VIA MEDICA

ORIGINAL ARTICLE

Vol. 65, No. 2, pp. 105–110

ISSN 0015–5659
www.fm.viamedica.pl

A comparative LM and SEM study of the structure of the mucosal glands of the gallbladder in two species of canids: the dog and the Chinese raccoon dog

H. Jackowiak, Sz. Godynicki

Department of Animal Anatomy, Agricultural University of Poznań, Poland

[Received 21 March 2006; Revised 24 April 2006; Accepted 24 April 2006]

The studies were performed on the mucosa of the body of the gallbladder in the dog and Chinese raccoon dog, species belonging to the Canidae family. The mucosal glands in both species mostly have the form of alveolar or short tubular secretory units without excretory ducts and are situated in the middle part at the bottom of the crypts surrounded by folds of the mucosa. Sporadically we observed the mucous intraepithelial glands.

The results of the light and scanning electron microscopic observations indicate interspecies differences in the density, type and size of secretory units and also their openings. In the raccoon dog the number of secretory units is 30 times greater than in the dog and the units are predominantly simple glands with small openings. In the dog mostly 2 or 3 secretory units with common wide openings were observed. The SEM images of the NaOH macerated mucosa of the gallbladders showed a connective tissue framework around the glands composed of flat lamina with an irregular pattern of fine collagen fibres and numerous fenestrations. The collagen network around the openings of the glands is more compact and provides mechanical support for the glands of the gallbladder.

Key words: mucosal glands, gallbladder, LM and SEM microscopy, dog, Chinese raccoon dog

INTRODUCTION

Most microscopic studies on the mucosa of the gallbladder in animals and in humans are focused on the structure of the epithelium covering the mucosa of the gallbladder and on the organisation of the blood vessel network in the gallbladder wall as structures directly connected with bile condensation processes [1, 4, 7, 9, 10–12, 15, 19, 20, 23–25, 29]. Subepithelial glands are permanent structural elements in the lamina propria of the gallbladder mucosa.

In the review of studies on the microstructure of the gallbladder in animals Möllendorf [21] reported that the distribution of subepithelial glands in the gallbladder mucosa is highly varied and species-specific. Generally it is assumed that in animals and humans the tubuloacinar mucous glands are found most frequently in the vicinity of the neck of the gallbladder [3, 8, 18, 21, 22, 28]. The greatest number of glands is reported in ruminants, where they may occur in a cluster between the epithelium and the muscle layer. According to Jurisch [13], in the gallbladder in cattle glands may form a continuous band, while in sheep and goats they are more dispersed. In carnivorous animals the glands in the

Address for correspondence: Dr. H. Jackowiak, Department of Animal Anatomy, Agricultural University of Poznań, Wojska Polskiego 71C, 60–625 Poznań, Poland, tel: +48 61 848 76 24, fax: +48 61 848 76 23, e-mail: hannaj@au.poznan.pl

mucosa are more scattered and, as reported by Cutore [5, 6], these glands may also occur in the form of endoepithelial glands. In rodents the glands have been found in the neck of the gallbladder in the guinea pig, and in lagomorphs such as rabbits they have not been observed [16]. In the human gallbladder compound tubuloalveolar glands are observed primarily in the area of the neck [3, 15].

Our preliminary light microscope (LM) observations on the arrangement of glands in the mucosa of the gallbladder in some species of carnivorous animals, such as the dog, raccoon dog, cat, mink and fox, showed that they are present both in the body and the neck of the gallbladder.

The aim of the microscopic examinations was to determine the distribution and structure of glands in the mucosa of the gallbladder in two species of the Canidae family, namely the dog and the Chinese raccoon dog.

