

HISTOMORPHOLOGICAL STUDIES ON STOMACH AND SMALL INTESTINE OF RABBIT (Oryctolagus cuniculus)



THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA, (SAMASTIPUR), BIHAR

(FACULTY OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY)

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

(VETERINARY ANATOMY)

By

Shri Narayan Parihar

(Registration No. - M/VAN/38/1997-98)

DEPARTMENT OF VETERINARY ANATOMY AND HISTOLOGY

BIHAR VETERINARY COLLEGE

PATNA - 800 014

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In partial fulfilment of the requirements
FOR THE DEGREE OF
MASTER OF VETERINARY SCIENCE
(VETERINARY ANATOMY)

BY
SHRI NARAYAN PARIHAR

(Registration No. M/VAN/38/1997-98)

Department of Veterinary Anatomy and Histology

BIHAR VETERINARY COLLEGE, PATNA-14.

2000

CHAM VET. & N. A. COLLEGE

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Date 30-3-2002

DEDICATED

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MY

RESPECTABLE

PARENTS

CERTIFICATE-I

This is to certify that the thesis entitled
"HISTOMORPHOLOGICAL STUDIES ON STOMACH AND
SMALL INTESTINE OF RABBIT (Oryctolagus cuniculus)"
submitted in partial fulfilment of the requirement for the degree
of Master of Veterinary Science (Veterinary Anatomy) of the
Faculty of Post-Graduate Studies, Rajendra Agricultural
University, Bihar is the record of bonafide research work carried
out by Dr. Shri Narayan Parihar (Regd. No. M/VAN/38/ 97 -
98) under my supervision and guidance. No part of this thesis
has been submitted for any other Degree or Diploma.

It is further certified that the assistance and help received
during the course of this investigation and preparation of the
thesis have been fully acknowledged.



(M.K. Roy)

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CERTIFICATE-II

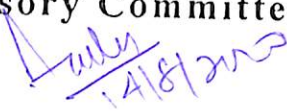
We, the undersigned, members of the Advisory Committee of **Dr. Shri Narayan Parihar** (Regd. No. M/VAN/38/97-98) a candidate for the **Degree of Master of Veterinary Science** with major in Veterinary Anatomy have gone through the manuscript of the thesis and agree that the thesis entitled **"HISTOMORPHOLOGICAL STUDIES ON STOMACH AND SMALL INTESTINE OF RABBIT (Oryctolagus cuniculus)"** may be submitted by **Dr. Shri Narayan Parihar** (Regd.No-M/VAN/38/97-98) in partial fulfilment of the requirements for the Degree.


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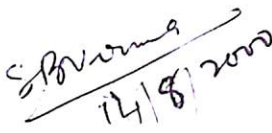
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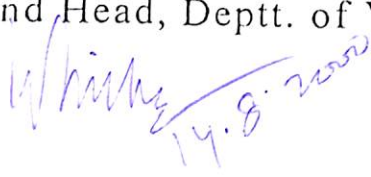
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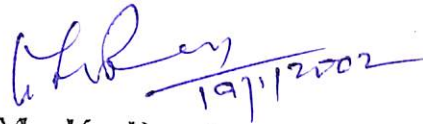

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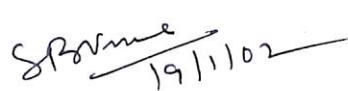
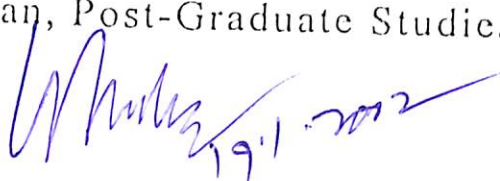
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INTRODUCTION

1. INTRODUCTION

In the recent past, special status has been provided for the maintenance of good quality of rabbit population in India. The economic value of rabbit population is directly dependent on the production of wool fibres, in addition to its meat quality for human consumption. Considering the economic value of rabbit in India, the Veterinary Council of India incorporated various aspects of this species in newly adopted Veterinary course curricula for under graduate programme.

Since there is no standard Veterinary Text Books in the field of Anatomy and Histology of rabbit's organ systems, it has become essential to explore the subjects extensively for fulfilment of the course requirement at under graduate level.

The digestive system forms one of the important system of the body. The digestion of food materials and absorption of micronutrients are associated with the physiological functions of stomach and small intestine. The functional activities of these organs are greatly depending on their structural components. Keeping in view the above facts, the histomorphological studies on stomach and small intestine have been under taken during the present project to fulfil the objectives. The findings of the project can be well utilized by the scientists working in various fields of veterinary science as well.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Stomach

Various authors described that wall of stomach consisted of four classical tunics. viz ; tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. They further subdivided tunica mucosa into lamina epithelialis , lamina propria and lamina muscularis mucosae (Trautmann and Fiebiger, 1960; Banks, 1981; Stinson and Calhoun, 1987 and Banerjee, 1993).

Tunica mucosa

Depending upon the histomorphological characters of tunica mucosa, the stomach in different animals had been described to contain a nonglandular or oesophageal part and a glandular part. Sloss (1954) described a small nonglandular part in pig stomach Stinson and Calhoun (1987) described that nonglandular part was small in pig, large in horse and greatest in ruminants. Nonglandular area was absent in carnivores. Banerjee (1993) also mentioned a small nonglandular oesophageal part in the stomach of rabbit.

Lamina epithelialis

Sloss (1954) studied on the microscopic anatomy of the digestive tract of pig and observed that the oesophageal portion of the stomach in the porcine was lined with a layer of stratified squamous epithelium. There was abrupt change of stratified squamous epithelium into simple columnar epithelium at the cardiac region of the stomach.

Trautmann and Fiebiger (1960) stated that the lamina epithelialis of the glandular stomach of domestic animals began abruptly at the zagged border of the thick stratified squamous epithelium of the oesophageal

mucosa. It consisted of a simple layer of high columnar cells with terminal bars. The ellipsoid nucleus lied in the basal half of the cell.

Rubin *et al.* (1968) discussed the role of immature or undifferentiated basophilic epithelial cells in the pit bases and upper most portion of mammalian gastric gland. They further discussed their transformation into surface cells, mucous neck cells and parietal cells.

Roy (1973) observed that the surface epithelium in the diverticulum and cardiac glandular zone of the porcine stomach was of columnar type with a single layer of cells showing an abrupt transition from the non-glandular oesophageal region of the stomach.

Roy (1974) also observed that the fundic mucosa of the porcine stomach was lined by simple columnar epithelium containing PAS- reactive cytoplasmic granules.

Malik and Prakash (1977) observed that the mucosa of the abomasum of buffalo and ox was lined by simple columnar epithelium. The free surface of these cells had a striated border which was obliterated at places in the fundic part of the abomasum. These cells extended down to line the gastric foveolae of pits and become shorter towards the bottom of the pits. Due to the profuse foldings of the epithelium, epithelium gave rise to the tubular glands.

Banks (1981) stated that the lamina epithelialis of the stomach of domestic animals, including the gastric pits, was a simple columnar epithelium. The lining cells were mucous secreting.

Chandramouly and Rao (1984) observed that the surface epithelial cells of fundic region of abomasum of buffalo lined the gastric pit and were

tall columnar with a broad apex and a narrow base. The apex presented a brush border.

Stinson and Calhoun (1987) reported that the mucosal surface of the glandular region of the stomach of domestic animals was lined with tall columnar cells whose mucous secretory product was released continuously and served as a protective coat that prevented autolysis of the mucosa.

Banerjee (1993) described that in rabbit, the glandular mucosa of stomach contained columnar epithelium.

Lamina propria

Trautmann and Fiebiger (1960) stated that the lamina propria of the stomach of domestic animals contained the gastric glands, supported by a delicate, sparse connective tissue framework. The connective tissue was predominantly reticular connective tissue.

Malik and Prakash (1977) observed that the lamina propria of the abomasum of buffalo and ox was made up of reticular areolar connective tissue. The tubular glands were distributed in the mucosa lying embedded in the reticular areolar connective tissue of the lamina propria which was relatively scanty in different glandular zones of the abomasum.

Banks (1981) stated that the lamina propria of the stomach of domestic animals was typical and consisted of loose connective tissue. Usually the connective tissue space had numerous mononucleated cells which impart a distinct hypercellularity. Scattered lymphatic follicles might be encountered.

Chandramouly and Rao (1984) stated that the lamina propria of fundic region of abomsum of buffalo showed loose connective tissue fibres, connective tissue cells including mast cells and smooth muscle cells.

Stinson and Calhoun (1987) reported that the most of the lamina propria of the glandular region of the stomach of domestic animals was occupied with gastric glands. So that only a few connective tissue fibres and cells were seen between them. Thus the glands were so extensive that it was often difficult to visualize the lamina propria in this area. However, the Lamina propria of nonglandular region was composed of irregularly arranged collagen, reticular and elastic fibres.

Gastric glands

Lamina propria accommodated different gastric glands. The mucosa of the glandular stomach has three distinct regions named according to the various glandular types present viz, cardiac, fundic and pyloric.

Cardiac glands

Bensley (1910) studied on the cardiac gland of mammalian stomach and observed that the cardiac glands were mucous glands. This conclusion was based on the fact that in every case examined, in staining the secretory contents of the cells of these glands with mucihaematin and mucicarmine. The cells of the cardiac glands were fundamentally different from the chief cells of the body of the fundus. The cardiac gland cells were closely related to the mucous cells of the neck of the fundus gland and to the pyloric gland cells.

Sloss (1954) studied on the microscopic anatomy of the digestive tract of Sus scrofa domestica and observed that the branched tubulo-acinar cardiac glands of the stomach in the porcine occupied a larger area in the stomach mucosa than either the fundic and pyloric glands.

Trautmann and Fiebiger (1960) stated that the cardiac glands of the stomach of domestic animals were highly branched and often coiled into a

ball. The body of the gland had a relatively wide lumen and was lined by pyramidal or cuboidal cells with a basally located nucleus. The cells contained granules in their distal portion. In the pig, the only domestic animal with a significant area of true cardiac glands, the cells in the deep portion of the gland were eosinophilic and did not give the characteristic reaction to mucin stains.

Roy (1973) observed that both the diverticulum ventriculi and cardiac glandular zone of the porcine stomach contained similar type of branched tubular glands. The glands opened in varying number into the deep gastric pits. The glandular epithelium was comprised of simple columnar cells which were arranged in the fine meshwork of reticular fibres. The glandular cells were more the cuboidal type than that of columnar towards the deeper parts of the glands. In the neck of the glands the cells were studded with numerous toluidine blue-reactive mucous granules at their supranuclear zone. The basally placed nuclei contained one or more prominent nucleoli and were frequently indented. The mucous granules were also strongly positive for PAS reaction.

Banks (1981) stated that the cardiac glands of the stomach of domestic animals were branched tubular coiled glands which consisted of a neck and body region. The neck and upper portion of the body was lined by cuboidal cells. The cuboidal lining cells were mucous secreting. The remaining cells of the gland were columnar mucous secreting cells. Some parietal cells might be present in the canine cardiac gland region, whereas some chief cells might be present in porcine cardiac glands.

Argentaffin cells were small, pyramidal cells with a clear cytoplasm that were located between the lining cells of the glands and the associated basement membrane.

Stinson and Calhoun (1987) reported that the cardiac gland region occupied a narrow strip at the junction of the glandular and nonglandular mucosae in all domestic animals except the pig, where it covered nearly half of the stomach, including the diverticulum ventriculi. The cardiac glands were branched coiled tubular glands that opened into the gastric pits. The body of the gland was relatively short and had a wide lumen than those of the fundic or pyloric glands. The mucous secretory epithelium was cuboidal with the nuclei located in the basal portion of the cells.

Banerjee (1993) described that in rabbit, the gastric glands of cardiac region were simple glands, formed by only mucin cells.

Fundic glands

Sloss (1954) studied on the microscopic anatomy of the digestive tract of Sus scrofa domestica and observed that the tubules in the fundic gland region of the stomach in the porcine appeared less branched than those in the cardiac gland region. The gastric crypts were lined by a cuboidal type of cell. The true fundic gland cells were of two types-the chief or peptic cells and parietal cells. In the neck of the fundic glands, the parietal cells appeared to surround the tube to the exclusion of most of the chief cells. In the body of the gland the chief cells assumed the position nearest the lumen and the parietal cells formed a complete band around them in a collar-like arrangement. The chief cells and mucous cells were in majority in the fundic portion of the glands. When a parietal cell did appear in the fundus of the gland, it lied outside the chief cells or mucous cells lining the tube,

although at times the cytoplasm of these cells projected towards the lumen between the chief and mucous cells.

Trautmann and Fiebiger (1960) stated that the body of the fundic glands of stomach of domestic animals contained two kinds of cells viz; chief cells and parietal cells.

The chief cells were cuboidal to columnar or wedge-shaped and contained rather coarse secretory granules. In hemalum-eosin preparations, the cytoplasm of the chief cells remained nearly unstained. Their nuclei were spheroid and were located near the basal end.

The parietal cells were larger than the chief cells. The central nucleus was occasionally double. The parietal cells stained deeply with acid dyes. The parietal cells were most numerous in the upper part of the body and in the neck of the gland, where they often occurred in groups. They were rarer in the gland fundus.

Deane and Padykula (1966) described that the surface mucous cells in human stomach covering the entire surface and lining the pits, were high columnar with basal nuclei. The apical cytoplasm appeared usually empty or foamy because the mucous droplets stained poorly with routine histological dyes. The mucous stained with PAS suggesting the neutral mucopolysaccharides. The neck mucous cells were smaller than the surface cells and contained mucicarmine and PAS-positive few droplets. These cells also stained with basic dyes suggesting the presence of acid mucopolysaccharides. They further described the chief cells as serozymogenic cells containing large quantity of basophilic materials in the basal cytoplasm.

Bloom and Fawcett (1968) described that chief or zymogenic cells in human stomach were arranged in lower half or third of glandular tubules.

After the death they began to disintegrate almost immediately, so that adequate preservation was difficult to achieve, although if there was no acid in stomach they might remain for sometime. In fresh condition, specially after a period of fasting, the cells were full of coarse, refractile granules. The mucous neck cells were reactive to mucicarmin, mucihaematin and PAS at their apical parts. The deep distribution of mucous neck cells were prominent in the glands near pyloric region. The glands of narrow intermediate zone contained usually mucous neck and parietal cells and was devoid of zymogenic cells.

Roy (1974) observed that the porcine fundic glands comprised neck mucous cells, chief cells and parietal cells.

The columnar shaped neck mucous cells appeared in more numbers in the neck and the base of the glands. The study revealed that the neck mucous cells were predominantly present in the base of the glands towards the pyloric region.

The chief cells were of low columnar type, having centrally located spherical nucleus and lightly stained basophilic cytoplasmic granules. The cells appeared in more numbers in the body and base portions of the fundic glands.

The large and nearly triangular parietal cells contained eosinophilic granules and spherical nucleus. They were more in number than the chief cells and appeared right from the neck of the glands. Rarely the cells reached the lumen of the glands. In the body, the parietal cells were numerous and were almost encircling the neck mucous and chief cells from outside. In the base of the glands, the cells had flattened appearance.

Malik and Prakash (1977) observed that the fundic glands of abomasum of buffalo and ox had mainly two kinds of cells in their epithelium viz; the chief cells and the parietal cells with a few mucous neck cells confined to the neck portions of the gastric glands. The chief cells were relatively smaller than the parietal cells and lined the tubular lumen of the glands. The parietal cells were present between the chief cells and the basement membrane and were spheroid to pyramidal in shape with rounded centrally placed nuclei. These cells were numerous in the upper part of the body and in the neck of fundic gland. However, they were less numerous in the fundus of the gland.

Banks (1981) stated that the fundic glands of the stomach of domestic animals were branched tubular glands which were longer than but less frequently branched than their cardiac region counterparts. The fundic gland was divisible into four regions : base, body, neck and isthmus. The isthmus or opening of the gland into the gastric pit was continuous with a constricted part of the gland, the neck. The body or main tubular portion of the gland continued from the neck and terminated as a slightly dilated and bent adenomere, the base.

Three cell types were distinguishable readily in routine preparations of fundic glands viz; chief cells (Zymogen cells) , parietal cells and mucous neck cells.

Mucous neck cells lined the constricted portions or neck of the gland and were interspersed among the parietal cells . The mucous neck cells were cuboidal or low columnar cells with a pale staining cytoplasm.

The chief cells were the predominant cells of the fundic glands. They were pyramidal shaped cells with a basally positioned round nucleus.

