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THE EFFECT OF HIGH CONCENTRATE DIET ON THE HISTOLOGICAL STRUCTURE OF THE SMALL INTESTINE OF EARLY WEANED LAMBS

(With 2 Tables and 19 Figures)

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تأثير تغذية الحملان حديثة الفطام على عليقة عالية المركزات على التركيب النسيجي للامعاء الدقيقة

منى عبد الفتاح على

اجري هذا العمل لدراسة تاثير تغذية الحملان حديثة الفطام بعلائق عالية المركـــزات علـــى النمو وعلى التركيب النسيجي للامعاء الدقيقة. أجريت هذه التجربة على ١٨ حمـــل قســـمت على ثلاث مجموعات. المجموعة الأولى واعتبرت المجموعة الضابطة تكونت من عـــدد ٦ حملان عمر ١٢٠ يوم وغذيت على ٨٠% مركزات و٢٠% سيلاج الذرة. المجموعة الثانيـة تكونت من ٦ حملان حديثي الفطام عمر ٦٠ يوم وغذيت علـــى ٩٠% مركـــزات و ١٠% سيلاج الذرة والمجموعة الثالثة تكونت من ٦ حملان حديثي الفطام عمر ٦٠ يوم وغذيـــت على ١٠٠% مركزات فقط ــ تركت الحملان لتتغذى دون قيد على المركزات مـــن خـــلال عذايات. وقد أظهرت الدراسات ان مخاطية الامعاء الدقيقة للمجموعة الضابطة تتكون مـــن خملات ضيقة وقصيرة مع غدد معوية عديدة وطويلة. ووجد ان الظهارة المبطنـــة للامعـــاء تكون من خلاية كأسية تكثر في الغدد المعوية عن الخملات وخلايا عمادية وخلايـــة محبـــة للفضة. وجد ايضا عقد ليمفاوية موجودة في الطبقة تحت المخاطية في الجزء العلـــوي مـــن الامعاء (Duodenum) اما الخلايا المعوية داخلية الافراز فكانت عديدة في الجزء العلـــوي من الامعاء الدقيقة عنها في الجزء السفلى وكذلك كانت عديدة فـــي الغـــدد المعويـــة عنـــها فيالخملات. ظهرت الخلايا المعوية داخلية الافراز في غدد Brunner كانت الخلايا المعويـــة داخلية الافراز متعددة الاشكال فمنها المثلث والمخروطى والكأسي. وقد اظهرت الدراســـات الهستولوجية انه هناك تحلل في الطبقة الطلائية المبطنة لخملات الامعاء الدقيقة في الحمـــلان التي تغذيت على مركزات عالية. وكانت التغيرات الهستولوجية في امعـــاء الحمـــلان التـــى تغذيت على ٩٠% مركزات عبارة عن اتساع واحتقان شديد في الاوعيـــة الدمويـــة مـــع علامات تحلل في الطبقة الطلائية المبطنة للخملات وقد تطورت مظاهر التحلل فـــي امعـــاء الحملان التي تغذيت على ١٠٠% مركزات حيث ازداد عـــدد خلايـــا البلازمـــا والخلايـــا الليمفاوية. ومن هذه النتائج ننصح المربى الذي يريد فطام الحملان الصغيرة بعـــد ٦٠ يـــوم

فقط من تاريخ الولادة واستبدال اللبن بالعلائق عالية التركيز أن يتم ذبح الخراف عندما تصل إلى وزن ٤٣ كجم حتى لا يزداد الضرر بالحيوان.

SUMMARY

The mucosa of the small intestine of the lambs was formed of short, narrow finger like villi and numerous long intestinal glands. The goblet cells were more abundant in the crypts than on the sides of villi. Lymphatic nodules were seen between the ubmucosal glands in the duodenum. The enteroendocrine cells showed a descending pattern of distribution from the duodenum to the ileum and more abundant in the crypts and base of the villi than in the upper parts of the villi. The enteroendocrine cells may occasionally found scattered between the cells linning the submucosal glands. (Brunner's glands). The enteroendocrine cells were mainly triangular, spindle or flask-shaped. The dark brown granules of the enteroendocrine cells were basally located and occasionally forming a crescent-like appearance around the unstained nucleus. The lining epithelium of the intestinal villi of early-weaned lambs fed 90% concentrate diets showed various degrees of desquamation and degencration. The signs of degeneration increased in the small intestine of early-weaned lambs fed on 100% concentrate diets.

