau-S (see Appendix).

In all staining procedures dehydration was completed with two

changes of absolute alcohol. Slides were placed in a 50/50 absolute ethyl alcohol - xylene solution for one minute and transferred to two changes of xylene before mounting (see Appendix).

The mounting media was DPX; a mixture of distrene (a polystyrene), a plasticizer (tricresyl phosphate), xylene. DPX is a colourless synthetic resin (Drury et al., 1967).

Cover glasses used were number one thickness.

OBSERVATION TECHNIQUES

OPTICAL MICROSCOPY

Observations and measurements were made with a model ECE Bi Olympus binocular microscope. An ocular micrometer was placed in the front focal plane of the WF 10x ocular and calibrated using a stage micrometer.

MEASUREMENTS

Tissue Measurements

Various aspects of the oesophagus were measured with the calibrated microscope as shown in Figure 1. In some cases it was difficult to distinguish the exact boundaries of a tissue layer or establish a definite point at the base of a mucosal convolution from which its length (height) may be measured.

The accuracy of the histological measurements were estimated as follows:

a) Between 5,000 and 12,000 microns - 50 microns accuracy at 40X magnification
b) Between 1,000 and 5,000 microns - 10 microns accuracy at

TRANSVERSE SECTION OF BIRD OESOPHAGUS

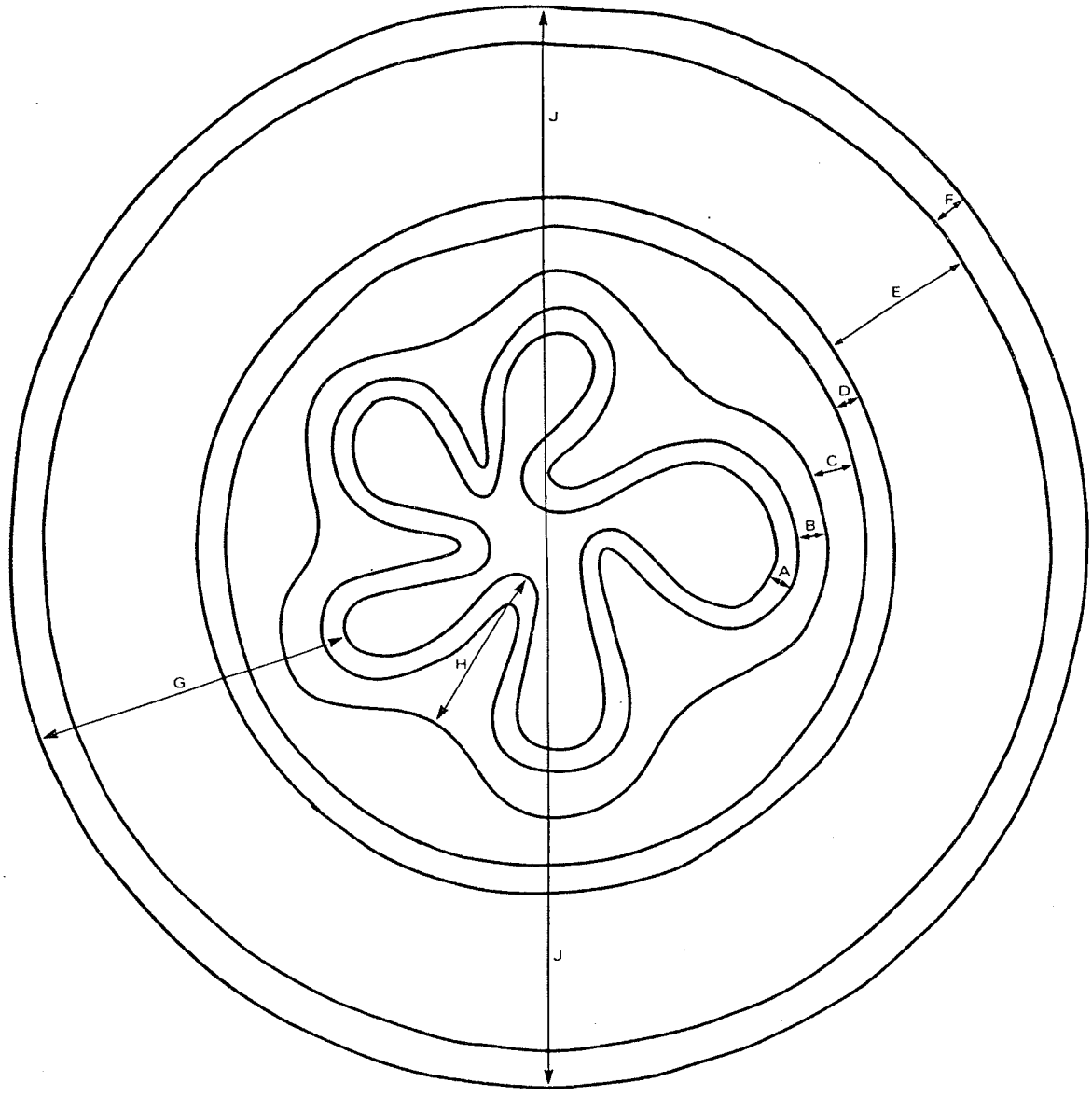
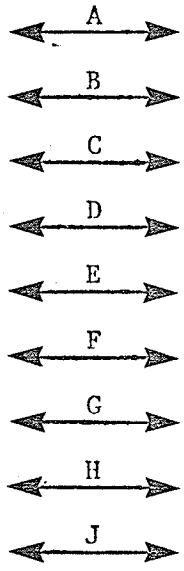


Figure 1. Schematic representation of transverse section of bird oesophagus to show sites used for histological measurements. See legend on page 31.

LEGEND FOR FIGURE I



A — Thickness of the Mucous Membrane in Microns

B — Thickness of the Lamina Propria in Microns

C — Thickness of the Muscularis Mucosae in Microns

D — Thickness of the Submucosa in Microns

E — Thickness of the Muscularis Externa in Microns

F — Thickness of the Adventitia in Microns

G — Thickness of the Oesophageal Wall in Microns

H — Length of the Mucosal Convolutions in Microns

J — Diameter of the Oesophagus in Microns

100X magnification
c) Less than 1,000 microns - accuracy to the nearest micron using 400X magnification.

Errors never exceeded 1% of the measured distance and would not affect the accuracy of significance testing. Measurements of type (a) above included oesophageal diameters which were not considered as a reliable method to determine oesophageal size.

Organ Measurements

The oesophageal diameter alone is not considered to be a reliable method to indicate "size" of an histologically fixed oesophagus. The diameter of hollow organs may become mechanically distorted while resting on the bottom of containers during periods of fixation, dehydration and infiltration. Three histological characters of diameter, wall thickness, and length of mucosal convolutions, were all considered as contributing to the "size" of the oesophagus. Data for these comparisons are included in the section of "Results".

Bird Measurements

The sample number for each species was limited by the comparative nature of the study. One of the aims of this study was to examine as many species as possible. At least two specimens were taken for most species with the exception of the white pelican, ring-billed gull, and great horned owl for which one specimen of each was taken.

Body lengths as listed by Godfrey (1966) were used as a criterion of species size. Godfrey's (1966) measurements of bird length

were reported as:

> "From the tip of the bill to the tip of the tail, with the bird flat on its back, its bill parallel with the ruler, its neck extended but not unduely stretched".

In his glossary Godfrey defined length as:

> "the distance in a straight line from the tip of the bill to the tip of the longest tail feather".

He stated also:

> "Although this measurement is not of great scientific value, it is useful in indicating relative size".

Table 1 in the Appendix lists the ranges in bird lengths and the median lengths, in inches, according to Godfrey (1966).

PHOTOGRAPHY

A Zeiss automatic photomicroscope equipped with planachromat objectives was used to take 35 mm photomicrographs.

Agfa IFF 35 mm film (ASA 25) was used. This film was developed in a Rodinal-water (1 : 30) solution, fixed with Kodak fixer and washed.

Prints of the negative were made using single weight Kodak Polycontrast paper developed with Kodak dektol solution.

STASTISTICAL ANALYSIS

Significance tests were used for grouping several oesophageal measurements by a) arbitrarily deciding that differences which approximated twice the distance between the standard errors of two compared groups and b) by student-t-tests constituted significant differences for separate groupings.

RESULTS

GROSS ANATOMICAL OBSERVATIONS

The oesophagus in each of the birds studied did not show any signs of gross morphogenic abnormalities. The adventitia was in close juxtaposition to the underlying tissues with no gross evidence of encapsulated fluid vesicles. There was no evidence of hemorrhagic discoloration as a result of earlier bruises.

Each digestive organ was examined at the gross level for parasitic infections. Parasites were found only in a ring-billed gull where a few unidentified trematode eggs were observed in the prepared slides of the oesophageal mucosa.

EVALUATION OF STAINING PROCEDURES

The hematoxylin-eosin staining procedure gave good observable results. The nuclei stained dark blue and the cytoplasm stained orange to red.

The Mallory staining procedure of Pantin (1946), as outlined by Humason (1962), required that the Mallory II staining solution be kept separate from the mordant of phosphomolybidic acid solution. The stain was difficult to differentiate when the phosphomolybdic acid was mixed with aniline blue and orange G.

With Pantin's method the following results were obtained: nuclei, red; muscle and some cytoplasmic elements, red to orange; nervous system, lilac; collagen, dark blue; myelin and red blood cells, yellow and orange (Humason, 1962).

There was some doubt as to the consistency and reliability of Verhoeff's elastin stain as a means of identifying elastin fibres. In some species of birds elastin fibres were not observed in connective tissues of the oesophageal wall. In some instances fibres with the physical appearance of elastin fibres were observed but did not stain black as characteristic for this staining procedure. The critical evaluation for the strength of the elastin stain was made on arterial tissues. A fresh staining solution was prepared if elastin did not appear in the connective tissues of arteries. The exact chemical mechanism for this staining process is unknown but Baker (1958) stated that the elastin fibres were dense and able to retain large amounts of dye. Fe_2Cl_3, as a mordant, resulted in the tissues staining rapidly and intensely with hematein. Ferric chloride was found to be a good oxidizer and rapidly oxidized unripened (unoxidized) hematoxylin when these were brought together in the staining solution. It was found that overripened hematoxylin would not stain the tissues. The effectiveness of the staining solution lasted for two weeks (Humason, 1962).

Nuclei and elastin fibres retained more dye than other surrounding structures. Chemical differentiation of the stain was difficult as the dye was easily removed from the nuclei and elastin fibres.

Ponceau-S had a strong affinity for collagen, staining it bright red. This provided good contrast for the darkly stained elastin fibres. The red stained collagen was contrasted from smooth muscle which was stained by the picric acid and had a yellow color. The tissues were counterstained for one minute and differentiated in 95% ethyl alcohol.

The results of the stain were as follows: elastin fibres, brilliant blue to black; nuclei, blue to brownish black; collagen, red; other tissue, yellowish (Humason, 1962).

HISTOLOGICAL OBSERVATIONS

ARRANGEMENT OF THE TISSUE LAYERS OF THE OESOPHAGUS

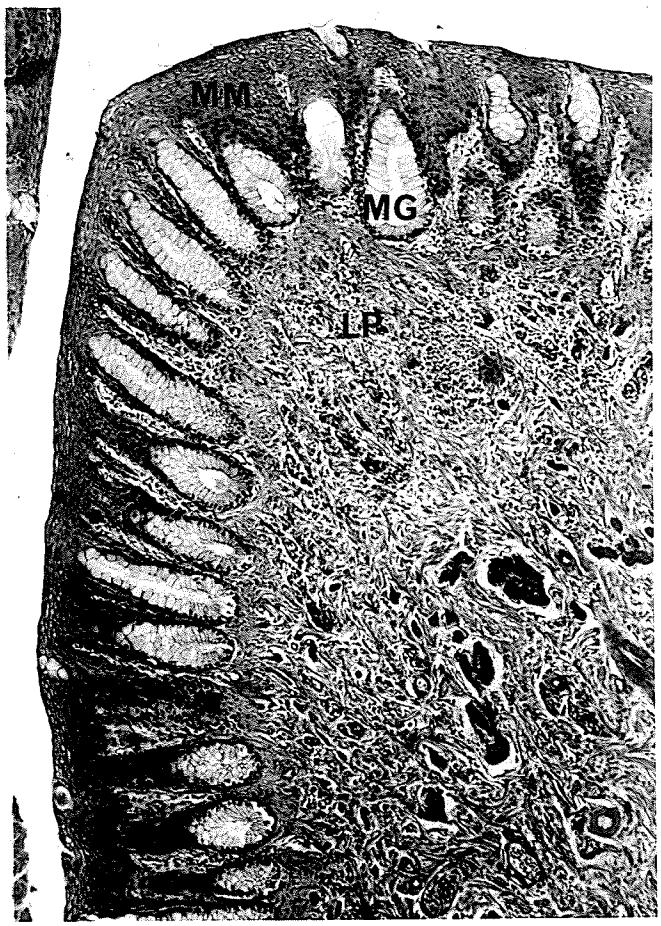
The four major tissue layers; mucosa, subumcosa, muscularis externa, adventitia, were recognized in the avian oesophagus. The mucosa was composed of a mucous membrane of stratified squamous epithelium, a lamina propria and a muscularis mucosae. The mucosa was typically folded into longitudinal convolutions. The longitudinally oriented muscularis mucosae was well developed and because of a thin submucosa the former was sometimes included as part of the muscularis externa.

Several mucous glands were present within the mucosa. In all birds examined in this study the submucosa was found to be thin with thicker regions to accommodate blood vessels, lymph vessels and nerves. The muscularis externa was observed to consist of a circularly arranged layer of muscle. An outer longitudinal muscle layer which is characteristic of other vertebrates and of the domestic fowl was not present in the birds used in this study. The outer layer of adventitia encircled the muscularis externa and this was found to contain blood vessels, lymph tissue and nerves.

Pelecanus erythrorhynchos (white pelican)

Mucosa

Mucous membrane. The mucous membrane was composed of stratified

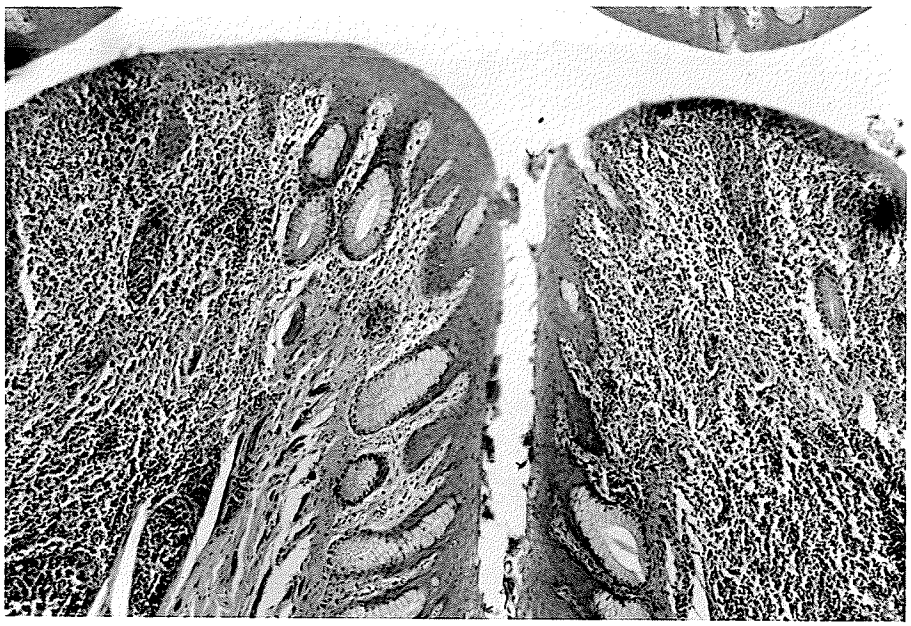


250u

Plate I. Photomicrograph of convolution of the mucosa showing mucous membrane (MM), mucous glands (MG) and lamina propria (LP) in the white pelican. 100X. T.S.

squamous epithelium which showed no cornification at the surface (Plates I, II, III and IV). This layer was of medium thickness in com-

parison to other birds studied. Its junction with the lamina propria was well papillated.



250u

Plate II. Photomicrograph of convolutions of mucosa showing lymph nodes and lymphatic tissue invading the mucous membrane in the white pelican. The lymphatic tissue displaces the mucous membrane and the mucous glands. 100X. T.S.

The mucosa was greatly convoluted and "secondary convolutions" were often present on the "primary convolutions". The convolutions of the mucosa were so great that they occupied most of the lumen (Plate II). The epithelium was of uniform thickness throughout the bases and the apices of the convolutions.

No previously undescribed cell types were observed in the mucous membrane. Often lymphatic tissue from the lamina propria penetrated into the mucous membrane (Plates II, III and IV). There was some

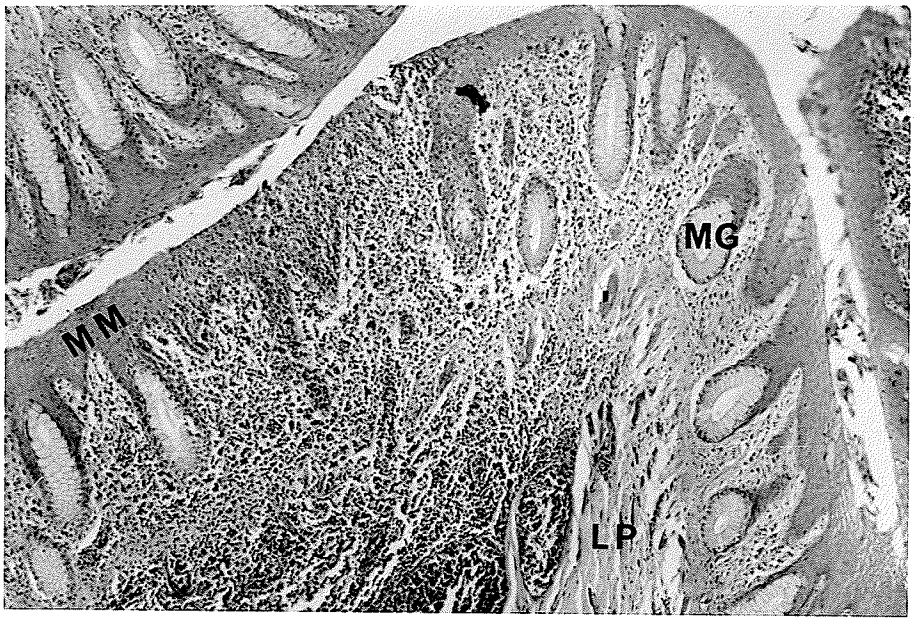


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Plate III. Photomicrograph of convolution of mucosa showing mucous membrane (MM), mucous glands (MG), lamina propria (LP) and lymphatic tissue in the white pelican. 100X. T.S.

evidence of sloughing off of superficial cells (Plates II and III). The surface of the mucosa was typically smooth with flat surface cells.

Lamina propria. This was a thick and well developed layer composed of areolar connective tissue (Plate I).

Lymph nodes were often present and in most cases the lymphatic tissue displaced the mucous membrane and mucous glands (Plates II, III and IV).

Muscularis mucosae. This formed a well developed layer of longitudinally disposed smooth muscle about the mucosa. This layer was about half as thick as the muscularis externa (Plates V, VI and VII).

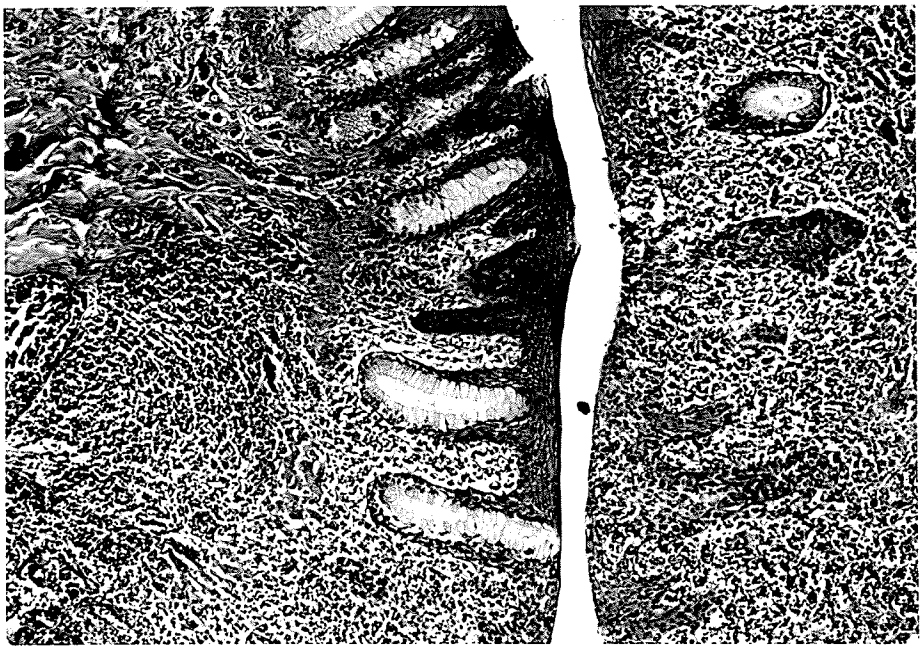

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Plate IV. Photomicrograph of mucosa showing mucous membrane, mucous glands, lamina propria and lymphatic tissue in the white pelican. 100X. T.S.

Submucosa

The submucosa was a well developed continuous layer containing many collagenous fibres and some elastic tissue. It had many blood vessels which were more numerous in thickened areas (Plates V, VI, and VII).

Muscularis externa

The muscularis externa was composed of a thick layer of circular muscles with no evidence of an outer longitudinal layer of muscle fibres. The concentric layers of muscle were separated by connective tissue; blood vessels were observed also between the muscle layers (Plates V, VI and VII).

