Assiut Vet. Med. J. Vol. 44 No. 87, October 2000

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COMPARATIVE MORPHOMETRICAL AND SCANNING ELECTRON MICROSCOPICAL STUDIES ON THE GLANDULAR STOMACH OF THE CHICKEN (GALLUS DOMESTICUS), PIGEON (COLUMBIA LIVIA), DUCK (ANAS DOMESTICUS) AND CATTLE EGRET (BUBULCUS IBIS)

(With 4 Tables, 2 Histograms and 22 Figures)

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(Received at 30/9/2000)

دراسات مقارنة مورفومترية وبالماسح الإلكتروني علي المعدة الغدية في الدجاج والحمام والبط وأبوقردان

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في هذا البحث تم فحص السطح المخاطي للمعدة الغدية مورفومتريا وكذلك بالمجهر الإلكتروني الماسح في كل من الفراخ ، الحمام ، البط وأبوقردان. أظهرت الدراسة أن المعدة الغدية تكون مغزلية الشكل في الفراخ والحمام والبط أما في أبوقردان فكانت عدسية الشكل. كانت هناك علاقة عكسية بين كل من المسافة بين فتحات الغدد وكثافتها في المليمتر المربع فكانت عالية في الحمام وأبوقردان والبط عنها في الفراخ. أظهر الميكروسكوب الإلكتروني الماسح وجود العديد من الثنايات المخاطية. هذه الثنايات كانت مرتبة دائريا حول فتحات الغدد مكونة الحلمات المخاطية و كذلك موزعة بغير انتظام بين هذه الحلمات. في الفراخ كان النوع الشائع من الحلمات يشبه القرص المعي في شكله العام، بينما وجدت أشكال أخرى مثل قبة الفرن و كذلك مثلثة المركز في بعض الأحيان. في الحمام كانت الحلمات المخاطية تشبة عصيان عجلة العربة الكارو وتأخذ شكل الحفرة في البط، أما في أبوقردان فكانت تشبه الوردة ويدل اختلاف أشكال هذه الحلمات المخاطية في الطيور موضع الدراسة علي علاقتها بنوع الطائر.

SUMMARY

The mucosal surface of the glandular stomach of chicken, pigeon, duck and cattle egret was examined morphometrically and by scanning electron microscope. The proventriculus was spindle-shaped in chicken, pigeon and

duck as well as lens-shaped in cattle egret. There was a reversed relationship between the distance between two successive gland openings and their number per mm^2. The density of these openings per mm^2 was higher in pigeon, cattle egret and duck than in the chicken. Scanning electron microscopy revealed numerous mucosal folds. The latter were concentrically arranged around the gland opening forming the mucosal papillae and irregularly distributed between them. The mucosal papillae of chicken resembled nearly the intestinal disc, but some of them were dome-shaped or had a triangular-shaped center. They resembled spokes of a cartwheel in pigeon, pit-like invagination in duck and rose-shaped in cattle egret, indicating species dependent.

Key words: *Mucosal papillae, SEM, morphology, morphometry, glandular stomach, chicken, pigeon, duck, cattle egret.*

INTRODUCTION

There is a considerable literature on the development, macro-and microscopic anatomy of the avian digestive tract specially the glandular stomach, which received a lot of works (for review see, Calhoun, 1954; Bradley and Grahame, 1960; Romanoff, 1960; Hodges, 1974; Salem, 1982 & 1985; Salem, 1991). The glandular stomach of the chicken has an elaborate system of the proventricular glands, which make up most of the thickness of its wall (Toner, 1963a). The excretory ducts of these glands open at the apex of the raised papillae, which scattered over the mucosal surface of the proventriculus (Hodges, 1974; Banks, 1993). According to the available literature, the morphometrical studies of the mucosal surface of the proventriculus in birds in relation to the gland openings were lacked, while scanning electron microscopical investigation of the mucosal surface of the avian glandular stomach is very rare except of Salem's (1997) work on chicken. Therefore, this study focuses on the investigation of the mucosal surface of the proventriculus morphometrically and morphologically by using scanning electron microscope specially the shape of the gland openings (mucosal papillae) in chicken, pigeon, duck and cattle egret.