MATERIAL AND METHODS

Gallbladders from five shepherd dogs and seven raccoon dogs were dissected immediately after the deaths of the animals and prepared for observation using LM and scanning electron microscope (SEM) observations. For LM study the tissues were fixed in Bouin solution and then dehydrated and embedded in paraplast. The 4 μm sections were stained by the Masson-Goldner method, periodic acid Schiff (PAS) and Alcian Blue pH = 2.5 reaction [27]. The histological slides were examined and documented under an Axioscope Plus light microscope (Zeiss, Germany).

For observations in SEM the fixed samples were dehydrated in a series of ethanol (70–99.8%) and acetone and subsequently dried at critical point using CO_2 (Critical Point Dryer K850, EMITECH). In order to remove cellular elements and visualise the three-dimensional structure of the connective tissue of the gallbladder mucosa, the tissues were macerated according to the method of Ohtani [24]. Some of the glutaraldehyde-fixed tissues were washed in distilled water and then macerated in a 15% NaOH solution at 20°C for 5 days. The tissues were washed in distilled water and immersed in 2% tannic acid for 5 hours and washed in water. Then the samples were postfixed in 1% osmium tetroxide for one hour. After being washed in several changes of distilled water (for 1 hour) the tissues were dehydrated in the ethanol and acetone series and critical-point dried. All the dried specimens were mounted on aluminium stubs covered with carbon tabs, sputtered with gold (Sputter Coater S 150B, EDWARDS) and observed under the SEM LEO 435 VP (ZEISS) at the accelerating voltage of 10–15 kV. The number of secretory units per 1 mm^2 surface of the gallbladder was recorded on the 10 scanning electron images from each animal at a magnification of × 70.

RESULTS

The gallbladder mucosa in the dog and the raccoon dog is composed of folded connective tissue lamina propria covered with a simple cylindrical epithelium (Figs. 1, 2, 5, 7). The folds of the mucosa anastomose and form polygonal structures termed "crypts" of the mucosa. The subepithelial glands in the gallbladder mucosa are located at the bottom of the crypts (Figs. 5, 7).

The number of secretory units in the body of the gallbladder in the dog is one or two per 1 mm^2, whereas in the raccoon dog it is higher, amounting to 26–40 per 1 mm^2. In both species scarce intraepithelial glands were observed (Fig. 2).

The mucous glands in the dog and the raccoon dog are composed of secretory units in the form of rounded alveoli or short tubuli located under the epithelium of the mucosa (Figs. 1–4). In the dog the diameter of the gland cross-section is approx. 30–50 μm, while in the raccoon dog it is 40–65 μm.

The cells of the secretory units in both species are similar in height, approximating to 20–23 μm. Round nuclei are located in the basal part of these cells, while in the apical part of the cytoplasm in both species secretory granules were observed, exhibiting positive PAS and Alcian Blue reactions at a pH of 2.5 (Figs. 1, 3, 4). Figures 5–7 present images of the surface of mucosal crypts with the openings of the subepithelial glands observed under a SEM. In both species glandular openings are most frequently found in the central part of the mucosa of a crypt. Sporadically the openings releasing the secretion were found on the folds of the mucosa (Fig. 5).

In the dog the glands are composed of 2 or 3 separate secretory units with a common wide opening with a diameter of approximately 50–60 μm (Figs. 1, 3, 9). Smaller openings with a diameter of approximately 32–40 μm belonging to one secretory unit are observed less often. In turn, in the Chinese raccoon dog at the bottom of the mucosal crypt there are frequently numerous openings of single secretory units with a diameter of approximately 10–15 μm (Figs. 4–6). In some places in the mucosa the diameter of wider openings of the glands reaches 25–30 μm.