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Plate V. Photomicrograph showing lamina propria (LP) and muscularis mucosae (MUS M) of the mucosa, the submucosa (SUB) and the muscularis externa (MUS E) of the white pelican. 100X. T.S.

Adventitia

The adventitia was easily distinguished (Plate VII) and was composed mainly of collagenous tissue with some elastic fibres and adipose tissue. The adventitia contained many nerves, blood vessels and some lymphoid tissue.

Glands

The mucosal glands of the white pelican were simple tubular and

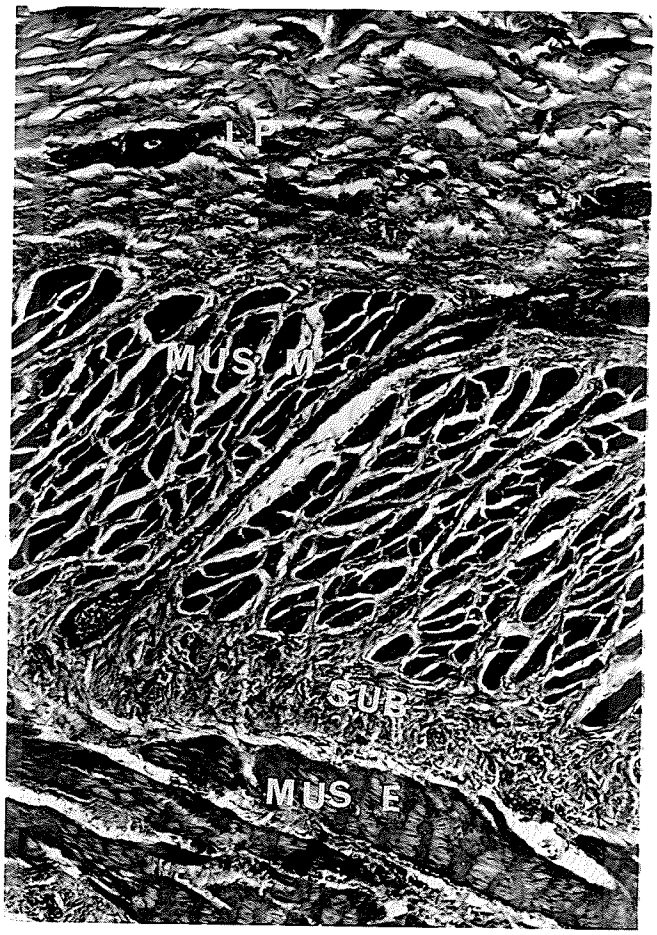

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Plate VI. Photomicrograph of section showing lamina propria (LP), muscularis mucosae (MUS M), submucosa (SUB) and a portion of the muscularis externa (MUS E) in the white pelican oesophagus. 100X. T.S.

simple alveolar and were, for the most part, set in the lamina propria (Plates I, II, III and IV). Lymphoid tissue was not generally associated with the mucous glands. Where lymph nodes arose the lymphatic tissue penetrated the epithelium and obliterated the mucous glands (Plates II, III and IV).

The glands were numerous and formed a continuous layer beneath

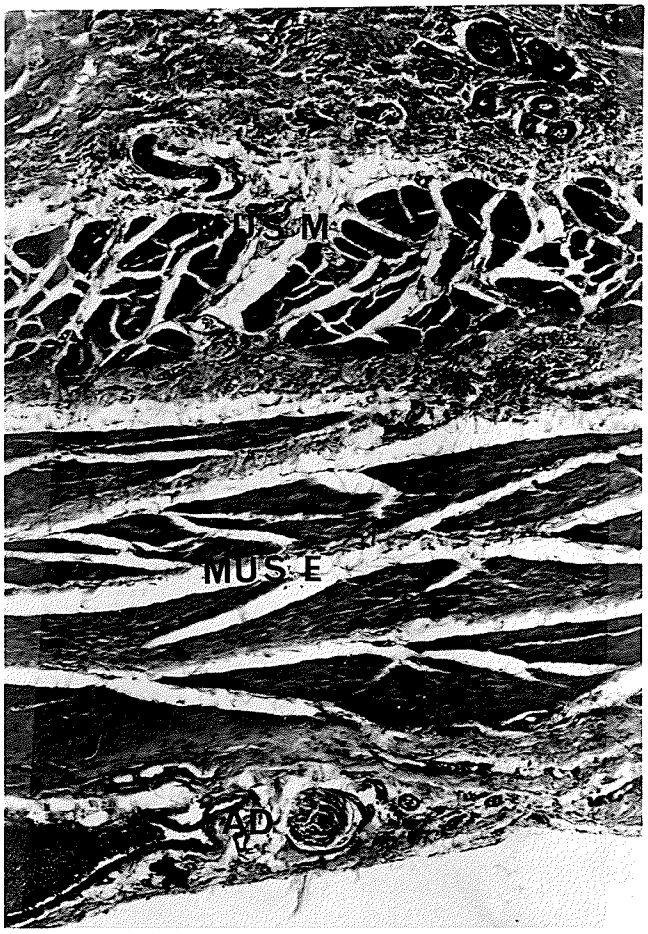

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Plate VII. Photomicrograph showing section through a thin region of the oesophageal wall demonstrating the relative thickness of the muscularis mucosae (MUS M) and muscularis externa (MUS E) in the white pelican. Also shown is the lamina propria, submucosa and adventitia (AD). 100X. T.S.

the mucous membrane. The individual glands were small and arranged side-by-side.

Buteo jamaicensis (red-tailed hawk)

Mucosa

Mucous membrane. The mucous membrane consisted of a thick layer of stratified squamous epithelium which met the lamina propria at a greatly papillated junction (Plates VIII and IX). The

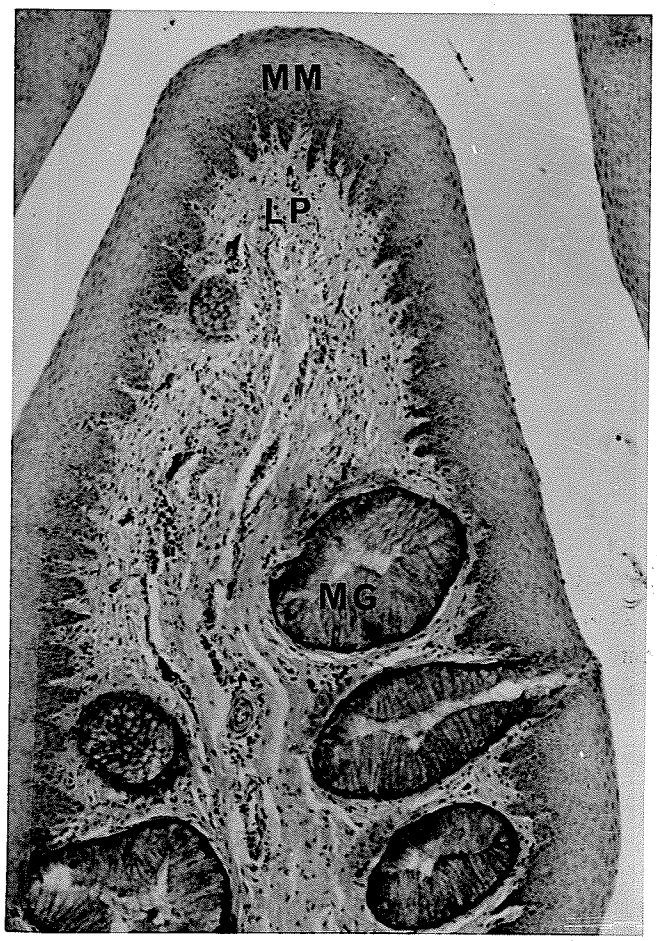

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Plate VIII. Photomicrograph of convolution of mucosa showing mucous membrane (MM), mucous glands (MG) and lamina propria (LP) in the red-tailed hawk oesophagus. 100X. T.S.

mucosa was prominently convoluted with the mucous membrane being thickest at the bases of the convolutions and thinnest at the apices.

Surface cells were noted to slough off (Plate IX). However, the surface was consistently smooth (Plates VIII and IX).

Lamina propria. This layer was well developed and was slightly

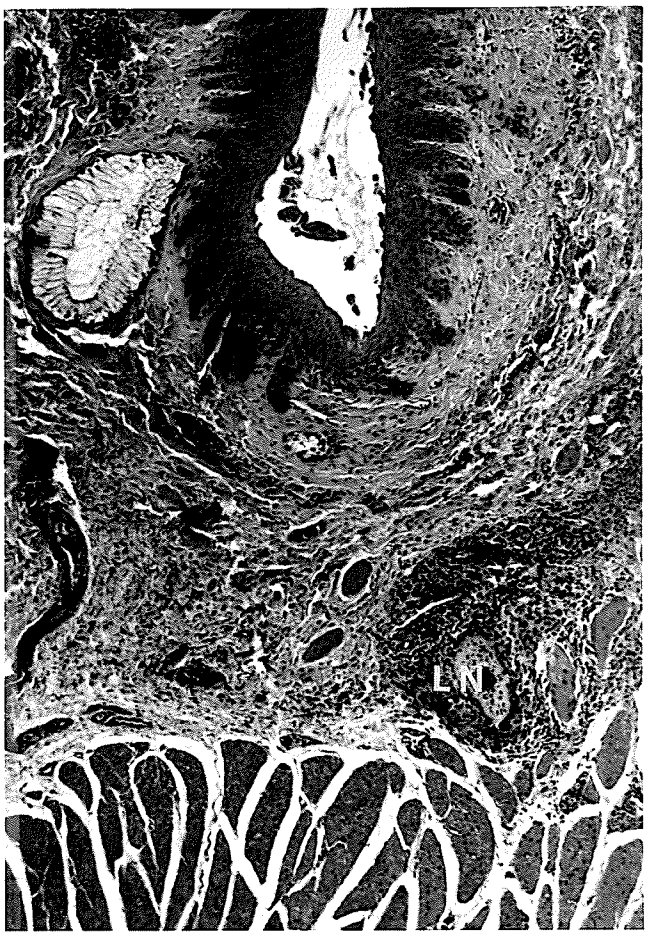

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Plate IX. Photomicrograph showing the three layers of the mucosa of the red-tailed hawk oesophagus. A small lymph node (LN) may be seen in the lower right corner. 100X. T.S.

thicker than either the mucous membrane or the muscularis mucosae (Plates IX and X). The lamina propria contained areolar connective tissue, numerous blood capillaries and lymph nodes which were present beneath the mucous membrane (Plates IX and X). Muscle fibres were not observed within the core of the lamina propria.

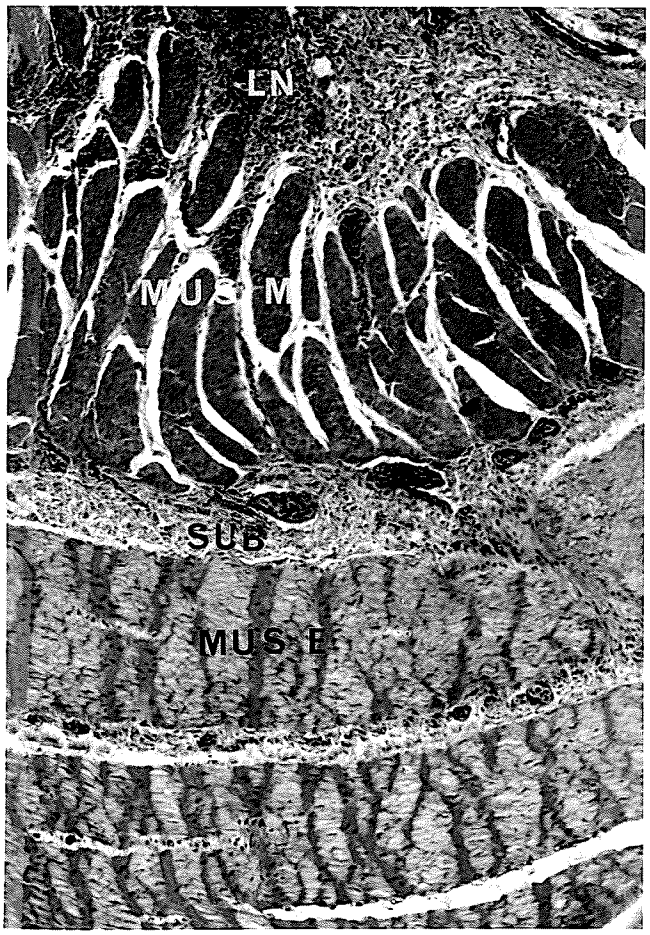

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Plate X. Photomicrograph showing lamina propria containing some lymphatic tissue (LN), muscularis mucosae (MUS M), submucosa (SUB) and muscularis externa (MUS E), in the red-tailed hawk oesophagus. 100X. T.S.

Muscularis mucosae. This layer was well developed and calcu-

lated to be approximately half as thick as the muscularis externa (Plates IX and X, and Table 1). Only longitudinally disposed smooth muscle fibres were observed in this layer.

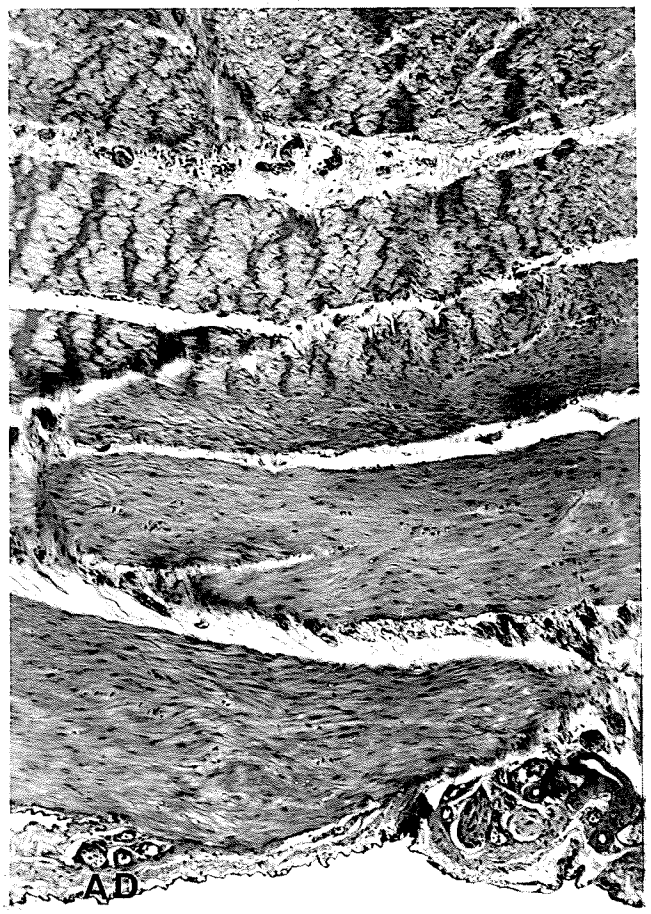

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Plate XI. Photomicrograph showing section of the muscularis externa in a thick area of the oesophageal wall in the red-tailed hawk. Outer adventitia (AD) is also shown. 100X. T.S.

Submucosa

The submucosa contained areolar connective tissue with a predominance of collagenous fibres. This layer was usually quite thin and

in some areas was hardly discernible. Thickened areas of the submucosa usually contained blood vessels (Plate X). Small blood vessels and capillaries were found throughout the submucosa.

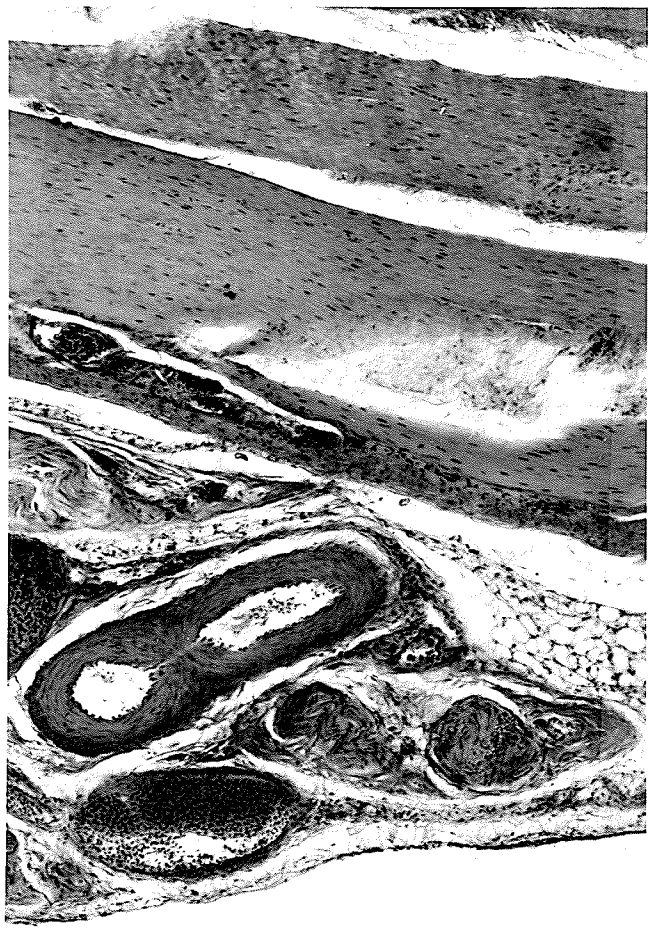

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Plate XII. Photomicrograph of red-tailed hawk oesophagus showing adventitia containing blood vessels and possible nerve plexuses. The muscularis externa lies in the top half of the photomicrograph. 100X. T.S.

Muscularis externa

This layer was composed of a thick circular muscle band with no clear evidence of an outer muscle band of longitudinally disposed fi-

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Plate XIII. Photomicrograph showing mucous glands in the lamina propria of the red-tailed hawk. 100X. T.S.

bres (Plates X, XI and XII). It was calculated that this layer was more than twice as thick as the muscularis mucosae (Table 1).

Adventitia

The adventitia was thin and composed mainly of collagenous fibres with lesser amounts of elastic fibres and adipose tissue. Many blood vessels were observed and smaller and poorly stained fibres which were tentatively defined as nerve plexuses (Plates XI and XII).

Glands

The mucous glands of the oesophagus of the red-tailed hawk were tubuloalveolar glands located in the lamina propria and provided with ducts lined by low columnar epithelium (Plates VIII, IX and XIII).

Lymphatic tissue was often noted to be dispersed between mucous glands. The mucous glands were large when compared to similar glands in some of the other birds (Plates XXXIX and XL).

Fulica americana (American coot)

Mucosa

Mucous membrane. The mucous membrane of the oesophagus was made up of two layers; a layer of stratified squamous epithelium predominantly displayed at the bases of mucosal convolutions which was continuous with another layer of connective tissue fibres found at the apices (Plates XIV, XV and XVI). The mucosa was greatly convoluted.

The stratified squamous epithelium was thickest at the bases of the convolutions and terminated at the apices (Plates XIV, XV and

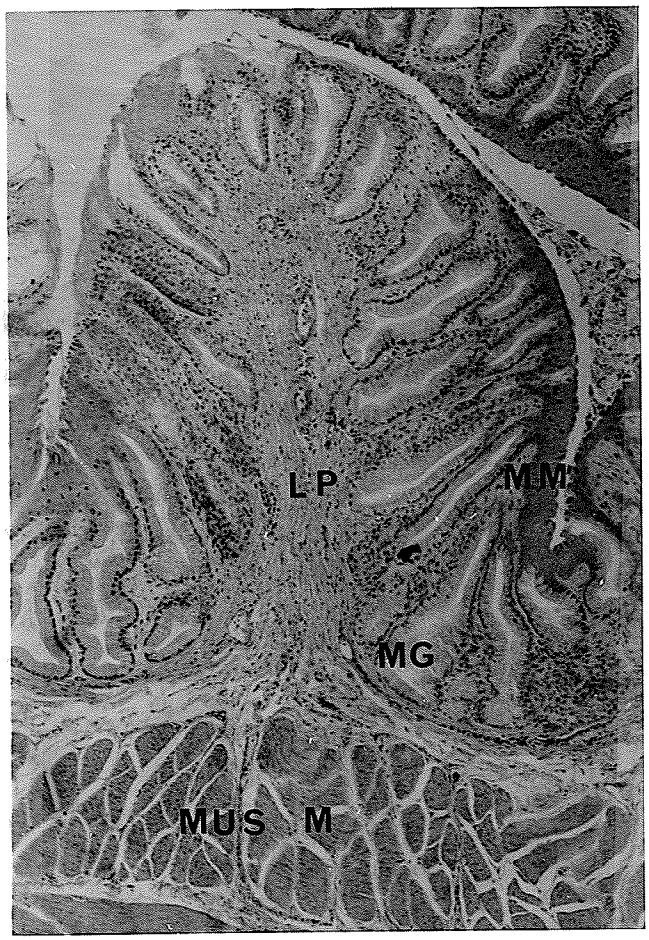

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Plate XIV. Photomicrograph of American coot oesophageal convolution of the mucosa showing mucous membrane (MM), mucous glands (MG), lamina propria (LP) and muscularis mucosae (MUS M). Note that the stratified squamous epithelium is not a continuous layer and is confined to the bases of the convolutions. The apices are lined by a connective tissue membrane which is continuous with the lamina propria. 100X. T.S.

XVI). It was composed of connective tissue which was continuous with the lamina propria (Plates XIV, XV and XVI).