MATERIALS and METHODS

This study was carried on adult apparently healthy birds. They include 13 chicken (Fayoumi), pigeon, duck and cattle egret each. For macroscopical and morphometrical studies, 10 birds of each species were

used. The birds were weighed, then slaughtered, evascerated and the glandular stomach was cut at both junctions with the esophagus and the gizzard. The diameter at the widest part of the stomach, its length and weight were calculated (after longitudinal cutting of the stomach and washing in normal saline to remove the content). Then after the glandular stomach was temporarily pinned on a cork plate for 30 minutes to prevent fixation contracture. The stomach was covered with fresh fixative for initial fixation. By using Leica Q 500 MC Image analyzer (connected with dissecting microscope) the following measurements were carried out on 5 birds each species:

1. The total surface area of the glandular stomach.
2. The number of the glandular openings in three different random fields each bird was counted and consequently the number of the openings per mm^2 was obtained.
3. The distance between two successive openings was measured in 10 fields of each bird

The values were given as means $\pm$ standard error. The data was statistically analyzed using Anova and t-test.

For scanning electron microscopy, pieces of the glandular stomach (5 mm^2) were obtained from 3 chickens, 3 pigeons, 3 ducks and 3 cattle egrets. After washing in normal saline, the pieces were fixed in Karnovesky solution (Karnovesky, 1965) for 24 hours at 4°C. They were washed in 0.1 M cacodylate buffer pH 7.2 and post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for further 2 hours at 4°C. Then after the pieces were dehydrated through ascending ethanol series and amyle acetate. They were then critical point dried using carbon dioxide, mounted on cupper stubs and coated with gold prior to examination in JOEL 5400 LV scanning electron microscope at 15 KV.

RESULTS

1. Macroscopical and morphometrical investigation:

The glandular stomach of chicken, pigeon and duck was spindle-shaped, while that of the cattle egret was lens-shaped. When the stomach was opened along their longitudinal axis to expose their mucosal surface, they appeared nearly trapezoid in chicken, duck and cattle egret, while that of the pigeon was nearly rectangular (Fig. 1). The esophageal folds terminated gradually in the glandular stomach in the studied birds, while there was an isthmus between it and the gizzard in chicken, pigeon and duck

which was absent in cattle egret and replaced internally by ridge-like elevation (Fig. 1).

The mucosal surface of the pigeon and duck proventriculus showed four longitudinal folds, while that of the cattle egret had one large fold and another three-four small ones. These folds were not recognized in the chicken. The mucosa revealed numerous papillae (openings of the excretory duct of proventricular glands) which were easy identifiable in the chicken (Fig. 1).

Morphometrically, the weight of the glandular stomach reached about 3.09±0.18, 0.77±0.05, 6.07±0.81 and 3.26±0.25 gm in the chicken, pigeon, duck and cattle egret, respectively. Its length was 21.41± 0.87 in the chicken, 17± 1.5 in the pigeon, 38 ±3.9 in the duck and 19.4± 0.7 mm in the cattle egret (Table 1-4). The glandular stomach of duck possessed the greatest surface area which reached 1528.9 mm^2 ± 168.6, while that of pigeon was the smallest (274.45 mm^2 ± 31.68). In chicken and cattle egret the total surface area of the proventriculus reached 556.63 mm^2 ± 33.05 and 660.83 mm^2 ± 14.99, respectively (Table 1-4). The distance between two successive gland openings was the longest in chicken (2.76 mm ± 0.13) and the shortest in pigeon (0.69 mm ± 0.02). In duck and cattle egret, these values were slightly greater than in pigeon (Table 1-4 & Histogram 1). The glandular openings were densely distributed upon the mucosal surface of the proventriculus of pigeon and cattle egret, where one mm^2 contained 1.52 ± 0.09 and 1.4 ± 0.05 openings, respectively. In the chicken the gland openings were less densely distributed, where 0.14±0.02 opening was observed in one mm^2 (Table 1-4 & Histogram 2).

2. Scanning electron microscopical investigation:

I- Chicken:

Scanning electron microscopical investigation of the proventriculus of the chicken revealed that the luminal surface was formed of variable forms of mucosal folds. Some of them were concentrically arranged around the openings of the proventricular glands giving them the commonly known mucosal papillae, which took nearly the form of intestinal disc. In between these papillae the mucosal folds were long finger-like. Both of them were lined with nearly hexagonal-shaped cells with prominent cell boundaries and numerous microvilli (Figs. 2 & 3).