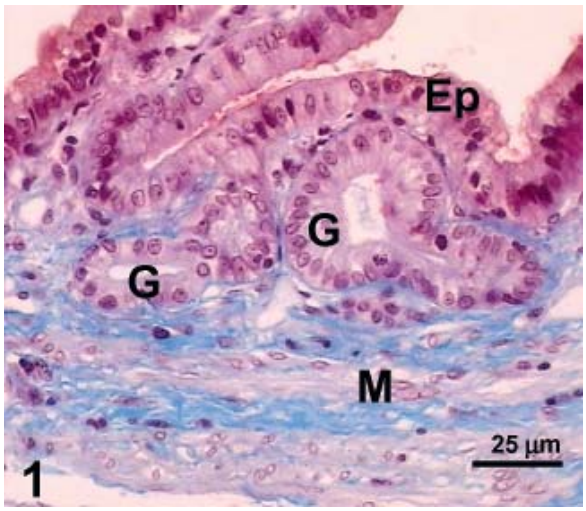

Figure 1. LM micrograph of the wall of the gallbladder in the dog. The glands (G) in the mucosa have the form of alveolar and tubular secretory units; Ep — mucosal epithelium, M — bands of muscle cells, Masson-Goldner staining.

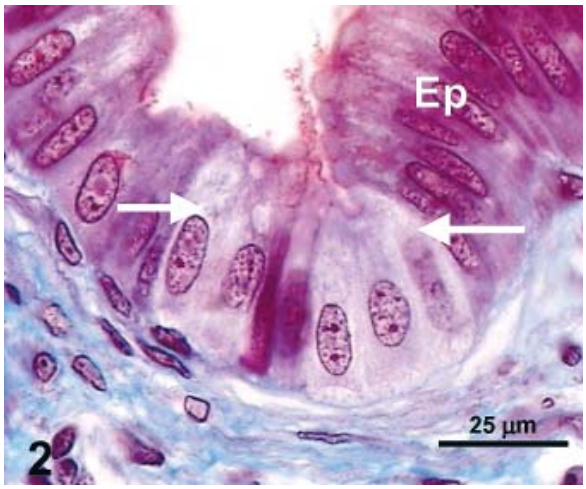

Figure 2. LM micrograph of the mucosa in the dog. Between the epithelial cells (Ep) light cells of the intraepithelial gland are present (arrows). Masson-Goldner staining.

The SEM images of macerated samples of the gallbladder mucosa in the dog and Chinese raccoon dog show a fine fibrillar skeleton around the concavities of the glands and around their openings (Figs. 8, 11). In both species the collagen fibrils surrounding the secretory units of glands form a fine lamina with a smooth surface with fenestrations (Fig. 9). The collagen fibres are orientated randomly in all directions (Fig. 11). The distribution and density of collagen fibres change on the surface of the mucosa around the openings of glands, where a ring is present around the glandular openings composed of bands of compactly arranged collagen fibrils (Figs. 9, 10).

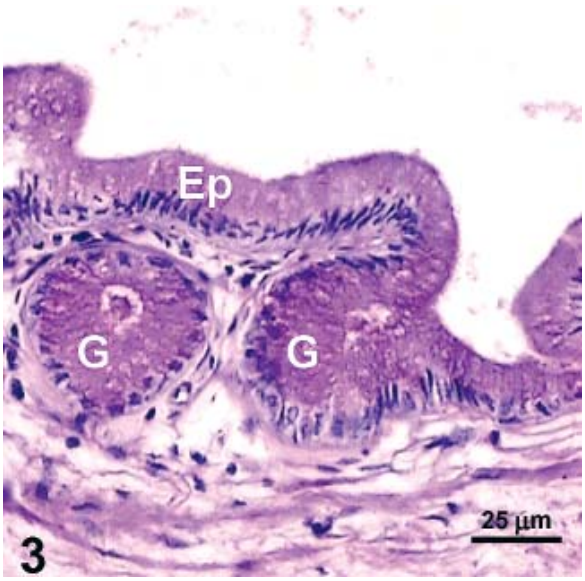

Figure 3. LM micrograph of the mucosa in the dog. Note the positive PAS staining result in the cytoplasm of the cells in the glands (G) and in mucosal epithelium (Ep). PAS reaction.