The junction between the epithelial mucous membrane and the lamina propria was not always papillated and the interface between these two layers is best described as uneven (Plates XIV, XV, XVI

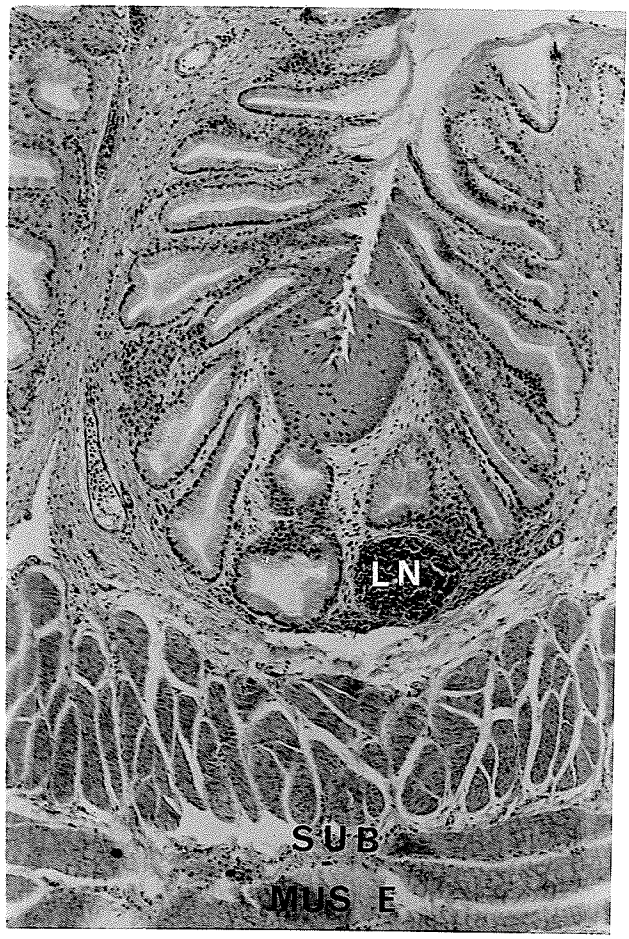

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Plate XV. Photomicrograph of mucosal convolutions showing mucous membrane, mucous glands, lamina propria and muscularis mucosae in the American coot oesophagus. Also shown is the submucosa (SUB) and the inner muscles of the muscularis externa (MUS E). In the lamina propria is a lymph node (LN). 100X. T.S.

and XVII).

In some of the histological sections studied considerable loosening of the superficial layers was apparent.

Lamina propria. This layer was composed of areolar connective tissue, blood capillaries, and lymph nodes which were small and

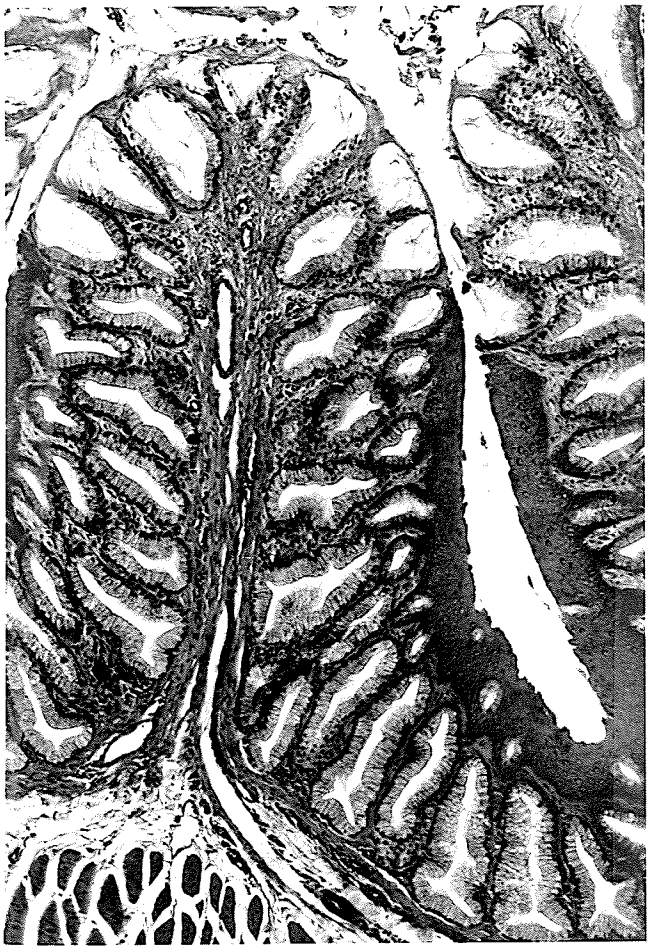

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Plate XVI. Photomicrograph of mucosal convolution in American coot oesophagus showing discontinuous nature of the epithelial mucous membrane at the apex of the convolution. The free surface at this apex consists of connective tissue fibres which are continuous with the lamina propria. Lower portion of the convolution is lined by stratified squamous epithelium which is thickest at the base. 100X. T.S.

oval and did not penetrate into the mucous membrane (Plates XV and XVIII).

Submucosa

The submucosa was a thin and sometimes indistinct layer which

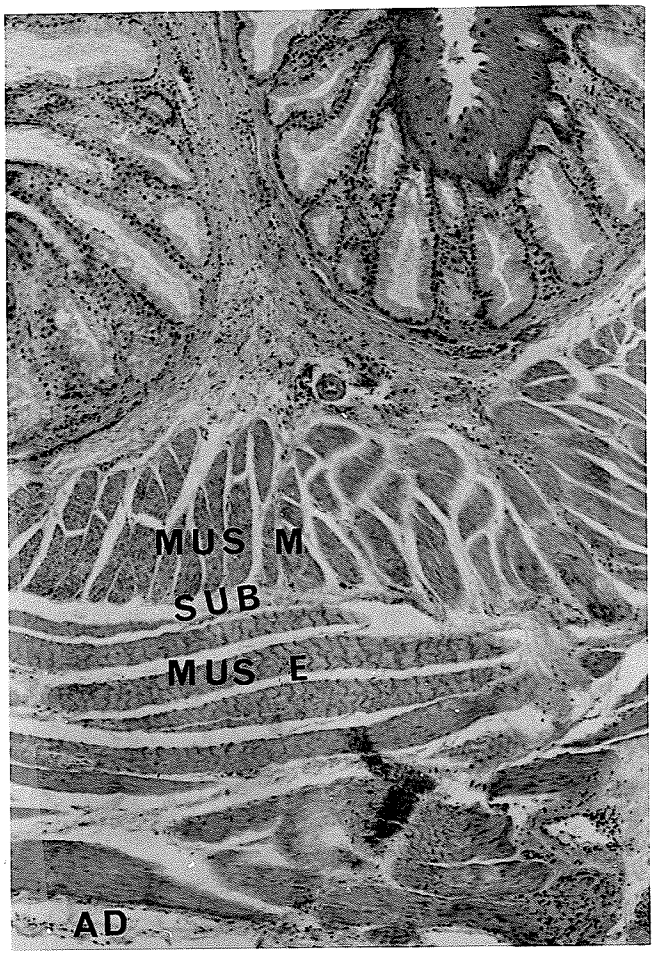

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Plate XVII. Photomicrograph of oesophagus of the American coot showing four tissue layers. The (1) mucosa showing mucous membrane, mucous glands, lamina propria and muscularis mucosae (MUS M). Note also (2) submucosa (SUB), (3) muscularis externa (MUS E) and (4) adventitia (AD). 100X. T.S.

showed areas of thickening for blood vessels (Plate XV).

Muscularis externa

This was a thick and well developed circular layer of smooth muscle (Plates XV, XVII and XVIII). No outer longitudinal muscle fibres were observed in the muscularis externa.

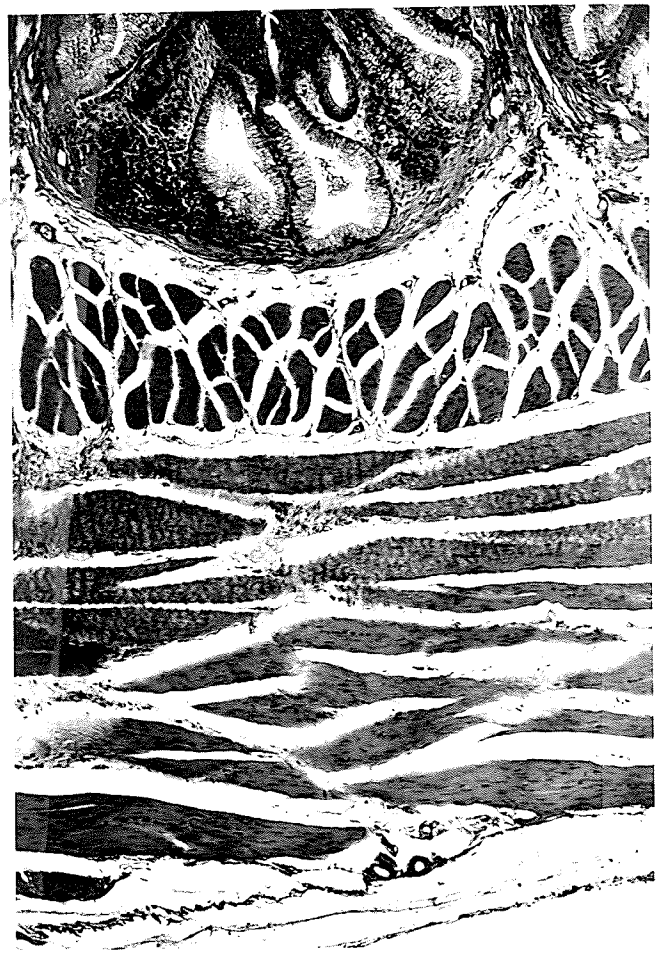

Plate XVIII. Photomicrograph of American coot oesophagus showing the following muscle layers: upper muscularis mucosae and beneath it the muscularis externa. At the upper left corner a small lymph node can be seen. 100X. T.S.

Adventitia

The adventitia consisted of a thin layer of collagenous connective tissue which contained blood vessels, nerves and some lymphatic tissue (Plates XVII and XVIII).

Glands

Simple tubular and simple branched alveolar mucous glands lay

entirely within the lamina propria with ducts lined by low columnar epithelium leading to the surface (Plates XIV, XV, XVI and XVII). Small patches of lymph nodes were often found between the mucous glands (Plates XV and XVI).

Larus delawarensis (ring-billed gull)

Mucosa

Mucous membrane. The mucous membrane consisted of a thick layer of non-cornified, stratified squamous epithelium (Plates XIX and XX). Between the mucous membrane and the lamina propria was a linear row of mucous glands (Plates XIX, XX and XXI). Papillae of the lamina propria lay in contact with the mucous membrane between adjacent glands (Plates XIX and XX).

The mucosa was convoluted with the mucous membrane showing a tendency towards increased thickening at the base of the convolutions (Plates XIX and XX).

Only epithelial cells were present in the mucous membrane. A tendency towards a sloughing off of the superficial cells was observed (Plate XX). The surface cells were generally flattened and gave a smooth surface (Plate XIX).

Lamina propria. This layer was well developed and clearly defined within the core of the convolutions (Plate XIX, XX and XXI).

The lamina propria was composed of areolar connective tissue. Muscle fibres were not observed in this layer. Small lymph nodes were

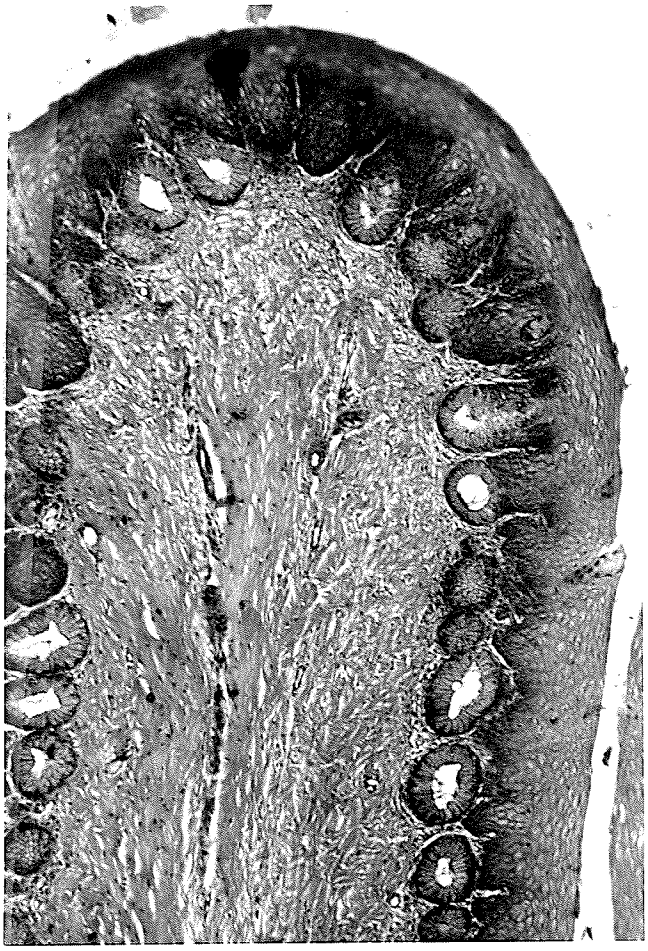

Plate XIX. Photomicrograph of mucosal convolution showing mucous membrane, mucous glands and lamina propria in the ring-billed gull oesophagus. 100X. T.S.

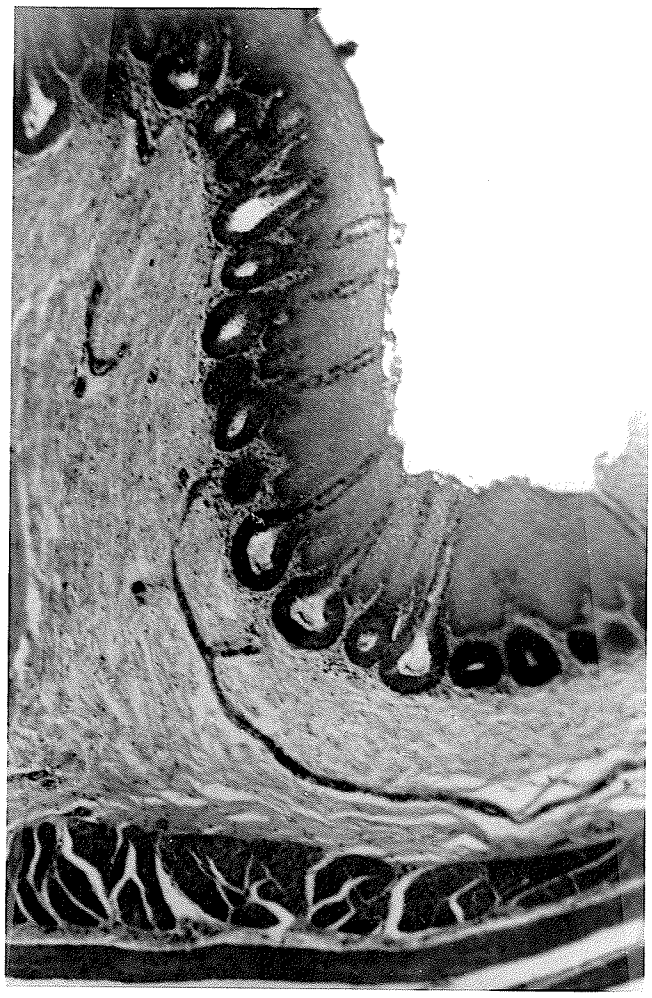

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Plate XX. Photomicrograph showing three layers of the mucosa; mucous membrane, lamina propria and muscularis mucosae in the ring-billed gull oesophagus. Note increased thickening of mucous membrane at the base of the convolution. 100X. T.S.

infrequently observed in the lamina propria and when present extended into the surface epithelium. Blood capillaries were also present.

Muscularis mucosae. This layer of longitudinally disposed smooth muscle was well developed and was approximately half as thick as the muscularis externa (Plates XX and XXI, and Table 1).

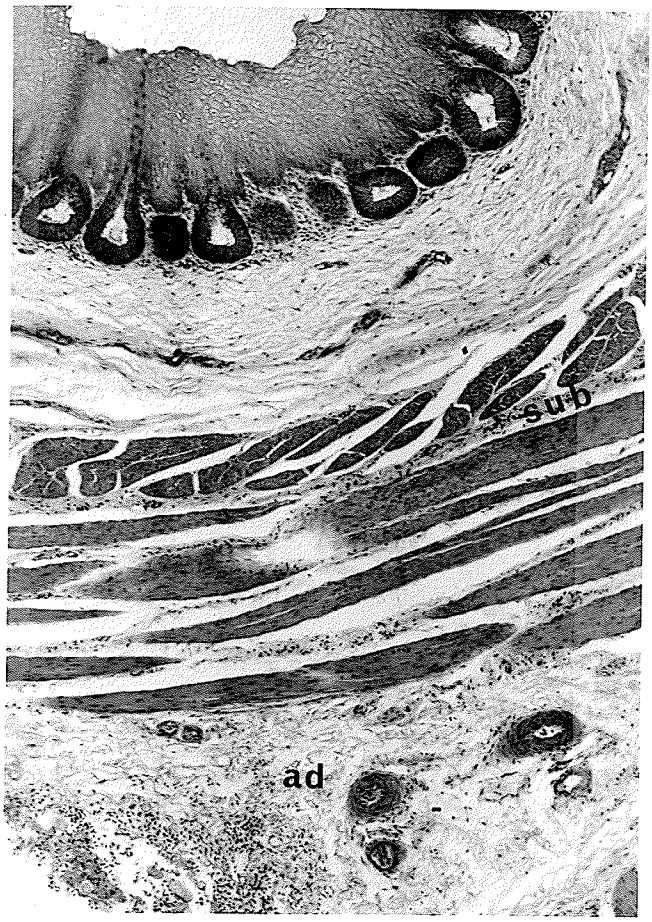

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Plate XXI. Photomicrograph of four layers of the oesophageal wall of ring-billed gull. The submucosa (SUB) is very thin and hardly discernible. The outer muscularis externa is the next layer and is surrounded by the adventitia (AD). 100X. T.S.

Submucosa

The submucosa was of variable thickness and formed a distinct layer beneath the muscularis mucosae (Plate XXI). The enlarged areas were noted by the presence of blood vessels.

Muscularis externa

Only circularly oriented smooth muscle fibres were observed

in the muscularis externa with muscular bands separated by fibrous connective tissue (Plate XXI).

Adventitia

This outer layer consisted of predominantly collagenous and elastic connective tissue fibres containing blood vessels, lymphatic vessels and nerves.

Glands

The mucosal glands were noted to consist of simple alveolar glands with the secretory portion beneath the mucous membrane. A duct lined by simple low columnar epithelial cells traversed the mucous membrane to open onto the surface (Plates XIX, XX and XXI).

Larus pipixcan (Franklin's gull)

Mucosa

Mucous membrane. The mucous membrane of the Franklin's gull was noted to consist of a thick layer of stratified squamous epithelium (Plates XXII and XXIII). The junction between the mucous membrane and lamina propria was marked by papillae of the lamina propria and by mucous glands (Plates XXII and XXIII).

The mucous membrane of the convoluted mucosa was thickest at the base of the convolutions and thinnest at the apex. The surface cells tended to show sloughing, however the mucosal surface was smooth (Plates XXII and XXIII).

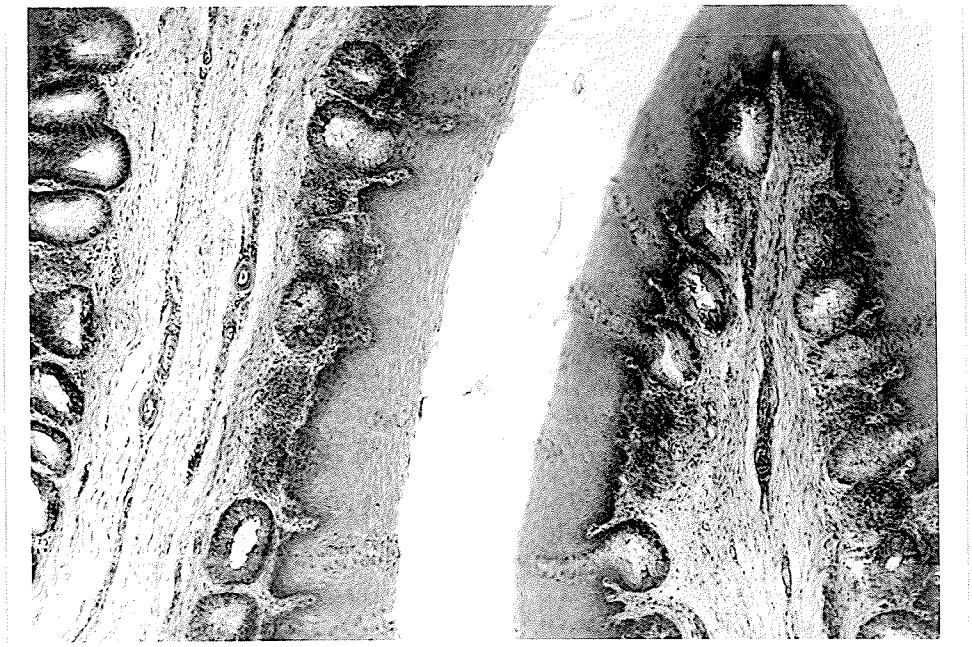

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Plate XXII. Photomicrograph of mucosal convolutions of Franklin's gull oesophagus showing mucous membrane, mucous glands and lamina propria. 100X. T.S.