Key words: *Diet, Histological Structure, Small Intestine*

INTRODUCTION

Sheep numbers have declined markedly for several reasons, including lack of profitability compared to beef cattle production. New production techniques could reverse this pattern. Lambs can be early-weaned to dry lot and finished on high concentration diets (Bakr, and Hegazi, 1997). High-energy diets with a low roughage contents (0-10%) are efficient to promote high growth rate, (Thérize, 1975).

The small and large intestines form together a long tube adapted for the digestion, absorption of nutrients and absorption of salt and water (Smith et al., 1988).

The small intestine has two principal functions (1) to complete the digestion of food by the action of appropriate enzymes, (2) to absorb selectively the final products of digestion into the blood and lymph vessels. (Turk 1982, Bank 1993 and Matthew and Robert 1996).

The aim of the present study is to illustrate the changes in the histological structure of small intestine of early-weaned lambs finished on 100% or 90% concentrate diets or feeder lambs finished on 80% concentrate diet.

MATERIALS and METHODS

Eighteen lambs were purchased from the grazing flock in Kafr El-Sheikh Governorate. These lambs were divided into 3 groups: (each one contains 6 lambs) (Table 2).

Group I: (Considered as a control group) feeder lambs, 120 days old, fed on 80% concentrate and 20% corn silage.

Group II: Early-weaned lambs, 60 days old, fed on 90% concentrate and 10% corn silage.

Group III: Early-weaned lambs, 60 days old, fed on 100% concentrate.

Ingredient compositions of concentrate finishing diets were shown in Table (1). 14-days adaptation period was used to adjust lambs to their respective high-concentrate diets. At day 15 lambs were weighed (initial weight) and switched to be self-fed the high concentrate diets. The lambs were individually weighed in early morning every 21 days throughout study. Immediately after weighing at the final conclusion of the feed trial, three lambs from each group were slaughtered.

Specimens from the small intestine of lambs were taken just after slaughtering. The specimens were fixed either in Bouin's fluid or in 10% neutral buffered formalin. After a proper fixation, the specimens were dehydrated, cleared and embedded in paraffin wax. Sections of 5 microns thick were obtained and stained with Haematoxylin and Eosin, Crossman's trichrome stain, periodic acid Schiff's technique and Grimelius silver nitrate impregnation method (Grimelius 1968). All stain techniques were adapted to that reported by Bancroft and Stevens (1990).

Table 1: Ingredients composition of high concentrate finishing diets.

Item	100% concentrate	90% concentrate	80% concentrate
Ingredient, % of DM			
Whole white corn	73.30	65.40	55.00
Soybean meal	21.00	21.00	21.00
Corn silage	-	10.00	20.00
Ground limestone	2.10	2.00	2.00
Trace-mineralized salt	1.00	1.00	1.00
Ammonium sulfate	0.50	0.50	0.50
Neomycin sulfate	0.10	0.10	0.10
Vitamin mix	0.002	0.0004	0.0002

DM = Dry matter

RESULTS

The average values of the initial and final body weights and daily gain are presented in Table (2). Early-weaned lambs fed 100% concentrate diet were reached to 43.4 kg more earlier than those fed 90% concentrate diet. With 100% concentrate diet the final body weight (43.4 kg) of early-weaned lambs was increased nearly 3 folds the initial body weight (15.40 kg) while with feeder lambs fed 80% concentrate diet (41.5 kg) was nearly 2 folds the initial body weight (23.7 kg) in the same period 83 days reflecting a faster average daily gain over(83 day) feeding period.

Table 2: Average values of performance parameters of early-weaned and feeder lambs fed high concentrate diets.

Item	Group I	Group II	Group III
No. of lambs	6	6	6
% of concentrate	80%	90%	100%
Initial body wt. Kg	23.70	15.00	15.40
Final body wt. kg	41.50	43.40	43.40
Experimental period (days)	83	125	83
Average daily gain/kg	0.21	0.23	0.34

Histological findings:

The wall of the small intestine of control group was formed of mucosa, submucosa, muscular layer and serosa.

The mucosa of the small intestine was formed of short, narrow finger like villi and numerous long intestinal glands filled nearly all lamina propria to reach muscularis mucosa (Fig. 1).