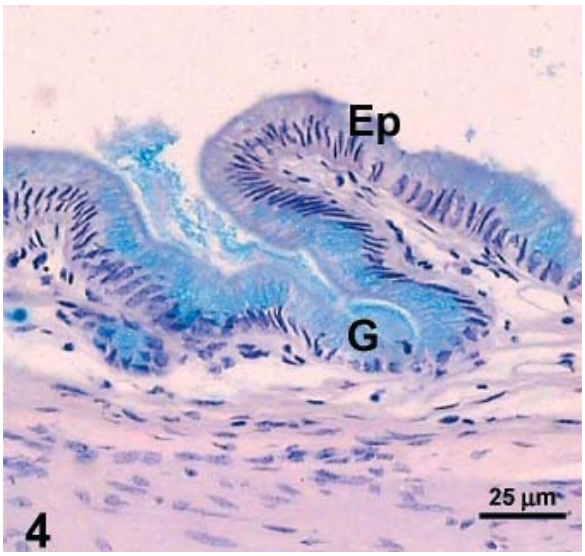

Figure 4. LM micrograph of the mucosa in the Chinese raccoon dog. Note the positive Alcian Blue staining results in the cytoplasm of the glands (G) and mucosal epithelium (Ep). Alcian Blue (pH 2.5) staining.

DISCUSSION

The results of our microscopic observations on the distribution of glands in the mucosa of the body of the gallbladder in the dog and the raccoon dog confirmed the earlier opinion of Cutore [5, 6] on the dispersed arrangement of the subepithelial mucous glands in Carnivora. In both species differences were observed in the density of glands, the size of the secretory units and the

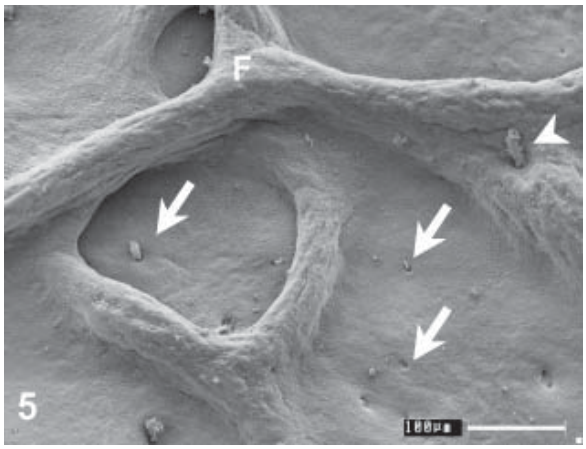

Figure 5. SEM micrograph of the surface of the gallbladder in the Chinese raccoon dog. On the bottom of the crypts surrounded by mucosal folds (F) numerous openings of single secretory units are observed (arrows). Arrowhead marks the opening of the gland on the mucosal fold.

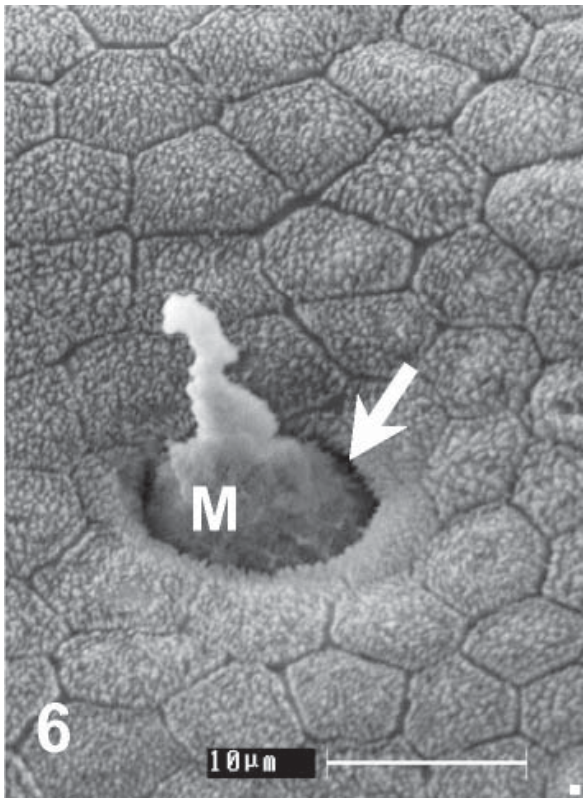

Figure 6. A higher magnification of the surface of the epithelial cells covered with numerous microvilli and the opening of the mucosal gland filled by mucus (arrow) in the Chinese raccoon dog. SEM.