In the most commonly observed type of the mucosal papillae, the gland opening was surrounded circumferentially in one row by 6-8 finger-like mucosal folds (most inner ones), which intern surrounded by another 10-12 similar ones. Outside the latter, 6-8 concentrically arranged large

interrupted or notched mucosal folds were seen. They were separated from each other by clefts containing mucous (Fig. 4). Some papillae were also observed with closed gland opening, where the most inner folds were seen closely adhere to each other (Fig. 5).

Another two forms of the mucosal papillae were uncommonly seen. In the first form, the inner most folds were fused together in a circular manner around the gland opening forming dome-like elevation (Fig. 6). This form was also observed with partially closed proventricular gland opening (Fig. 7). The second form was characterized by a triangular-shaped appearance of its center containing the gland opening. Small irregular bridge-like secondary folds were observed crossing this opening giving it a fenestrated appearance.

II- Pigeon:

The mucosal surface of the glandular stomach of pigeon showed irregular densely distributed mucosal papillae in between them irregularly, interrupted mucosal folds were seen (Figs. 9 &10). The mucosal papillae resembled nearly spokes of a cartwheel. Each papilla consisted of a central opening of the excretory duct of the proventricular gland and 2-3 layers of semicircularly arranged discontinuous mucosal folds. The folds of the most inner layer (10 – 12 folds) were arranged in a circular row around the gland opening in a diverging manner with fused apices forming an external rim. These diverging folds were separated laterally by parallel clefts (Fig. 11). Some of the papillae were also seen with nearly closed gland opening.

III- Duck:

The luminal surface of the glandular stomach projected into longitudinal folds separated by parallel furrows (Fig. 12). It displayed numerous gland openings, which were arranged somewhat in parallel lines. The openings were round or oval-shaped invaginations of the mucosa simulating the mammalian gastric pits (Fig. 13). At higher magnification (Fig. 14), closely packed mucosal folds possessing irregular flat apices, which lined with nearly hexagonal-shaped cells, surrounded the opening.

IV- Cattle egret:

The mucosal surface of the glandular stomach of the cattle egret possessed bricklayer-like appearance with interposition of the rose-shaped mucosal papillae; in between them the folds were randomly distributed (Figs. 15 &16). The latter possessed triangular, rectangular-, hexagonal - and bean-shaped apices with one or two depressions (Fig. 17).

The rose-shaped mucosal papillae were formed of 4 – 6 circularly arranged mucosal folds around the gland opening. They were fused together

laterally and surrounded further by another 3– 4 turns of mucosal folds (Figs.16 &18). The papillae took flat form at the same level of the mucosa (Fig. 18) or were raised above the mucosal surface (Fig. 19). Commonly, the fused mucosal folds formed an inverted continuous rim resembling nearly an inverted edge of a sack (Fig. 20). The gland openings were nearly H-, star-, or triangular-shaped (Figs. 18 – 21).

Another two forms of fusion were rarely seen. In the first form, two of the inner folds fused laterally in horse-shoe manner, capped externally in the opposite side by fusion of the another ones (Fig. 21). In the second form, fusion of the most inner folds were interwoven (Fig. 22). Some of the mucosal papillae appeared nearly closed.

DISCUSSION

In the present study, the glandular stomach of chicken, pigeon and duck was spindle-shaped simulating that mentioned by Calhoun (1954), Hodges (1974); Nickel, Schummer and Seiferle (1977); Dyce, Sack and Wensing (1996) in chicken, and Ibrahim (1992) in chicken, duck and pigeon. Contrary to that mentioned by Ibrahim (1992), the cattle egret glandular stomach was lens-shaped. The interior of the glandular stomach has variable appearance in the studied birds. It was trapezoid in chicken, duck and cattle egret and rectangular- shaped in pigeon and showed variable number of gland openings. There was an isthmus between it and the gizzard in chicken, pigeon and duck as stated by the above mentioned authors as well as Parasad and Kakade (1992) in duck. The isthmus was absent in cattle egret as recorded by Ibrahim (1992) in the same bird and replaced internally by ridge-like elevation, which was not mentioned before.

The present study revealed a positive relationship between the total surface area and the number of gland openings in the proventriculus of the duck. Kolda and Komarek (1958) who mentioned that the proventricular gland openings of duck were more numerous confirmed these results, as the proventricular glands of this bird are unilobular (Das and Biswal, 1967a; Parasade and Kakade, 1990).