The epithelium linning the intestinal villi and intestinal crypts consisted of tall columnar cells, goblet cells and argyrophil cells.

The tall columnar cells contained vacuolated eosinophilic cytoplasm with rounded basally located nucleus. The apical surface of these cells showed a pronounced striated border (Fig. 2).

The goblet cells were scattered among the tall columnar cells. They were more abundant in the crypts than on the sides of the villi (Fig. 3).

The central core of the intestinal villi and the subepithelial lamina propria contained blood capillaries, smooth muscle fibres, fibroblasts, lymphocytes, plasma cells, eosinophils and central lacteals (Fig. 4). The central lacteals were numerous, dilated and occupied nearly the length of the villi (Fig. 3).They were lined with one layer of endothelial cells. (Fig. 5).

The muscularis mucosa consisted of thin layer of longitudinally arranged smooth muscle fibres (Fig. 6).

The submucosa of the duodenum contained clusters of branched, coiled tubular glands (Brunner's; glands) that open into the intestinal crypts (Fig. 6). These glands were lined with cuboidal to low columnar cells contained vacuolated cytoplasm with basally located oval or flat nuclei (Fig. 7). Few argyrophilic cells were demonstrated scattered among the mucus cells of Brunner's glands (Fig. 8a, b).

Secondary lymphatic nodules with germinal centers were seen between the submucosal glands in the duodenum (Fig. 9).

The muscular layer was formed of inner circular and outer longitudinal layers of smooth muscle fibres. The inner layer was thicker than outer layer (Fig. 9).

In sections impregnated with silver, the enteroendocrine cells granules were stained dark brown. The distribution and shape of the enteroendocrine cells were variable in different regions of the intestine. Moreover, these cells showed a descending pattern of distribution from the duodenum to the ileum and more abundant in crypts and base of villi than the upper parts of villi (Fig. 10a, b, c).

The enteroendocrine cells were mainly triangular, spindle or flask-shaped. The cytoplasm of these cells demonstrated fine dark brown granules which were located in the basal parts of cells and

occasionally forming a crescent-like appearance around the unstained nucleus (Fig. 11a & b & c & d & e). All enteroendocrine cells rest on the basement membrane of the crypts and villi but, some cells occupied the whole length of the epithelium and showed a luminal contact, while other cells did not reach the lumen.

The linning epithelium of the intestinal villi of early-weaned lambs fed 90% concentrate diets showed various degrees of desquamation and degeneration. (Fig. 12a & b). The cytoplasm of the linning epithelium of the villi was more acidophilic than that in control group and the apical surface of the columnar cells appeared strongly acidophilic (Fig. 13), but the linning epithelium of the intestinal glands was still contact and their cytoplasm was less acidophilic than that of the villi and the apical surface of the columnar cells gave a marked positive reaction with acid fuchsin in trichrone stain (Fig. 14 & 15). At this group there was more lymphocytic infiltration and caseated acidophilic materials surrounded by inflammatory cells in the lamina propria (Fig. 16), also, the blood vessels were enlarged and engorged with blood.

The signs of degeneration increased in the small intestine of early-weaned lambs fed on 100% concentrate diets (Fig. 17). These changes were associated with an increase in the number of plasma cells and lymphocytes (Figs. 18 a & b). The cells linning the intestinal glands were also acidophilic (Fig. 19).

DISCUSSION

Our investigation revealed that the epithelium linning the intestinal villi and crypts consisted of tall columnar, goblet and argyrophil cells. The apical surface of columnar cells of control group showed a pronounced striated border, where the apical surface of the columnar cells of early-weaned lambs fed 90% or 100% concentrate were strongly acidophilic and gave a strong reaction with acid fuchsin stain. Humphrey and Turk (1974) and Telford and Bridgman (1990), revealed that the striated border is composed of parallel rows of microvilli of even height. These microvilli increase the luminal surface of each enterocyte, (Bayer et al., 1975), from which extend a network of hair-like structures termed the glycocalyx. The glycocalyx and the microvilli known as the epithelial brush border, contain many of enzymes responsible for the terminal digestion of carbohydrate and

protein while the special carrier protein necessary for the absorption of hexose sugars, amino acids, vitamins, minerals and electrolytes situated on the surface of microvilli (Smith et al., 1988).