The parietal cells were large cells that were scattered throughout the gland from neck to base. Their spheroidal or pyramidal configuration with a round nucleus was a distinctive feature.

Chandramouly and Rao (1984) observed that the fundic glands of abomasum of buffalo were straight branched and tubular glands.

The chief cells were found in the body of the gland: they were cuboidal and were in cords along the lumen of the gland. The chief cells formed the largest group of cells. The PAS and PAS- alcian blue techniques demonstrated negative reactions revealing the absence of neutral mucopolysaccharides. Aldehyde fuchsin-alcian blue technique demonstrated a moderate concentration of acid mucopolysaccharides .

The parietal cells were confined to the neck and the body of the glands. Two types of parietal cells, viz; type 'A' and type 'B' were observed.

Type 'A' parietal cell was large, irregular with an indistinct contour. The cytoplasm was coarsely granular, vacuolated and eosinophilic . Its nucleus was spherical with dispersed chromatin and an indistinct nucleolus. The type 'A' parietal cells were regarded as mature parietal cells. PAS and PAS- alcian blue staining resulted in a very mild reaction.

Type 'B' parietal cell was small with a distinct contour. The cytoplasm was densely granular and eosinophilic. A small compressed oval or spindle shaped nucleus with condensed chromatin was located in the centre. These cells were considered as immature parietal cells.

Stinson and Calhoun (1987) reported that the fundic glands of the stomach of all domestic animals were straight branched tubular glands. Four structurally and functionally distinct cell types comprised the secretory

epithelium of the fundic gland : mucous neck cells, chief cells, parietal cells and argentaffin cells.

The mucous neck cells occupied the neck of the gland. They were typical mucous cells with the flat nucleus located towards the cell base.

The chief cells were the most numerous of the gastric gland cell. They were cuboidal or pyramidal with the spherical nucleus near the base of the cell. The basal area of the chief cell had a well developed rough ER resulting in a basophilic staining reaction.

The parietal cells were larger and less numerous than the chief cells. They had a tendency to occur singly, were pyramid shaped and were located peripheral to the chief cells. The parietal cell had a spherical nucleus.

Argentaffin cells were moderately abundant in the fundic gland as single cells wedged between the basement membrane and the chief cells. They were best demonstrated with silver stains which revealed small grannules in the cytoplasm.

Banerjee (1993) described that in rabbit, the gastric glands of fundic part were 'compound' or 'complex' gastric glands, formed by mucin cells, oxyntic cells and zymogen cells.

Pyloric glands

Sloss (1954) observed that the tubular pyloric glands of the stomach in the porcine did not branch to any considerable extent and were lined by simple columnar epithelium which gave a mucous stain when mucicarmin was employed.

Trautmann and Fiebiger (1960) stated that the pyloric glands of the stomach of domestic animals opened into much deeper pits and were also more branched than the fundic glands. In the deepest portion of the gastric

pits there was a gradual transition to glandular epithelium. The cells of the glandular epithelium were cuboidal or pyramidal. They contained a few dark granules but chiefly much larger, light mucigenous granules. The deeply staining nucleus was basal and often strongly flattened .

Malik and Prakash (1977) stated that the pyloric glands of abomasum of buffalo and ox opened in much deeper pits. The cells of the glandular epithelium were low columnar mucous type with flattened basal nuclei.

Banks (1981) stated that the pyloric glands of the stomach of domestic animals were short, simple or branched tubular glands. The predominant cells were the mucous secreting cells similar to those that occurred in the cardiac glands.

Stinson and Calhoun (1987) reported that the pyloric glands of the stomach of all domestic animals were branched, coiled and relatively short compared to the other gastric glands. The gastric pits were considerably deeper than those in the cardiac and fundic gland regions. The cells were mucous secreting and stained slightly basophilic. The flat nuclei were located at the base of the cell.

Banerjee (1993) mentioned that gastric glands of pyloric part in rabbit were simple gland similar to those of cardiac part.

Lamina muscularis mucosae

Trautmann and Fiebiger (1960) stated that the lamina muscularis mucosae of the stomach of domestic animals lied under the propria. The muscle fibres were irregularly interwoven or stratified. Fibre bundles extended into the propria to the vicinity of the surface epithelium.

Banks (1981) stated that the lamina muscularis mucosae of the stomach in domestic animals was present but its arrangement was variable.

Two to four muscle layers might comprise the lamina. The smooth muscle fibres were oriented longitudinally and circularly. Thin strands of smooth muscle extended into the lamina propria between the glands.

Stinson and Calhoun (1987) reported that the lamina muscularis mucosae of the glandular region of stomach of domestic animals was relatively thick usually comprising three layers ; an inner and outer circular layer and a middle longitudinal layer. Small bundles of smooth muscle fibres extended into the mucosa coursing through the connective tissue fibres between the gastric glands. This layer was, however, distinct in non-glandular part of tunica mucosa as well.

Banerjee (1993) described inner circular and outer longitudinal muscle layers in lamina muscularis mucosae in rabbit's stomach and considered the lamina to occupy submucosa.

Tunica submucosa

Trautmann and Fiebiger (1960) stated that the tunica submucosa of the stomach of domestic animals was composed of loose connective tissue and many elastic nets. It often contained numerous adipose cells as well as the large vessels, nerves and ganglia.

Malik and Prakash (1977) observed that the submucosal zone of abomasum of buffalo and ox consisted of loose areolar connective tissue with blood vessels.

Banks (1981) stated that the tunica submucosa of stomach in domestic animals was present and typical. Adipose tissue, loose connective tissue, blood vessels, nerve processes, ganglion cell cytons and lymphatics were present. The nerve fibres and ganglion cell cytons formed the submucosal plexus (Meissner's plexus).

Stinson and Calhoun (1987) reported that the submucosa of the stomach of domestic animals contained collagen fibres, fat and the submucosal nerve plexuses. At pylorico-duodenal junction, the submucosal intestinal glands were found in the submucosa of pyloric region.

Banerjee (1993) described that the submucosa of rabbit's stomach was made up of loose connective tissue containing blood vessels, nerves and fat cells. He further mentioned that in the submucosa, the thick lamina muscularis mucosae was located. The submucosa of nonglandular part contained mucous-secreting oesophageal glands in rabbit's stomach.

Tunica muscularis

Trautmann and Fiebiger (1960) stated that the tunica muscularis of the stomach of domestic animals was made up of an incomplete outer longitudinal layer and an outer oblique layer occurring near the cardia. In addition there is an inner circular layer, which was reinforced in the pyloric part of the stomach.

Malik and Prakash (1977) stated that the tunica muscularis of abomasum of buffalo and ox consisted of an inner thick circular and an outer thin longitudinal smooth muscle layer. Tunica muscularis in both, the fundic and pyloric parts of abomasum was significantly thicker in buffalo than in ox.

Banks (1981) stated that the tunica muscularis of stomach in domestic animals was present and typical. The nerves formed the myenteric plexus (Auerbach's plexus) between the inner and outer laminae of smooth muscle.

Stinson and Calhoun (1987) reported that the tunica muscularis externa of the stomach of domestic animals had three coats: an inner oblique, a middle circular and an outer longitudinal layer.

Banerjee (1993) reported outer longitudinal, middle circular and inner oblique muscle layers in rabbit.

Tunica serosa

Malik and Prakash (1977) observed that the tunica serosa of abomasum of buffalo and ox was thin and consisted of coarse areolar connective tissue with many elastic fibres and was covered with a single layer of squamous mesothelial cells.

Banks (1981) stated that the tunica serosa of stomach in domestic animals was present and typical.

Stinson and Calhoun (1987) reported that the tunica serosa of the stomach of domestic animals was composed of mesothelium overlying a layer of loose connective tissue.

Banerjee (1993) also described tunica serosa outside the muscular layer in case of stomach of rabbit.

2.2 Small Intestine

As a continuation of the digestive tube the small intestine of rabbit was modified highly for the secretion and absorption of materials. The small intestine was composed of duodenum, jejunum and ileum. The wall of the small intestine was composed of four layers i.e. tunica mucosa containing intestinal villi, tunica submucosa, tunica muscularis and tunica serosa.

Tunica mucosa

The tunica mucosa was the inner most layer of the small intestine. It was composed of lamina epithelialis, lamina propria and a lamina muscularis mucosae. The tunica mucosa of small intestine presented numerous finger-like mucosal projections, the intestinal villi throughout the entire length.

Jacobson and Noer (1952) found gradual transition from closely packed, broad, blunt villi in jejunum to sparsely packed, flattened, leaf-like roughly triangular villi in the lower ileum of rabbit. They also found ribbon-like variety of villus in upper part of the bowel. In dog, the villi were more uniform. They were cylindrical with rounded ends and were lying close together. On the antimesenteric border and occasionally in other portions of the circumference, short relatively broad villi were seen lying between the larger and more typical forms. In man, the villi of ileum resembled to those of rabbit and were sparsely distributed and usually conical in shape with an occasional broad stubby variant.

Stinson and Calhoun (1987) reported that the finger-like intestinal villi varied in length depending on the regions and species of the animals. They further reported presence of a single lymphatic capillary, the lacteal, located in the centre of lamina propria of the villus.

Lamina epithelialis

Trautmann and Fiebiger (1960) stated that the surface epithelium of the small intestine of domestic animals was simple and consisted of columnar and goblet cells. The columnar cells were narrow and high and reached their greatest height on the villi. Their free surface showed a clearly defined border which frequently appeared to be striated.

Suzuki *et al.* (1963) studied the histological structure of intestinal epithelium of loach. They found that the epithelium was composed of single layer of columnar cells. These cells were slender and had a striated border. The nucleus was round or oval being placed on the basal part of the cell.

Barnwal and Yadava (1975) reported columnar epithelium lining the villi of small intestine of buffalo. They further reported the columnar absorptive cells and goblet cells in the epithelium.

Banks (1981) stated that the lining cells of the small intestine of domestic animals were typical columnar epithelial cells.

Stinson and Calhoun (1987) stated that the lumen of the small intestine of domestic animals was lined by simple columnar epithelium with numerous goblet cells interspersed among the columnar cells.

Lamina propria

Blank (1950) found that the lamina propria of duodenum in cotton rat contained diffuse lymphoid tissue. In many areas of the tunica mucosa the lymphatic tissue were dense, forming solitary ovoid nodules.

Barnwal and Yadava (1975) reported that the lamina propria of small intestine in buffalo was made up of loose connective tissue. The mucosal glands present in the propria did not present paneth cells.

Banks (1981) stated that the accumulation of reticular fibres, reticular cells, granulocytes and agranulocytes in the lamina propria of the small intestine of domestic animals.

Stinson and Calhoun (1987) stated that the lamina propria of small intestine of domestic animals was of loose connective tissue that formed the core of the villi and surrounded the intestinal glands. It was composed of collagen and elastic fibres supported by a reticular fibre framework. They further described the simple tubular intestinal glands were lined by a variety of cell types. The undifferentiated cells were thought to multiply and differentiate and finally were migrated into the villus giving rise to absorptive columnar cells and goblet cells.

Lamina muscularis mucosae

Calhoun (1954) stated that the muscularis mucosae in chicken was comprised of an outer circular and an inner longitudinal layer. The latter sent fibres into the villi. In places the outer circular layer appeared to fuse with the circular layer of the lamina muscularis.

Titkemeyer and Calhoun (1955) found that in all cases viz ; cow, horse, pig, sheep, dog, cat and goat, muscularis mucosae comprised of two layers – an inner circular and outer longitudinal one.

Trautmann and Fiebiger (1960) stated that in domestic animals muscularis mucosae of the small intestine was composed of smooth muscle fibres arranged in two sheets perpendicular to one another. In areas of duodenal glands and in some cases in the area of Peyer's patches this layer was discontinuous and even absent in part because it splited in separate strands which dipped into the glandular layer.

Stinson and Calhoun (1987) stated that the muscularis mucosae of the small intestine of domestic animals was composed of inner circular and outer longitudinal layers of smooth muscle.

Tunica submucosa

Trautmann and Fiebiger (1960) stated that the tunica submucosa of the small intestine of domestic animals consisted of loose fibrillar connective tissue and elastic fibre nets. It contained fat cells, lymph nodules, autonomic ganglia, nerves and vessels.

Banks (1981) stated that the tunica submucosa in the small intestine of domestic animals was present and typical. Submucosal glands were simple branched tubuloacinar glands that opened into the crypts. They might be mucous (ruminant, dog), mixed (cat) or serous (horse, pig). These glands

were variously referred to as Brunner's glands or duodenal glands. They were most properly referred to as intestinal submucosal glands.

Binson and Calhoun (1987) stated that the submucosa of small intestine of domestic animals was a connective tissue layer of collagen and elastic fibre bundles located between the muscularis mucosae and the tunica muscularis. The tubuloalveolar submucosal glands (Brunner's glands), located within this connective tissue network, opened into the base of the intestinal mucosal glands. They were mucous in the dog and ruminants, serous in the pig and horse and mixed in the cat. The lumen of the glands was largest in the goat and smallest in the pig. Since the glands were variable in distribution in different species, the term "Submucosal gland" was more appropriate than the term "Duodenal gland".

Tunica muscularis

Titkemeyer and Calhoun (1955) found that in dog there was an extra oblique layer of muscle fibres in between the submucosa and inner circular layer. In other animals there was only two distinct layers.

Trautmann and Fiebiger (1960) stated that the tunica muscularis of the small intestine of domestic animals consisted of an outer thinner longitudinal layer and an inner circular layer. The two layers were connected by the inter-muscular connective tissue.

Kakharov (1963) found that in man the intramuscular network of lymphatic capillaries gave origin to lymphatic vessels of the lateral aspect of the intestine. The vascular supply of lamina muscularis were by straight and recurrent branches as well as by a number of arterioles coming directly from the mesentry.

Banks (1981) stated that the tunica muscularis of the small intestine in the domestic animals was present with Auerbach's plexus and was typical. The contraction of this smooth muscle was responsible for peristalsis.

Stinson and Calhoun (1987) stated that the tunica muscularis of small intestine of the domestic animals consisted of inner circular and outer longitudinal smooth muscle layers. They further reported the thickest tunica muscularis in horse, in which the two layers were nearly equal in thickness.

Tunica Serosa

Finerty and Cowdry (1960) and Bloom and Fawcett (1968) stated that the serosa in the small intestine in man comprised of loose connective tissue, covered by a layer of mesothelium.

Banks (1981) stated that the tunica serosa of the small intestine in the domestic animals was present and was typical.

Stinson and Calhoun (1987) stated that the tunica serosa of small intestine in domestic animals was a layer of loose connective tissue invested by a mesothelium of the visceral peritoneum.

Banerjee (1993) reported tunica serosa in rabbit's stomach to be lined with mesothelium.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation was conducted on twelve adult rabbits which included six male and six female. The animals were apparently healthy and free from diseases.

The stomach and small intestine were collected from each animal after humane slaughter. Immediately after the slaughter the tissue samples from stomach and small intestine were collected in desired fixative solutions. All together tissue samples were collected from seven areas of stomach and from eight areas of small intestine to cover up all the representative areas of the organs.

The following fixative solutions were used during the study (Luna, 1980).

- (1) 10% Neutral buffered formalin solution
- (2) Zenker's solution
- (3) Helley's solution
- (4) Bouin's solution
- (5) Chilled acetone

After the proper fixation, the tissues were processed for various histological and histochemical methods such as washing, dehydration, clearing, paraffin infiltration and microtomy (Humason, 1967 and Luna, 1968). The paraffin sections were cut at 5 to 7 μm thickness with the help of rotary microtome. The paraffin sections were then stained with different standard staining procedures.

The following histological and histochemical staining methods were employed.

- (1) Haematoxylin and Eosin stain for routine study (Luna, 1968).
- (2) Van Gieson's stain for collagen and muscle fibres (Luna, 1968).
- (3) Verhoeff's elastin stain for elastic fibres (Humason, 1967).
- (4) Gomori's reticulin stain for reticular fibres (Humason, 1967).
- (5) Mallory-Heidenhain's Azan stain for connective tissue, muscle fibres etc. (Humason, 1967).
- (6) Periodic Acid Schiff for mucosubstances. (Pearse, 1968).
- (7) Modification of Mowry's colloidal iron stain for acid mucopolysaccharides. (Luna, 1968).
- (8) Periodic Acid Schiff orange-G stain for mucoprotein (Pearse, 1968).
- (9) Meyer's mucicarmine method for mucin (Luna, 1968).
- (10) Gomori's method for alkaline phosphatase (Davenport, 1969).