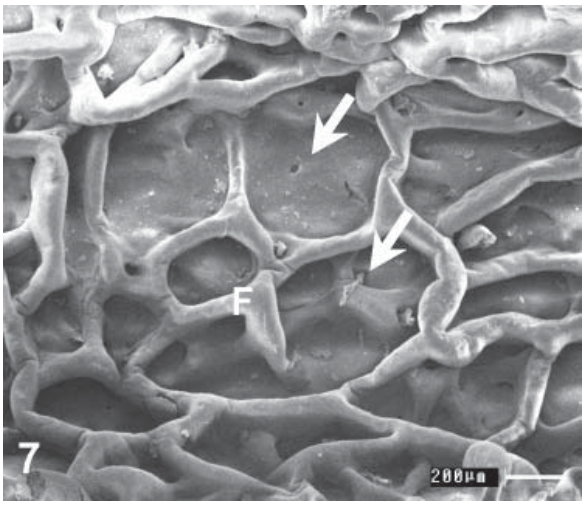

Figure 7. SEM micrograph of the surface of the mucosa in the gallbladder in the dog. Arrows show openings of glands; F — folds of mucosa.

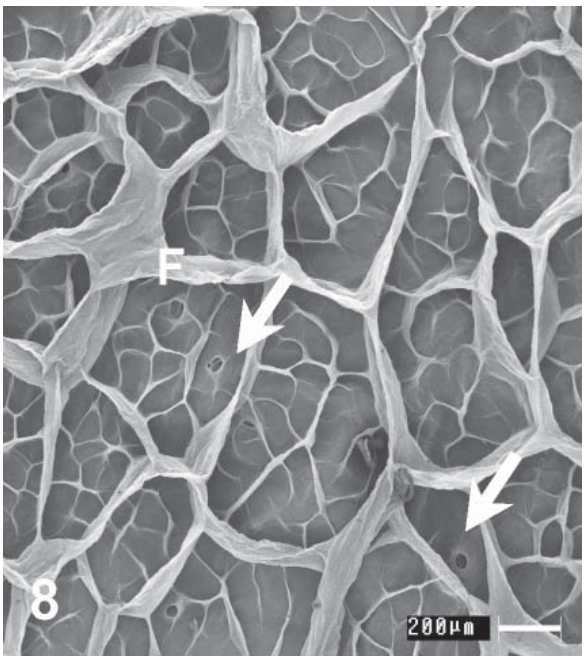

Figure 8. SEM micrograph of the surface of mucosa in the dog after NaOH maceration Arrows show the openings of glands; F — fibrillar framework of mucosal folds.

structure and size of the glandular openings of the glands.

In the raccoon dog the density of the glands in the mucosa of the gallbladder body is on average 30 times higher than in the dog. The microscopic structure of the glands and the histochemical characteristics of the cytoplasm of the glandular cells are similar in the two species. The glands in the mucosa of the gallbladder in both species, as in other animals, do not have excretory ducts [21]. The microscopic structure of the secretory units is similar to that in other animals, although there are no distinct compound branched glands of the tubuloalveolar type [3, 18, 21, 28]. In the dog the secretion from several secretory units is released through a common wide opening