The present study revealed that the goblet cells were scattered among the tall columnar cells. Teford and Bridgman (1990), reported that these cells release copious amount of mucus to protect the linning cells from abrasion and to lubricate the passage of material along the digestive tract. They may also serve to assist in absorption (Turk, 1982). It has been emphasized that, the layer of mucus secreted by the goblet cells constitutes the first line of defence for the intestine by its tenacious adherence to the underlying tissue and through its general impermeability to the destructive chemical agents (Holander, 1954, Cecil, 1990 and El-Gengehy, 1990).

The present investigation showed that the Brunner's glands were lined with cuboidal to low columnar cells contained vacuolated slightly basophilic cytoplasm. Lesson et al. (1985) and Telford and Bridgman (1990), reported that the secretion of Brunner's glands is strongly alkaline which neutralize the acidic chyme of the stomach and thus protects the duodenal mucosa from autodigestion. Also, to bring the intestinal contents to the optimum pH for pancreatic enzyme action (Junqeira et al., 1989). Banks (1993), suggested that these glands may be the aboral continuations of pylóric glands displaced to the submucosa.

Grimelius silver impregnation, in our study, showed few argyrophilic cells scattered among the mucus cells linning the Brunner's gland. Recent imunofluorescence studies suggest that Brunner's glands contains urogastrone, a peptide hormone, that inhibits HCl production in the stomach (Lesson et al., 1985,and Telford and Bridgman, 1990) and stimulate the epithelial cell proliferation (Junqueira et al., 1989).

The muscular layer of the small intestine of lambs was formed of inner circular and outer longitudinal smooth muscle fibres. The inner circular layer serves to constrict and dilate different regions of the intestinal length and acting to mix the chyme with the digestive enzymes while the outer longitudinal layer is responsible for the peristaltic waves that slowly drive the chyme forward (Sheeler, 1996).

Some of the enteroendocrine cells stimulate, and others inhibit certain alimentary processes. Their overlapping activities collectively regulator and itegrate the activities of the GI tract, (Telford and Bridgman 1990).

The present studies revealed some variabilities in the distribution and shape of the enteroendocrine cells in different the regions of the intestine. They showed a descending pattern of distribution from the duodenum to the ileum. Banks (1993), reported that the argentaffin cells secrete serotonin, motilin, pancreatic glucagon, cholecystokinin, vasoactive intestinal peptide, somatostatin, gastrin, neurotensin, secretin and enteroglucagon. Also, Matthew and Robert (1996), added that these cells synthesize and store histamine and release it when stimulated by acetylecholine or gastrin. Histamine diffuses to nearly parietal cells to stimulate HCl secretion. On other hand, Kirkegeard et al. (1982), reported that glucagon and other related peptides secreted by the endocrine cells act as powerful inhibitors of gastric acid secretion. This fact could explain the abundance of endocrine cells in the proximal part of intestinal tract as observed in the present study.

Our results revealed that the enteroendocrine cells were more concentrated in the crypts and base of villi than the upper part of villi. This location could be attributed to the slow rate of migration of the newly formed enteroendocrine cells from the bases of the crypts to their superficial parts (Ferreira, 1971).

In the present study, different shapes of enteroendocrine cells could be demonstrated. Their cytoplasm showed fine dark brown granules, mainly basally located and occasionally forming a crescent like appearance around the unstained nucleus. In agreement to that reported by Schofield and Silva (1968), Nossier (1992) and Kassab (1996), in which the argyrophilic granules either occupied basal position, or sparsely distributed in some cells and densely packed in others.

The basal accumulation of the argyrophilic granules indicates the possibility of secretion by exocytosis across the basal membrane to the lamina propria (Forssman et al.1969, Nichols et al., 1974, Cristina et al.,1978, Sjolund et al., 1983 and Luis and Thompson, 1988).

The present study revealed that the linning epithelium of the intestinal villi of early-weaned lambs fed 90% concentrate diets showed various degrees of desquamation and degeneration. The signs of degeneration increased in the small intestine of early weaned lambs fed on 100% concentrate diets. McGee et al. (1992), stated that the dietary fibre is an important but not essential constituent of the diet. Fibre passes along the gastrointestinal tract acting as a sponge, and modulates function by delaying gastric emptying, slowing the absorption of

nutrients from the jejunum, possibly modifying bile acid absorption from the ileum, providing nutrition to caecal bacteria and increasing weight.