The stained sections were mounted with DPX. Micrometry of different parameters of stomach and small intestine were conducted with the help of calibrated ocular micrometer as detailed below.

| Organs | Structures | Parameters |
|---|---|------------|
| A. Stomach | I. <u>Tunica mucosa</u> | Thickness |
| | of three different | |
| | glandular regions. | |
| | II. <u>Lamina</u> | Thickness |
| | <u>muscularis</u> | |
| | <u>mucosae</u> of three | |
| | different glandular regions | |
| | I. <u>Lamina epithelialis</u> | Height |
| | of three different | |
| | segments | |
| B. Small intestine | II. <u>Lamina muscularis</u> | Thickness |
| | <u>mucosae</u> of three | |
| | different segments | |
| | III. <u>Tunica muscularis</u> of | |
| | three different segments | |
| | including that of sacculus rotundus | |
| | separately. | |
| | a. Inner layer | Thickness |
| | b. Outer layer | Thickness |
| | Statistical analysis of different measurements were done as | |
| per Snedecor and Cochran (1967). | | |
| Photomicrographs were taken to substantiate the | | |
| observations made during the study. | | |

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Stomach

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RESULTS AND DISCUSSION

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4. Results and Discussion

4.1 Stomach

The tunica mucosa of stomach in rabbit had a smaller non-glandular and a larger glandular parts. The nonglandular part was localised at craniodorsal position of the organ just close to its junction with oesophagus. The glandular portion was comprised of cardiac glandular zone, fundic glandular zone and pyloric glandular zone. Of the three glandular zones, the cardiac and pyloric glandular zones were adherent to nonglandular area of the stomach of rabbit.

Banerjee (1993) also described oesophageal, cardiac, fundic and pyloric parts in the stomach of rabbit.

Histologically, the wall of the stomach in rabbit presented four different tunics viz; tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. Similar histological tunics had been described by Trautmann and Fiebiger (1960), Banks (1981), Stinson and Calhoun (1987) and Banerjee (1993) in the stomach of different domestic animals including rabbit.

Nonglandular part

Tunica mucosa

The tunica mucosa was comprised of three classical sub-layers namely lamina epithelialis, lamina propria and lamina muscularis mucosae.

Lamina epithelialis

The surface epithelium of nonglandular part of stomach of rabbit was made up of stratified squamous epithelium of non-

keratinised variety. The transition between nonglandular and glandular epithelia was abrupt. Occasionally obliquely oriented mucosal glands, particularly of cardiac glandular region, invaded the stratified squamous epithelium at certain level (Figs. 1, 2 & 3). The present findings were in agreement with the findings of Sloss (1954) in pig, Trautmann, and Fiebiger (1960) in certain domestic animals and Roy (1973) in pig, who reported abrupt transition between the nonglandular and glandular epithelia of stomach.

Lamina propria

It was scanty, made up of coarsely arranged collagen fibres alongwith few elastic fibres (Figs. 1&3). Connective tissue cell population was significantly low with few heterochromatic fibrocytes and lymphocytes. Stinson and Calhoun (1987) also described that the lamina propria of nonglandular region of stomach in different domestic animals was composed of irregularly arranged connective tissue fibres of all the three varieties.

Lamina muscularis mucosae

It was made up of irregularly arranged smooth muscle fibres which were possibly oriented in oblique manner. The continuous layer was lacking as the muscle bundles were separated from each other by interfascicular connective tissue (Figs. 1&3). Stinson and Calhoun (1987) described a distinct lamina muscularis mucosae in the nonglandular part of stomach of different domestic animals. Banerjee (1993) also reported

lamina muscularis mucosae in rabbit's stomach but he considered this layer as a part of tunica submucosa.

Tunica submucosa

The tunica submucosa was comprised of loose connective tissue predominantly made up of collagen fibres and connective tissue cells. Apart from blood vessels and nerves the submucosa contained occasionally wide lumened glandular alveoli of seromucous nature. These glandular units were supposed to be extension of oesophageal submucosal gland in the nonglandular part of stomach (Figs. 1&3). The present observations were in agreement with the descriptions made by Trautmann and Fiebiger (1960) in the stomach of different domestic animals. Banerjee (1993) described mucous secreting oesophageal glands in the oesophageal part of stomach in rabbit.

Tunica muscularis

The tunica muscularis of nonglandular region of stomach was extensively thick containing irregularly oriented smooth muscle fibres in association with striated muscle fibres. Such intermingling of muscle fibres was predominated near the tunica submucosa (Figs. 1 & 4). The outer part of tunica muscularis was mainly made up of smooth muscle fibres which were regularly arranged in their long axes. The myenteric plexuses were randomly distributed between the muscle bundles surrounded by densely arranged collagen fibres.

The invasion of striated muscle fibres in the tunica muscularis of nonglandular part of stomach was probably due to

extension of striated muscle fibres from the tunica muscularis portion of oesophagus from the point of junction between oesophagus and stomach.

Tunica Serosa

The tunica serosa of nonglandular part of stomach was continuous with tunica serosa of glandular part. It was made up of loose connective tissue, rich in blood vessels and lymph vessels and was lined with single layer of flattened mesothelial cells.

Glandular part

The glandular part of the stomach microscopically presented similar layers and sublayers in its wall like those of nonglandular part.

Tunica mucosa

The mean, standard error and C.V.% of thickness of tunica mucosa of different glandular regions of rabbit's stomach have been presented in Table No. 1. The tunica mucosa of different glandular regions of stomach measured differently. The average thickness of tunica mucosa of cardiac, fundic and pyloric glandular regions was measured as $463.56 \pm 9.49 \mu\text{m}$, $401.13 \pm 3.25 \mu\text{m}$ and $392.65 \pm 8.59 \mu\text{m}$ respectively.

Analysis of variance (Table No. 2) showing the effect of different glandular regions of stomach on mucosal thickness revealed highly significant difference ($P < 0.01$). The mean thickness of tunica mucosa of cardiac glandular region was significantly ($P < 0.01$) higher by $62.43 \mu\text{m}$ and $70.91 \mu\text{m}$ than

the mean thickness of tunica mucosae of fundic and pyloric glandular regions respectively. No significant difference was noticed between the mean thickness of fundic and pyloric glandular regions.

The tunica mucosa of pylorico-duodenal junction presented irregular line of junction. A part of tunica mucosa resembled like that of proper pyloric glandular area whereas adjoining area at the same level showed mucosal villi suggesting the beginning of small intestine. Therefore, the line of junction between pyloric glandular area and the duodenal mucosa appeared to be interdigitating (Fig. 14).

Lamina epithelialis

The lamina epithelialis was formed by simple columnar epithelium that constituted surface epithelium and the epithelium of gastric pits (Figs. 5, 7, 10 & 13). The cells were tall columnar with basally placed oval or elliptical nucleus. The supranuclear cytoplasm was clear with routine haematoxylin and eosin stain. The cells of the pit epithelium were slightly narrower and shorter than the cells of surface epithelium. Histochemically, the surface epithelium showed mild positivity with mucicarmine and PAS stain suggesting the presence of epithelial mucin and neutral mucopolysaccharides. The pit epithelium rarely showed positivity for mucicarmine and PAS stain. The secretory materials, adhered with the free borders of these cells of both surface and pit epithelium, were mild to moderately reactive for mucicarmine, PAS and colloidal iron reaction. The epithelium

did not react for alkaline phosphatase. The present investigations on lamina epithelialis of rabbit's stomach could be well compared with the findings of Sloss (1954) in the cardiac region of porcine stomach, Trautmann and Fiebiger (1960) in the glandular stomach of domestic animals, Roy (1973, 1974) in cardiac and fundic part of pig stomach, Malik and Prakash (1977) in glandular mucosa of abomasum in buffalo and ox, Banks (1981) in the stomach of domestic animals, Chandramouly and Rao (1984) in abomasum of buffalo, Stinson and Calhoun (1987) in abomasum of buffalo, Stinson and Calhoun (1987) in the glandular region of stomach in domestic animals and Banerjee (1993) in the glandular parts of rabbit's stomach.

Majority of nonmucinogenous cells in the pit epithelium, recorded during the present study, might be involved in the replacement of surface epithelium and mucous secreting cells in the different gastric glands as discussed by Rubin *et al.* (1968) in normal human gastric epithelium.

Lamina Propria

The lamina propria was made up of loose connective tissue and was more distinct towards the surface epithelium and near the lamina muscularis mucosae (Figs. 5, 13 & 15). It was made up of fine collagenous and a few reticular fibres along with scattered fibroblasts and lymphocytes. In between the surface epithelium and lamina muscularis mucosae, the lamina propria condensed due to distribution of various types of gastric glands. A few smooth muscle fibres derived from lamina muscularis

mucosae invaded the lamina propria around the glandular tubules along with connective tissue fibres (Fig. 13). The lamina propria of cardiac glandular region usually presented accumulation of lymphocytes at its deepest part. Such lymphocytic accumulation was also occasionally observed at the deepest part of lamina propria of pyloric glandular region.

The presence of loose connective tissue in the lamina propria of rabbit's stomach was in accordance to the report made by Trautmann and Fiebiger (1960) in domestic animals, Malik and Prakash (1977) in buffalo and ox, Banks (1981) in domestic animals, Chandramouly and Rao (1984) in buffalo abomasum and Stinson and Calhoun (1987) in the domestic animals. Banks (1981) also reported distribution of scattered lymphatic follicles in the lamina propria of stomach in different domestic animals.

Gastric Glands

As per histomorphology of tunica mucosa, the stomach of rabbit showed three different types of gastric glands. These glands were distributed in the lamina propria and were eventually formed by inpocketing of surface epithelium through gastric pits. According to the distribution of these three types of glands, the tunica mucosa of glandular part of rabbit's stomach was differentiated into cardiac gastric gland region, fundic gastric gland region and pyloric gastric gland region.

Cardiac gastric gland

The cardiac gastric glands were simple branched coiled tubular glands usually extended from the level of gastric pits up

to the level of lamina muscularis mucosae (Figs. 2, 5 & 6). The glandular cells were cuboidal or low columnar type having spherical nucleus placed slightly basal to the centre. The supra-nuclear cytoplasm was pale stained during routine preparation with haematoxylin eosin. Histochemically, these cells showed mild reaction for mucicarmine and PAS suggesting the positivity for neutral mucopolysaccharides. The cardiac glands closer to oesophageal junction where cells were mild to moderately reactive for PAS stain with PAS-orange G schedule, suggesting the presence of glycoprotein. The cardiac gastric glands were negative for colloidal iron stain and alkaline phosphatase (Table No. 3).

The present observation did not agree with the findings of Sloss (1954) who described the cardiac gastric glands in pig stomach as branched tubulo-acinar type of gland. Trautmann and Fiebiger (1960) described highly branched and often coiled cardiac glands in the domestic animals. Roy (1973) recorded branched tubular glands in the cardiac diverticulum and the cardiac glandular zone of porcine stomach. The variable reaction with PAS and mucicarmine stains in the glandular cells of cardiac glands in rabbit's stomach could be well compared with the similar findings of Bensley (1910) in cardiac glands of mammalian stomach, Roy (1973) in pig, Banks (1981) and Stinson and Calhoun (1987) in domestic animals. Banerjee (1993) also described that the gastric glands of cardiac region in

rabbit's stomach were simple glands and were formed by only mucin cells.

Fundic gastric gland

The fundic gastric glands in the stomach of rabbit were simple straight tubular glands occasionally showing branching. They extended from the base of the gastric pits up to the level of lamina muscularis mucosae (Fig. 7). Each tubular gland was comprised of short neck, a long body and a base or fundus. Occasionally the deepest part of the tubular gland near the base appeared to be slightly coiled with an wider central lumen (Figs. 7 & 8).

Three different cell types were observed viz; mucous neck cells, parietal cells and chief cells as special staining for argentaffin cells was not employed during present studies.

Roy (1974) in pig, Banks (1981) and Stinson and Calhoun (1987) in domestic animals reported mucous neck cells, chief cells and parietal cells apart from the argentaffin cells in the fundic gastric glands. Banerjee (1993) described the gastric gland of the fundic part in the stomach of rabbit as "compound" or "complex" gastric glands formed by mucin cells, oxyntic cells and zymogen cells. He probably opined such nomenclature due to the distribution of several different cell types in the fundic gastric gland which were not so in case of cardiac and pyloric gastric glands in rabbit's stomach.

Mucous neck cells

The mucous neck cells were identified as low columnar cells with parabasally placed oval nucleus. The supranuclear cytoplasm was slightly eosinophilic whereas basal cytoplasm appeared mildly basophilic with routine haematoxylin-eosin stain (Fig. 7). These cells were usually distributed at the neck and in the upper part of the body in association with parietal cells in the fundic gastric glands, located towards the cardiac glandular region. The mucous neck cells were also found at the bases of fundic gastric gland which were located towards the pyloric glandular area. There the cells were large pyramidal and contained coarse basophilic granules (Fig. 7).

Histochemically, the apical cytoplasm was mildly reactive for mucicarmine and PAS stain suggesting the presence of mucin and neutral mucopolysaccharides (Fig. 11). The presence of acid mucopolysaccharide was also recorded in these cells when stained with colloidal iron technique (Fig. 10). It did not react for alkaline phosphatase (Table No. 3). The present observations were in accordance with the descriptions of Deane and Padykula (1966) who reported mucicarmine and PAS positive cytoplasmic droplets in "neck mucous cell" in the human fundic glands. They also mentioned the presence of acid mucopolysaccharides in these cells as the droplets were also stained with basic dyes. Similarly Bloom and Fawcett (1968) described that the apical part of these cells were reactive for mucicarmine, mucihaematin and PAS. They further reported deep distribution of mucous neck

cells prominently in the fundic gastric gland near the pyloric region. Roy (1974) also recorded mucous neck cells at the bases of fundic gastric gland of pig stomach.

Parietal cells

The parietal cells predominated all the cell types in the fundic gastric gland of rabbit. The neck and the body of the glands remained thickly populated with large oval or triangular parietal cells having large spherical or oval nucleus. The cytoplasm was highly acidophilic which took the stain of eosin during routine haematoxylin-eosin stain (Figs. 7, 8 & 9). The cytoplasm appeared orange when stained with PAS-orange G technique. With Azan stain, the cytoplasm of parietal cells appeared reddish orange in colour. Histochemically, the parietal cells were negative for mucicarmine, PAS, colloidal iron stain and stain for alkaline phosphatase (Table no. 3). The present observations were in agreement with the findings of Sloss (1954) in fundic gland of pig stomach, Trautmann and Fiebiger (1960) in domestic animals and Malik and Prakash (1977) in buffalo and ox who described chief and parietal cells as two glandular cells of fundic gastric gland. During present observations only one type of parietal cells were observed. In contrast, Chandramouly and Rao (1984) reported two varieties of parietal cells in the fundic gastric gland of abomasum of buffalo as type "A" mature cells and type "B" immature cells. The mature parietal cells of buffalo abomasum reacted mildly for PAS and PAS-alcian blue stainings. Stinson and Calhoun (1987) also reported that parietal

cells were larger and less numerous than chief cells in the fundic gastric gland of domestic animals.

Chief cells

The chief cells were of low columnar type having slightly oval nucleus. The cytoplasm was faintly basophilic. These cells were more frequently found at the body of the fundic gastric gland though their distribution was occasionally at the base area also (Figs. 8 & 9). The cytoplasm was negative for PAS, mucicarmine-colloidal iron reaction and alkaline phosphatase (Table no. 3). Deane and Padykula (1966) reported the distribution of chief cells at the lower portion of the gland and were more numerous towards cardiac end of the organ. They further described these cells as sero- zymogenic cells containing large quantity of basophilic materials in the basal cytoplasm. Bloom and Fawcett (1968) described that in human stomach, the chief cells were arranged in lower half or third of the glandular tubules. They opined that after death they began to disintegrate immediately making the preservation of zymogen granules more difficult. They further reported that the zymogenic granules might remain for sometime if there was no acid in the stomach. During the present observation the granules of the chief cells failed to show distinct basophilia probably due to high acidic medium of the stomach caused by the secretion of HCl through predominantly distributed parietal cells in the fundic region of the rabbit. The present observation was in contrast to the findings of Chandramouly and Rao (1984) who observed the

chief cells as largest group of cells in the fundic gastric glands of abomasum in buffalo with moderate concentration of acid mucopolysaccharides.