On the other hand, there is negative (reversed) relationship between the increased distance between the gland openings and their number/mm^2 in the studied birds. The distance between two successive gland openings was the shortest in pigeon (0.69 mm $\pm$ 0.02), indicating the high densely distributed openings upon the smallest total surface area (274.45 mm^2 $\pm$ 31.68). On the contrary, the proventriculus of chicken had the longest

distance between the gland openings. This could be confirmed by McLelland (1975) who stated that the proventricular glands of chicken are multilobular. This relationship was highly significant in all studied birds ($P \geq 0.001$).

Scanning electron microscpical investigation of the luminal surface of the glandular stomach of the studied birds revealed variable forms of mucosal folds. The projection of the mucosa into numerous folds in all examined birds leads to increase the surface area and consequently the amount of mucin secreted by their lining cells in order to give more protection against the harmful effects of the gastric juice and ingested materials. Some of these folds were concentrically arranged around the openings of the excretory duct of the proventricular glands forming the mucosal papillae as mentioned by Stinson and Calhoun (1993) and Salem (1997) in the chicken. In between these papillae, the mucosal folds were irregularly distributed. These folds were finger-like in chicken as observed by Salem (1997) or possessed flat apices as in pigeon, duck and cattle egret. In all studied birds they were lined with hexagonal-shaped mucus secreting cells (Hodges, 1974; Salem, 1997).

The concentric manner of arrangement of the mucosal folds around the openings of the proventricular glands probably gives more support and protection of the gland opening, better than the finger-forms, against their probably damage produced by variable stretching conditions of this organ. On the other hand, the irregular distribution of the mucosal folds in between the papillae probably play a role in the easy spreading of the mucin upon the mucosal surface by the aid of movement of the ingesta. They also retain certain amount of mucin above and in between them to protect the underlying tissue from the effects of the internal milieu.

The present study revealed different forms of the mucosal papillae upon the luminal surface of the glandular stomach of chicken, pigeon, duck and cattle egret. These papillae resembled nearly the intestinal disc in the chicken. However, dome-shaped papillae as well as those with triangular-shaped center were uncommonly seen. On the other hand, they were similar to spokes of a cartwheel in pigeon or pit-like invagination in the duck and rose-shaped in the cattle egret. These forms may consider as species-dependant.

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LEGENDS

Figs. 1a-d: Photographs of the interior of the stomach of the chicken (a), pigeon (b), duck (c) and cattle egret (d) showing gizzard (G); proventriculus (P); esophagus (O); isthmus (I); mucosal folds (arrow); elevated ridge (double arrow). Corresponding inset showing mucosal papillae with the glandular opening (arrowhead).

Figs. 2-8: Scanning electron micrographs of the mucosal surface of the proventriculus of the chicken showing:

2. Concentrical mucosal folds arranged parallel to each other and separated by grooves (arrow).

3. Finger-like mucosal folds. Inset: Higher magnification showing hexagonal-shaped cells with prominent cell boundaries and microvilli.

4&5. Mucosal papillae with opened (4) and closed (5) gland opening (asterisk); finger-like mucosal folds (arrow); concentrically arranged large mucosal folds (thick arrow); clefts (arrowhead).

6&7. Dome-like papillae with opened (6) or partially closed (7) proventricular gland opening (asterisk).

8. Triangular-shaped center of the papilla with fenestrated appearance (arrow); small bridge-like mucosal fold (arrowhead); concentrically arranged large mucosal folds (thick arrow).

Figs. 9-11: Scanning electron micrographs of the mucosal surface of the proventriculus of the pigeon showing:

9. Irregularly distributed mucosal papillae (arrow). Mucosal folds (arrowhead).
10. Higher magnification showing interrupted mucosal folds (arrow) and the mucosal papillae (asterisk).
11. Mucosal papilla with a central opening (asterisk); diverging inner folds (arrowhead), external rim (thick arrow) and parallel clefts (double arrow).

Figs. 12-14: Scanning electron micrographs of the mucosal surface of the proventriculus of the duck showing:

12. Luminal surface with longitudinal folds (arrow) and parallel furrows (arrowhead).
13. Pit-like invagination of the gland openings (arrow).
14. Higher magnification of the invagination (asterisk) with irregular flat apices of the mucosal folds (arrow).