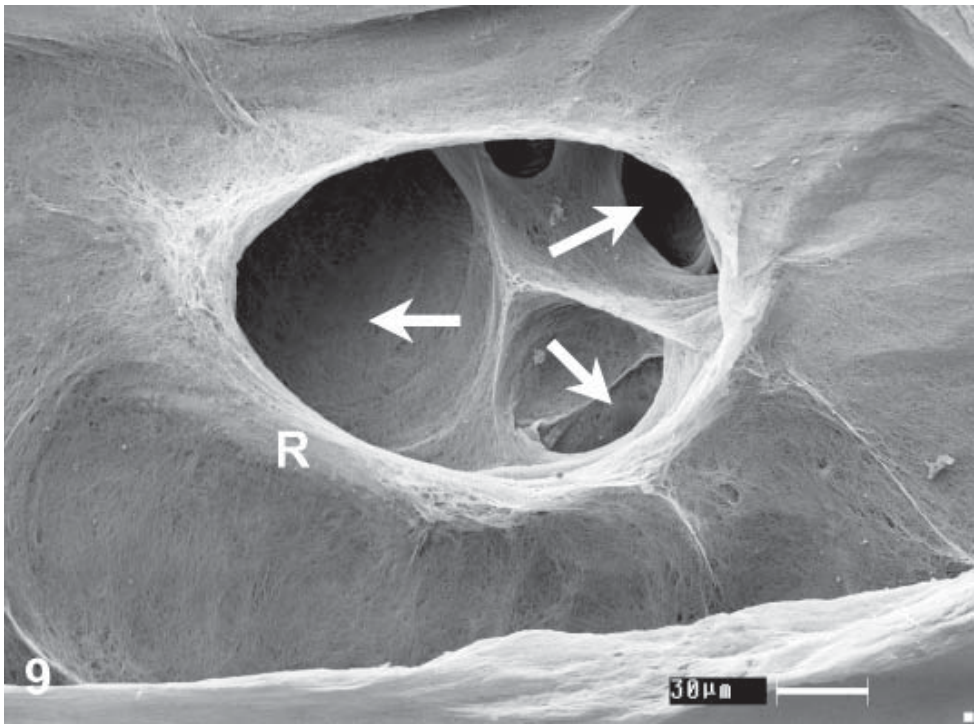

Figure 9. SEM micrograph of the opening of the glands in the gallbladder dog after NaOH maceration. The arrows show the entrance to the separate secretory units; R — fibrillar ring around opening of the gland.

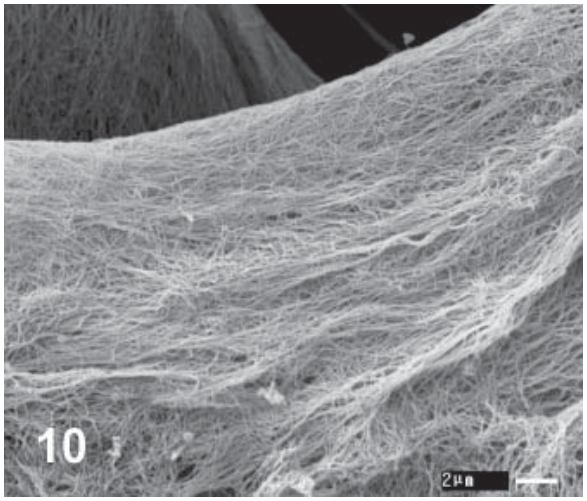

Figure 10. A higher magnification of the part of the fibrillar ring around the glandular opening. Note the dense arrangement of collagen fibrils. SEM.