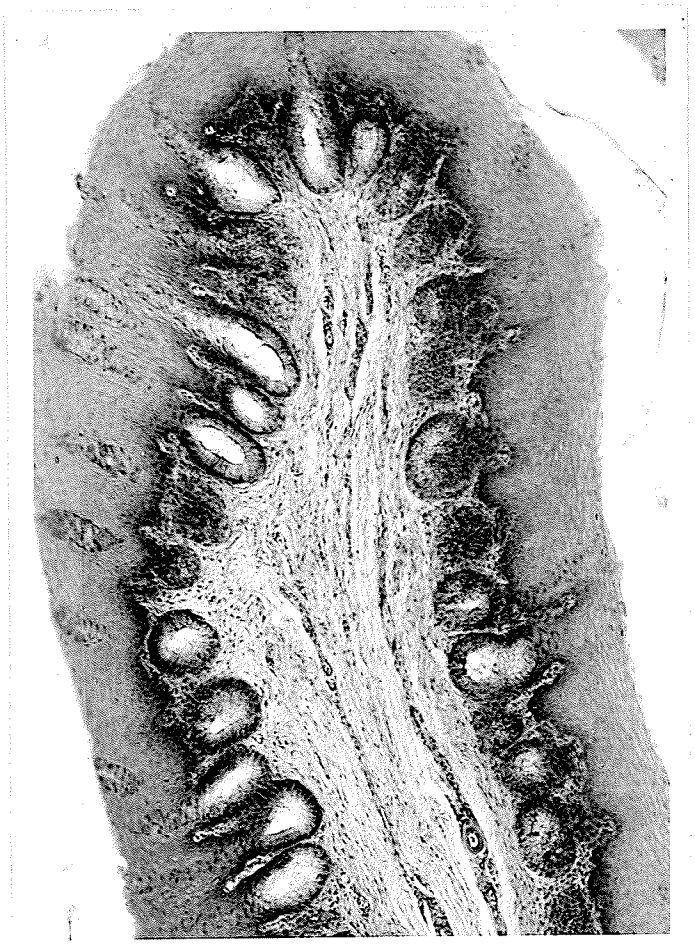

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Plate XXIII. Photomicrograph of mucosal convolution of Franklin's gull oesophagus showing mucous membrane, mucous glands and lamina propria. 100X. T.S.

Lamina propria. The lamina propria was wider in the core of the convolutions (Plate XXII) when compared to the lamina propria at the base of the convolutions. Blood capillaries and small patches of lymphatic tissue were observed in this region.

Muscularis mucosae. This layer of longitudinally arranged smooth muscle was well developed (Plate XXIV). It was almost as thick as the

outer muscularis externa. The muscularis mucosae in this bird was prominently subdivided into muscle bands by connective tissue septa.

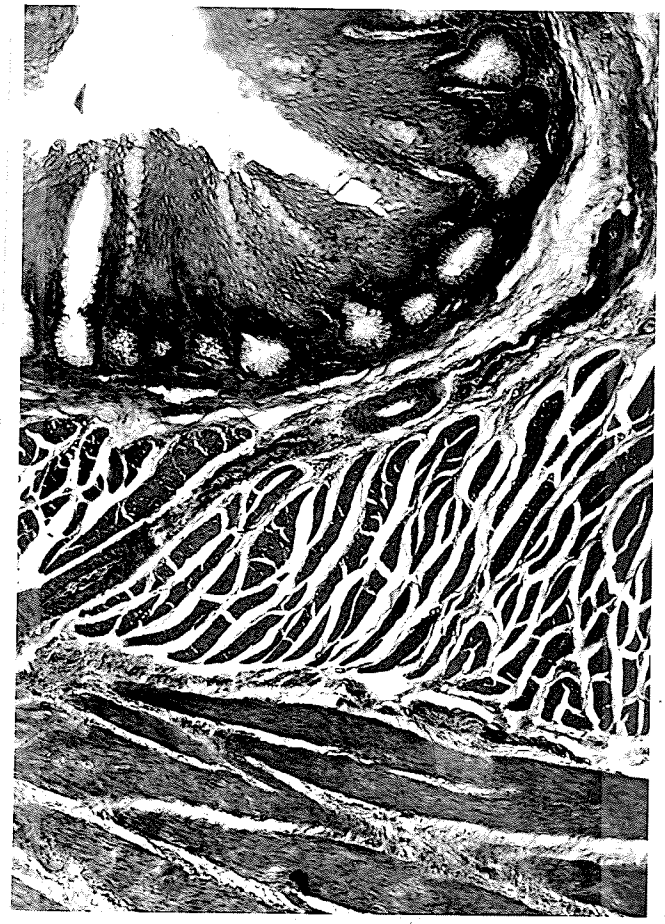

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Plate XXIV. Photomicrograph of the mucosa, submucosa and muscularis externa of the Franklin's gull oesophagus. The base of the convolution shows that the mucous membrane is quite thick and the lamina propria (LP) is thin. The submucosa (SUB) is thin. The muscularis externa lies beneath the submucosa. 100X. T.S.

Submucosa

The submucosa was thin and in some places not recognizable

(Plates XXIV and XXV). It was composed of areolar connective tissue with blood vessels occurring in some of the wider areas.

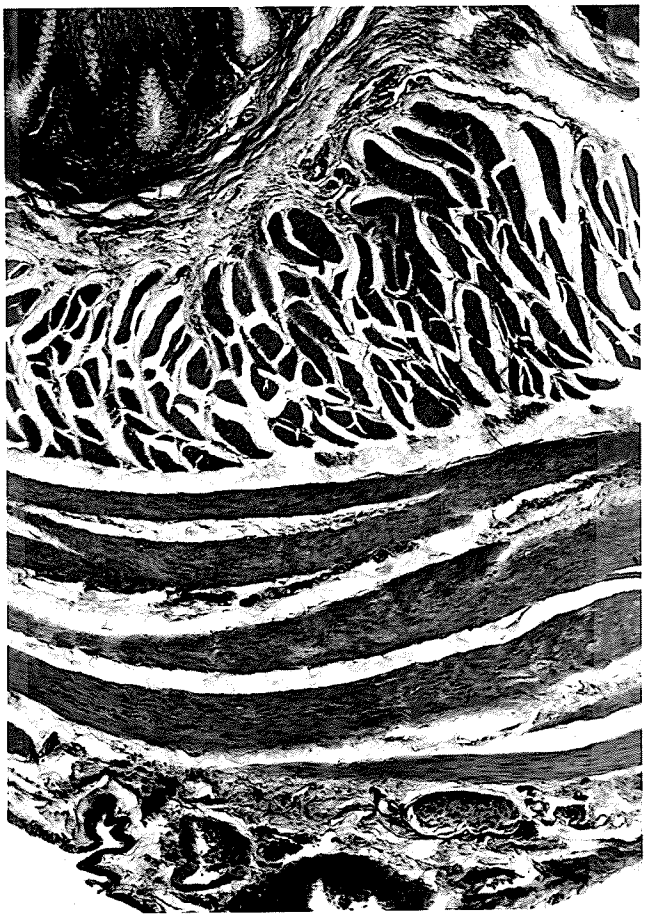

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Plate XXV. Photomicrograph showing all tissue layers in the Franklin's gull oesophagus. The submucosa consists of a very thin layer. Note large blood vessels in the adventitia. 100X.

Muscularis externa

This layer consisted of a circularly arranged smooth muscle band (Plates XXIV and XXV). An outer longitudinal muscle layer was not present. Connective tissue septae were observed to lie

between the bands of muscle which sometimes showed the presence of blood vessels.

Adventitia

This outer connective tissue layer was quite thin with the exception of those areas in which blood vessels, lymph vessels and nerves were observed to be present (Plate XXV).

Glands

Mucosal glands were simple alveolar in structure with the secretory portion lying beneath the mucous membrane (Plates XXII, XXIII and XXIV).

The individual glands were small in size and arranged in a linear row beneath the mucous membrane.

Lymph nodes were seldom observed but some lymphatic tissue was found near the bases of the glands (Plates XXII and XXIII).

Bubo virginianus (great horned owl)

Mucosa

Mucous membrane. The mucous membrane was quite thin and consisted of only a few cells in thickness (Plates XXVI and XXVII). No sign of cornification of the mucous membrane was observed. The mucous glands formed a tightly packed continuous row which separated the lamina propria from the mucous membrane (Plates XXVI, XXVII and XXVIII).

The epithelium was equally thick at the bases and at the

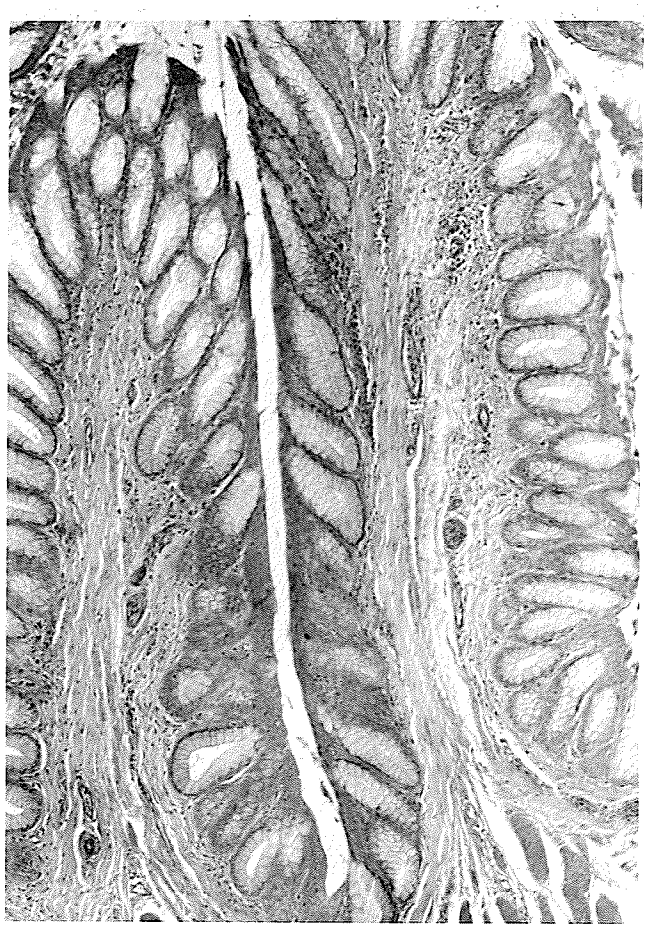

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Plate XXVI. Mucosal convolutions of the great horned owl oesophagus showing mucous membrane, mucous glands and lamina propria. 100X. T.S.

apices of the mucosal convolutions (Plates XXVI, XXVII and XXVIII). The surface layer of epithelium showed considerable sloughing off of cells.

Lamina propria. At the base of the convolution the lamina propria had a tendency to be thinner when compared to the core. Lymphatic tissue was observed to lie adjacent to the mucous glands (Plate XXVII).

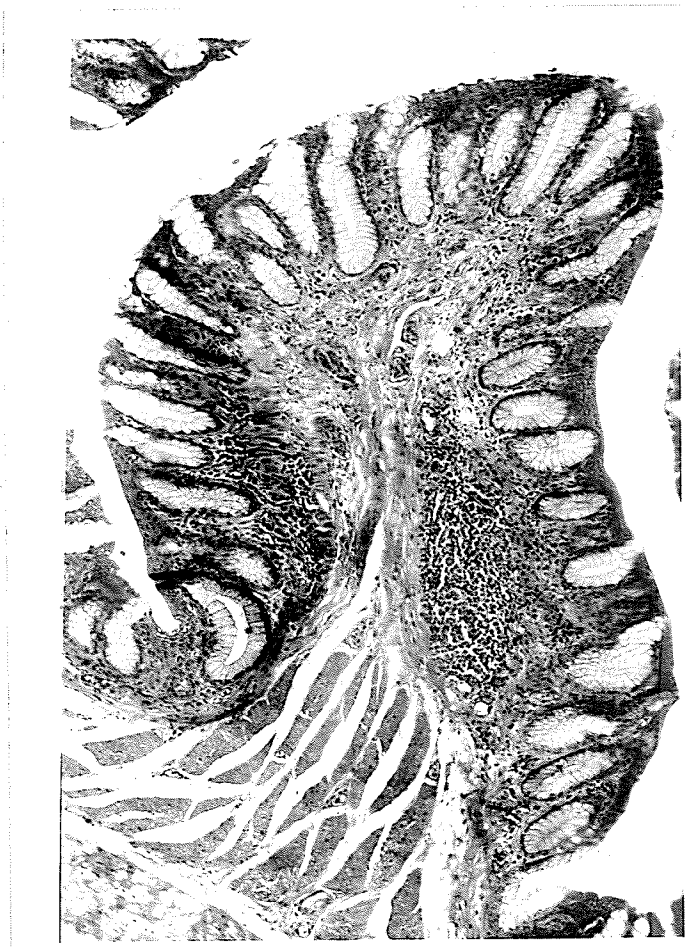

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Plate XXVII. Photomicrograph of mucosal convolution of great horned owl oesophagus showing muscularis mucosae extending into core of lamina propria. Note patch of lymphatic tissue lying adjacent to muscularis mucosae. 100X. T.S.

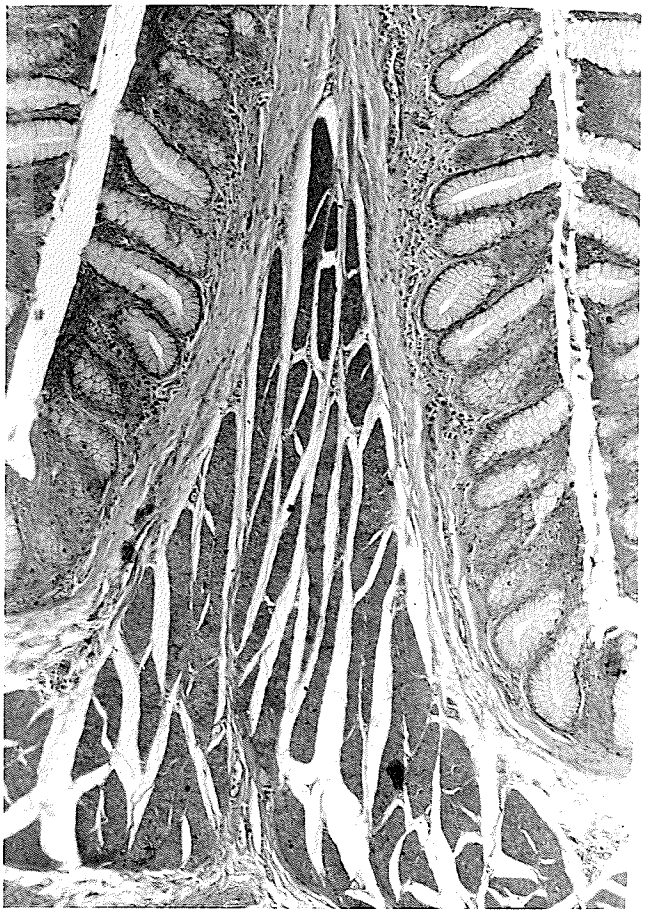

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Plate XXVIII. Photomicrograph showing the muscularis mucosae (MUS M) as a conical thickening which penetrates into the lamina propria of the mucosal convolution in the great horned owl oesophagus. 100X. T.S.

In some instances the lymphatic tissue had penetrated into the mucous membrane. Blood vessels were abundant in the lamina propria.

Muscularis mucosae. The muscularis mucosae was well developed and consisted of longitudinally oriented muscle fibres. In this study only the owl showed prominent extensions of the muscularis mucosae into the core of the mucosal convolutions (Plates XXVII, XXVIII

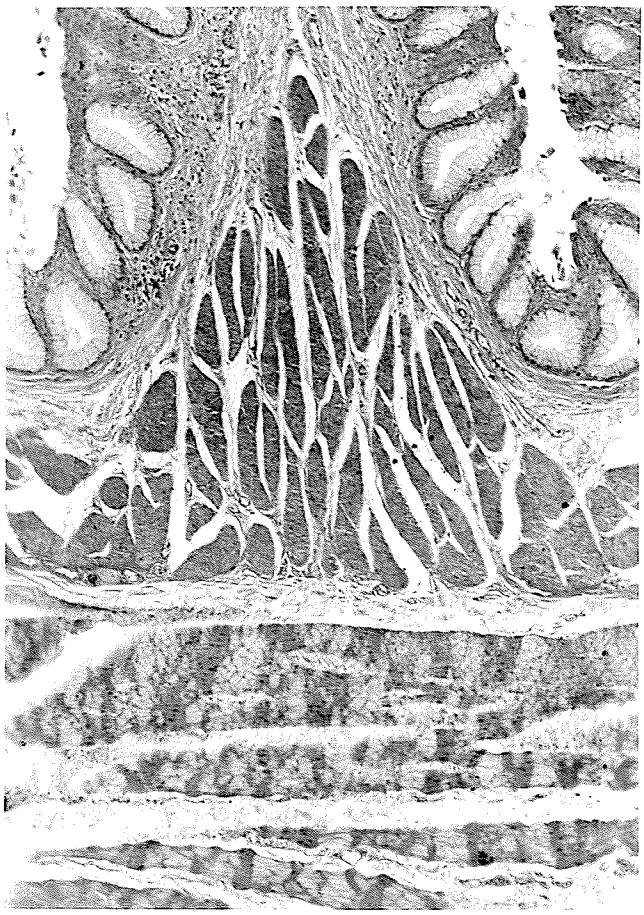

Plate XXIX. Photomicrograph of the mucosa, submucosa (SUB) and muscularis externa in the great horned owl oesophagus. 100X.

and XXIX). These projections of muscularis mucosae into the mucosal convolutions may be functionally related to the common practice of regurgitating waste pellets of non-digested material such as bones and teeth.

Submucosa

This layer consisted of areolar connective tissue with small

thickenings in which blood vessels were present (Plate XXIX).

Muscularis externa

This muscle layer, when compared to similar muscle layers in seed-eating sparrows (Plates XXIX, XXX, XXXIX and XL), was observed to be thicker in the carnivorous owl.

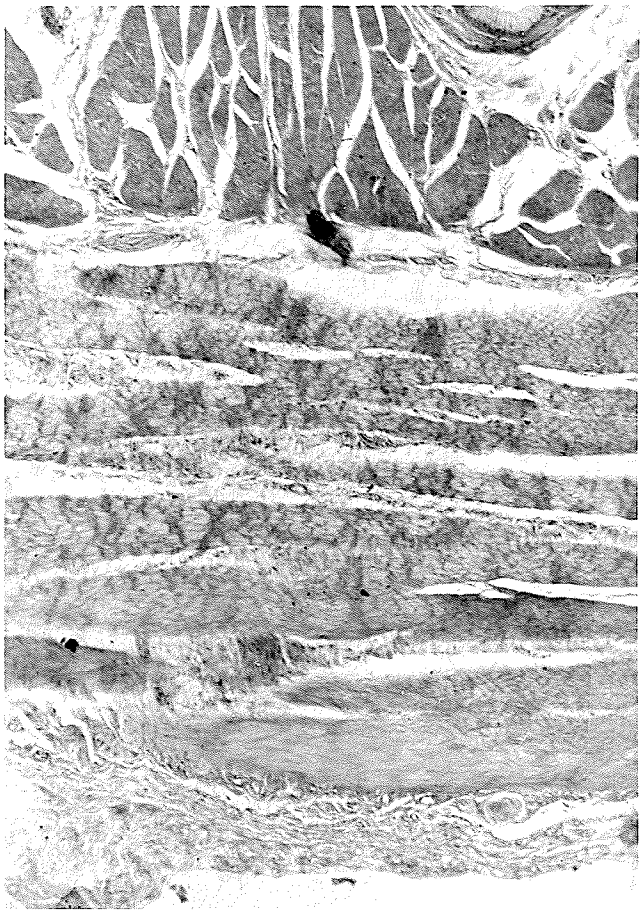

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Plate XXX. Photomicrograph of the four oesophageal tissue layers showing mucosa, submucosa, muscularis externa and adventitia in the great horned owl. 100X. T.S.

Adventitia

This thin layer of connective tissue with collagenous fibres contained many blood and lymph vessels as well as nerves. The lymph nodes in the adventitia, when compared to most of the other birds, were observed to be much larger in the owl.

Glands

The oesophageal glands were simple tubular and simple alveolar in structure (Plate XXVI and XXVII). The glands extended deeply into the lamina propria with a short duct leading through the thin mucous membrane to open onto the surface. Lymphatic patches of tissue were observed to lie in juxtaposition to the mucous glands (Plate XXVII). In some areas the mucous glands gave the appearance of being branched alveolar, however, each gland had its own duct which opened onto the surface. These mucous glands were therefore classified as simple alveolar in structure (Plate XXVI).

Tyrannus tyrannus (eastern kingbird)

Mucosa

Mucous membrane. The mucosal convolutions were capped by a relatively thick layer of stratified squamous epithelium (Plate XXXI). The junction between the mucous membrane and the lamina propria was interrupted regularly by oesophageal mucous glands (Plates XXXI and XXXII).

The layer of stratified squamous epithelium at the bases and apices of the convolutions tended to be of uniform thickness (Plates XXXI and XXXII).