Figs. 15-22: Scanning electron micrographs of the mucosal surface of the proventriculus of the cattle egret showing:

15. Bricklayer-like appearance of mucosal folds (arrow head) with interposition of the mucosal papillae (arrow).
16. Rose-shaped papilla (asterisk) with concentrically arranged mucosal folds (arrow).
17. Higher magnification of rectangular-and triangular-shaped apices of the mucosal folds with one or two depressions (arrowhead).
18. Rose-shaped mucosal papilla formed by fusion of 4 folds with H-shaped opening.
19. Rose-shaped mucosal papilla formed by fusion of 6 folds with star-shaped opening (raised above the surface).
20. The apices of the mucosal folds resembling an inverted edge of a sack (arrow) with an H-shaped opening.
21. Fusion of the folds in horse-shoe manner capped externally in the opposite side with fusion of another ones.
22. Fusion of folds in an interwoven form.

Table 1: Different measurements of the proventriculus of the chicken.

N.	Sex	Body weight (gm)	Provent. weight (gm)	Provent. length (mm)	Provent. diameter (mm)	Surface area (mm^2)	Openings number/ mm^2	Distance between two successive openings.		
								Min.	Max.	Mean ±SE
1	♀	1039.23	3.30	21.09	10.20	492.24	0.16	2.11	4.60	3.02 ± 0.23
2	♂	1100.00	2.63	22.71	12.40	594.90	0.16	1.68	2.93	2.31 ± 0.13
3	♀	1300.00	3.33	22.84	13.20	659.93	0.11	1.75	5.38	2.92 ± 0.24
4	♂	964.20	3.52	22.25	10.41	554.00	0.11	2.29	3.70	2.92 ± 0.14
5	♀	962.60	2.67	18.18	10.20	482.08	0.15	2.09	3.53	2.62 ± 0.13
Mean		1073.2± 62	3.09± 0.18	21.41± 0.87	11.28 ±0.63	556.63±33.05	0.14±0.02	1.98	4.03	2.76±0.13

Table 2: Different measurements of the proventriculus of the pigeon.

N.	Sex	Body weight (gm)	Provent. weight (gm)	Provent. length (mm)	Provent. diameter (mm)	Surface area (mm^2)	Openings number/ mm^2	Distance between two successive openings.		
								Min.	Max.	Mean±SE
1	♀	259.80	0.65	12	5.3	180.29	1.28	0.54	0.79	0.76±0.02
2	♂	308.60	0.87	20	6.5	261.24	1.64	0.53	0.91	0.72±0.03
3	♀	295.30	0.74	15	5.5	241.10	1.80	0.54	0.75	0.66±0.02
4	♂	311.11	0.71	20	5.9	348.07	1.55	0.54	0.82	0.68±0.03
5	♂	308.5	0.88	18	5.5	341.53	1.35	0.45	0.82	0.64±0.04
Mean		296.7 ±9.6	0.77± 0.05	17±1.5	5.74 ±0.21	274.45±31.68	1.52±0.09	0.54	0.82	0.69±0.021

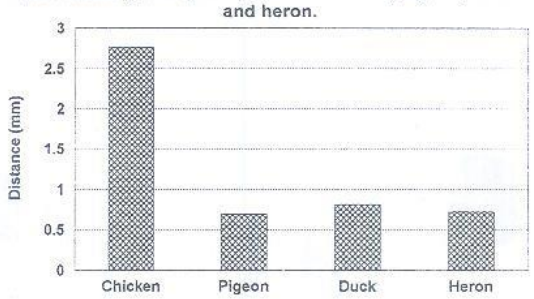
Table 3: Different measurements of the proventriculus of the Duck.

No.	Sex	Body weight (gm)	Provent. weight (gm)	Provent. length (mm)	Provent. diameter (mm)	Surface area (mm^2)	Openings number/ mm^2	Distance between two successive openings.		
								Min.	Max.	Mean±SE
1	♀	1590	4.30	30	10.07	1125	1.20	0.53	0.88	0.69±0.03
2	♀	1540	4.23	28	10.07	1122	0.94	0.77	1.17	0.93±0.04
3	♂	2111	6.11	40	10.05	1700	0.94	0.67	1.09	0.87±0.04
4	♂	2400	7.40	43	10.02	1794	0.91	0.65	1.10	0.86±0.04
5	♂	2520	8.30	49	10.05	1903.5	0.96	0.53	0.88	0.69±0.03
Mean		2032.2±202	6.07± .81	38 ±3.9	10.05± 0.5	1528.9±168.6	0.99±0.05	0.63	1.02	0.81±0.05

