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Sven Nikander

College of Veterinary Medicine, Department of Pathology, Helsinki, Finland

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This thesis is based on the following papers, which will be referred to in the next by their Roman numerals:

I **Nikander, S.** 1990. On the anatomy and topography of the pancreas and the pancreatic duct in reindeer (*Rangifer tarandus tarandus L.). Rangifer,* 10: 25–29.

II **Rahko, T.** and **Nikander, S.** 1990. Macroscopical and microscopical studies of the common bile duct in reindeer (*Rangifer tarandus tarandus L.*). *Rangifer,* 10: 3–8.

III **Rahko, T.** and **Nikander, S.** 1990. Histochemical studies of the common bile duct in reindeer. *Rangifer,* 10: 9–15.

IV **Rahko, T.** and **Nikander, S.** 1990. Electron microscopical studies of the common bile duct in reindeer. *Rangifer,* 10: 17–23.

V **Nikander, S.** and **Rahko, T.** 1990. Ultrastructure of granulated cells in the bile duct of reindeer. *Rangifer, Special Issue No. 3,* 10: 363–367.

RANGIFER

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Contents

Abbreviations

AB = Alcian blue
AB-S = Alcian blue – safranin
AF = Aldehyde fuchsin
CTMC = Connective tissue mast call
d-PAS = Periodic acid Schiff with diastase
GL = Globule leukocyte
HE = Hematoxylin and eosin
MC = Mast cell
MMC = Mucosal mast cell
PAS = Periodic acid Schiff
SEM = Scanning electron microscope
TEM = Transmission electron microscope

I. INTRODUCTION

Although literature dealing with the macro- and topographical anatomy of the pancreas and liver of the reindeer is limited in extent, it has a long history beginning already in the Middle Ages with a statement by a priest in Sweden that «there is no gall: only a small black stripe in the liver» (Tornaeus, 1672). A Russian publication «Anatomy of the Reindeer» from 1939 mentions that the bile is secreted directly through the ductus hepaticus communis into duodenum and that the opening of ductus pancreaticus is in the bile duct but pays only little attention on the anatomy and topography of the pancreas (Akaevskij, 1939). The first report on topographic anatomy of the reindeer was published in 1975 but the pancreas was only illustrated with one horizontal section of a female reindeer (Engebretsen, 1975).

The histology and histochemistry of different organs of the reindeer have not been documented at all. This holds true also for the pancreas and for the ducts of the pancreas and liver. Many details concerning the histogenesis and function of the different cells, especially the globule leukocytes, in the bile ducts are not elucidated. A study of the composition, function, and interaction of the mucous covering the epithelium in the common bile duct and also of the globule leukocytes probably would be of interest from a parasitological point of view.

II. GENERAL REVIEW OF THE LITERATURE

1. Phylogeny of the pancreas and the pancreatic and bile ducts

The pancreas is a specific organ for vertebrates and cephalocordates. A homologous organ has not been identified in the lowest chordates or invertebrates. The pancreas consists of two distinct organs both derived from the endoderm. The main part of the organ is involved directly with digestion secreting its products into the intestine. The remaining portion consists of small cell aggregates called the islets of Langerhans spread throughout the whole organ and releasing into the blood stream.

The pancreas is thus both an exocrine and an endocrine gland. It originates from two symmetrical lateroventral buds and a dorsal bud of the undifferentiated endoderm tube in the embryo. The pancreatic tissue in the mesenteron is divided into the pars mesenterica cranialis, intermedii, and caudalis. In young Amphioxus the pancreas is a triangular thickening of the wall of the intestine close to the liver primordium, a disk-like structure which develops and extends into the liver (van Wijhe, 1914). However, this organ appears to be absent in adults (Hill, 1926).

In cyclostomes the pancreas is formed by a cluster of cells originating in the transition between the foregut and the midgut (Boenig, 1929). The cranial and intermedial parts develope from the epithelium of the gut and a caudal part from the epithelium of the ductus choledochus. There are no real ducts from the pancreas (Keibel, 1927).

In sharks the pancreas usually is a massive compact gland (Stannius, 1853). But in many ganoids (sturgeons, gars, etc.), it is spread out into the whole abdomen and called the pancreas disseminatum, (Alessandrini, 1833, Balfour & Parker, 1882, MacCallum, 1886).

In the higher boney fishes, three types of pancreases have been indentified (Legouis, 1873) with one duct from the right ventral pancreas bud, as in the ganoids, but one or two other ducts may be present (Siwe, 1927).

The pancreas of the lungfish is a small organ close to the pylorus (Laguesse, 1889). The dorsal bud is connected to the right ventral bud and has a duct to the bile duct (Neumayer, 1904).

In the amphibians the pancreas is located between the stomach and duodenum. It is embedded in the ligamentum hepato-gastricum. Three ducts are present in larval forms but the dorsal disappears. In frogs only one of the ventral ducts persist (Göppert, 1891). In salamanders the number of pancreatic ducts varies from a few up to even 47 (Oppel, 1889, Göppert, 1891).