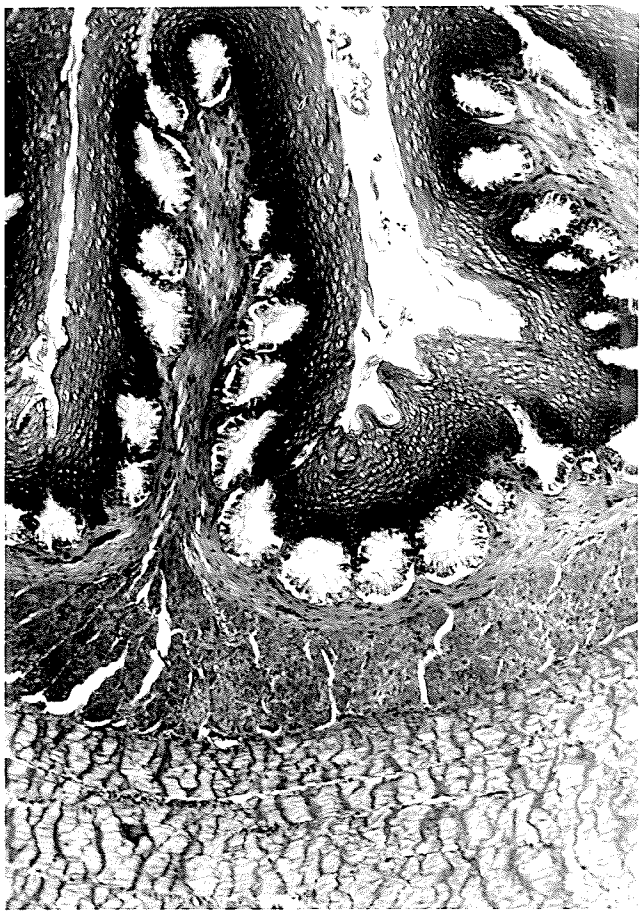

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Plate XXXI. Photomicrograph showing mucosal convolution, submucosa and muscularis externa of oesophagus in the eastern kingbird. The mucosal convolution consists of mucous membrane, mucous glands, lamina propria and basal layer of muscularis mucosae (MUS M). The submucosa is barely perceptable as a connective tissue layer between muscularis mucosae and muscularis externa. 100X. T.S.

The layer of stratified squamous epithelium at the bases and apices of the convolutions tended to be of uniform thickness (Plates XXXI and XXXII).

Lamina propria. This layer of collagenous connective tissue

tended to be slightly thicker in the core of the convolution when compared to the basal region (Plate XXXI).

Many small blood vessels were observed in this layer while lymphatic tissue and muscle fibres were notably absent.

Muscularis mucosae. This smooth muscle layer was made up of longitudinal fibres which did not invade the core of the convolutions.

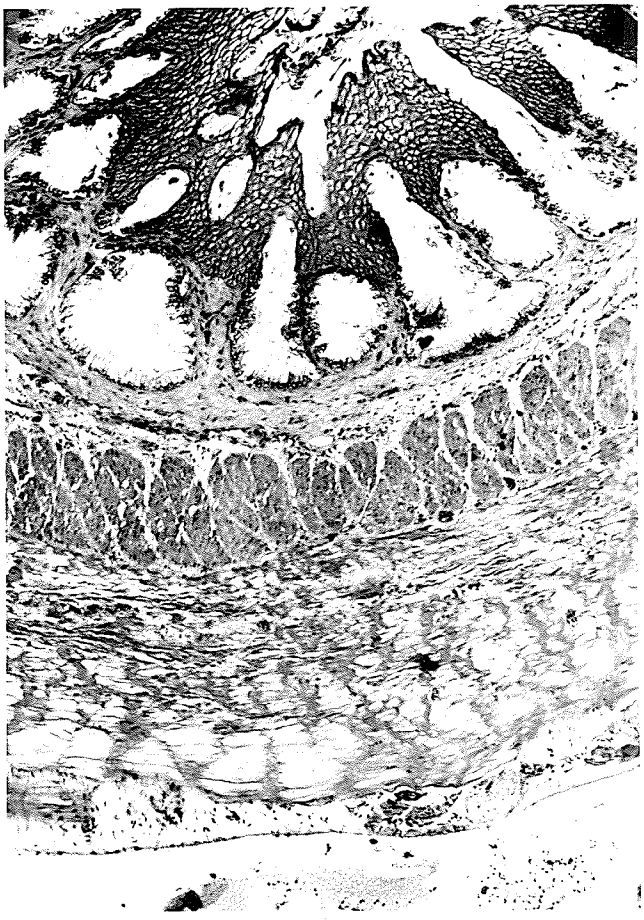

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Plate XXXII. Photomicrograph showing four tissue layers; mucosa, submucosa, muscularis externa and adventitia in the eastern kingbird. 100X. T.S.

It was observed that small papillae of muscle were present at the base of the convolution (Plates XXXI and XXXII).

Submucosa

This layer was barely perceptable as a connective tissue layer which was situated between the muscularis mucosae and the muscularis externa (Plates XXXI and XXXII). In some areas it was thickened by the presence of densely packed blood vessels.

Muscularis externa

Thisthick circular layer of smooth muscle gave the appearance of reticular fibres spirally arranged around the individual muscle fibres (Plate XXXI). These apparent reticular fibres resemble similar observations of smooth muscle from the human intestine described by Bloom and Fawcett (1968).

Adventitia

This layer of fibrous connective tissue contained numerous blood vessels, nerves and in general resembled similar layers in other birds examined in this study.

Glands

The oesophageal mucous glands were simple alveolar in structure and were observed to lie in the lamina propria and partially within the mucous membrane (Plates XXXI and XXXII). The ducts were simple and opened directly onto the surface of the mucous membrane.

Iridoprocne bicolor (tree swallow)

Mucosa

Mucous membrane. Mucosal convolutions were not observed to be present in the oesophagus of the tree swallow. The mucous membrane consisted of a relatively thick layer of stratified squamous epithe-

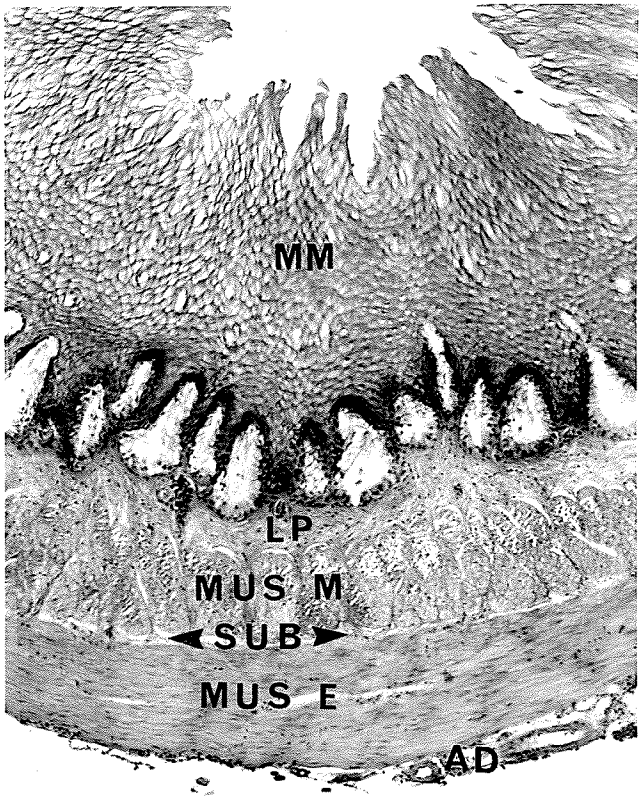

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Plate XXXIII. Photomicrograph showing mucosa, submucosa, muscularis externa and adventitia in the tree swallow oesophagus. 100X. T.S.

lium with no evidence of surface cornification. The surface was marked by irregularly shaped conical projections which were capped by squamous cells which tended to be larger than the basal layer of cells (Plates XXXIII and XXXIV).

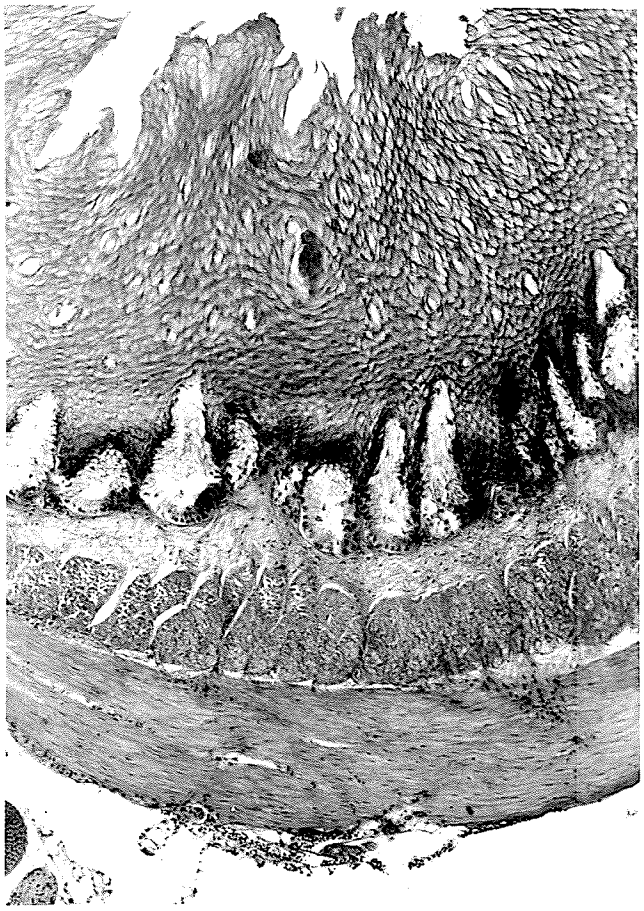

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Plate XXXIV. Photomicrograph showing mucosa, submucosa, muscularis externa and adventitia in the tree swallow oesophagus. 100X. T.S.

In some instances mucous glands appeared to lie entirely within the mucous membrane. Surface cells tended to slough off and gave an

irregular surface.

Lamina propria. This connective tissue layer, though well developed, showed no evidence of either muscle fibres or lymphatic tissue (Plate XXXIII).

Muscularis mucosae. The thickness of this longitudinal muscle layer appeared to be only slightly less than the muscularis externa. The arrangement of vertical connective tissue septa gave the general impression that the muscularis mucosae was regularly subdivided into vertical muscle bands (Plates XXXIII and XXXIV).

Submucosa

This thin layer of areolar connective tissue had several thickenings where blood vessels were found to be present (Plates XXXIII and XXXIV).

Muscularis externa

This smooth muscle coat was arranged in a circular layer with no sign of an outer longitudinal muscle layer (Plates XXXIII and XXXIV).

Adventitia

This layer of outer connective tissue was very thin and contained blood vessels (Plates XXXIII and XXXIV).

Glands

The oesophageal mucous glands were simple alveolar in structure. The secretory end portions were located at the junction between the mucous membrane and lamina propria (Plates XXXIII and XXXIV). The

total gland tissue was not as great as that found in other species examined in this study.

Corvus brachyrhynchos (common crow)

Mucosa

Mucous membrane. The basal layer of the mucous membrane showed several conical invaginations which were formed by papillae of the lamina propria (Plates XXXV and XXXVI). The layer of stratified squamous epithelium appeared thickest at the basal region of the mucosal convolution (Plate XXXVI). Straight secretory tubules leading from the mucous glands were often observed passing through the mucous membrane.

Lamina propria. This layer was of uniform width throughout the height of the mucosal convolutions. Several patches of lymphatic tissue were distributed throughout the lamina propria (Plates XXXV and XXXVI). Mucous glands were unevenly distributed in the lamina propria.

Muscularis mucosae. This muscle layer was subdivided by connective tissue septae into bands of uneven size (Plate XXXVII). Conical projections of the muscularis mucosae were observed invaginating the basal cores of the mucosal convolutions (Plate XXXVIII).

Submucosa

This was poorly defined in this bird and consisted of a thin

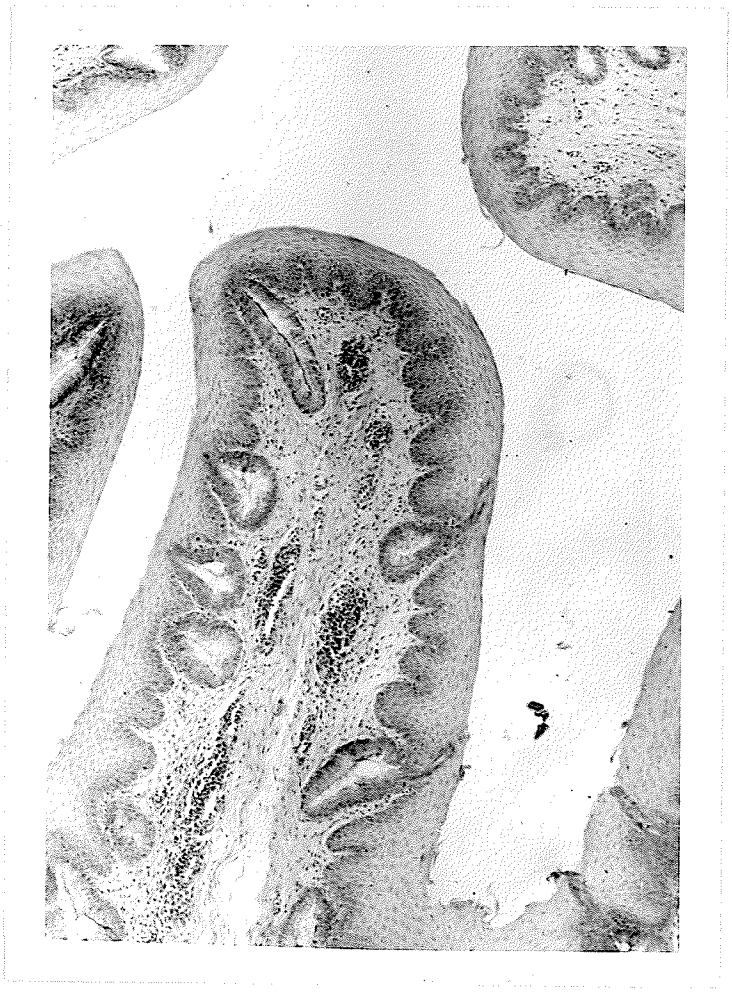

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Plate XXXV. Photomicrograph of mucosal convolutions showing the mucous membrane, lamina propria and mucous glands in the crow oesophagus. 100X. T.S.

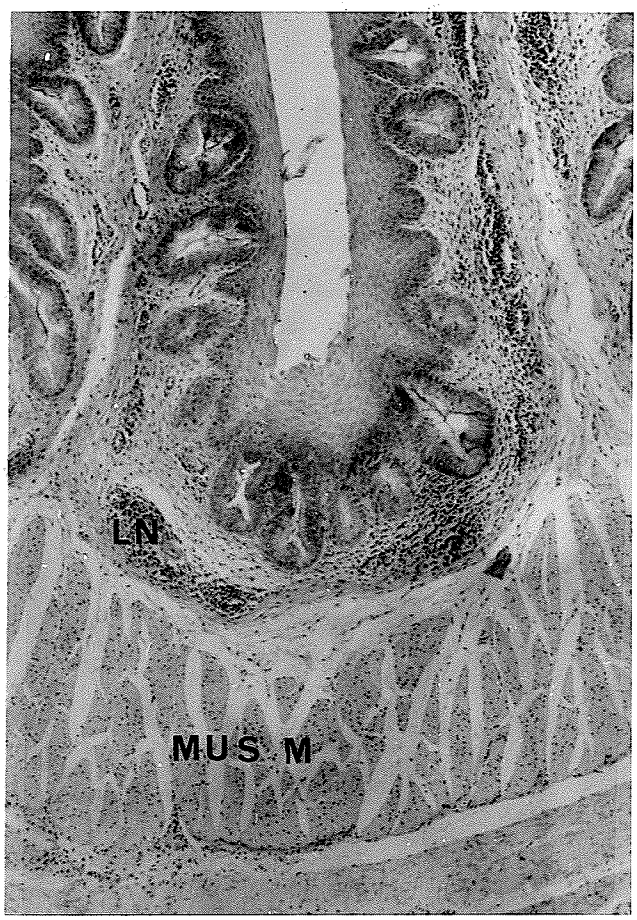

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Plate XXXVI. Photomicrograph of section through crow oesophagus showing mucosal convolutions, lymphatic tissue (LN) in the lamina propria and thickened portion of submucosa. 100X. T.S.

connective tissue band with a few blood vessels (Plate XXXVI).

Muscularis externa

This circular layer of smooth muscle lay in close juxtaposition to the muscularis mucosae. Several circularly disposed blood capillaries and thin stands of connective tissue

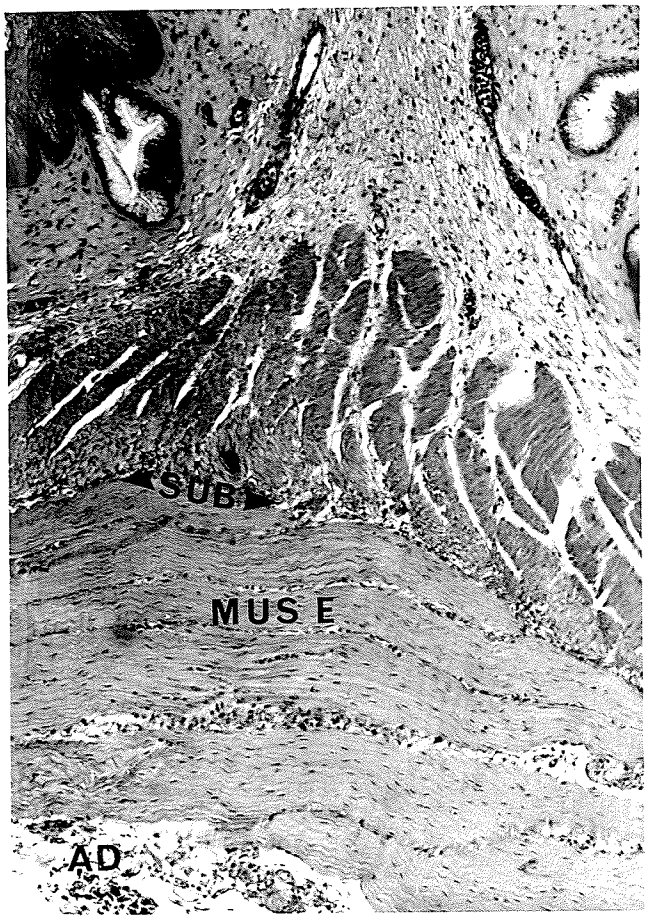

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Plate XXXVII. Photomicrograph showing the four tissue layers; mucosa, submucosa, muscularis externa, and adventitia in crow oesophagus. 100X. T.S.

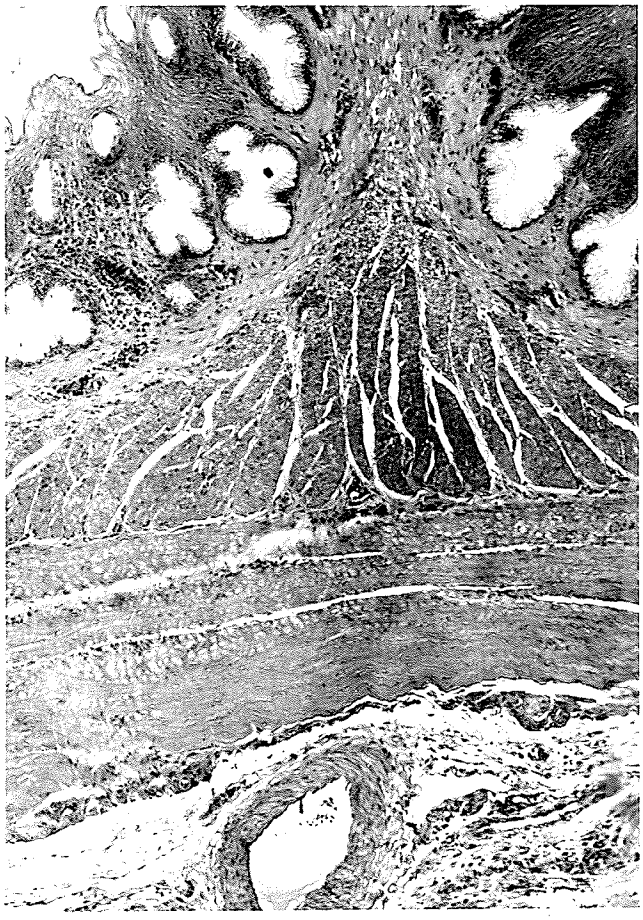

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Plate XXXVIII. Photomicrograph of crow oesophagus. Note conical projection of muscularis mucosae invaginating the basal core of the mucosal convolution. 100X. T.S.

fibres gave the appearance that the muscle was subdivided into bundles (Plate XXXVII).

Adventitia

This layer of connective tissue contained large blood vessels and several nerve fibres. The connective tissue formes a prominent layer around the larger blood vessels (Plate XXXVIII).

Glands

These were simple branched alveolar glands in structure and were located in the lamina propria with ducts leading to the surface. The glands showed some evidence of forming a branched pattern (Plate XXXVIII). The glands were often separated from one another in the lamina propria.

Passer domesticus (house sparrow)

Mucosa

Mucous membrane. The mucous membrane was composed of a relatively thick layer of stratified squamous epithelium (Plates XXXIX and XL).

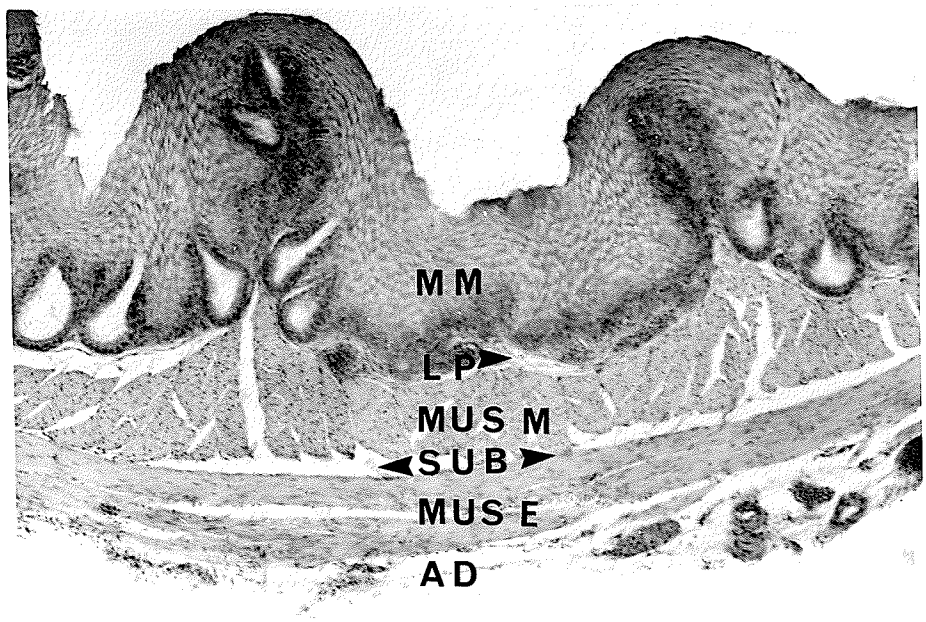

Plate XXXIX. Photomicrograph of transverse section of oesophagus in sparrow showing mucosa, submucosa, muscularis externa and adventitia. Note: the lamina propria is represented by a very thin core. 100X. T.S.