Pyloric gastric gland

The pyloric gastric glands were remarkably shorter and were simple branched coiled tubular glands (Fig. 13). They opened into the gastric pits which were very deep covering more than half of the total thickness of the tunica mucosa. The pyloric glandular cells were pyramidal or columnar in shape and contained basally placed flattened nucleus. The supranuclear cytoplasm was pale. The glandular cells were mildly positive for PAS & mucicarmine and moderately positive for colloidal iron reaction (Figs. 15 & 16; Table No. 3). The pyloric glandular cells did not react for alkaline phosphatase. Near the junction with duodenum, the pyloric gastric mucosa contained comparatively shorter gastric pits with more coiled pyloric gastric glands. These glands were intensely reactive for PAS, mucicarmine and colloidal iron reactions than the glands of the other part of the pyloric gastric area (Figs. 14 & 16).

The present findings were in agreement with the findings of the Sloss (1954) who observed mucicarmine positive, simple columnar epithelial cells in the tubular pyloric glands of porcine stomach. Trautmann and Fiebiger (1960) in domestic animals and Malik and Prakash (1977) in abomasum of buffalo and ox also described flattened basal nuclei in the mucous secreting cells of the pyloric gastric glands.

During present investigation the mucous secreting cells of pyloric gastric glands were histomorphologically different from the cells of cardiac gastric glands of the stomach of rabbit. This observation was in contrast to the descriptions made by Banks (1981) in domestic animals and Banerjee (1993) in rabbit where the pyloric and cardiac gastric glandular cells were reported to be similar histologically. However, Stinson and Calhoun (1987) reported branched coiled and relatively short pyloric glands in domestic animals.

Lamina muscularis mucosae

The lamina muscularis mucosae was made up of smooth muscle cells which were irregularly arranged in inner circular and outer longitudinal layers. Frequently the smooth muscle cells from inner circular layer extended in the lamina propria between the glandular tubules (Figs. 5, 6, 7, 8, 11, 13 & 15). The mean, standard error and C.V.% of the thickness of the lamina muscularis mucosae have been presented in Table No-4. The average thickness of muscularis mucosae was highest in pyloric glandular region and lowest in the fundic glandular region. The mean thickness of lamina muscularis mucosae measured $53.46 \pm 3.37 \mu\text{m}$ in cardiac glandular region, $28.60 \pm 1.88 \mu\text{m}$ in fundic glandular region and $95.26 \pm 3.77 \mu\text{m}$ in pyloric glandular region of rabbit's stomach.

The analysis of variance (Table No. 5) showing the effect of different glandular regions on thickness of lamina muscularis mucosae revealed highly significant difference ($P < 0.01$). The

mean thickness of pyloric glandular region was significantly ($P < 0.01$) higher by $41.80\ \mu\text{m}$ and $66.66\ \mu\text{m}$ than the mean thickness of lamina muscularis mucosae of cardiac and fundic glandular regions respectively. Similarly, mean thickness of lamina muscularis mucosae of cardiac glandular region was significantly ($P < 0.01$) higher by $24.86\ \mu\text{m}$ than the mean thickness of lamina muscularis mucosae of the fundic glandular region.

The present investigation of lamina muscularis mucosae could be well compared with the descriptions made by Trautmann and Fiebiger (1960), Banks (1981) and Stinson and Calhoun (1987) in the stomach of domestic animals. Banks (1981) recorded two to four muscle layers whereas Stinson and Calhoun (1987) described three layers of smooth muscles in the lamina muscularis mucosae of the stomach of domestic animals. Banerjee (1993) however described two sublayers of lamina muscularis mucosae considering them as parts of submucosa in the rabbit's stomach.

Extension of smooth muscle fibres in the lamina propria had also been reported by Stinson and Calhoun (1987). These fibres might be playing some role for the expulsion of glandular secretion as well as to enhance the circulation of blood in the area.

Tunica submucosa

The tunica submucosa of the stomach of rabbit was made up of loose connective tissue. A few elastic fibres were also

occasionally observed. The connective tissue of submucosa appeared to be continuous peripherally with the connective tissue components surrounding the muscle bundles of tunica muscularis (Figs. 5, 13, 14 & 16). The blood vessels, submucosal nerve plexuses and lymph spaces were usually distributed in all the regions of stomach. A few lymphocytic aggregations were seen in the submucosa adjoining the lamina muscularis mucosae. Trautmann and Fiebiger (1960) in domestic animals, Malik and Prakash (1977) in abomasum of buffalo and ox, Banks (1981) and Stinson and Calhoun (1987) in domestic animals and Banerjee (1993) in rabbit also reported similar description of submucosa. During present investigation fat cells were not observed in the tunica submucosa of rabbit stomach. However, at the pylorico-duodenal junction the submucosa contained submucosal glands (Figs. 14 & 16) which were mucous secreting in nature. These submucosal glands were thought to be extended from interdigitating duodenal submucosa. Stinson and Calhoun (1987) have also described the presence of submucosal duodenal glands in the submucosa of pyloric region near the junction of the stomach and small intestine.

Tunica muscularis

The tunica muscularis of glandular part of the stomach was made up of smooth muscle cells. Near the cardiac glandular region, the tunica muscularis was comprised of an inner oblique, a middle circular and an outer longitudinal smooth muscle layers (Fig. 5). Near the middle of the fundic region, the three muscle

layers converged showing inner circular and outer longitudinal muscle layers (Fig. 12).

Present investigation was in contrast with the descriptions made by Trautmann and Fiebiger (1960) in domestic animals who mentioned only two layers of *tunica muscularis* in general. They further mentioned a third layer near the pyloric end. Malik and Prakash (1977) in the abomasum of buffalo and ox and Banks (1981) in the stomach of domestic animals reported two layers of *tunica muscularis*. Stinson and Calhoun (1987) however described three coats in the *tunica muscularis externa* of stomach in domestic animals. Banerjee (1993) also reported outer longitudinal, middle circular and inner oblique muscle layers in the *tunica muscularis* of rabbit stomach.

The myenteric plexuses were frequently present between the circular and longitudinal muscle layers surrounded by inter-muscular connective tissue (Fig. 12).

Tunica Serosa

The *tunica serosa* of rabbit's stomach was made up of loose connective tissue containing blood vessels and was lined by flattened mesothelial cells exteriorly (Fig. 14). Similar descriptions were made by Malik and Prakash (1977) in the abomasum of buffalo and ox, Banks (1981) and Stinson and Calhoun (1987) in the stomach of domestic animals and Banerjee (1993) in the stomach of rabbit.

4.2 Small intestine

Small intestine of rabbit was comprised of cranial duodenum, middle jejunum and caudal ileum. The caudal ileum was comparatively straight, terminating over caecum by an expanded end known as Sacculus rotundus of ileum. By gross observation, the three segments of small intestine could not be demarkated from each other very sharply. Banerjee (1993) however, described duodenum and ileum as two distinct part of small intestine in rabbit.

Histomorphologically, the wall of small intestine presented tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The tunica mucosa was further comprised of lamina epithelialis, lamina propria and lamina muscularis mucosae.

Tunica mucosa

The small intestine presented numerous finger-like mucosal projections, the intestinal villi throughout the entire length (Figs. 17, 18, 25, 26, 27, & 28). These projections were leaf-like or tongue-shaped in most of the area of duodenum. At the level of plica circulares, present at the cranial part of duodenum, the villi were comparatively longer and tortuous, as compared to the villi of the other part of the mucous membrane. Such variation was probably due to the contraction of the villi at certain levels of intestinal wall exhibiting their physiological activities. The villi of jejunum were regularly distributed as long finger-like projections. However, those present on the longitudinal mucosal folds were smaller than the villi of other areas. The height of the

villi diminished towards the caudal segment of the small intestine and at the sacculus rotundus of the ileum the villi appeared as small pyramids with broad bases (Fig. 29). The measurement of the height of villi could not be ascertained due to their irregular profiles and tangential sections caused by contraction and relaxation of villi for physiological purposes. These type of irregular profile might be due to fixation of the tissue as well.

The present finding was in agreement with the findings of Jacobson and Noer (1952) who reported gradual transition from closely packed, broad blunt villi in jejunum to sparsely packed flattened leaf-like roughly triangular villi in the lower ileum of rabbit. The ribbon-like variety of villi in the upper part of bowel as observed by them could be well compared with the long tortuous villi found in the cranial segment of duodenum during the present investigation. Stinson and Calhoun (1987) also reported variation in the height of villi was according to the region of small intestine and species.

Each villus of rabbit's small intestine was comprised of a central core of connective tissue and a peripheral epithelial lining. The connective tissue of the central core was comprised of fine collagen and reticular fibres along with fibroblasts and lymphocytes. A fine calibered lymph vessel, the lacteal, lined with endothelium was present at the centre of the connective tissue core of the each villus. The lacteals were more prominently observed at the apices of villi than the body and the bases. Isolated smooth muscle fibres were also distributed along

the long axis of villi within the connective tissue core. Stinson and Calhoun (1987) also reported similar type of descriptions in the central connective tissue core of villi in the small intestine of different domestic animals.

Lamina epithelialis

The lamina epithelialis was made up of single layer of columnar cells covering the villi throughout. Two definite cell types were observed viz; columnar absorptive cells and goblet cells. The columnar absorptive cells contained oval nuclei placed parabasally (Figs. 18 & 25). The free border of these cells presented a striated border which was prominently stained by aniline-blue with Azan stain (Fig. 27). The supranuclear cytoplasm appeared clear. The goblet cells were distributed between columnar absorptive cells and were less in number in the duodenal segment as compared to the jejunal and ileac segments (Figs. 20, 21, & 29). Histochemically, goblet cells were reactive for mucicarmine, PAS and colloidal iron reaction and negative for alkaline phosphatase. However the striated border of absorptive columnar cells showed inconstant mild positivity for alkaline phosphatase (Table No. 6). The intraepithelial lymphocytes were also present in the epithelial lining which were supposed to be migrated from central core of intestinal villi.

The present observations could be well compared with the descriptions made for small intestine of domestic animals (Trautmann and Fiebiger, 1960; Barnwal and Yadava, 1975; Banks, 1981 and Stinson and Calhoun 1987). Suzuki et al. (1963)

also reported a single layer of columnar cell in the intestinal epithelium of loach. The cells were slender having striated border.

The mean, standard error and C.V.% of epithelial height of different segments of small intestine of rabbit have been presented in Table No. 7. The epithelial height of small intestine of rabbit differed in duodenum, jejunum and ileum. The average height of the epithelium measured $27.46 \pm 0.65 \mu\text{m}$ in duodenum, $34.40 \pm 1.36 \mu\text{m}$ in jejunum and $24.49 \pm 0.57 \mu\text{m}$ in ileum.

Analysis of variance (Table No. 8) of different segments of small intestine of rabbit on epithelial height revealed significant difference ($P < 0.01$). The mean epithelial height of jejunum was significantly ($P < 0.01$) higher by $6.94 \mu\text{m}$ and $9.91 \mu\text{m}$ than the mean epithelial height of duodenum and ileum respectively. At the same time mean epithelial height of duodenum was significantly ($P < 0.05$) higher by $2.97 \mu\text{m}$ than the mean epithelial height of ileum.

Lamina propria

The lamina propria of the small intestine was made up of loose connective tissue which extended into the central core of intestinal villi. The connective tissue components were rich in fibroblasts and lymphocytic aggregations (Figs. 18 & 22). Within the lamina propria short straight tubular glands extended from the level between the bases of two adjoining villi. These mucosal glands were lined with simple columnar type of epithelial cells which did not present striated border. The cell cytoplasm was

moderately basophilic. Occasionally a few goblet cells were also observed within the lining epithelium of mucosal glands (Figs. 18, 19 & 22). These goblet cells were mild to moderately reactive for mucicarmine, PAS and colloidal iron stain and negative for alkaline phosphatase (Table No. 6). The paneth cells were not recorded in these glands of rabbit. At the sacculus rotendus of the ileum the lamina propria was invaded by group of lymph nodules which extended from submucosa, disrupting the lamina muscularis mucosae to reach into the subepithelial lamina propria (Fig. 29). These lymphatic nodules present in the group could be compared with Peyer's patches.

The present findings were in consonance with the findings on cotton rat and domestic animals (Blank, 1950; Barnwal and Yadava, 1975; Banks, 1981 and Stinson and Calhoun, 1987). Stinson and Calhoun (1987) further opined that undifferentiated low columnar or cuboidal cells in the mucosal glands gave rise to absorptive columnar cells and goblet cells of intestinal villi. They also reported the presence of paneth cells near the base of intestinal crypts in the small intestine of ruminants, horse and man. These cells were marked by the distribution of acidophilic granules in apical cytoplasm. Such type of cells were lacking in the mucosal glands of small intestine of rabbit during the present investigation.

Lamina muscularis mucosae

The lamina muscularis mucosae in the small intestine of rabbit was composed of smooth muscle cells arranged in

indistinctly as inner circular and outer longitudinal layers (Figs. 17, 18, 19 & 23). The few smooth muscle cells from inner circular layer frequently extended into the lamina propria which further ascended through connective tissue core of intestinal villi. The continuity of lamina muscularis mucosae was frequently disrupted at the region of duodenum by the ducts of the submucosal glands that opened at the base of mucosal glands (Figs. 17 & 18). This muscular layer was also discontinued at the level of lymphatic nodules that extended from submucosa towards the lamina propria particularly at sacculus rotendus of ileum. The present findings were contrast to the observations made by Calhoun (1954) in chicken where she reported an outer circular and an inner longitudinal layer in lamina muscularis mucosae. The present observations on lamina muscularis mucosae of rabbit's small intestine were similar to those of cow, horse, pig, sheep, dog, cat and goat (Titkemeyer and Calhoun, 1955). Trautmann and Fiebiger (1960) and Stinson and Calhoun, (1987) also reported inner circular and outer longitudinal layers of smooth muscles in the lamina muscularis mucosae of small intestine in domestic animals.

Mean, standard error and C.V% of thickness of lamina muscularis mucosae at different segments of small intestine of rabbit have been presented in Table No. 9. The average thickness of lamina muscularis mucosae was $10.80 \pm 0.91 \mu\text{m}$ in duodenum, $8.19 \pm 0.62 \mu\text{m}$ in jejunum and $8.19 \pm 0.56 \mu\text{m}$ in ileum.

The analysis of variance (Table No. 10) showing the effect of different segments of small intestine on thickness of lamina muscularis mucosae revealed significant difference ($P < 0.05$). The lamina muscularis mucosae of duodenum was significantly ($P < 0.05$) higher by $2.61 \mu\text{m}$ and $2.61 \mu\text{m}$ than mean thickness of lamina muscularis mucosae of jejunum and ileum respectively.

Tunica submucosa

The tunica submucosa of small intestine in rabbit was made up of loose connective tissue (Figs. 17, 20 23, 24, 26, 27, 28 & 29). The fibroblasts were predominating connective tissue cells in the submucosa. Loosely dispersed connective tissue fibres were mainly comprised of collagen fibres with a few elastic fibres. The submucosa contained isolated clusters of lymphocytic cells in the most of the areas. The solitary lymph nodules were observed occasionally in different segments of small intestine. Most of such lymph nodules presented germinal centres. At the sacculus rotundus of ileum the lymphatic nodules aggregated similar to those of Peyer's patches (Fig. 29). Nodules of these patches usually appeared triangular with a broader base facing towards the tunica muscularis. The apices were conical which invaded in the lamina propria disrupting the lamina muscularis mucosae. Most of these aggregated lymph nodules were of secondary type as they presented distinct germinal centres. The tunica submucosa also contained isolated clusters of adipocytes, blood vessels, lymph spaces and submucosal plexuses. The tunica submucosa of duodenum was marked by the presence of simple

tubuloalveolar submucosal glands. These glands were distributed in the cranial two third of the duodenum. The glands opened in the bases of mucosal glands through single excretory duct (Fig. 23). Near the junction with pyloric end with the duodenum, the submucosal glands were absolutely of mucous type (Figs. 14 & 16). In the remaining part of the duodenum the glands were of mixed type containing mucous and serous secreting cells separately in the adenomeres (Figs. 17, 18, 19, 20 & 23). The serous demilunes were however absent. The mucous secreting cells were foamy in appearance with flattened basal nucleus. The serous cells presented spherical nucleus with lightly stained eosinophilic cytoplasm all around. The ducts of the submucosal glands were lined with columnar type of cells which were very similar to the cells of pyloric gastric glands. The density of submucosal glands gradually decreased from cranial end to caudal end in the duodenum. Histochemically, the mucous secreting cells of submucosal glands and their ducts were mildly reactive for PAS and moderately reactive for mucicarmine and colloidal iron stains. They were negative for alkaline phosphatase. The serous cells did not react for mucin, acid mucopolysaccharides and alkaline phosphatase (Table No. 6). The submucosal glands were however absent in the caudal third of the duodenum and entire segments of the jejunum and ileum.