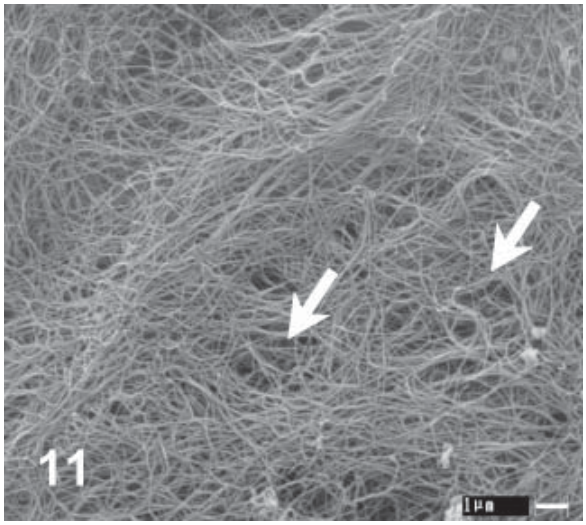

Figure 11. A higher magnification of the collagen network in the lamina of the secretory units. Between randomly distributed fibrils the fenestrations are present (arrows). SEM.

and in the raccoon dog openings 2 or 3 times smaller excrete mucus from a single secretory unit.

The SEM pictures showed the specific localisation of the glands mainly in the middle of the gallbladder crypts, away from the folds of the mucosa. This location is probably associated with changes in the morphology of the gallbladder mucosa linked with the size of the gallbladder. Our previous observations of the changes in the structure of the gallbladder wall in both species demonstrated that in the filled gallbladder the height of the connective tissue folds of the mucosa decreases by approximately 40% to as much as 70% at the gradual expansion of the crypts [the authors' unpublished data]. The location of the mucosal gland away from the folds of the mucosa does not expose them to the action of forces stretching the gallbladder wall and prevents translocation during rhythmical

changes in the capacity of the gallbladder. We are of the opinion that the fibrillar ring around each opening of the glands, revealed thanks to the maceration technique, may play a role in the stabilisation of the position of the glands and the mucosal epithelium located at the margin of the openings. As observations of macerated samples of the mucosa revealed, the fibrillar skeleton of the collagen framework around the secretory units is very thin with fenestrae, which ensure the close contact of the glandular cells with the rete of the periglandular capillaries.

The results of the histochemical reactions in the dog and the raccoon dog showed that the main components of secretion are neutral and sulphate glycoproteins. As with results from studies with the application of lectins, secretory granules in the mucosal glands have a characteristic pattern of oligosaccharide composition and differ in the particular segments of the bile tract [8]. The glycoproteins secreted by the epithelial cells of the mucosa of the gallbladder and by the subepithelial glands on the surface of the gallbladder mucosa in animals and in humans act as protecting agents against the macerating action of bile. The mucous film on the surface of the gallbladder also plays a lubricant role. In the human gallbladder mucins, along with the desquamated epithelial cells, promote nucleation of cholesterol and may be elements in the formation of crystallisation centres for gallstones [2, 14, 17, 26].

ACKNOWLEDGEMENTS

The authors would like to thank Mrs Katarzyna Jackowiak for her skilful technical assistance. This study was supported by grant from KBN 3PO6D 00425.