The mucosal convolutions were relatively small with most of the mucous glands lying within the area of the mucous membrane. Convoluted invaginations of the basal layer of the mucous membrane were formed by papillae from the lamina propria. The mucous membrane was thickest at the bases of the convolutions and thinnest at the apices (Plate XXXIX).

The surface cells showed little evidence of sloughing or cornification.

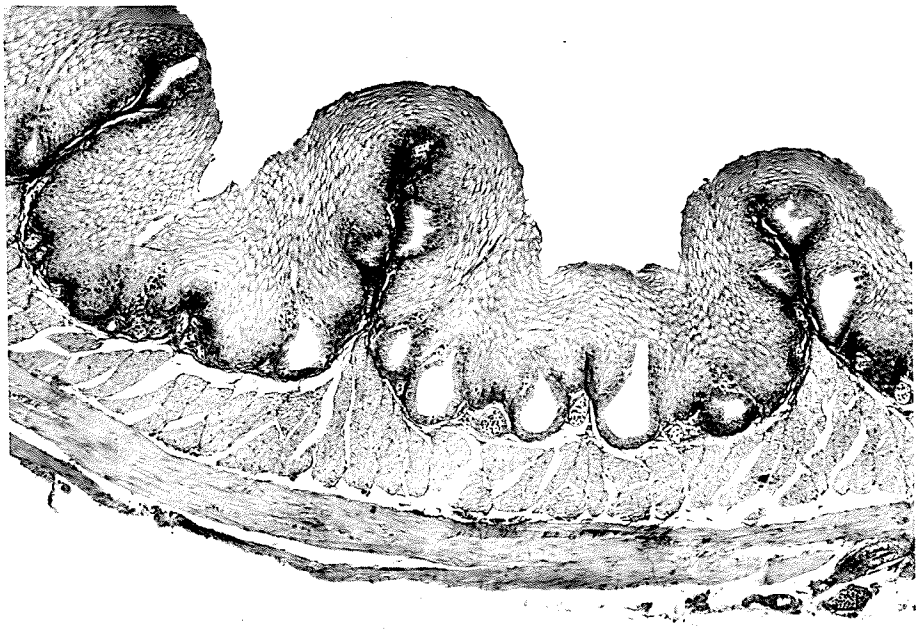

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Plate XL. Photomicrograph of oesophagus in sparrow. Note that the lamina propria consists of a thin core with few mucous glands. 100X. T.S.

Lamina propria. The lamina propria was hardly discernible in the oesophagus of this bird (Plate XL). Small blood vessels were numerous but lymphatic tissue was sparsely evident (Plate XL).

Muscularis mucosae. This longitudinal smooth muscle layer was subdivided by many connective tissue septae which were made up of both epimysium and endomysium. The muscle, in general, was well developed and appeared thicker than the muscularis externa.

Submucosa

The submucosa was a thin and poorly developed layer which was hardly distinguishable in certain areas. Collagenous fibres usually predominated in this layer. Large blood and lymph vessels were not observed in this layer and thickened areas were not found.

Muscularis externa

Circularly disposed smooth muscles were observed in this layer (Plate XL). Only a small amount of connective tissue and few blood capillaries were located between the bands of circular muscle. There was no evidence of an outer longitudinal layer.

Adventitia

This outer connective tissue layer, in relation to the thickness of the oesophageal wall, was well developed and many blood vessels and some patches of lymphatic tissue were observed.

Glands

The oesophageal glands were simple alveolar in structure and were located almost entirely within the mucous membrane with ducts leading to the surface. The individual glands were sparsely distributed. Lymph tissue was not observed in this area.

HISTOLOGICAL MEASUREMENTS OF THE OESOPHAGUS

Measurements were made from transverse histological sections taken from the mid-length region of the oesophagus from eighteen different birds of six taxonomic orders.

The results of all histological measurements with their standard errors are presented in Table 1.

OESOPHAGEAL DIAMETER

The largest oesophageal diameter (11,160±310 microns) was in the white pelican while the smallest oesophageal diameter (1910±24 microns) was in the tree swallow. Birds were categorized into four groups and are listed in a descending order of size. The pelican had the largest oesophageal diameter. No significant differences occurred between each of the following birds: red-tailed hawk, ring-billed gull, Franklin's gull, great horned owl, common crow. The oesophageal diameter in each of the tree swallow, eastern kingbird and house sparrow, as shown in Table 1 and Figure 2 did not significantly differ from each other.

The oesophageal diameter of the American coot, when compared to other birds examined in this study, was intermediate in size.

The grouping of birds according to their oesophageal diameters is listed in Table 2.

MEAN HISTOLOGICAL MEASUREMENTS IN MICRONS

SPECIES	DIAMETER OF OESOPHAGUS	THICKNESS OF WALL	LENGTH OF CONVOLUTION	THICKNESS OF MUCOUS MEMBRANE	THICKNESS OF LAMINA PROPRIA	THICKNESS OF MUSCULARIS MUCOSAE	THICKNESS OF SUBMUCOSA	THICKNESS OF MUSCULARIS EXTERNA
Pelecanus erythrorhynchos	11,160±310	1,780±132	3,720±158	78±6	337±18	330±27	113±22	788±97
Buteo jamaicensis	6,590±223	1,570±95	1,670±121	92±6	258±28	321±29	45±14	748±51
Fulica americana	3,945±61	900±51	970±51	49±5	249±13	169±10	22±3	376±10
Larus delewarensis	6,570±349	1,170±58	1,765±163	107±9	205±31	183±11	28±5	344±33
Larus pipixcan	6,310±320	1,060±54	1,420±64	119±10	169±13	184±18	38±7	331±28
Bubo virginianus	6,810±508	870±58	1,280±85	45±6	137±10	162±15	22±3	396±29
Tyrannus tyrannus	2,945±40	665±26	655±11	117±13	72±6	102±5	5±1	255±16
Iridoprocne bicolor	1,910±24	685±38	CONVOL. ABSENT	352±39	75±8	67±6	4±.8	117±8
Corvus brachyrhynchos	5,000±429	915±43	835±45	101±13	158±14	169±18	18±3	281±21
Passer domesticus	2,060±88	420±16	345±13	126±14	35±4	72±5	5±1	69±6

Table 1. Mean histological measurements at mid-length of oesophagus in ten different species of birds.

BIRD GROUPS				
HISTOLOGICAL PARAMETER OF OESOPHAGUS	GROUP I	GROUP II	GROUP III	GROUP IV
DIAMETER	Pelican	Hawk R. B. Gull F. Gull Owl Crow	Coot	Kingbird Swallow Sparrow
THICKNESS OF WALL	Pelican Hawk	Coot R. B. Gull F. Gull Owl Crow	Kingbird Swallow	Sparrow
LENGTH OF CONVOLUTIONS	Pelican	Hawk R. B. Gull F. Gull Owl	Coot Kingbird Crow	Sparrow
THICKNESS OF MUCOUS MEMBRANE	Swallow	Pelican Hawk R. B. Gull F. Gull Kingbird Crow Sparrow	Coot Owl	
THICKNESS OF LAMINA PROPRIA	Pelican	Hawk Coot	R. B. Gull F. Gull Owl Crow	Kingbird Swallow * GROUP V Sparrow
THICKNESS OF MUSCULARIS MUCOSAE	Pelican Hawk	Coot R. B. Gull F. Gull Owl Crow	Kingbird Swallow Sparrow	
THICKNESS OF SUBMUCOSA	Pelican	Hawk R. B. Gull F. Gull	Coot Owl Crow	Kingbird Swallow Sparrow
THICKNESS OF MUSCULARIS EXTERNA	Pelican Hawk	Coot R. B. Gull F. Gull Owl Kingbird Crow	Swallow Sparrow	

Table 2. Birds were categorized into histological groups which were determined by significance testing.

* *Group V recognized only for Lamina Propria.*

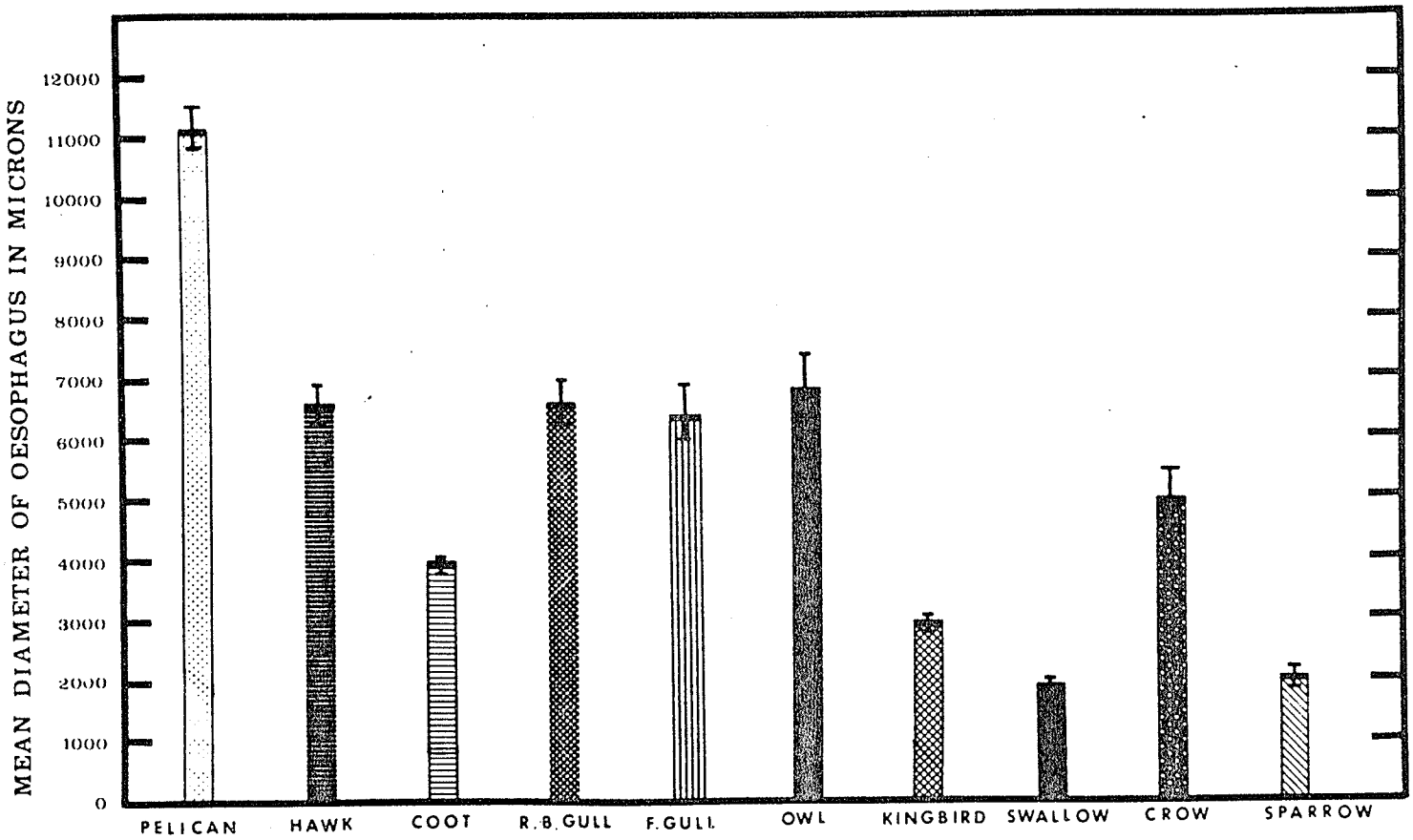

Figure 2. Histogram of mean diameter of oesophagus in ten different bird species.

THICKNESS OF THE OESOPHAGEAL WALL

The birds studied could be categorized into four groups on the basis of statistical differences in their oesophageal wall thickness and are listed in descending order and shown in Table 2. The oesophageal wall thickness in each of the white pelican and red-tailed hawk did not significantly differ from each other and were therefore grouped together (Table 2 and Figure 3). A second group of birds whose oesophageal wall thickness did not significantly differ from each other consisted of the American coot, ring-billed gull, Franklin's gull, great horned owl and common crow.

The oesophageal wall thickness in the tree swallow and eastern kingbird were grouped as being intermediate in small size when compared to other birds examined in this study. The house sparrow with an oesophageal wall thickness of 420±16 microns was compared to other birds and was found to have the thinnest oesophageal wall.

The histogram of Figure 3 illustrates graphically these significant differences.

LENGTH OF MUCOSAL CONVOLUTIONS

A fresh piece of oesophagus from a chicken which had long mucosal convolutions was tested for expansibility. Glass probes of various diameters were passed down the oesophageal tube. It was observed, with the aid of a dissection microscope, that the oesophageal convolutions did not retain their structural integrity during the distended condition. The functional significance of this finding is discussed in the next section of the thesis.

The birds could be categorized into four groups based on the

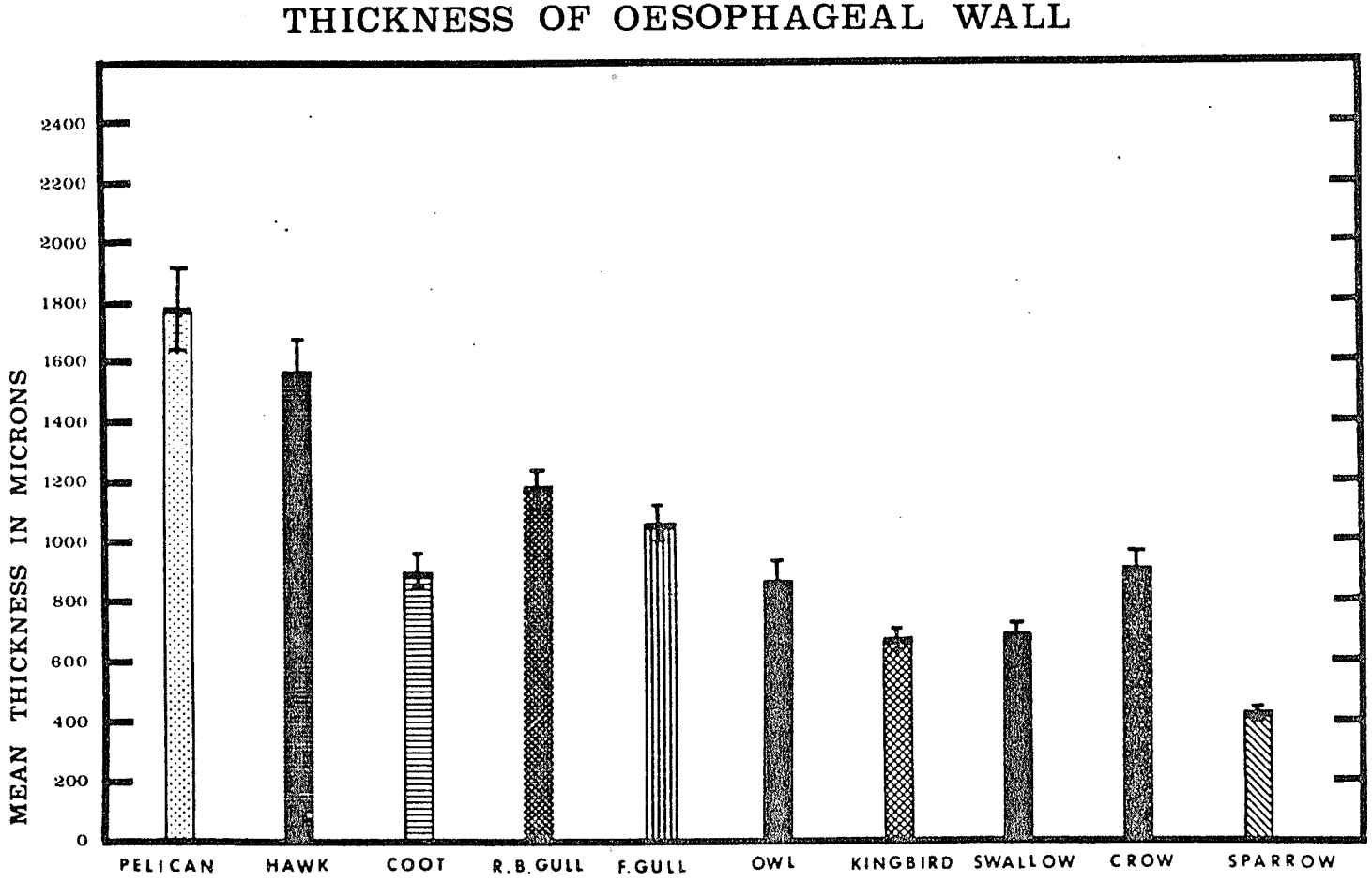

Figure 3. Histogram of mean thickness of oesophageal wall in ten different bird species.

descending order of the length of their mucosal convolutions (Table 2 and Figure 4).

The longest mucosal convolutions were found to exist in the white pelican in which the mean length was 3720±158 microns. The white pelican was singularly grouped for this histological measurement as a result of significance testing. A second group, next smaller in order from the white pelican, was tested for significant difference from other birds examined.

The mean length of convolutions in each of the red-tailed hawk, ring-billed gull, Franklin's gull and great horned owl were compared to each other. This second group was compared to the rest of the birds examined in this study. In this second group it was found that although the mean convolution size varied between birds the differences were small and were not statistically significant. The convolution size in the second group was next smaller in order and was found also to differ significantly when similarly compared to other birds. A third group of birds, and next in order of convolution size, consisted of the American coot, eastern kingbird and common crow. The mean size of the convolutions in the sparrow was 420±16 microns which was noted to be the smallest size and was placed in a group by itself. These groups are shown graphically in Figure 4.

THICKNESS OF OESOPHAGEAL MUCOUS MEMBRANE

Three groups of birds were recognized on the basis of thickness of the mucous membrane and are listed in a descending order. The thickest layer, 352±39 microns, was found to occur in the tree swallow and

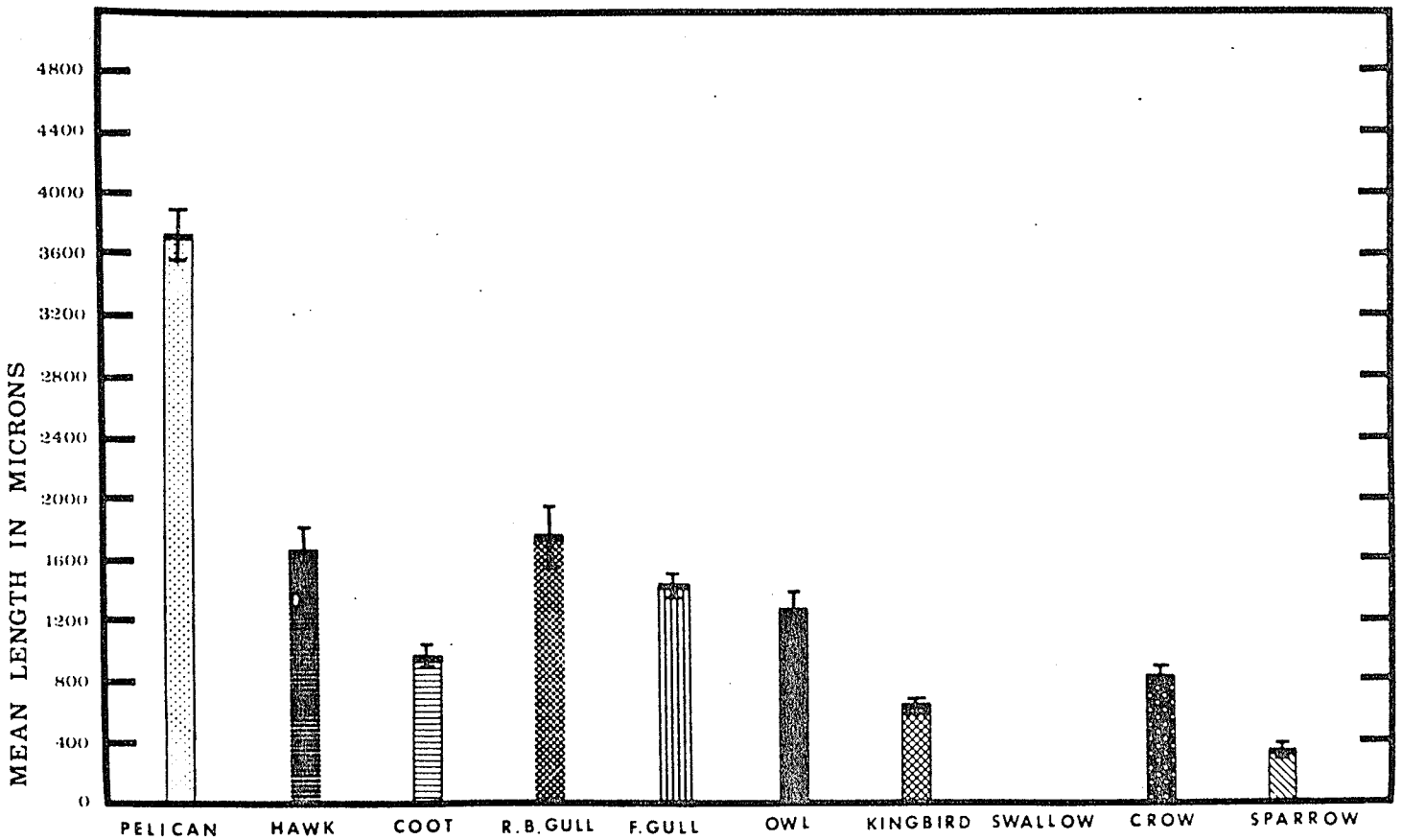

Figure 4. Histogram of mean length of mucosal convolutions in ten different bird species.