In the reptiles the pancreas also orginates from three buds, but only two, the dorsal and the right ventral, form the final organ. In some reptiles parts of the ventral pancreas spread into the liver, forming pancreas intrahepaticum (Siwe, 1927). The ducts join together and the pancreas discharge its secretions eventually through one duct.

In birds the pancreas develops from all three buds. It is located in the loop formed by the ascending and descending duodenum. There is usually one duct but occasionally two from the pancreas to the duodenum. The right ventral and the dorsal bud form a ring around vena portae in certain birds.

There is a large variation in the form of the pancreas among mammals. Rodents have a diffuse pancreas; humans have a compact pancreas,

and all intermediates between these two extremes can be found in other mammals. Usually the main part of the organ is located in the first loop of duodenum.

The pancreas in ruminants develops from a dorsal and a ventral bud which grow together (Bonnet, 1891, Nickel et al., 1960, Getty, 1975). Stoss stated that the ventral pancreas bud in the embryo of the sheep was paired and that the dorsal bud with its duct became atrophied. In some cases, however, the ventral bud degenerated and pancreas would develop only from the dorsal part. Usually only one duct persists but there are individual variations (Stoss, 1891).

The pancreas in the reindeer is located in a loop of duodenum, close to the right wall of the rumen. It touches the vena portae and the liver and is 20–25 cm long (Akaevskij, 1939).

The opening of ductus pancreaticus into duodenum is rather distal in some animals such as the rabbit in which the dorsal pancreas bud in the embryo had an accessory duct to the ductus choledochus (Krölliker, 1884).

The anatomy of the billiary tract varies greatly among different species of animals (Schache, 1907, Mann et al., 1920, Bevandic et al., 1967, Elias & Sherric, 1969, Dellman & Brown, 1976.). The mouth part of the common bile duct originates in the same cluster of cells as the pancreas (Romer, 1959). The biliary ducts of the liver join into an extrahepatic duct (= ductus hepaticus), which may empty into the ductus choledochus, or into the gall bladder through the ductus hepato-cysticus or directly into the intestine by the ductus hepato - entericus (=ductus hepaticus communis). Ductus cysticus connects the gall bladder with the ductus choledochus (Schimkewitsch, 1910). If the gall bladder does not develop as in reindeer, the bile is secreted directly into the duodenum through the ductus hepaticus communis, the common bile duct (Akaevskij, 1939).

Ultrastructural differences of the common bile duct in the rat and the mouse are probably attributable to the lack or presence of the gall bladder (Yamada, 1969, Luciano, 1972). In ruminants the gall bladder is richly endowed with serous and mucous glands (Dellman & Brown, 1976). The gall bladder thus contains a mixture of bile and mucous fluids. The wall of main bile ducts also is profoundly glandular in cattle (Rahko, 1971, 1973).

2. Cytology of the pancreatic and bile ducts

2.1 *Epithelium of the duct system*

Although the epithelial cells of the bile ducts are derived from a common cell cluster, they differentiate into various types of bile ductular cells. An autoradiography study showed that the ductules produced by chronic hepatic injury do not derive from hepatocytes, mesenchymal cells, or bile ductal epithelial cells, but rather from a special cell type which probably originates from pre-existing ductular cells in the canals of Hering. In contrast, the tubular structures produced by bile duct ligation did not originate from the canals of Hering but were a proliferation of the epithelium of the bile duct. The ductular cells disappear by cell death and are not transformed into other cell types (Rubin, 1964).

2.2. *Mast cells*

There are two specific phenotypes of mast cells (= MC): the connective tissue mast cell (= CTMC) and the mucosal mast cell (= MMC) (Enerbäck & al., 1986). Both CTMC and MMC may possibly originate from the hemopoietic tissue. The CTMC is found in connective tissues, such as in the skin and the tongue. The MMC probably is present in the mucosa of the alimentary and respiratory tracts of most vertebrates. The metachromatic granules of MC contain characteristic proteoglycans, such as heparin in the CTMC and chondroitin sulphate in the MMC. MC probably interact with the cells of many tissues and organs. They can produce and release biologically - active compounds (Enerbäck & Norrby, 1989). MMC are also involved in the «self-cure» expulsion of parasites (Jarrett et al., 1968).

2.3. *Globule leukocytes*

Globule leukocytes (= GL) are infrequent cells in the epithelium of the respiratory, alimentary, urinary, and reproductive tracts, probably in all vertebrates (Clara, 1926, Törö, 1931). The origin of GL is unclear. Many precursors, such as erythrocytes, Russel body cells, plasma cells, and mast cells have been proposed, but GL seems to be an independent cell population of mesenchymal origin (Kirkman, 1950). The nucleus of GL is often indented because of presence of large globules in the cytoplasm; the granu-

les in the MMC are usually smaller and such indentation is not observed (Kent, 1952, Kellas, 1961). The GL is associated in an unknown way with the immune response to parasitic infections and also in their expulsion (Hole, 1937, Sommerville, 1956, Whur, 1967, Nikander, 1991).