In our study, the number of plasma cells was increased. This increase is a hallmark of subacute and chronic inflammation (Telford and Bridgman, 1990). This inflammation may be due to that the whole corn, consumed by sheep, is crushed during eating and ruminating and an amount may escape degradation in the rumen and pass intact or coarse crushed to the small intestine (Qrskov, 1986).

Mona (1997) found a similar results in the liver and kidney of lambs fed on 90% or 100% concentrate where the histological studies of liver and kidney revealed signs of degenerative changes.

In conclusion, finishing of early-weaned lambs on a diet based on 100% concentrate diet resulting in greater daily gain compared with those finishing on 90% concentrate diet, and 10% corn silage or feeder lambs finished on 80% concentrate diet and 20% corn silage, but the histological studies of small intestine of early weaned lambs finished on 100% or 90% concentrate diet showed signs of inflammation and degeneration in the mucosa, so, we advice that these lambs must be slaughter immediately after this period of fattening (when reach to 43 kg).

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FIGURES

Fig. 1 & 2: Small intestine of control lambs showing: mucosa (A), submucosa (E), muscular layer (O) muscularis mucosa (K) intestinal villi (V), crypts (J), central lacteal (N), goblet cell (T), columnar cells (R), striated border (arrow) and lymphocytes (S) (H & E stain x: (1) 40, (2) 1000.

Fig. 3: Small intestine of control lambs showing: the distribution of goblet cells (T), crypts (J) and central lacteal (N) (PAS-H tech., x: 100).

Fig. 4 & 5: Small intestine of control labms showing: plasma cells (S), eosinophil (x), blood capillary (W) lymphocyte (Z) and central lacteal (N) (H & E stain x: 1000).

Fig. 6 & 7: The duodenum of control lambs showing: Brunner's glands (U), mucularis mucosa (K). (H & E stain, x (6): 100, (7): 1000.

Fig. 8a & b: Small intestine of control lambs showing: The enteroendocrine cells (two arrows) in Brunner's gland (U) (Grimelius stain x: (a): 100, (b) 1000.

Fig. 9: Small intestine of control labms showing: Lymphoid follicles (Q) with germinal center (m) embedded in Brunner's glands (U) (H & E stain, x: 100).

Fig. 10a & b & c: Sections in duodenum , Jejunum and ileum respectively showing distribution of enteroendocrine cells (arrows). (Grimelius stain x 100.

Fig. 11a & b & c & d & e: Sections in intestinal tract of lambs showing different shapes of enteroendocrine cells (Grimelius stain).
a, b: In crypts of small intestine (x: 400).
c, d: In villi of small intestine (x: 1000).
e: In Brunner's glands (x: 400).

Fig. 12a & b: Small intestine of lambs fed 90% concentrate diet (H & E stain x: (a) 100, (b) 400.

Fig. 13: Small intestine of lambs fed 90% concentrate diet showing acidophilia in the apical border of columnar cells (arrows) (H & E stain x: 400).

Fig. 14: Small intestine of lambs fed 90% concentrate diet showing acid fuchsin positive apical surface (arrow) (Crossmon's trichrome stain x 1000).

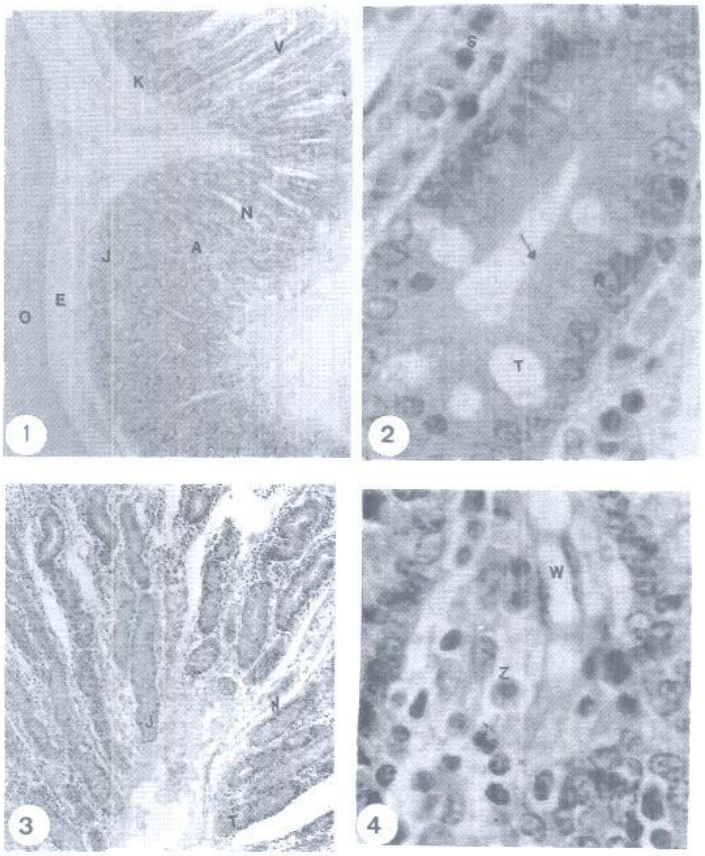
Fig. 15: Small intestine of lambs fed 90% concentrate diet showing intestinal glands arrows (H & E stain x 400).