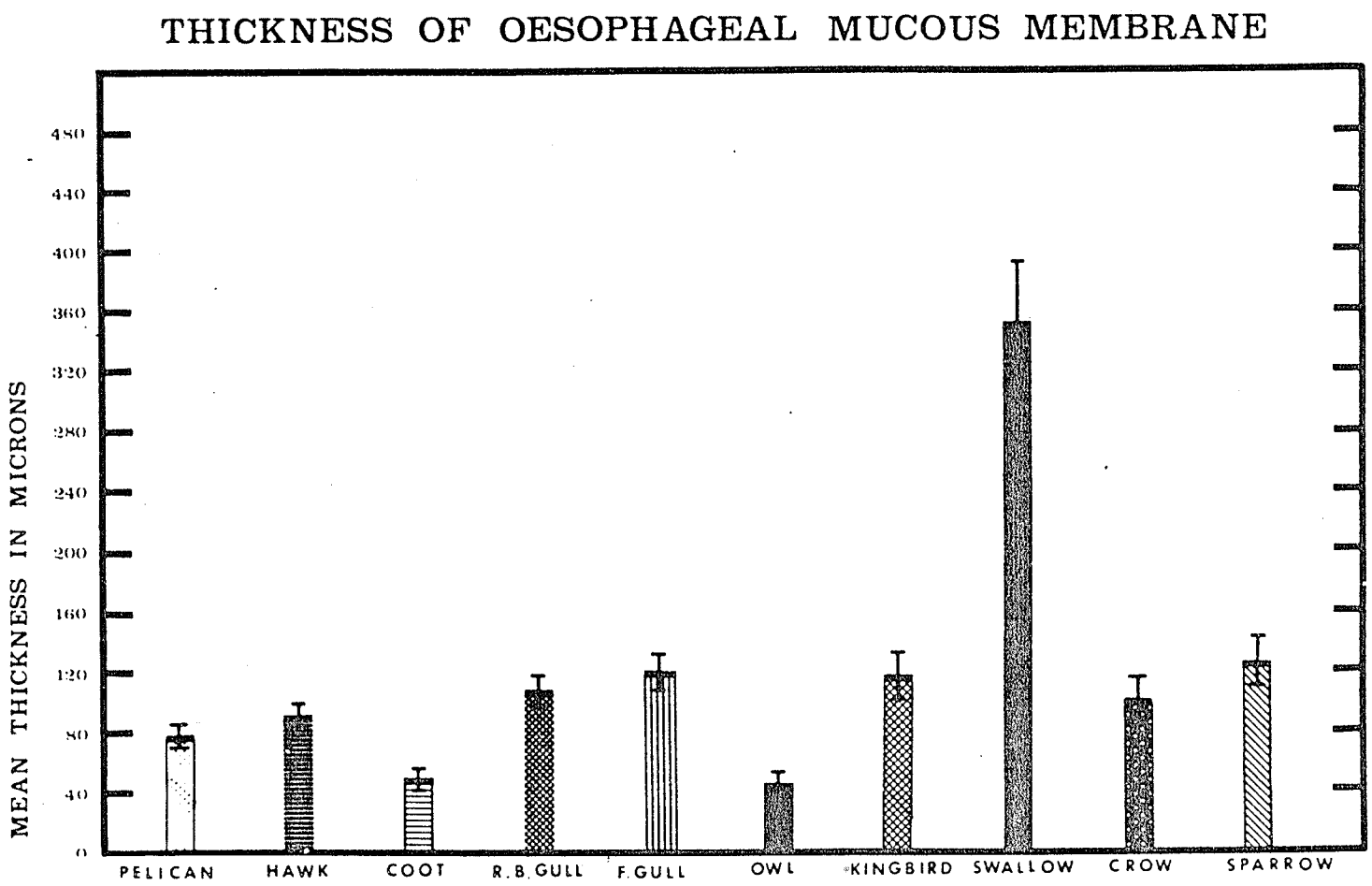

Figure 5. Histogram of mean thickness of oesophageal mucous membrane in ten different bird species.

is shown graphically in Table 1 and Figure 5. A second group of birds whose oesophageal mucous membrane was next thinner in order from the tree swallow and which did not significantly differ from each other consisted of: white pelican, red-tailed hawk, ring-billed gull, Franklin's gull, eastern kingbird, common crow and house sparrow. A third group consisting of the American coot and the great horned owl was noted to have the thinnest mucous membrane.

The significant difference between the second and third groups was estimated to be at the 5% level ($p \leq 0.05$).

The significant differences between the bird groups are shown graphically in the histogram of Figure 5.

THICKNESS OF OESOPHAGEAL LAMINA PROPRIA

The mean thickness of this layer was found to vary widely from one bird to another. However, the birds could be categorized into five groups and are listed in a descending order (Table 2 and Figure 6). The oesophageal lamina propria of the white pelican, when compared to other birds in this study, was found to be the thickest, 337±18 microns (Table 1). A second group of birds in which the thickness of oesophageal lamina propria did not significantly differ from each other consisted of the red-tailed hawk and American coot. A third category, which was arbitrarily grouped on the basis of observations with the aid of light microscopy included the ring-billed gull, Franklin's gull, great horned owl and common crow. A fourth group which consisted of the eastern kingbird and the tree swallow did not significantly differ from each other. The house sparrow was found to have the thinnest oesopha-

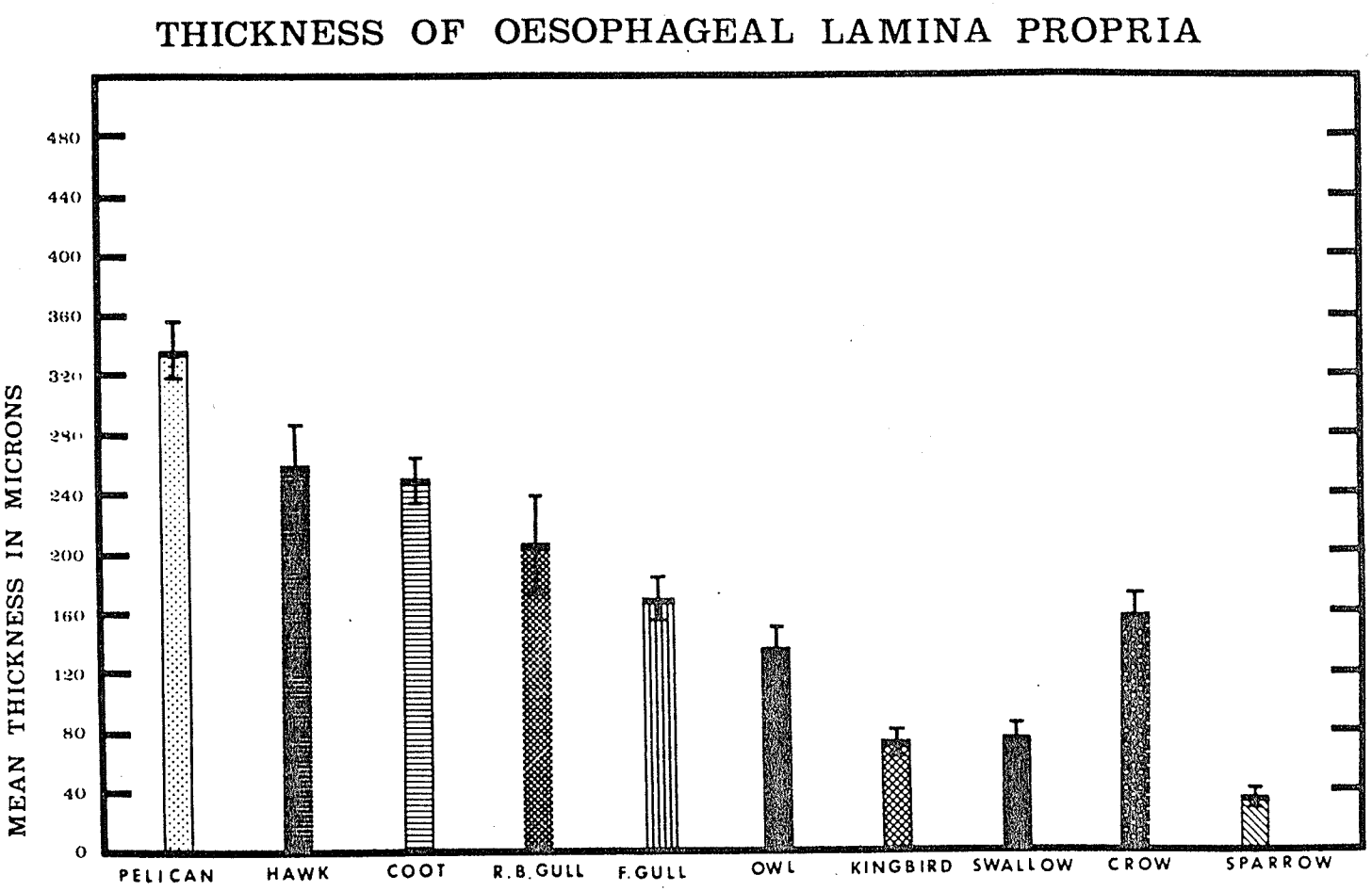

Figure 6. Histogram of mean thickness of oesophageal lamina propria in ten different bird species.

geal lamina propria, 35±4 microns, when compared to other birds in this study. The differences between bird groups are shown graphically in the histogram of Figure 6.

THICKNESS OF THE OESOPHAGEAL MUSCULARIS MUCOSAE

Three bird groups could be categorized on the basis of the thickness of their oesophageal muscularis mucosae and are listed in a descending order. The first group, which had the thickest oesophageal muscularis mucosae and did not significantly differ from each other, consisted of the white pelican and red-tailed hawk. The mean thicknesses of the oesophageal muscularis mucosae in the American coot, ring-billed gull, Franklin's gull, great horned owl and common crow were found not to significantly differ from each other and were therefore categorized as the second group. The third group consisted of those birds with the thinnest oesophageal muscularis mucosae and included the eastern kingbird, tree swallow and house sparrow. The eastern kingbird was arbitrarily included in this latter group. These results are shown graphically in Table 1 and Figure 7.

THICKNESS OF THE OESOPHAGEAL SUBMUCOSA

The birds could be categorized into five groups on the basis of the thickness of the oesophageal submucosa and are listed in a descending order. The oesophageal submucosa of the white pelican was thickest, 113±22 microns, when compared to other birds and was singularly grouped for this histological measurement. A second group of birds in which the oesophageal submucosa did not significantly differ from each other consisted of the red-tailed hawk, ring-billed gull and Franklin's

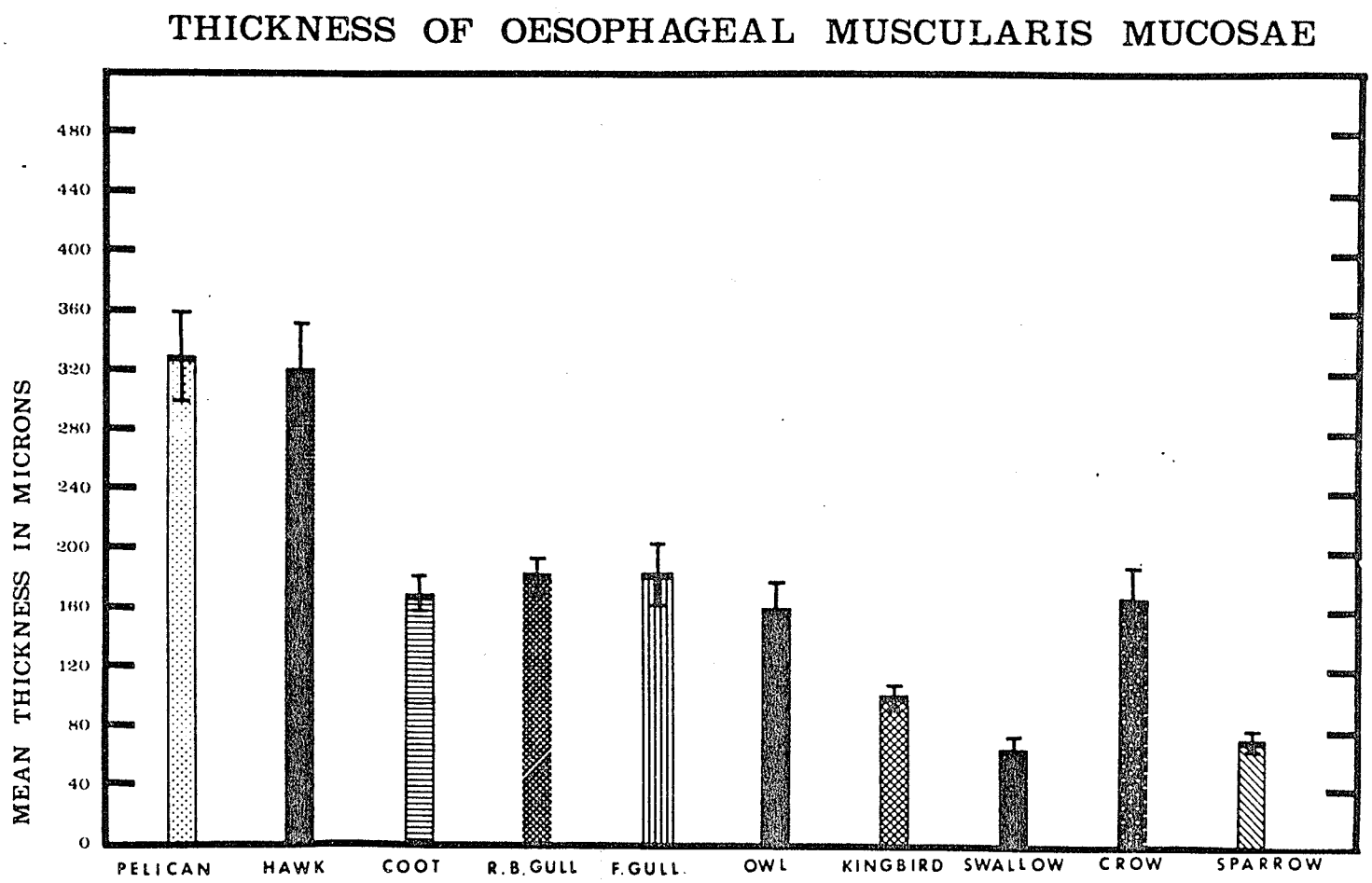

Figure 7. Histogram of mean thickness of oesophageal muscularis mucosae in ten different bird species.

gull. The oesophageal submucosa of the ring-billed gull was found to be intermediate in thickness when compared to the second and third groups and was therefore arbitrarily included in the second group. The third group consisted of the American coot, great horned owl and common crow. A fourth group consisted of the eastern kingbird, tree swallow and house sparrow. These results are shown graphically in Table 1 and Figure 8.

THICKNESS OF THE OESOPHAGEAL MUSCULARIS EXTERNA

The birds could be categorized into three groups on the basis of the thickness of the oesophageal muscularis externa and are listed in a descending order. The first group consisted of the white pelican and the red-tailed hawk. A second group whose thickness of oesophageal muscularis externa did not significantly differ from each other consisted of the American coot, ring-billed gull, Franklin's gull, great horned owl, eastern kingbird and common crow. The eastern kingbird was arbitrarily included in the second group. The third group consisted of the tree swallow and house sparrow. These results are shown graphically in the histograms of Figure 9.

THICKNESS OF OESOPHAGEAL ADVENTITIA

The histological thickness of the adventitia in the prepared slides appeared to be dependent upon the method of surgical excision and was therefore not analyzed.

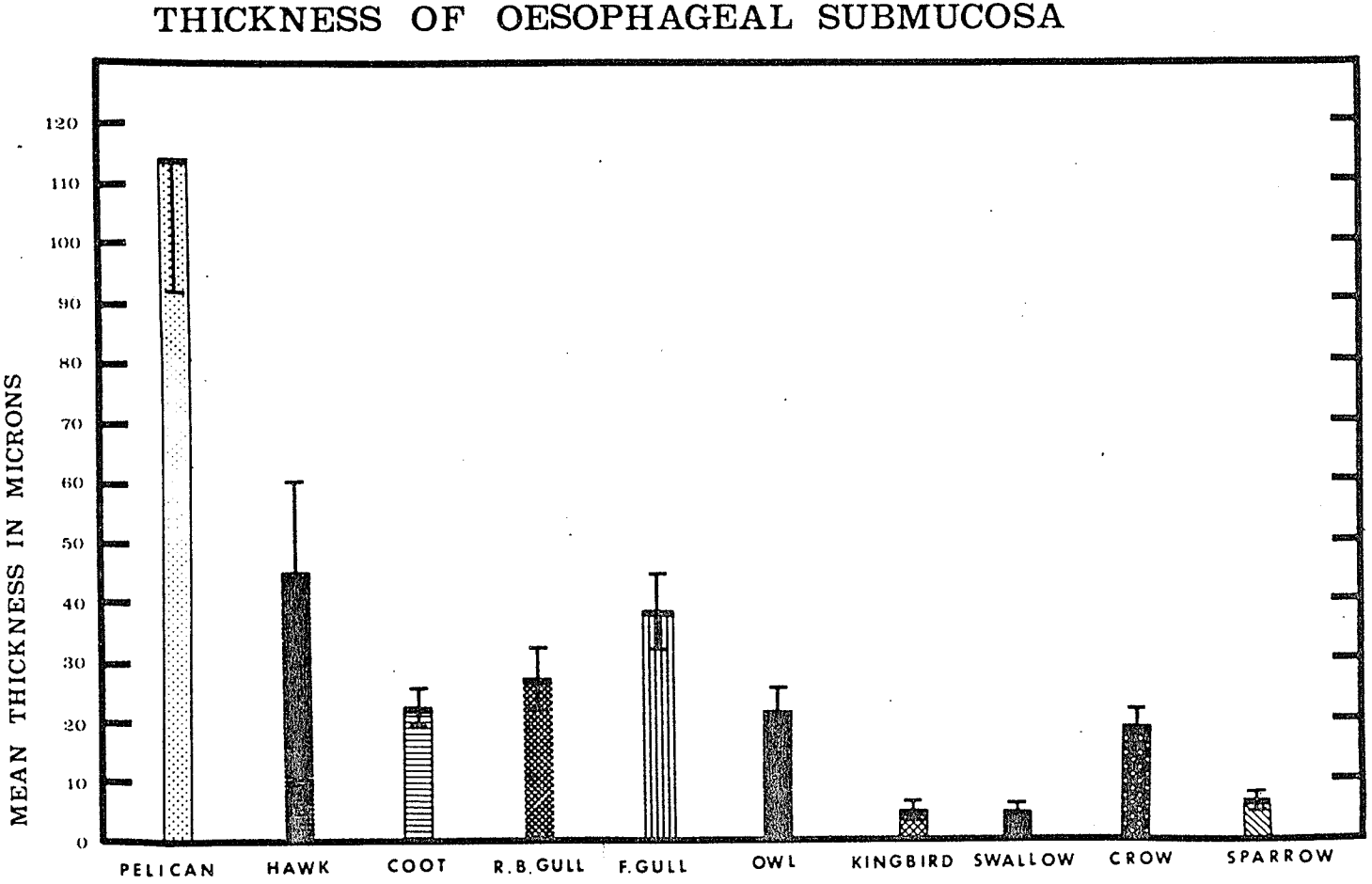

Figure 8. Histogram of mean thickness of oesophageal submucosa in ten different bird species.