Trautmann and Fiebiger (1960) reported loose fibrillar connective tissue and elastic fibre nets in the tunica submucosa of small intestine of domestic animals. They also reported the

distribution of fat cells, lymph nodules, autonomic ganglia, nerves and vessels. The present observations on the histomorphological characters of submucosal glands appeared to be very similar to that of cat intestine. Banks (1981) and Stinson and Calhoun (1987) reported that the submucosal glands (Brunner's glands) were mucous type in dog and ruminants, serous in horse and pig and mixed in case of cat. Stinson and Calhoun (1987) further reported the variation in the extent of distribution of submucosal glands in different species. In case of dog the glands were confined at the cranial portion of the duodenum whereas in horse they extended beyond the gross anatomical limit of the duodenum.

The presence of solitary lymph nodules in the different segments of small intestine of rabbit and aggregated lymph nodules in the sacculus rotundus of ileum were in agreement with the descriptions made by Banks (1981) and Stinson and Calhoun (1987) in different domestic animals. They considered the occurrence of large masses of lymphatic nodules as characteristic features of ileum.

Tunica muscularis

The tunica muscularis was made up of distinct inner circular and outer longitudinal layers of smooth muscles (Figs. 17, 19, 24 & 27). These two layers were separated from each other by a distinct connective tissue components which contained closely distributed myenteric plexuses. Both inner and outer layers of tunica muscularis were thickest at the sacculus rotundus

part of ileum than the other regions of small intestine. The mean, standard error and C.V.% of thickness of inner circular layer of tunica muscularis at the different segments of rabbit's small intestine have been presented in Table No. 11. The average thickness of inner circular layer of tunica muscularis was $38.57 \pm 1.15 \mu\text{m}$ in duodenum, $47.23 \pm 1.53 \mu\text{m}$ in jejunum, $36.13 \pm 0.95 \mu\text{m}$ in the straight part of ileum and $112.86 \pm 2.92 \mu\text{m}$ in sacculus rotundus part of ileum.

Analysis of variance (Table No. 12) showing the effect of different segments of small intestine on thickness of inner layer of tunica muscularis revealed significant difference ($P < 0.01$). Mean thickness of inner layer of tunica muscularis of sacculus rotundus of ileum was significantly ($P < 0.01$) higher by $74.29 \mu\text{m}$, $65.63 \mu\text{m}$ and $76.73 \mu\text{m}$ than the mean thickness of inner layer of tunica muscularis of duodenum, jejunum and straight portion of ileum respectively. The mean thickness of this layer of tunica muscularis of jejunum was also significantly ($P < 0.01$) higher by $8.66 \mu\text{m}$ and $11.10 \mu\text{m}$ than the mean thickness of this layer in duodenum and straight part of ileum respectively. The difference in the mean thickness of inner circular layer of tunica muscularis of duodenum and straight part of ileum was nonsignificant.

The mean, standard error and C. V.% of thickness of outer longitudinal layer of tunica muscularis at different segments of rabbit's small intestine have been presented in Table No. 13. The average thickness of outer layer of tunica muscularis measured $27.80 \pm 0.75 \mu\text{m}$ in duodenum, $20.35 \pm 0.84 \mu\text{m}$ in jejunum,



27.80 \pm 0.75 μ m in straight part of ileum and 57.66 \pm 4.39 μ m in sacculus rotundus of ileum.

The analysis of variance (Table No. 14) revealed significant difference ($P < 0.01$). The mean thickness of outer longitudinal layer of tunica muscularis of sacculus rotundus of ileum was significantly ($P < 0.01$) higher by 29.86 μ m, 37.31 μ m and 29.86 μ m than the mean thickness of outer longitudinal layer of tunica muscularis of duodenum, jejunum and straight part of ileum respectively. The mean thickness of outer longitudinal layer of duodenum and also straight part of ileum appeared significantly ($P < 0.01$) higher by 7.45 μ m than the mean thickness of this layer of tunica muscularis in jejunum.

The present observation revealed only two layers of smooth muscles in the tunica muscularis of rabbit's small intestine. Titkemeyer and Calhoun (1955) found an additional oblique muscle layer in between the submucosa and circular muscle layer in dog. However, they reported two distinct layers of tunica muscularis in small intestine of other domestic animals.

Intramuscular network of lymphatic capillaries as reported in man by Kakharov (1963) could not be observed in the tunica muscularis of rabbit's small intestine with the routine staining techniques adopted during the present investigation.

Trautmann and Fiebiger (1960), Banks (1981) and Stinson and Calhoun (1987) described inner circular and outer longitudinal layers of smooth muscles in the tunica muscularis of small intestine in various domestic animals. Stinson and Calhoun

(1987) further reported that the connective tissue between the two muscle layers contained the myenteric plexuses. They also reported thickest tunica muscularis in case of horse where both inner and outer layers were nearly equal in thickness.

Tunica Serosa

The tunica serosa of small intestine in rabbit was comprised of loose connective tissue lined with mesothelium (Fig. 24). It was richly distributed with blood vessels and nerves.

The present observation was in agreement with the findings of Finerty and Cowdry (1960) and Bloom and Fawcett (1968) in man, Banks (1981) and Stinson and Calhoun (1987) in domestic animals and Banerjee (1993) in rabbit who reported the tunica serosa of small intestine as a layer of loose connective tissue lined by a layer of mesothelium.

Table No – 1

Mean \pm S. E. along with C. V. % of the thickness of tunica mucosa of different glandular parts of stomach of rabbit :-

| | Mean \pm S. E. (μm) | C. V. % |
|--------------------------|------------------------------------|---------|
| Cardiac glandular region | 463.56 ^a \pm 9.49 | 8.69 |
| Fundic glandular region | 401.13 ^b \pm 3.25 | 3.44 |
| Pyloric glandular region | 392.65 ^b \pm 8.59 | 9.28 |

Means with different superscripts differ significantly ($P < 0.01$).

Table No – 2

Analysis of variance showing the effect of different glandular parts of stomach on thickness of tunica mucosa in rabbit :-

| Sources of variation | D. F. | M. S. | F |
|----------------------|-------|-----------|---------------------|
| Between treatment | 2 | 26994.565 | 25.76 ^{xx} |
| Error | 51 | 1047.925 | |

^{xx} Significant at $P < 0.01$

Table No - 3
HISTOCHEMICAL PROPERTIES OF GLANDULAR CELLS IN THE STOMACH OF RABBIT.

| Stains | | Cardiac glandular cells | Fundic glandular cells | | | Pyloric glandular cells |
|---|----------|-------------------------|------------------------|-------------|----------------|-------------------------|
| | | | Mucous neck cells | Chief cells | Parietal cells | |
| PAS - orange G Stain for glycoprotein | PAS | + to +++ | + | - | - | + |
| | Orange G | - | - | - | ++ | - |
| Muller's colloidal iron stain for acid mucopolysaccharide | | - | + | - | - | ++ |
| Mucicarmine stain for epithelial mucin | | + | + | - | - | + |
| Gomori's stain for alkaline phosphatage | | - | - | - | - | - |
| PAS reaction for neutral mucopolysaccharide | | + to +++ | + | - | - | + |

Scales for positivity :- + Weak or mild ; ++ Moderate ; +++ Intense ; - Negative.

Table No - 4

Mean \pm S. E. along with C. V. % of the thickness of lamina muscularis mucosae of different glandular parts of stomach of rabbit :-

| | Mean \pm S. E. (μ m) | C. V. % |
|--------------------------|-------------------------------|---------|
| Cardiac glandular region | 53.46 ^a \pm 3.37 | 26.75 |
| Fundic glandular region | 28.60 ^b \pm 1.88 | 27.92 |
| Pyloric glandular region | 95.26 ^c \pm 3.77 | 16.80 |

Means with different superscripts differ significantly ($P < 0.01$).

Table No - 5

Analysis of variance showing the effect of different glandular parts of stomach on thickness of lamina muscularis mucosae in rabbit :-

| Sources of variation | D. F. | M. S. | F |
|----------------------|-------|-----------|----------------------|
| Between treatment | 2 | 20430.105 | 116.84 ^{xx} |
| Error | 51 | 174.842 | |

^{xx} Significant at $P < 0.01$

Table No - 6
HISTOCHEMICAL PROPERTIES OF EPITHELIAL AND GLANDULAR CELLS IN THE SMALL INTESTINE OF RABBIT.

| Stains | | Duodenum | | | | Jejunum | | Ileum | |
|---|----------|---------------------------|--------------|----------------------------|---|---------------------------|--------------|---------------------------|--------------|
| | | Columnar absorptive Cells | Goblet cells | Submucosal glandular cells | | Columnar absorptive cells | Goblet cells | Columnar absorptive cells | Goblet cells |
| PAS - orange G Stain for glycoprotein | PAS | - | ++ | Mucous cells | - | - | + | - | ++ |
| | Orange G | - | - | - | - | - | - | - | - |
| Muller's colloidal iron stain for acid mucopolysaccharide | | - | ++ | ++ | - | - | + | - | ++ |
| Mucicarmine stain for epithelial mucin | | - | ++ | ++ | - | - | + | - | ++ |
| Gomori's stain for alkaline phosphatase | | + (only striated border) | - | - | - | + (only striated border) | - | + (only striated border) | - |
| PAS reaction for neutral mucopolysaccharide | | - | ++ | + | - | - | + | - | ++ |

Scales for positivity :- + weak or mild ; ++ Moderate ; +++ Intense ; - Negative.

Table No – 7

Mean \pm S. E. along with C. V. % of the epithelial height of different segments of small intestine of rabbit :-

| | Mean \pm S. E. (μ m) | C. V. % |
|----------|-------------------------------|---------|
| Duodenum | 27.46 ^a \pm 0.65 | 10.09 |
| Jejunum | 34.40 ^b \pm 1.36 | 16.79 |
| Ileum | 24.49 ^c \pm 0.57 | 10.03 |

Means with different superscripts differ significantly ($P < 0.05$).

Table No – 8

Analysis of variance showing the effect of different segments of small intestine on epithelial height in rabbit :-

| Sources of variation | D. F. | M. S. | F |
|----------------------|-------|---------|---------------------|
| Between treatment | 2 | 465.769 | 29.67 ^{xx} |
| Error | 51 | 15.698 | |

^{xx} Significant at $P < 0.01$

Table No – 9

Mean \pm S. E. along with C. V. % of the thickness of lamina muscularis mucosae of different segments of small intestine of rabbit :-

| | Mean \pm S. E. (μm) | C. V. % |
|----------|------------------------------------|---------|
| Duodenum | 10.80 ^a \pm 0.91 | 36.10 |
| Jejunum | 8.19 ^b \pm 0.62 | 32.22 |
| Ileum | 8.19 ^b \pm 0.56 | 29.49 |

Means with different superscripts differ significantly ($P < 0.05$).

Table No – 10

Analysis of variance showing the effect of different segments of small intestine on thickness of lamina muscularis mucosae in rabbit :-

| Sources of variation | D. F. | M. S. | F |
|----------------------|-------|--------|-------------------|
| Between treatment | 2 | 40.734 | 4.36 ^x |
| Error | 51 | 9.336 | |

^x Significant at $P < 0.05$

Table No – 11

Mean \pm S. E. along with C. V. % of the thickness of inner layer of tunica muscularis of different segments of small intestine of rabbit :-

| | | Mean \pm S. E. (μm) | C. V. % |
|----------|-------------------|------------------------------------|---------|
| Duodenum | | $38.57^a \pm 1.15$ | 12.75 |
| Jejunum | | $47.23^b \pm 1.53$ | 13.80 |
| Ileum | Straight | $36.13^a \pm 0.95$ | 11.17 |
| | Sacculus rotundus | $112.86^c \pm 2.92$ | 10.98 |

Means with different superscripts differ significantly ($P < 0.01$).

Table No – 12

Analysis of variance showing the effect of different segments of small intestine on the thickness of inner layer of tunica muscularis in rabbit :-

| Sources of variation | D. F. | M. S. | F |
|----------------------|-------|-----------|----------------------|
| Between treatment | 3 | 23878.597 | 403.56 ^{xx} |
| Error | 68 | 59.169 | |

^{xx} Significant at $P < 0.01$

Table No – 13

Mean \pm S. E. along with C. V. % of the thickness of outer layer of tunica muscularis of different segments of small intestine of rabbit :-

| | | Mean \pm S. E. (μm) | C. V. % |
|----------|-------------------|------------------------------------|---------|
| Duodenum | | 27.80 ^a \pm 0.75 | 11.58 |
| Jejunum | | 20.35 ^b \pm 0.84 | 17.54 |
| Ileum | Straight | 27.80 ^a \pm 0.75 | 11.58 |
| | Sacculus rotundus | 57.66 ^c \pm 4.39 | 32.32 |

Means with different superscripts differ significantly ($P < 0.05$).

Table No – 14

Analysis of variance showing the effect of different segments of small intestine on the thickness of outer layer of tunica muscularis in rabbit :-

| Sources of variation | D. F. | M. S. | F |
|----------------------|-------|----------|---------------------|
| Between treatment | 3 | 4931.361 | 51.79 ^{xx} |
| Error | 68 | 95.211 | |

^{xx} Significant at $P < 0.01$

5. SUMMARY AND CONCLUSIONS

The present investigation

healthy rabbits, six

with histochromic

stomach - Histology

a small non-glandular

glandular part

The lamina propria

SUMMARY

AND

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5. SUMMARY AND CONCLUSIONS

The present investigation was conducted on twelve adult healthy rabbits, six male and six female, for the histological and certain histochemical studies of the stomach and small intestine.

Stomach – Histomorphologically, the stomach of rabbit consisted of a small nonglandular and a large glandular parts.

Nonglandular part

The lamina epithelialis of nonglandular part of stomach was made up of nonkeratinised stratified squamous epithelium showing abrupt transition into the glandular epithelium at the junction with glandular part. Lamina muscularis mucosae was represented by isolated smooth muscle fibres. The submucosa was occasionally containing dilated simple alveolar glands which extended from terminal portion of the oesophagus near the junction with the stomach. The tunica muscularis was made up of irregularly arranged smooth muscle fibres along with striated muscle fibres which were extended at the initial region of the stomach from the tunica muscularis of caudal segment of the oesophagus.

Glandular part

The lamina epithelialis of glandular part of stomach was lined with simple columnar epithelium with PAS-positive apical cytoplasm. The surface epithelium in-pocketed to form gastric pits from where gastric glands were continued within the lamina

propria at different regions. The gastric pits were deepest at the pyloric glandular region as compared to cardiac and fundic ones.

The average thickness of tunica mucosa of cardiac, fundic, and pyloric glandular regions of rabbit's stomach was measured as $463.56 \pm 9.49 \mu\text{m}$, $401.13 \pm 3.25 \mu\text{m}$ and $392.65 \pm 8.59 \mu\text{m}$ respectively. The analysis of variance showing the effect of different glandular regions of stomach on mucosal thickness revealed highly significant difference ($P < 0.01$). However, there was no significant difference between the mean thickness of fundic and pyloric glandular region. The thickness of tunica mucosa of cardiac glandular region was higher than the fundic and pyloric glandular regions.

Lamina propria was scanty due to heavy distribution of different types of gastric glands in the layer. The cardiac gastric glands were simple branched coiled tubular glands whose glandular epithelium presented supranuclear foamy cytoplasm suggestive of mucous secreting nature. The fundic gastric glands were simple straight tubular glands comprised of predominantly of parietal cells, the chief cells and a few mucous cells. The pyloric glands were comparatively short simple branched tubular glands. The glandular cells were pyramidal or columnar in shape and contained basally placed flattened nucleus with pale coloured cytoplasm which reacted for PAS, mucicarmine and colloidal iron stains. Lamina muscularis mucosae was prominent throughout and was thickest at the pyloric region. Frequently the

smooth muscle cells from inner circular layer extended in the lamina propria between the glandular tubules.

The mean thickness of lamina muscularis mucosae measured $53.46 \pm 3.37 \mu\text{m}$ in cardiac glandular region, $28.60 \pm 1.88 \mu\text{m}$ in fundic glandular region and $95.26 \pm 3.77 \mu\text{m}$ in pyloric glandular region of rabbit's stomach. The analysis of variance showing the effect of different glandular regions on thickness of lamina muscularis mucosae revealed highly significant difference ($P < 0.01$).

Tunica submucosa presented numerous submucosal plexuses. Tunica muscularis was made up of three distinct layers of smooth muscle fibre bundles near the cardiac glandular region. In remaining portion of the stomach there were only two layers of smooth muscle. The myenteric plexuses were well distributed. Tunica serosa was typical.