Fig. 16: Small intestine of lambs fed 90% concentrate diet showing lymphocytic infiltration (arrow) and caseated acidophilic material (two arrows) (H & E stain x 100).

Fig. 17: Small intestine of lambs fed 100% concentrate diets (H & E x 100).

Figs. 18a & b: Small intestine of lambs fed 100% concentrate diets showing plasma cell (arrow) intraepithelial and in the lamina propria, respectively (H & E stain x 1000).

Fig. 19: Small intestine of lambs fed 100% concentrate diet showing acidophitic apical border in the crypts and interepithelial plasma cell (arrow) (H & E stain x 400).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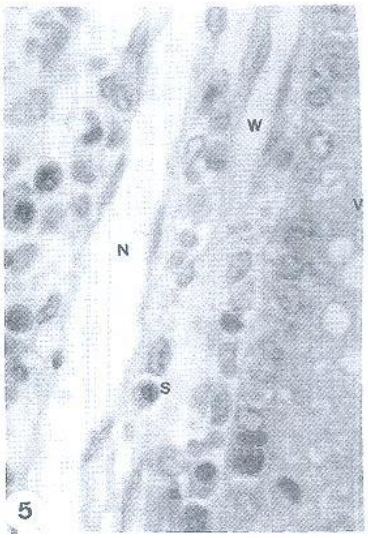
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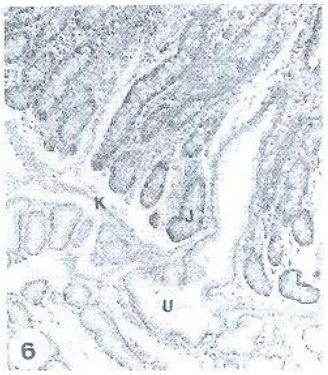
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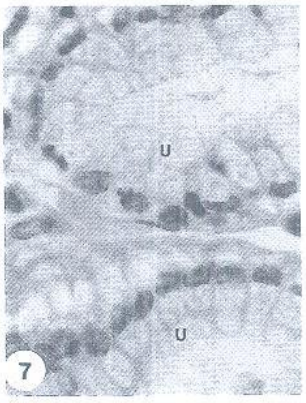
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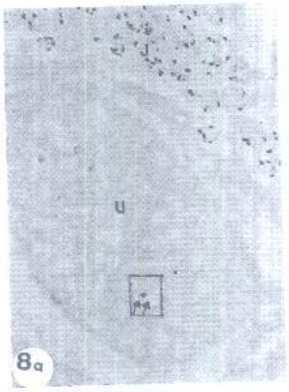

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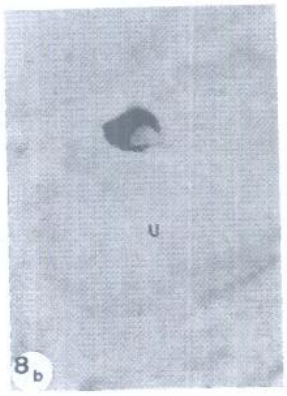