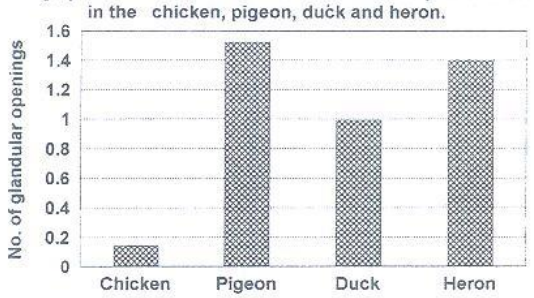
Table 4: Different measurements of the proventriculus of the cattle egret

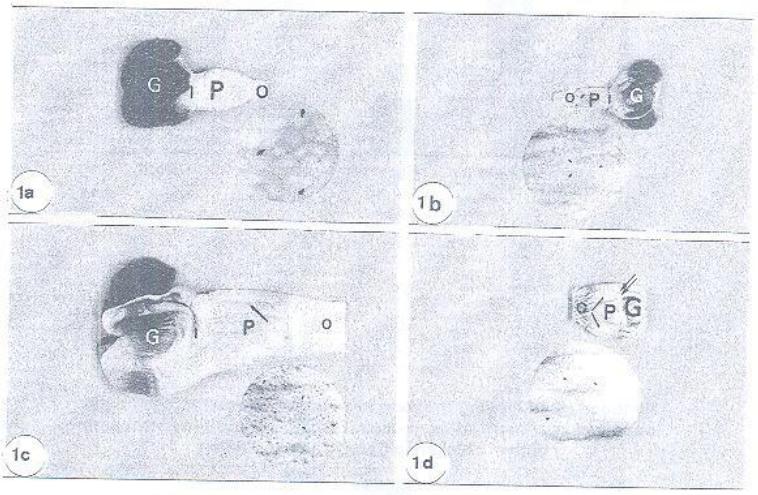
No.	Sex	Body weight (gm)	Provent. weight (gm)	Provent. length (mm)	Provent. diameter (mm)	Surface area (mm^2)	Openings number/ mm^2	Distance between two successive openings.		
								Min.	Max.	Mean±SE
1	♂	219.15	2.91	17	10.75	625.90	1.61	0.54	0.69	0.62±0.02
2	♀	314.85	3.95	20	10.00	713.75	1.39	0.55	0.84	0.72±0.03
3	♀	260.80	2.54	19	8.30	645.80	1.35	0.62	0.89	0.74±0.03
4	♂	269.12	3.33	20	11.00	648.20	1.32	0.60	0.91	0.76±0.03
5	♂	295.77	3.58	21	11.15	670.50	1.33	0.62	0.82	0.75±0.02
Mean		271.94±16	3.26± 0.25	19.4± 0.7	10.24± 0.5	660.83±14.99	1.40±0.05	0.59	0.83	0.72±0.03

Histogram 1 : Showing the distance "mm" between two successive gland openings in the chicken, pigeon, duck and heron.



Histogram 2 : Showing the density of distribution of the glandular openings per "mm^2" of the mucosal surface of the proventriculus in the chicken, pigeon, duck and heron.