Small intestine

The tunica mucosa of small intestine of rabbit presented numerous projections to constitute intestinal villi which were either leaf-like, finger-like or club-shaped. The lamina epithelialis of small intestine was comprised of simple columnar epithelium with striated borders which extended over the intestinal villi. The thickness of surface epithelium was highest in the jejunal region and lowest in the ileum region. In between the two subsequent intestinal villi the surface epithelium in-pocketed in the underlying lamina propria to form intestinal mucosal glands (Glands of Lieberkuhn). The surface epithelium

presented varying number of goblet cells which were reactive for PAS, mucicarmine and colloidal iron stains.

The average height of the epithelium of rabbit's small intestine measured $27.46 \pm 0.65 \mu\text{m}$ in duodenum, $34.40 \pm 1.36 \mu\text{m}$ in jejunum and $24.49 \pm 0.57 \mu\text{m}$ in ileum. The analysis of variance of different segments of small intestine of rabbit on epithelial height revealed significant difference ($P < 0.01$).

The lamina propria was made up of loose connective tissue which extended into the central core of intestinal villi. The connective tissue central core was marked by the presence of lacteal. The short intestinal mucosal glands distributed into the propria were lined with columnar cells which did not exhibit striated border. Paneth cells were not recorded in the intestinal mucosal gland. The epithelial cells of these glands were variably reactive for mucicarmine, PAS and colloidal iron stains. The lamina muscularis mucosae was made up of smooth muscle cells showing inner circular and outer longitudinal layers. Occasionally few smooth muscle cells were observed to extend into the lamina propria even reaching in the central connective tissue core of intestinal villi.

The average thickness of lamina muscularis mucosae of small intestine of rabbit was $10.80 \pm 0.91 \mu\text{m}$ in duodenum, $8.19 \pm 0.62 \mu\text{m}$ in jejunum and $8.19 \pm 0.56 \mu\text{m}$ in ileum. The analysis of variance showing the effect of different segments of small intestine on thickness of lamina muscularis mucosae revealed significant difference ($P < 0.05$).

Tunica submucosa was made up of loose connective tissue containing blood vessels, lymph spaces and submucosal plexuses. The tunica submucosa of cranial half of the duodenum contained simple tubular alveolar intestinal submucosal glands (Brunner's glands) which were seromucous in nature. Isolated solitary lymph nodules were occasionally found in the submucosa of the small intestine but at the sacculus rotundus of ileum the submucosa presented aggregated lymph nodules to constitute Peyer's patches. The tunica muscularis was made up of two distinct layers of smooth muscle cells as inner circular and outer longitudinal layers.

The average thickness of inner circular layer of tunica muscularis of rabbit's small intestine was $38.57 \pm 1.15 \mu\text{m}$ in duodenum, $47.23 \pm 1.53 \mu\text{m}$ in jejunum, $36.13 \pm 0.95 \mu\text{m}$ in the straight part of ileum and $112.86 \pm 2.92 \mu\text{m}$ in sacculus rotundus part of ileum. Whereas the average thickness of outer layer of tunica muscularis measured $27.80 \pm 0.75 \mu\text{m}$ in duodenum, $20.35 \pm 0.84 \mu\text{m}$ in jejunum, $27.80 \pm 0.75 \mu\text{m}$ in straight part of ileum and $57.66 \pm 4.39 \mu\text{m}$ in sacculus rotundus of ileum. The analysis of variance showing the effect of different segments of small intestine on thickness of inner and outer layers of tunica muscularis revealed significant difference ($P < 0.01$). However, the difference in the mean thickness between inner circular layer of tunica muscularis of duodenum and straight part of ileum was nonsignificant. The thickness of inner and outer layers of tunica

muscularis was highest in the sacculus rotundus part of ileum.
The tunica serosa was typical and was lined with mesothelium.

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Figure No – 1

Photomicrograph of section through nonglandular part of stomach of rabbit showing stratified squamous epithelium (a), tunica muscularis mucosae (b), oesophageal glands (c) in submucosa and tunica muscularis (d).

Haematoxylin and eosin ; X 48.

Figure No – 2

Photomicrograph of the section through the junction between nonglandular and cardiac glandular regions of the stomach of rabbit showing stratified squamous epithelium of nonglandular part (a) and cardiac glandular region (b) containing PAS – positive glandular tubule and lamina propria (c).

PAS – orange G ; X 150.

Figure No – 3

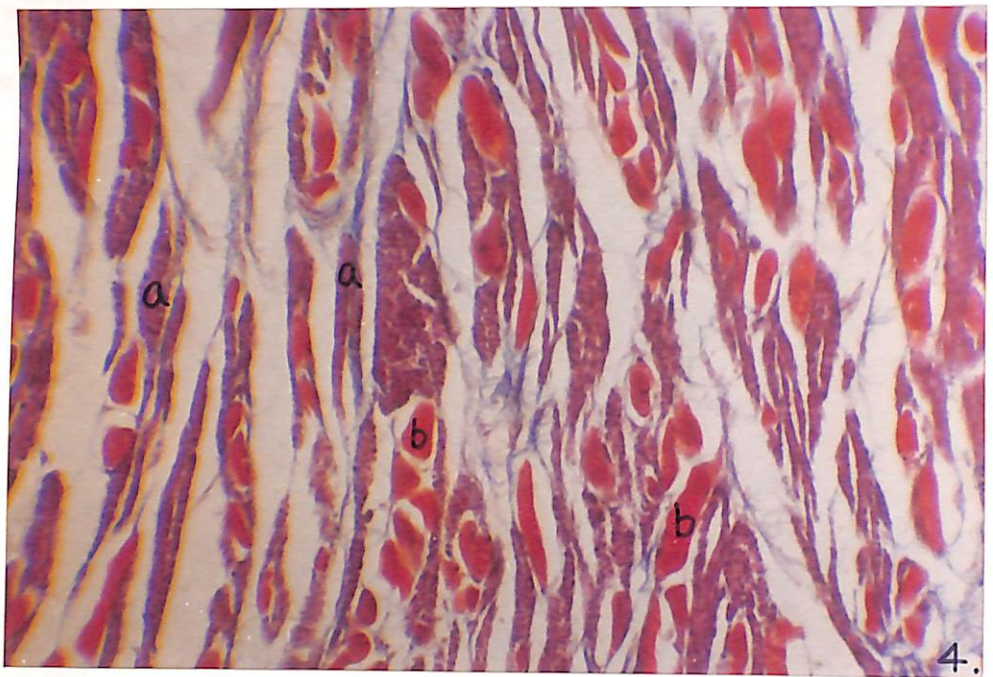
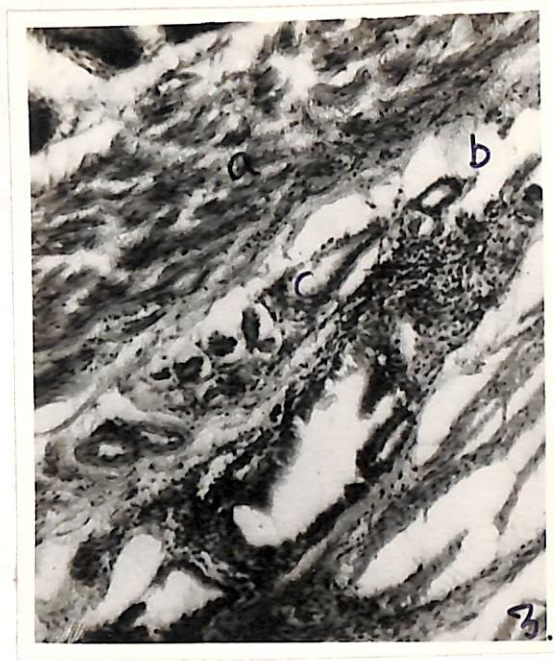
Photomicrograph of section through lamina muscularis mucosae (a) and submucosa (b) of nonglandular part of the stomach of rabbit, containing oesophageal glands (c).

Haematoxylin eosin ; X 100.

Figure No – 4

Photomicrograph of section through tunica muscularis of nonglandular part of stomach of rabbit showing admixture of smooth muscle fibres (a) and striated muscle fibres (b).

Azan stain ; X 150.



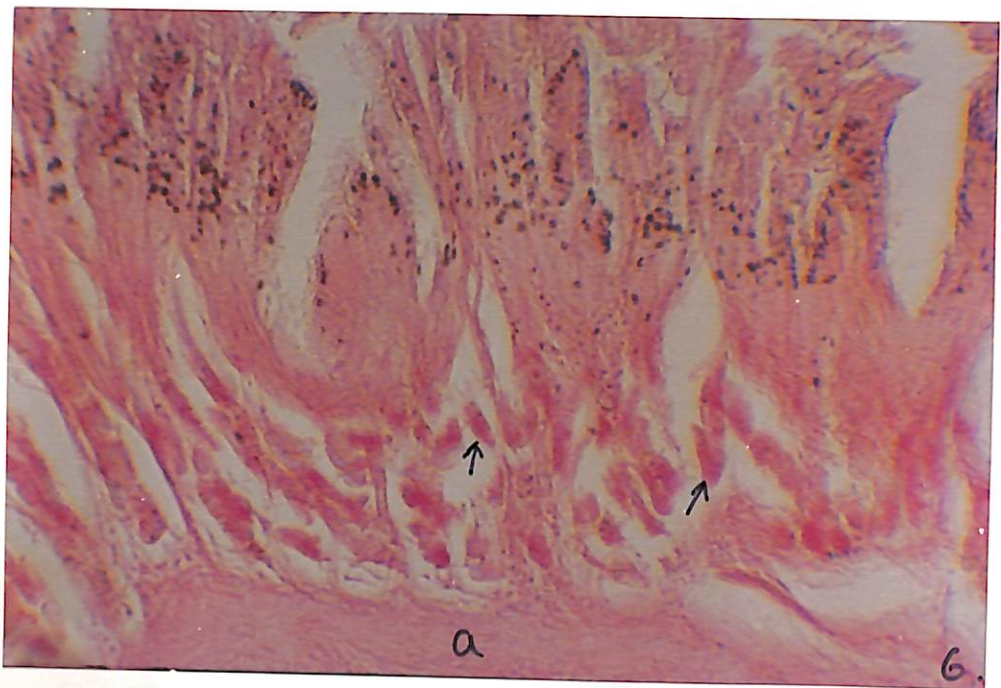
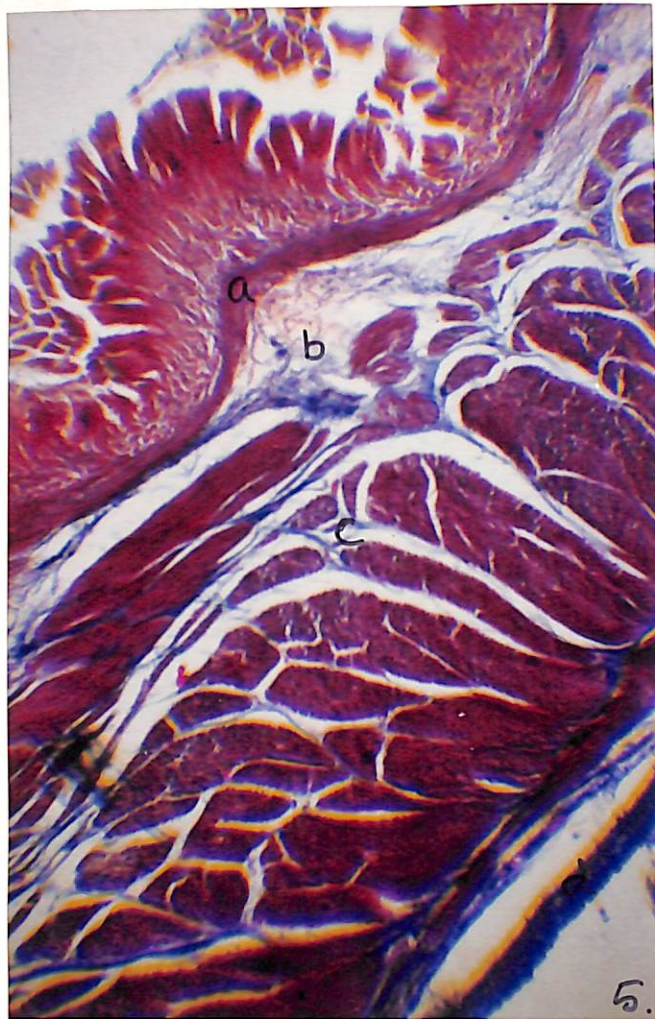


Figure No – 5

Photomicrograph of section through cardiac glandular region of rabbit's stomach showing tunica mucosa with prominent lamina muscularis mucosae (a), submucosa (b), tunica muscularis (c) and tunica serosa (d).

Azan stain ; X 48.

Figure No – 6

Photomicrograph of section through tunica mucosa of cardiac glandular region in rabbit's stomach showing positive reaction for mucin in glandular cells (arrow) and lamina muscularis mucosae (a). The black spots are artefacts.

Mucicarmin stain ; X 150.

Figure No – 7

Photomicrograph of section through tunica mucosa of fundic gastric glandular region in rabbit's stomach showing extensive distribution of eosinophilic parietal cells in the fundic gastric gland. The base of the gland contained few basophilic mucous secreting cells (a) and a continuous lamina muscularis mucosae (b).

Haematoxylin and eosin ; X 150.

Figure No – 8

Photomicrograph of section through the body and base of fundic gastric glands of rabbit showing parietal cells (a), chief cells (b) and mucous neck cells distributed at the base of the gland (c).

Haematoxylin and eosin ; X 600.

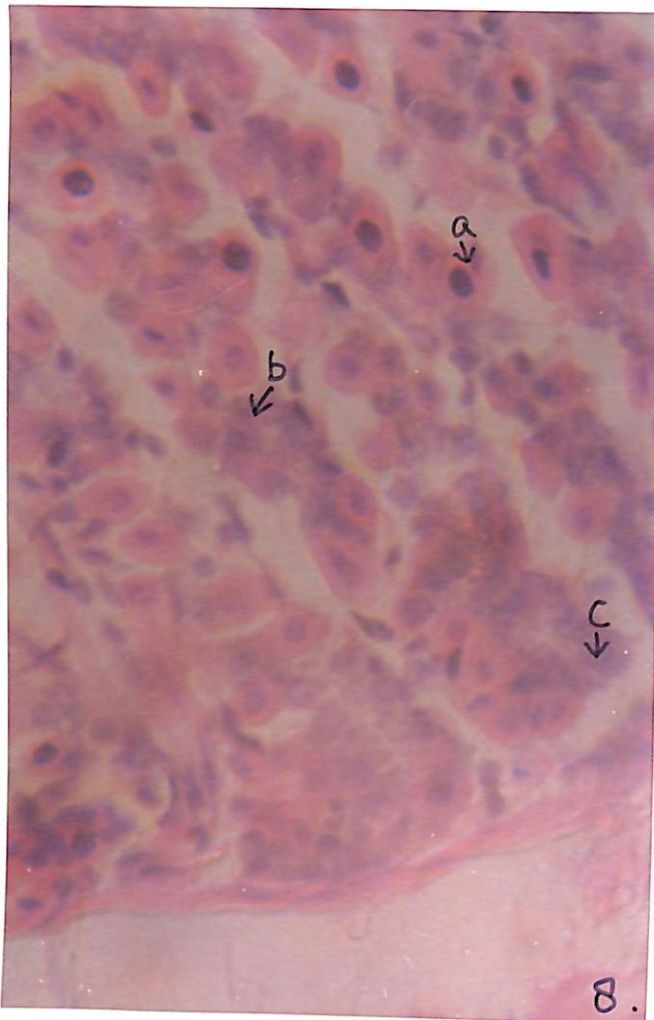
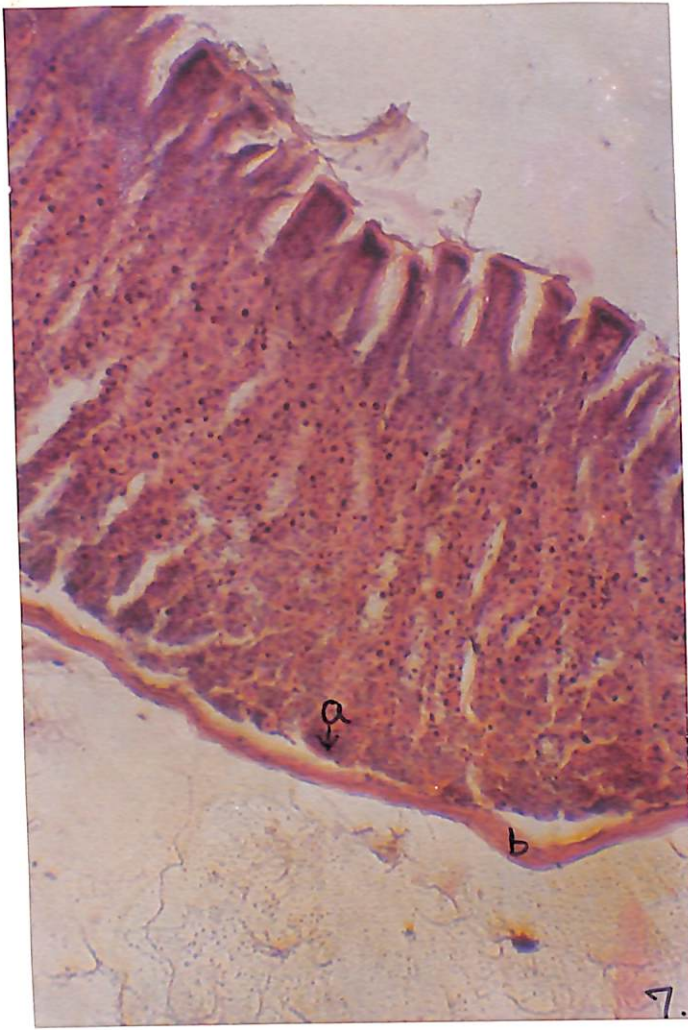


Figure No – 9

Photomicrograph of section through the body of fundic gastric glands in rabbit showing parietal cells (a) and chief cells (b).