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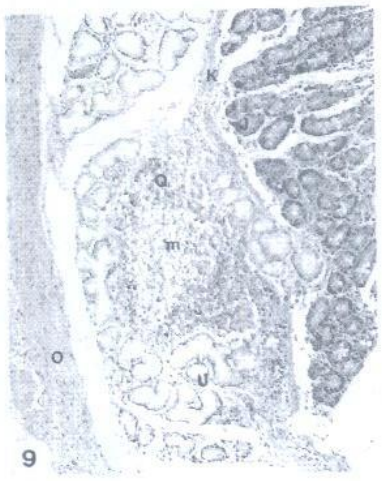

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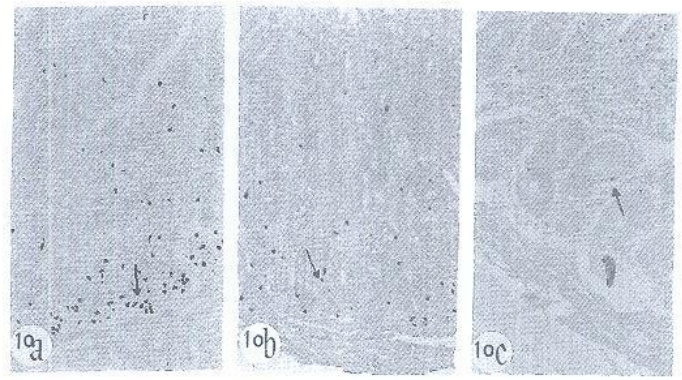
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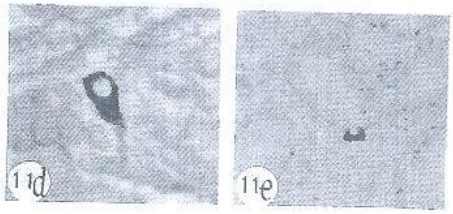
10c


11a
11b
11c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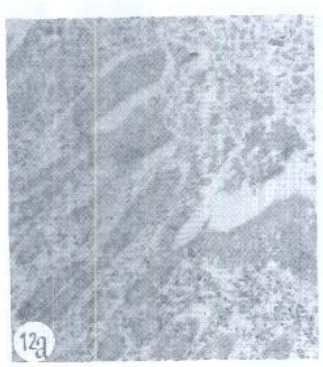
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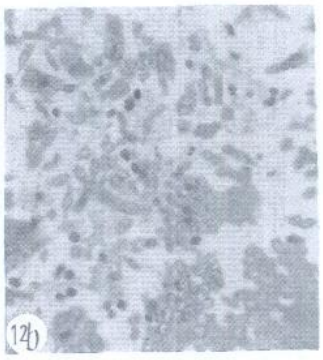
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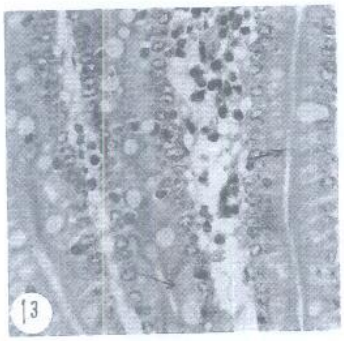
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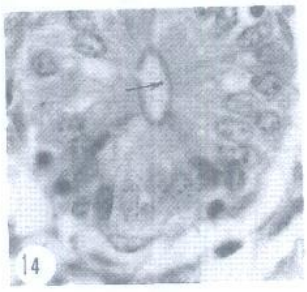
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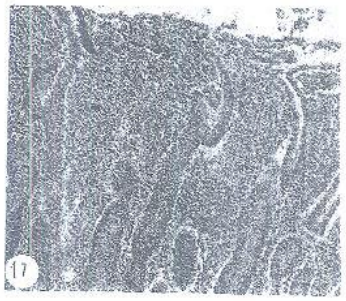
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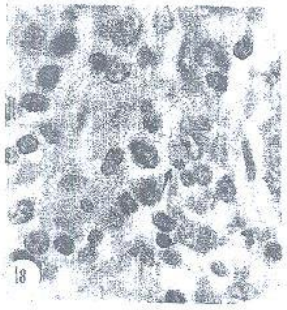
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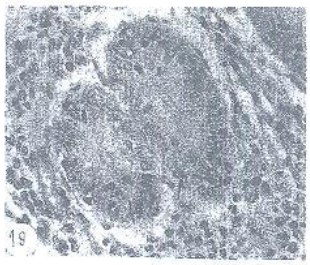
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