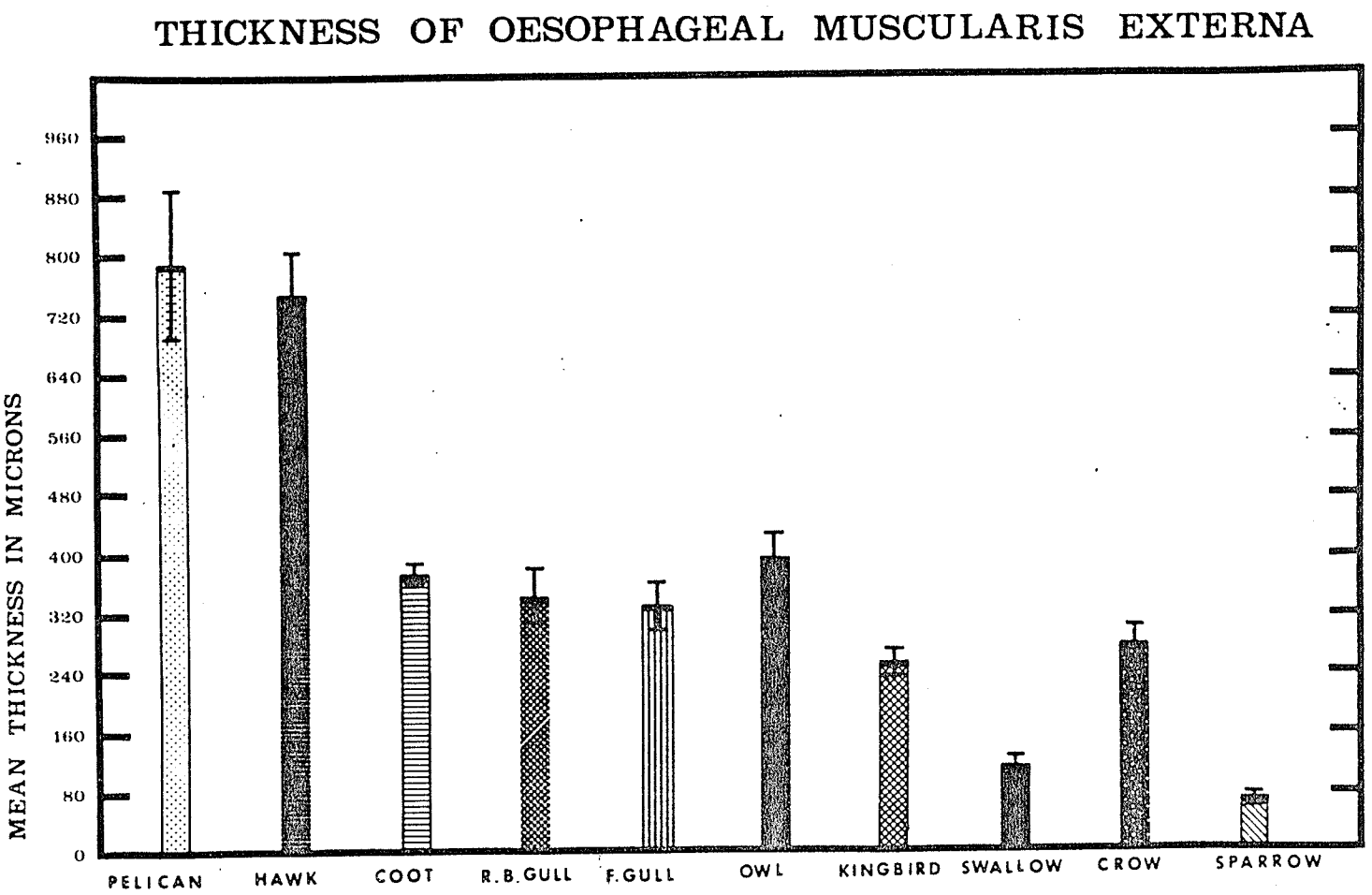

Figure 9. Histogram of mean thickness of oesophageal muscularis externa in ten different bird species.

RELATIVE THICKNESSES OF TISSUE LAYERS

PERCENT OF WALL THICKNESS / SPECIES	THICKNESS OF MUCOUS MEMBRANE IN PERCENT	THICKNESS OF LAMINA PROPRIA IN PERCENT	THICKNESS OF MUSCULARIS MUCOSAE IN PERCENT	THICKNESS OF MUSCULARIS EXTERNA IN PERCENT
Pelecanus erythrorhynchos	4.4	18.6	18.1	43.3
Buteo jamaicensis	5.6	16.1	19.8	46.4
Fulica americana	5.6	27.8	18.9	42.2
Larus delawarensis	11.3	21.6	18.6	35.1
Larus pipixcan	12.8	18.1	19.1	35.1
Bubo virginianus	5.6	15.7	18.0	45.0
Tyrannus tyrannus	19.2	11.2	16.0	41.6
Iridoprocne bicolor	53.8	11.5	10.8	18.5
Corvus brachyrhynchos	12.3	19.8	21.0	34.6
Passer domesticus	36.5	11.2	19.7	19.7

Table 3. Thickness of oesophageal layers expressed as percentile of oesophageal wall thickness.

DISCUSSION

The original purpose of this study was to describe and to compare the microscopic structure of the oesophagus in ten different bird species representing six taxonomic orders. An attempt would then be made to ascertain whether any histomorphological correlations existed between food utilized by each of the different birds. During the examination of the tissue sections it became apparent that other factors needed to be considered to account for the salient histological characteristics of the avian oesophagus.

PHYLOGENY

In a previous section of this thesis it was indicated that ornithologists could not agree upon any clear ancestral order for the birds used in the present study. Pelecaniformes was considered the most primitive while Passeriformes the most recent in phylogeny (Mayr and Amadon, 1951). No qualitative differences in the histology of the passerine oesophagus can be correctly interpreted as being more advanced when compared to the pelican oesophagus or even to that of any other group examined. The pelican oesophagus is histologically more complex when compared to the oesophagus found in each of the swallow and sparrow. Indeed, the oesophagus of the crow, a passerine, is histologically more comparable to the oesophagus of the pelican than to other taxonomically related passerines.

TAXONOMY

Birds in different taxonomic orders were grouped together on the basis of testing for significance for various histological measurements. The thicknesses of the mucous membranes in the Pelecaniformes, Falconiformes, Charadriiformes and Passeriformes does not significantly differ from each other. Groupings of birds from different taxonomic orders were made on the basis of the following:

a) thickness of the oesophageal wall included Gruiformes, Charadriiformes, Strigiformes and Passeriformes;
b) length of mucosal convolutions included Falconiformes, Charadriiformes and Strigiformes;
c) thickness of muscularis mucosae included Gruiformes, Charadriiformes, Strigiformes and Passeriformes;
d) thickness of muscularis externa included Gruiformes, Charadriiformes, Strigiformes and Passeriformes.

It is suggested therefore that the comparative histology of the avian oesophagus cannot be used to support nor reject the avian phylogenetic series or avian taxonomic divisions found in the current literature.

BIRD LENGTH

Bird length, as expressed by Codfrey (1966), could not be correlated with any of the histological parameters analyzed in this study. On the basis of testing for significance of oesophageal wall thickness the birds could be categorized into four groups (Table 2). When oesophageal wall thickness was plotted against bird length the coordinates were found to be scattered within the graph. It is concluded therefore that body length and oesophageal wall thickness do not exhibit a dependent relationship between each other.

FEEDING HABITS

A fundamental precept which was applied to this study can be summarized by the following:

> that all species of birds examined in this study were equally successful in their adaptation to their own feeding habits.

In the present study the various birds could be grouped also according to their basic feeding types: carnivorous, herbivorous, omnivorous. Insectivorous and piscivorous were categorized as subgroups of carnivorous, and graminivorous and granivorous as subgroups of herbivorous. The primiary food preferences utilized by the birds were grouped after a food-texture classification scheme by Szczesniak (1963). The relevant textural characteristics of food particles considered were gross geometrical size and shape of the food particle being swallowed. The histomorphological variations found in the various birds were then grouped, as shown in Table 2, and correlations with the above food categories were sought.

FUNCTION OF MUCOSA

The thickness of the mucous membrane does not increase in direct proportion with either the thickness of the oesophageal wall nor the lengths of the oesophageal convolutions (Table 2 and Figure 10). An excessively thick mucous membrane consisting of stratified squamous epithelium in an oesophagus lacking mucosal convolutions would narrow the diameter of the lumen and thus impede the mechanical flow (rheological) properties of the food. Excessively long mucosal convolutions which retain their structural integrity during maximum oesophageal

dilatation would similarily impede the passage of food. However, the presence of mucosal convolutions, as shown in an earlier section of this thesis, permits the oesophageal lumen to expand during the process of deglutition in order to accommodate the particle of food. On the basis of non-quantifiable microscopic observations it appears that in some birds, for example, pelican, kingbird, sparrow and swallow, the thickness of the mucous membrane has a tendency to be inversely proportional to the length of the mucosal convolutions. It is tempting to suggest also that in each bird an optimum size would be found for each of mucous membrane thickness and mucosal convolution length.

The birds used in this study were listed in a descending order according to the gross geometrical size of their primary food preferences (pages 19-22). The birds listed in the order are as follows: pelican, hawk, owl, gulls, crow, coot, kingbird, swallow and sparrow. This order parallels closely another listing of the same birds which was determined by the descending lengths of their mucosal convolutions (Table 2 and Figure 4). In this study it was found that only birds which lack pronounced mucosal convolutions, for example, swallow and sparrow, have primary food preferences which do not require oesophageal distention. It is interesting to note also that only in the latter birds does the mucosal lining consist of a relatively thick layer of non-distensible stratified squamous epithelium overlying a relatively sparse layer of mucous glands (Table 3). Some birds reduce the size of their food pieces with their bill, such as the hawk and the owl, so that the actual sizes and shapes of the food particles are therefore not constant. It can be expected that most material passing down the

avian oesophagus would be abrasive because of its unmasticated condition. This provides, in part, an explanation for the presence of many oesophageal mucous glands typically found in birds. Long mucosal convolutions are able to contain more mucous glands than short convolutions. These glands are known to actively secrete mucin which lubricates the inner exposed surfaces of the mucosa to facilitate the passage of large ingested materials. It is concluded, therefore, that in order to achieve adaptation at the histological level for the accommodation of large types of food in different bird species a direct relationship exists between length of mucosal convolution and oesophageal distensibility.

MUSCULATURE

In birds which utilize large food particles, for example, pelican, hawk, coot, both gulls, owl, kingbird and crow the two muscle layers of the oesophagus contribute between 54% and 66% of the wall thickness (Table 3). In the swallow and sparrow, which utilize small food particles, the two muscular layers contribute between 29% and 39% of the wall thickness. The thickness of the muscle layers between the two compared groups is significantly different ($p \leq 0.001$).

It is well known that the mechanism of peristaltic waves of muscular contraction of smooth muscle may be initiated through the innervation by the intramural nerve plexus or spontaneously as a result of distension of the gut tube. Oesophageal muscles are functionally important for the movement of ingested food materials. It is concluded, therefore, that a structure-function relationship exists be-

tween the extent of muscle thickness in the bird oesophagus and the capability of swallowing items included in its primary food preference.

SUMMARY

Several new and interesting adaptive characteristics have been demonstrated at the histological level in a study of the oesophagus in ten different species of birds. These characteristics are summarized as follows:

(1) The histology of the avian oesophagus does not show qualitative differences which can be interpreted as been structurally less differentiated or less adaptive in one group of birds.

(2) The comparative histology of the avian oesophagus cannot be used to support or reject an avian phylogenetic series or avian taxonomic divisions.

(3) Body length of birds cannot be correlated with histological measurements of the avian oesophagus.

(4) In the avian oesophagus the thickness of the mucous membrane has a tendency to be inversely proportional to the length of the mucosal convolutions.

(5) Mucosal convolutions facilitate expansion of the oesophageal lumen and are longest in those birds which swallow large food pieces.

(6) The number of mucous glands contained in the avian oesophagus is greatest in those birds which swallow large food pieces.

(7) Birds that lack mucosal convolutions have primary food preferences which do not require oesophageal distension.

(8) A structure-function relationship exists in the bird oesophagus between the extent of muscle thickness and the ability to

accommodate the gross geometrical size of the primary food preference.

(9) A histomorphological classification of the oesophagus in different birds closely parallels a classification scheme for the gross geometrical size of the primary food preference.

SUGGESTIONS FOR FUTURE INVESTIGATIONS

(1) In the present study the tussues to be examined was taken from the midregion of the oesophagus of each bird. An intensive comparative histological study of representative regions of the entire oesophagus might show additional structural variations.

(2) It is commonly believed that a crop is present in most birds. However, in the present study the crop was not readily identifiable at the gross level. Further studies at the histological level may ascertain whether this organ is always present even as a rudimentary structure.

(3) Histomorphological variations in the mucous membrane and mucosal convolutions, as well as some other features of the avian oesophagus, were shown to be related to the gross geometrical size of the primary food preference. It is suggested that a group of these same birds be reared from birth on a diet of an homogenous nutrient.

(4) The scope of the present study was designed to include an extensive range of birds in order to uncover general histomorphological variations in the avian oesophagus. It is suggested that this study be continued using representatives of other groups of birds.

(5) A suggestion for some future investigation, although not directly a part of this thesis, is a study of the gross structure of the ventriculus in birds. There appears to be great variation in the gross structure of this organ. This study would entail some aspects of embryonic development and histomorphological comparisons.

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APPENDIX

HISTOLOGICAL TECHNIQUES

FIXATIVES

Ten Percent Formalin Solution

37% - 40% formaldehyde	100 cc
Water	900 cc

(Armed Forces Laboratory Manual, 1960)

Tissues were fixed for 18 to 24 hours and then washed in running tap water for 8 hours.

Bouin's Fixative

Picric acid, saturated aqueous solution	750 cc
37% - 40% formaldehyde	250 cc
Glacial acetic acid	50 cc

(Humason, 1962)

Tissues were fixed for 18 to 24 hours and then washed in several changes of 50% ethyl alcohol to remove the picric acid. This removal was completed in the higher alcohols.

Zenker's Fixative

Potassium dichromate	25 gms
Mercuric bichloride	40 gms
Sodium sulfate	10 gms
Distilled water	1000 cc
Glacial acetic acid (added immediately before using)	50 cc

(Humason, 1962)

Tissues were fixed for 18 to 24 hours and then washed in run-

ning tap water for 8 hours.

FAA Fixative

37% - 40% formaldehyde.....	100 cc
95% ethyl alcohol.....	850 cc
Glacial acetic acid (added immediately before using).....	50 cc

(Galigher and Kozloff, 1964)

Tissues were fixed for 18 to 24 hours and then washed in running tap water for 8 hours.

DEHYDRATION PROCEDURES

Tissues were removed from running tap water and transferred to an ethyl alcohol series of increasing alcohol concentrations for complete dehydration.

ETHYL ALCOHOL SOLUTION	TIME
30%	1 hour
50%	1 hour
70%	1 hour
85%	1 hour
95% (1)	1 hour
95% (2)	1 hour
100% (1)	1.5 hours
100% (2)	1.5 hours

Tissues were dehydrated and then transferred, for a period of one hour, to a solution composed of one half absolute ethyl alcohol and one half toluene. The tissues were next transferred to toluene for clearing.

PREPARATION OF PARAFFIN BLOCKS - FLOW CHART

Tissues were removed from the bird by dissection, cut into small blocks not exceeding 10 mm^2 in size, and placed in fixative for a period of 18 to 24 hours.

SOLUTION	TIME	PROCESS
Fixative	18 - 24 hr	Fixation
*(Bouin's)		
Wash in running tap water	8 hours	Washing
30% Ethyl alcohol	1 hour	Dehydration
50% Ethyl alcohol	1 hour	
70% Ethyl alcohol	1 hour	
85% Ethyl alcohol	1 hour	
95% Ethyl alcohol (1)	1 hour	
95% Ethyl alcohol (2)	1 hour	
100% Ethyl alcohol (1)	1.5 hours	
100% Ethyl alcohol (2)	1.5 hours	
50/50 Alcohol/toluene	1 hour	
Toluene (1)	1.5 hours	Clearing
Toluene (2)	1.5 hours	
Melted paraffin (1)	1 hour	Infiltration
Melted paraffin (2)	1 hour	
Block tissue in paraffin		Embedding

*Tissues fixed in Bouin's fixative are washed in several changes of 50% ethyl alcohol.

PROCEDURE FOR DEPARAFFINIZATION AND HYDRATION OF SLIDES

Tissue sections were floated onto glass microscope slides and allowed to dry for a period of 72 hours.

SOLUTION	TIME
Xylene (1)	2 minutes
Xylene (2)	2 minutes
100% Ethyl alcohol (1)	1 minute
100% Ethyl alcohol (2)	1 minute
95% Ethyl alcohol	1 minute
85% Ethyl alcohol	1 minute
70% Ethyl alcohol	1 minute
50% Ethyl alcohol	1 minute
30% Ethyl alcohol	1 minute

Tissue sections were then rinsed in distilled water and next transferred to staining solutions.

STAINING

Ehrlich's Acid Alum Hematoxylin

Hematoxylin crystals	4 gms
95% Ethyl alcohol	200 cc
Distilled water	200 cc
Glycerin	200 cc
Ammonia alum	6 gms
Glacial acetic acid	20 gms

(Armed Forces Laboratory Manual, 1960; Humason, 1962)

The hematoxylin was dissolved in the 95% ethyl alcohol before the remaining materials were added. The stain was allowed to ripen for six weeks with exposure to light and air.

Alcoholic Eosin Solutions

A stock solution of alcoholic eosin was prepared from which working solutions could be made up when required.

Stock solution.

Eosin Y (water soluable)	1 gm
Distilled water	20 cc
Dissolve and add:	
95% Ethyl alcohol	80 cc

Working eosin solution.

Eosin: 1% stock solution	1 part
80% Ethyl alcohol	3 parts
Glacial acetic acid... 0.5 cc per 100 cc of stain	

(Armed Forces Laboratory Manual, 1960)

Hematoxylin stained the nuclear structures dark blue to blue whereas the cytoplasm and intercellular structures were stained varying shades of red and pink by the eosin.

Acid Alcohol

70% Ethyl alcohol	100 cc
Concentrated hydrochloric acid	1 cc

(Armed Forces Laboratory Manual, 1960)

Hematoxylin-Eosin Staining Procedure Following Hydration

SOLUTION	TIME
Ehrlich's hematoxylin	15 minutes
Distilled water	rinse
1% Acid alcohol	differentiate
Running tap water (blueing)	15 minutes
Eosin (working solution)	1 minute
70% Ethyl alcohol	differentiate
95% Ethyl alcohol	differentiate
100% Ethyl alcohol (1)	1 minute
100% Ethyl alcohol (2)	1 minute
50/50 Absolute ethyl alcohol/xylene	1 minute
Xylene (1)	1 minute
Xylene (2)	1 minute

Mount.

(Humason, 1962)

Lugol's Solution

Potassium iodide	2 gms
Iodine crystals	1 gm
Distilled water	100 cc

(Armed Forces Laboratory Manual, 1960)

Sodium Thiosulfate Solution (Hypo)

Sodium thiosulfate	5 gms
Distilled water	100 cc

(Armed Forces Laboratory Manual, 1960)

Mallory I Solution

Acid fuchsin, C.I. 42685	1 gm
Distilled water	100 cc

(Humason, 1962)

Mallory II Solution

Aniline blue (W.S.), C.I. 42780	0.5 gms
Orange G, C.I. 16230	1 gm
Distilled water	100 cc

(Humason, 1962)

Phosphomolybdic Acid Solution

Phosphomolybdic acid	1 gm
Distilled water	100 cc

(Humason, 1962)

Mallory Staining Procedure Following Hydration

SOLUTION	TIME
Mordant in saturated aqueous $HgCl_2$	10 minutes
Lugol's solution	3 minutes
Sodium thiosulfate solution	3 minutes
Distilled water	rinse
Mallory I solution	15 seconds
Distilled water	differentiate
Phosphomolybdic acid solution	1 to 5 mins.
Distilled water	rinse
Mallory II solution	2 minutes
Distilled water	rinse
90% Ethyl alcohol	differentiate
100% Ethyl alcohol (1)	1 minute
100% Ethyl alcohol (2)	1 minute
50/50 Absolute ethyl alcohol/Xylene	1 minute
Xylene (1)	1 minute
Xylene (2)	1 minute
Mount.	

(Humason, 1962)

Verhoeff's Staining Solution

Three grams of hematoxylin are dissolved in 66 cc of absolute ethyl alcohol which is heated on an electric hot plate. This solution is cooled and filtered. Next 24 cc of 10% aqueous ferric chloride and 24 cc of Verhoeff's iodide solution were added. The usefulness of this solution was limited to one to two weeks (Humason, 1962).

Verhoeff's Iodide Solution

Potassium iodide	4 gms
Distilled water	100 cc
Dissolve potassium iodide in water, then add	
Iodide crystals	2 gms

(Humason, 1962)

10% Ferric Chloride Solution

Ferric chloride	10 gms
Distilled water	100 cc

2% Ferric Chloride Solution

10% Ferric chloride solution	20 cc
Distilled water	100 cc

Picro-Ponceau Solution

Ponceau-S, C.F. 27195, 1% aqueous	10 cc
Picric acid, saturated aqueous	86 cc
Acetic acid, 1% aqueous	4 cc

(Humason, 1962)

Verhoeff's Staining Procedure

This staining procedure followed partial hydration to 70% ethyl alcohol.

SOLUTION	TIME
Verhoeff's staining solution	15 minutes
Distilled water	rinse
2% Ferric chloride solution	differentiate
5% Sodium thiosulfate	1 minute
Wash in running tap water	5 to 10 mins.
Picro-ponceau solution	1 minute
95% Ethyl alcohol (1)	differentiate
95% Ethyl alcohol (2)	differentiate
100% Ethyl alcohol (1)	1 minute
100% Ethyl alcohol (2)	1 minute
50/50 Absolute alcohol/xylene	1 minute
Xylene (1)	1 minute
Xylene (2)	1 minute
Mount.	

(Humason, 1962)

BIRD MEASUREMENTS

SPECIES	RANGE OF LENGTHS (inches)	MEDIAN LENGTH (inches)
Pelecanus erythrorhynchos	55.0 to 70.0	62.5
Buteo jamaicensis	19.0 to 24.0	21.5
Fulica americana	13.0 to 16.0	14.5
Larus delawarensis	18.0 to 20.0	19.0
Larus pipixcan	13.5 to 15.5	14.5
Bubo virginianus	18.0 to 25.0	21.5
Tyrannus tyrannus	7.8 to 8.7	8.3
Iridoprocne bicolor	5.0 to 6.2	5.6
Corvus brachyrhynchos	17.0 to 21.0	19.0
Passer domesticus	5.8 to 6.7	6.3

Appendix, Table 1. The above was taken from Godfrey (1966) and gives the ranges of bird lengths. From this information the median length of each species was determined and used as an indication of bird size. This is discussed in section four, Methods and Materials.