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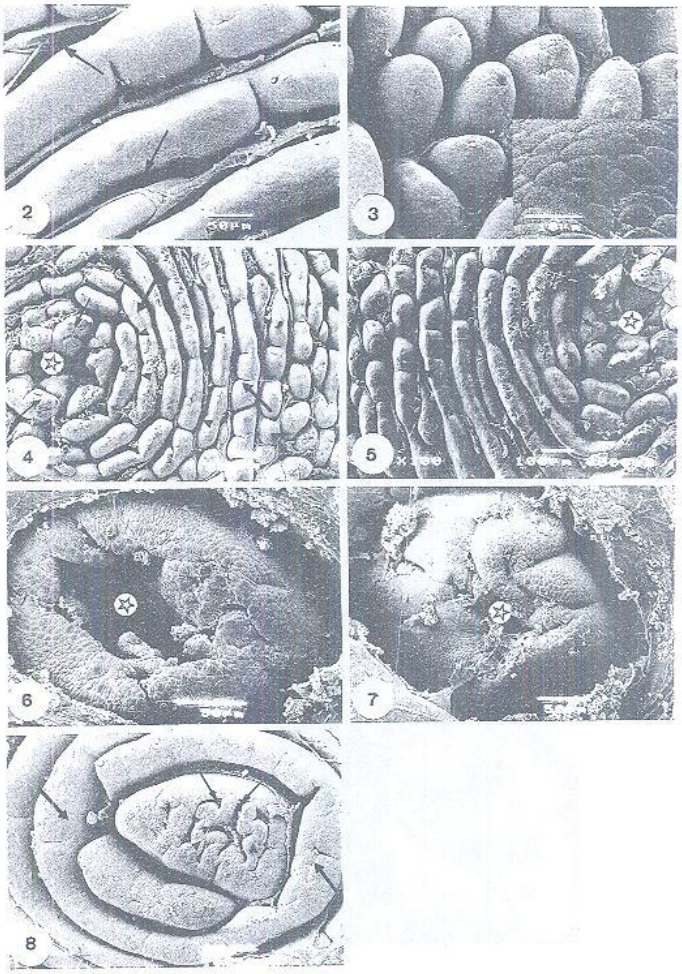
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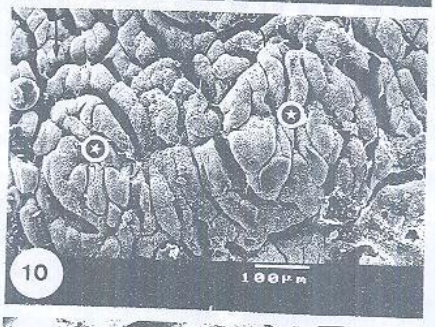
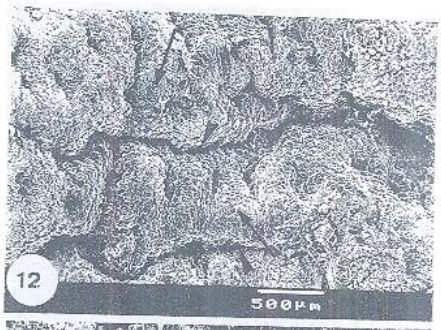
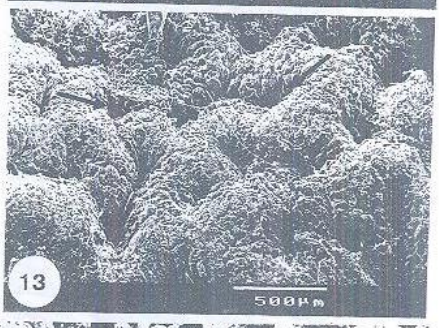
9
500µm

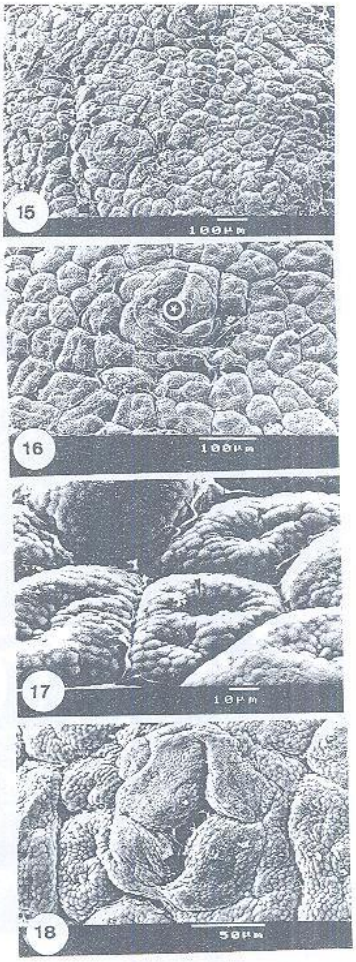

10
100µm

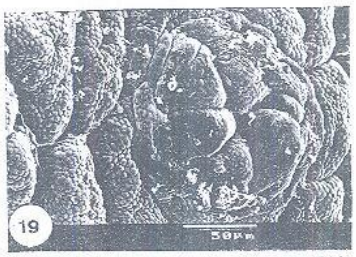

11
50µm


12
500µm


13
500µm

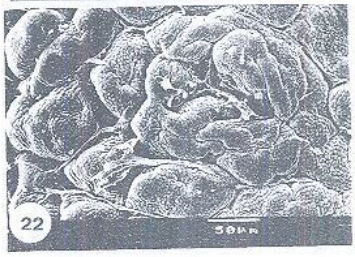

14
50µm


15
100µm
16
100µm
17
10µm
18
50µm


19
50µm


20
10µm


21
30µm


22
30µm