REFERENCES

1. Aharinejad S, Lametschwandtner A (1992) Microangioarchitecture of the guinea pig gallbladder and bile duct as studied by scanning electron microscopy of vascular corrosion casts. J Anat, 181: 89–100.
2. Bergmann F, Borgen H, Lindenlöf G, van der Linden W (1966) Influence of the carbohydrate source of the diet on gallstone formation in rabbits and mice. Acta Chir Scand, 132: 715–723.
3. Bucher O, Wartenberg H (1989) Cytologie, Histologie und Mikroskopische Anatomie des Menschen. 11 Auflage, Verlag Hans Huber, Bern, Stuttgart, Toronto.
4. Cagiatti A, Machiarelli G, Notolla SA, Vizza E, Familiari G (1992) Scanning electron microscopy of the rabbit gallbladder mucosal microvasculature. J Anat, 180: 275–280.
5. Cutore G (1906) Ghiandole intraepitheliali pluricellular nella cistifellea de cane. Arch Di Anat, 5: 454–465.
6. Cutore G (1910) Ancora delle ghiandole intraepitheliali pluricellulari nella cistifellea del cane. Anat Anz, 36: 100–103.
7. Evett RD, Higgins JA, Brown AL (1964) The fine structure of normal mucosa in human gallbladder. Gastroenterol, 47: 49–60.
8. Gelleff S, Böck P (1984) Lectin binding of surface epithelia and concomitant glands of gallbladder and biliary ducts in the guinea pig. Histochem, 81: 543–549.
9. Haley-Russell D, Husband KJ, Moody FG (1989) Morphology of the prairie dog gallbladder: normal characteristic and changes during lithogenesis. Am J Anat, 186: 133–143.
10. Hayward AF (1962) Aspects of the fine structure of the gallbladder epithelium of the mouse. J Anat, 96: 227–236.
11. Jackowiak H, Lametschwandtner A (2005) Angioarchitecture of the rabbit extrahepatic bile ducts and gallbladder. Anat Rec, 286: 974–981.
12. Johnson FR, McMinn MH, Birchenough RF (1962) The ultrastructure of the gallbladder epithelium of the dog. J Anat, 96: 477–487.
13. Jurisch A (1909) Beitrage zur mikroskopischen Anatomie und Histologie der Gallenblase. Anat H, 39: 395–467.
14. Konturek SJ (2001) Gastroenterologia i hepatologia kliniczna. PZWL, Warszawa.
15. Laito M, Nevalainen T (1975) Gland ultrastructure in human gallbladder. J Anat, 120: 305–325.
16. Lee SP (1980) The mechanism of mucous secretion by the gallbladder epithelium. Br J Exp Pathol, 61: 117–119.
17. Levy PF, Smith BF, Lamont JT (1984) Human gallbladder mucin accelerates nucleation of cholesterol in artificial bile. Gastroenterol, 87: 270–275.
18. Liebich HG (1990) Funktionelle Histologie. Schattauer, Stuttgart, New York.
19. Luciano L (1972) Die Feinstruktur der Gallenblase und der Gallengänge. I. Das Epithel der Gallenblase der Maus. Z. Zellforsch, 135: 87–102.
20. MacPherson BR, Scott GW, Lennon D (1983) Morphology of canine gallbladder; SEM observations of epithelium. Cell Tissue Res, 25: 161–174.
21. Möllendorf W (1932) Handbuch der mikroskopischen Anatomie des Menschen. Bd. 2. Verdauungsapparat. Springer Verlag.
22. Mueller JC, Jones AL, Long JA (1972) Topographic and subcellular anatomy of the guinea pig gallbladder. Gastroenterol, 63: 856–868.
23. Nickel R, Schummer A, Seiferle E (1999) Lehrbuch der Anatomie der Tiere. Parey Buch Verlag.
24. Ohtani O (1988) Three-dimensional organisation of collagen fibrillar framework of the human and rat livers. Arch Histol Cytol, 51: 249–261.
25. Ohtani O, Lee MW, Wang QX, Uchino S (1997) Organization of the blood and lymphatic microvasculature of the gallbladder in the Guinea pig: a scanning electron microscopic study. Microsc Res Tech, 38: 660–666.
26. Portincasa P, Moschetta A, Calamit G, Margari A, Palascino G (2003) Pathobiology of cholesterol disease: from equilibrium ternary phase diagram to agents preventing cholesterol crystallisation and stone formation. Curr Drug Targets Immune Endocr Metabol Disord, 3: 69–84.
27. Romeis (1989) Mikroskopische Technik. 17. Neubearb. Aufl, Urban and Schwarzenberg. München, Wien, Baltimore.
28. Ross MH, Romrell LJ, Kaye GI (1995) Histology: a text and atlas. 3rd Ed., Lippincott Wiliams & Wilkins.
29. Wahlin T, Bloom G, Carlsöö B (1974) Histochemical observations with the light and the electron microscope on the mucosubstances of the normal mouse gallbladder epithelial cells. Histochem, 42: 119–131.