Haematoxylin and eosin ; X 1000.

Figure No – 10

Photomicrograph of section through tunica mucosa of fundic gastric glandular region in rabbit's stomach showing acid mucopolysaccharide reactive mucous neck cells at the neck and upper part of the glandular tubules.

Colloidal iron stain ; X 150.

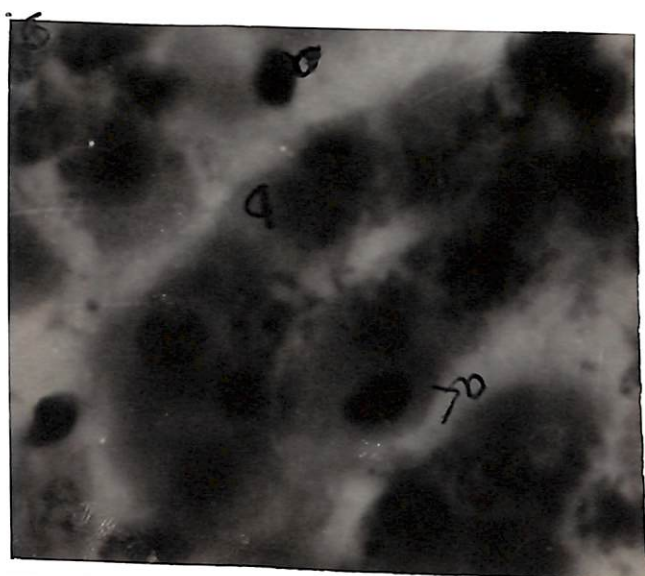
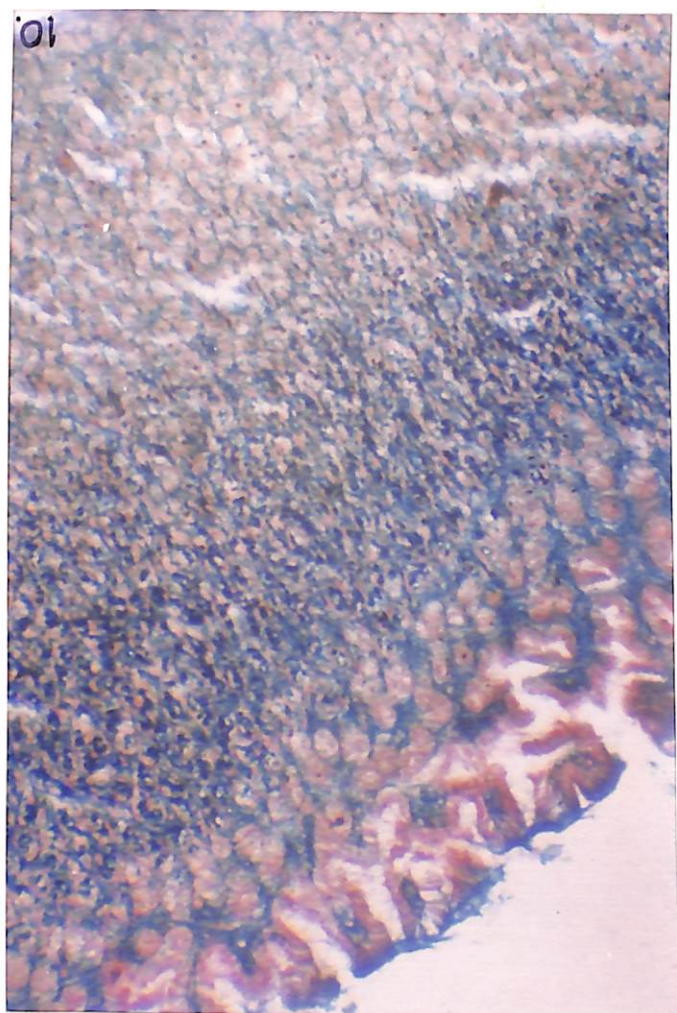


Figure No – 11

Photomicrograph of section through tunica mucosa of fundic gastric glandular region in rabbit's stomach showing mucicarmine positive surface epithelium and mucous neck cells of the glandular tubules. The black spots are artefacts.

Mucicarmine stain ; X 150.

Figure No – 12

Photomicrograph of section through tunica muscularis of fundic glandular region in rabbit's stomach showing myenteric plexuses (arrows) between inner circular (a) and outer longitudinal (b) muscle layers.

Colloidal iron stain ; X 150.

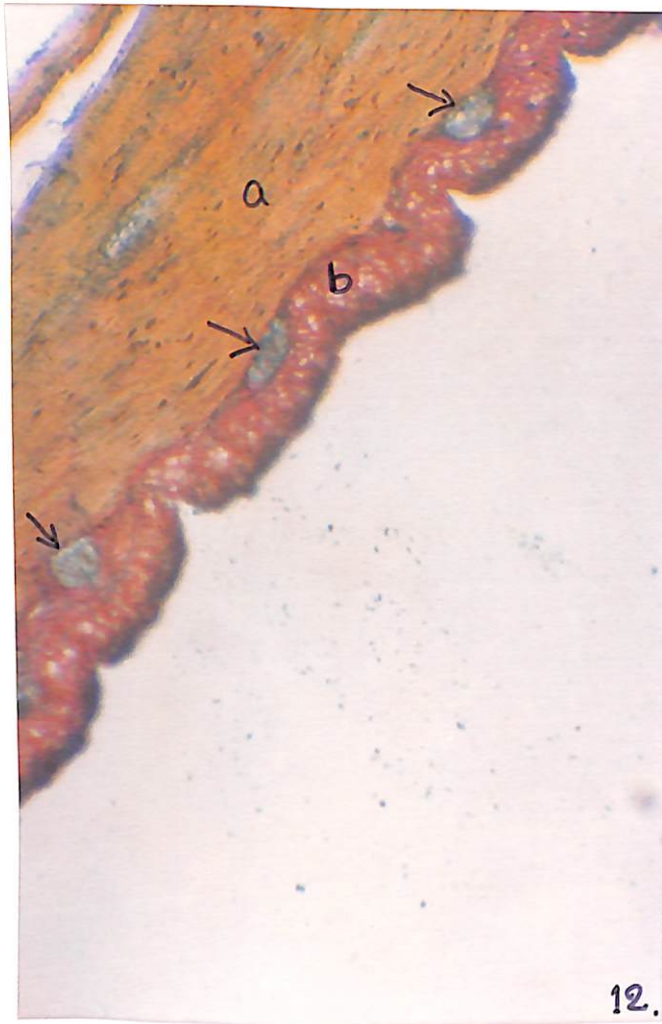
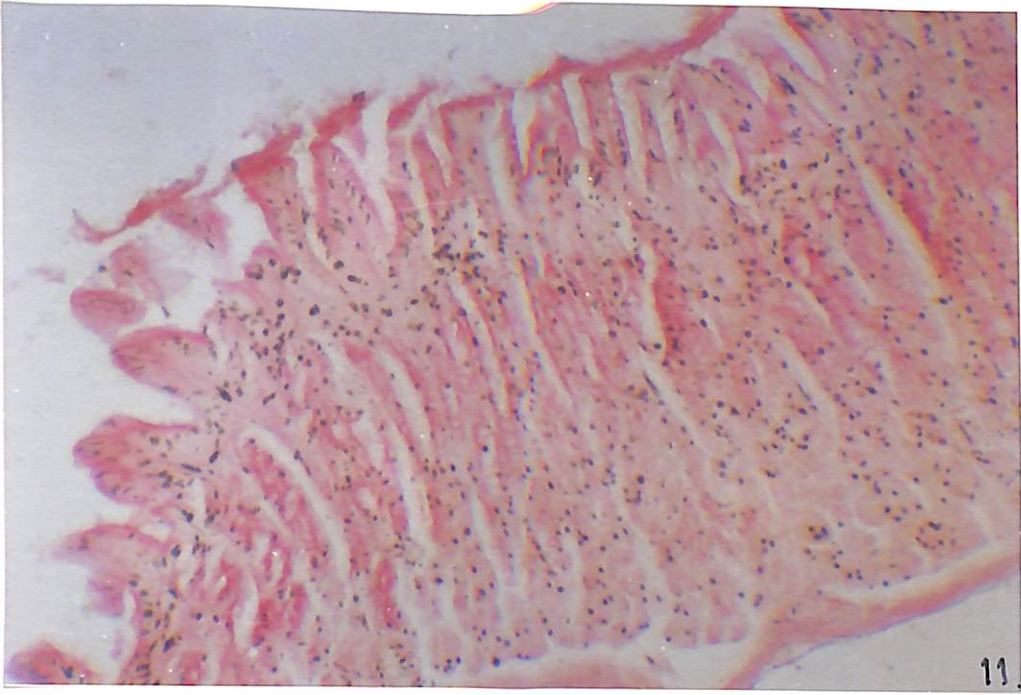


Figure No – 13

Photomicrograph of section through pyloric glandular region of rabbit's stomach showing gastric pits (a), pyloric gastric glands (b), lamina propria (c), lamina muscularis mucosae (d), submucosa (e) and tunica muscularis (f).

Haematoxylin and eosin stain ; X 150.

Figure No – 14

Photomicrograph of section through pylorico-duodenal junction of rabbit's stomach showing pyloric glandular region (a), duodenal mucosa (b), submucosa containing mucous secreting gland (c), tunica muscularis (d) and tunica serosa (e).

Heamatoxylin and eosin stain ; X 150.

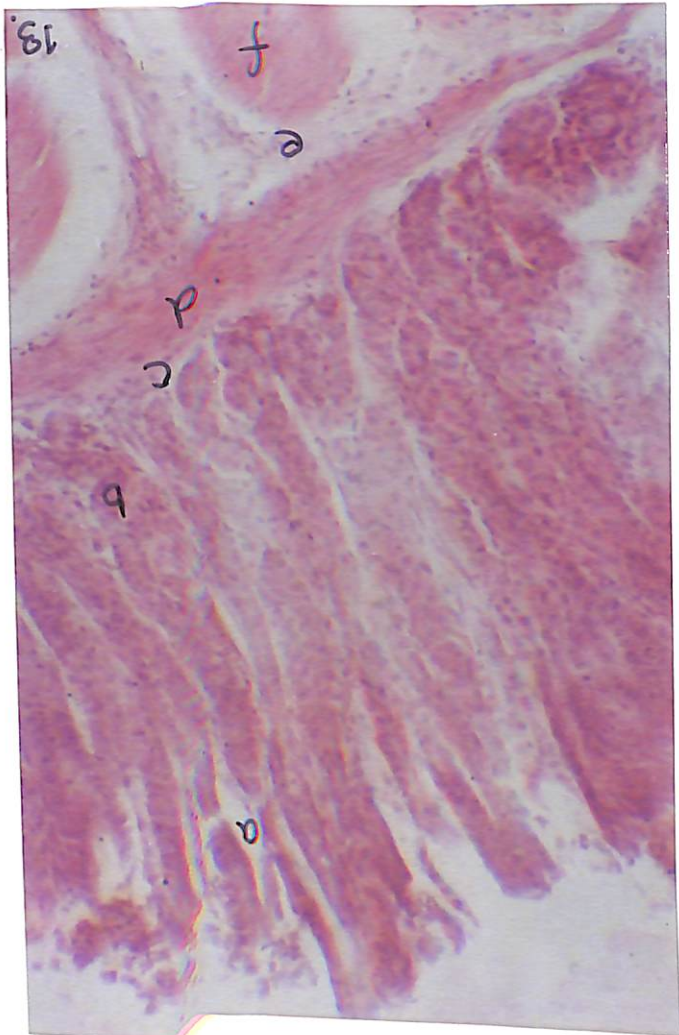
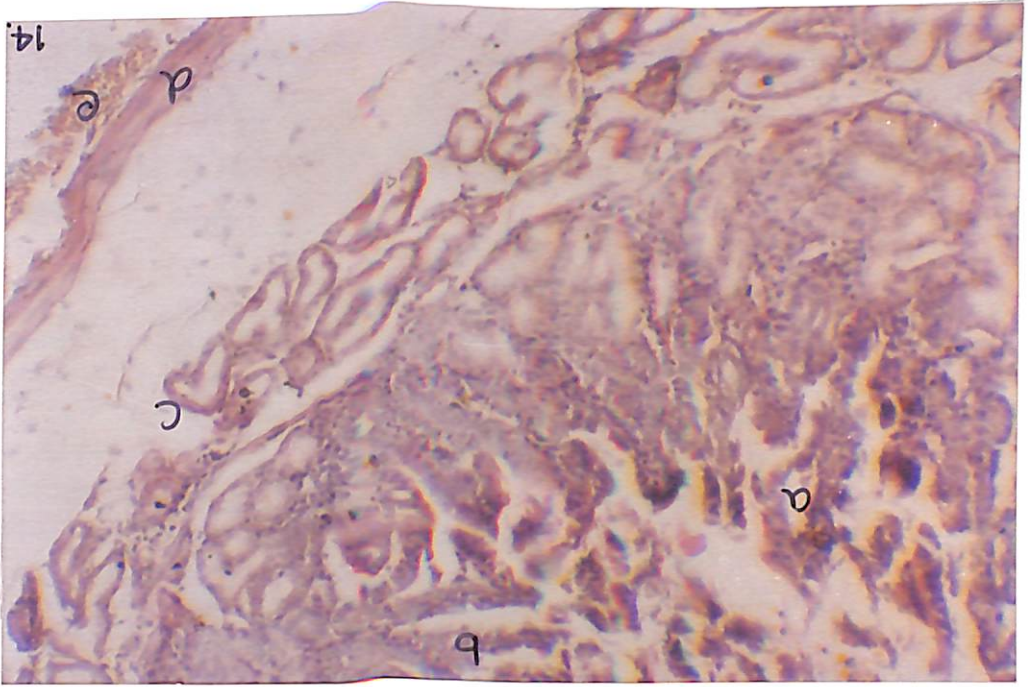


Figure No – 15

Photomicrograph of section through pyloric glandular region of rabbit's stomach showing long gastric pits (a), short pyloric glands (b), lamina propria (c), lamina muscularis mucosae (d), submucosa (e) and tunica muscularis (f).

Colloidal iron stain ; X 150.

Figure No – 16

Photomicrograph of section through pylorico – duodenal junction of rabbit's stomach showing pyloric glands (a), lamina muscularis mucosae (b), submucosa containing duodenal submucosal gland (c) and tunica muscularis (d).

Colloidal iron stain ; X 150.

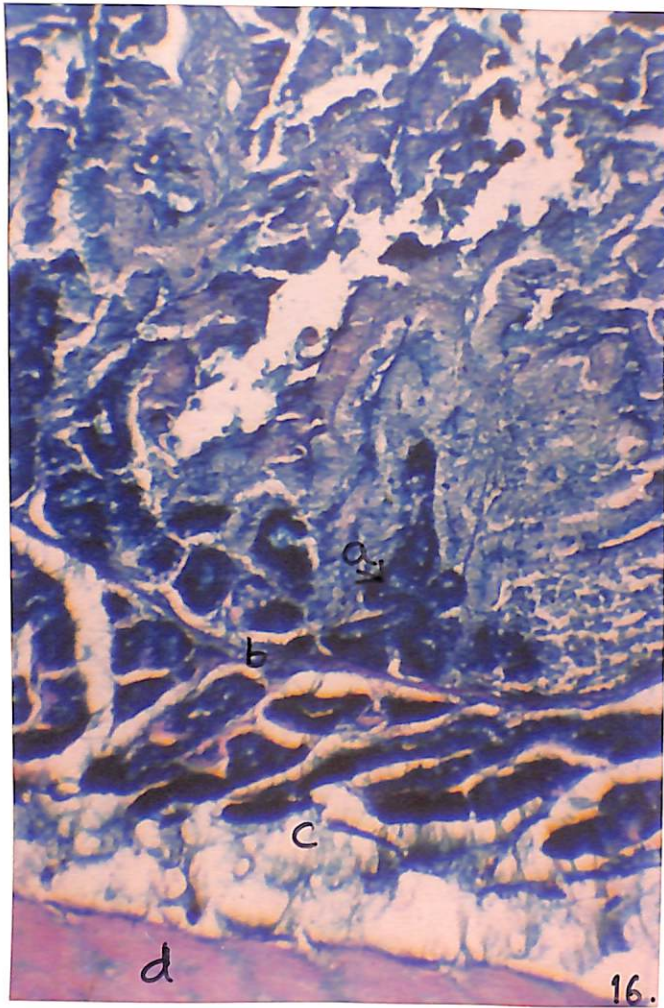
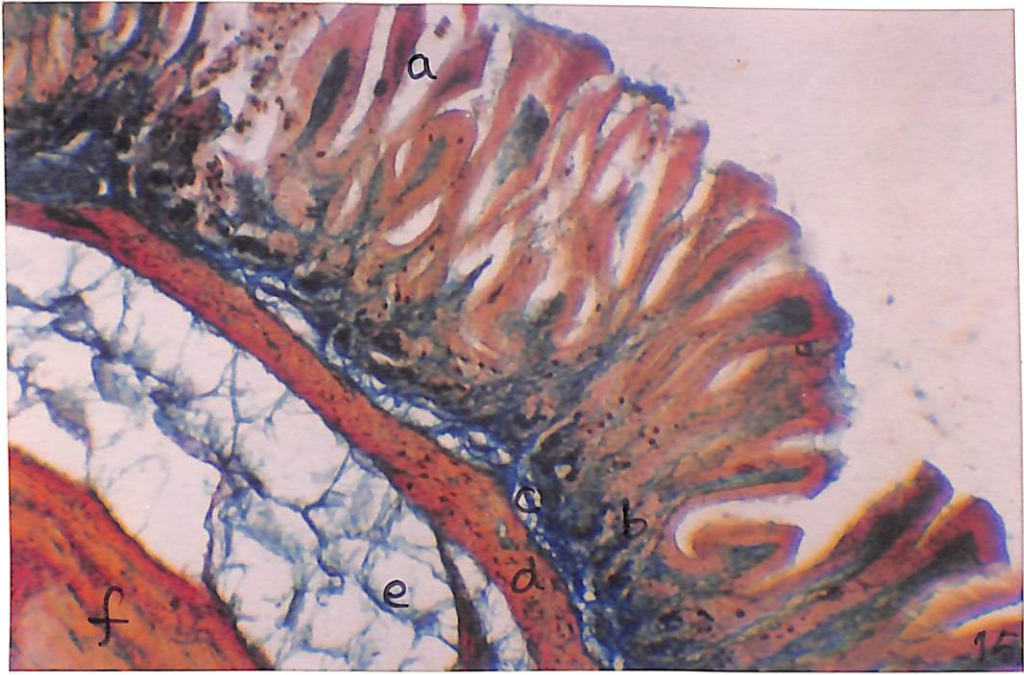


Figure No – 17

Photomicrograph of section through duodenum of rabbit showing intestinal villi (a), mucosal glands (b), submucosal glands (c) and tunica muscularis (d).
Haematoxylin and eosin ; X 48.

Figure No – 18

Photomicrograph of section through duodenum of rabbit showing epithelium of villi (a), lymph nodule (b), mucosal gland (c), submucosal gland (d).
Haematoxylin and eosin ; X 150.

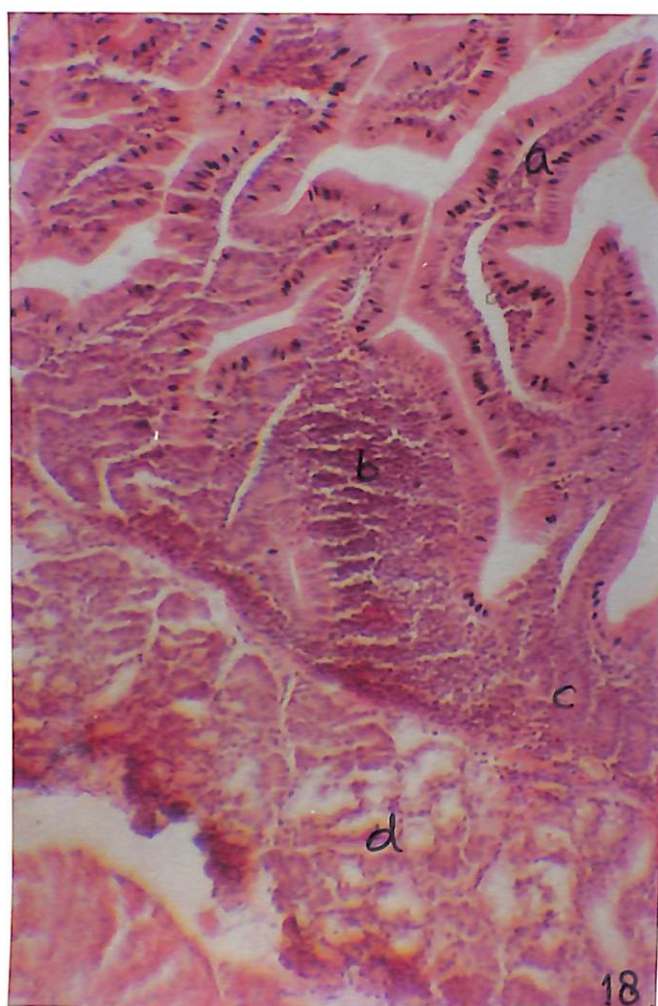


Figure No – 19

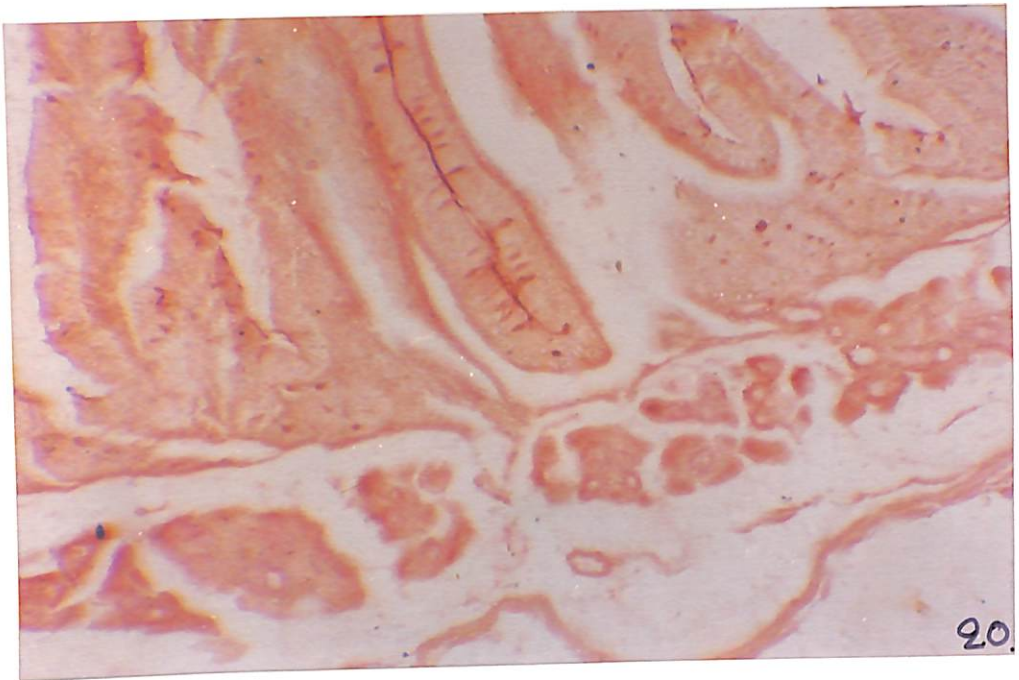
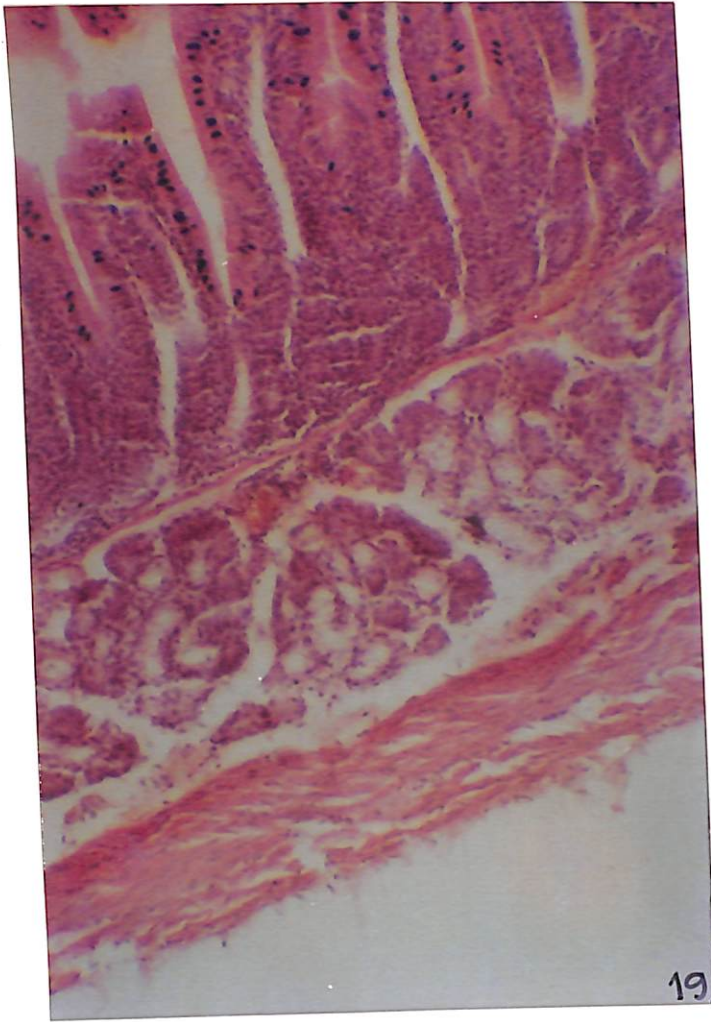
Photomicrograph of section through middle part of duodenum of rabbit showing distinct serous and mucous secreting cells in the submucosal glands.

Haematoxylin and eosin ; X 150.

Figure No – 20

Photomicrograph of section through the duodenum of middle part of rabbit showing few numbers of the goblet cells in the intestinal villi and PAS – positive cells in submucosal glands.

PAS – orange G ; X 150.



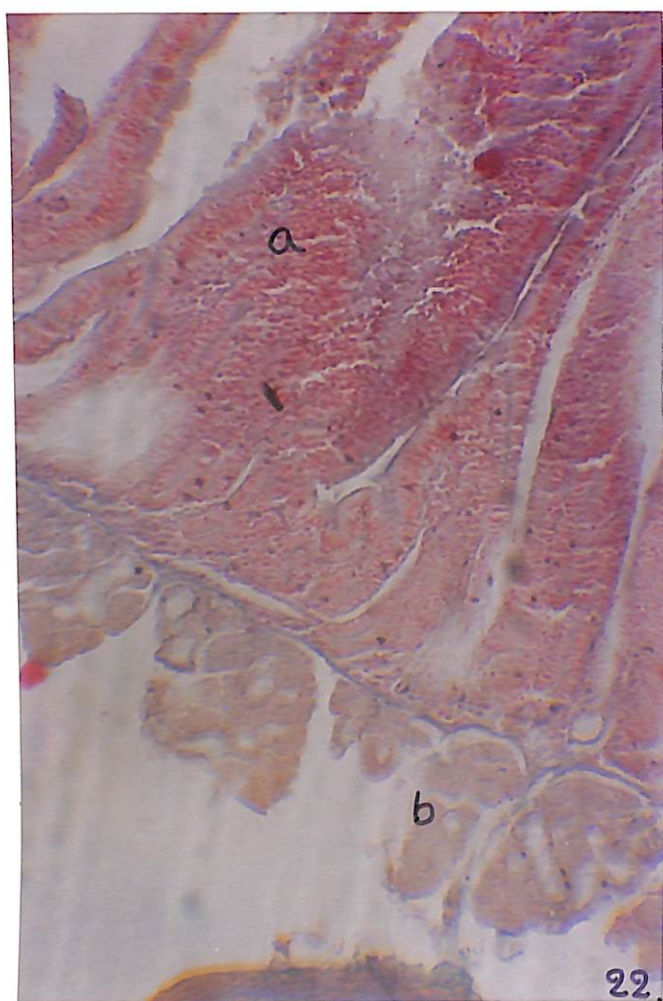
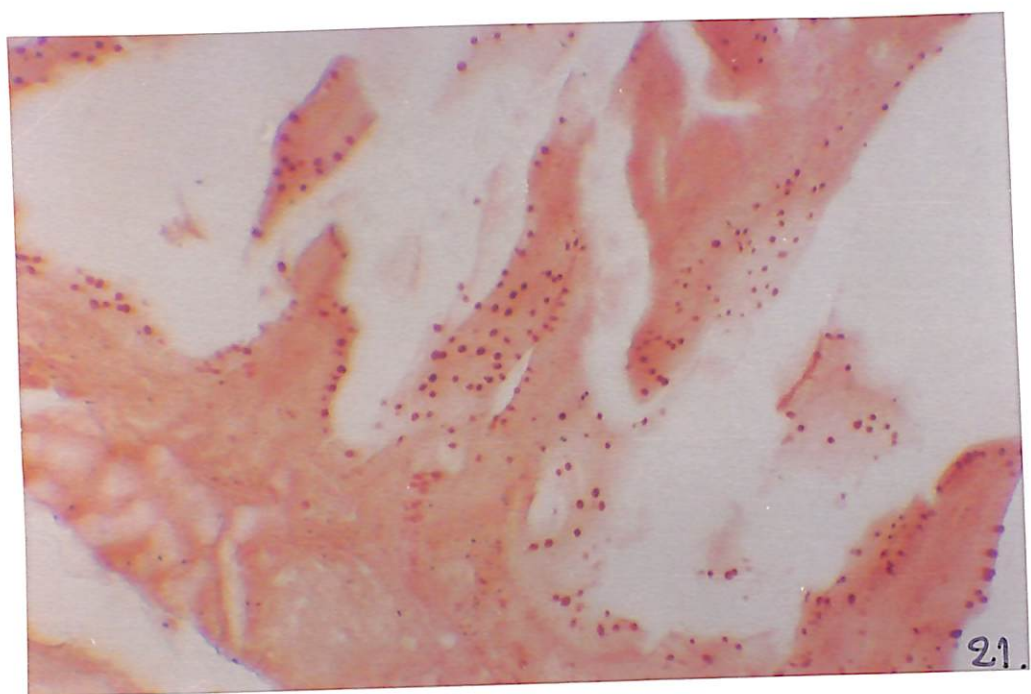


Figure No – 21

Photomicrograph of section through caudal to caudal flexor of duodenum of rabbit showing higher population of goblet cells in the intestinal epithelium. PAS – orange G ; X 150.

Figure No – 22

Photomicrograph of section through duodenum of rabbit showing lymph nodule surrounded by the epithelium of villi (a) and submucosal gland (b)
Azan stain ; X 150.

Figure No – 23

Photomicrograph of section through mid portion of duodenum of rabbit showing well developed submucosal glands of seromucous in nature. The duct of the gland (arrow) extending into the base of mucosal gland.

Mucicarmine counter stained with metanil yellow ; X 150.

Figure No – 24

Photomicrograph of section through duodenum of rabbit showing colloidal iron positive submucosal glands.

Colloidal iron stain ; X 150.