2.4. *Other granular cells*

Enterochromaffin cells are best known as moderately abundant cells in the fundic and pyloric glands. They are scattered between the basement membrane and the epithelial cells. They have a rounded and somewhat flattened form. The cytoplasm is filled with small granules. Two groups can be distinguished. First, the true argentaffin cells in which specific granules reduce silver salts without special pretreatment. Second, the argyrophilic cells which require reduction before the granules will react with silver (Penttilä, 1969).

The nucleus of the enterochromaffin cells has deep folds. The Golgi complex is small and a few profiles of a granular reticulum can be seen. The appearance of the small granules varies. In some cells they are very dense and spherical, being enclosed by a loose fitting membrane. In others they have a lower density and a tigther membrane. Enterochromaffin cells synthesize and store 5-hydroxytryptamine (Bloom & Fawcett, 1970).

Eosinophilic granulocytes are found in the blood and connective tissues of vertebrates. The nucleus of the eosinophils has two lobes. A characteristic feature of the cell is the eosinophilic refractile granules in the cytoplasm. The granules contain an electron-dense crystal. The form of the crystal varies from species to species (Bloom & Fowett, 1970). The cells contain histaminase. Eosinophils are involved in the defense against parasitic infections, allergic hypersensitivity, and the late phases of inflammatory reactions. It has also been shown that eosinophils are attracted by antigen-antibody complexes which destroy them by phagocytosis (Fernex, 1968).

III. PURPOSE OF THE STUDY

1. to contribute to the knowledge of the anatomy and topography of the pancreas, its exocrine ducts, and their connection with the intestine in the reindeer,

2. to describe the histology of the wall of the common bile duct and ultrastructure and histochemistry of different cells in the bile-duct epithelium, and

3. to acquire data especially about th controversial globule leukocytes, their ultrastructure and histochemistry for use in further studies on their significance in the defense against and expulsion of parasites.

IV. MATERIALS AND METHODS

1. Materials

The material includes the viscera and tissue samples from 61 reindeer slaughtered in the winter and spring of the years 1987–1989. Of these animals 25 were from the northern part of the Finnish Lapland (Kaamanen) and 36 from the eastern part (Savukoski).

The study in 1987 examined the anatomy and especially the topography of the pancreas and the pancreatic and bile ducts in 13 adult males, two females, and two newborn calves. In the following years, further topographic and macroscopical studies were performed on 24 reindeer. Twenty additional animals were used for studies of tissue specimens.

Tissue specimens of the ductus hepaticus communis used in light microscopical studies originated from 8 reindeer slaughtered at Kaamanen outdoors at -30°C and 12 reindeer slaughtered indoors at a slaughterhouse in Savukoski. Those samples studied with transmission electron microscope originated from 6 reindeer in Kaamanen, and those examined with a scanning electron microscope originated from 8 reindeer also slaughtered at Kaamanen. For light and transmission electron microscopy, the specimens of ductus hepaticus communis, 5–10 mm long, were taken and cut into small blocks and at once transferred into different fixatives. Care was taken to prevent freezing of the specimens.

For topographic studies, the pancreas and adjoining tissues were dissected after fixation in four animals.

For radiography, the pancreatic ducts were injected with latex in six cases and with a radio opaque silicon in four cases.

2. Methods

2.1. *Fixatives*

The organs dissected for topographic studies, were fixed in 4 % paraformaldehyde solution (Reima & al., 1987).

For light microscopical studies, specimens from six reindeer were fixed in both formalin (25 %) buffered at pH 7 by phosphate buffer (fixation time 4 days) and in Bouin's solution, for one day, then transferred into 70 % alcohol. Tissue specimens from another 14 reindeer were fixed 1–4 months in 4 % paraformaldehyde in 0.1 M phosphate buffer containing 0.1 % NaN_3 and 0.05–0.1 % glutaraldehyde (Reima & al., 1987).

For transmission electron microscopy (TEM) small samples of the common bile duct wall were sectioned in cold 2.5 % glutaraldehyde in 0.1 M phosphate buffer and fixed for 24 hours.

For scanning electron microscopy(SEM), the tissue blocks were fixed in a mixture of paraformaldehyde and glutaraldehyde.

2.2. *Radiography*

To visualize the ducts of Santorini and Wirsung, the opening of the ductus pancreaticus was searched as follows. A lobe of the pancreas was cut, and the largest interlobular duct was injected in the centrifugal direction with black latex until it flowed through the ostium pancreaticum into the common bile duct. Then a radio opaque contrast medium consisting of a mixture of silicon and red lead powder was injected through ostium pancreaticum into the pancreas. The ducts of Santorini and Wirsung

were visualized by a Siemens X-ray machine modified by Elema – Schonander, in the Roentgen Laboratory, College of Veterinary Medicine.

2.3. *Light microscopy*

The material for light microscopy was routinely embedded in paraffin and sectioned at 4 microns and stained with hematoxylin and eosin (HE). The stainings used were performed according to the descriptions presented by Romeis (1948), Roulet (1948), McManus & Mowry (1960), and Pearse (1968).

Herovic's staining and Ladewig's modification of Masson's trichromstaining were used in the studies of the mesenchymal tissues.

Glycogen and neutral glycoproteins were stained with periodic acid-Schiff with and without diastase (d-PAS, PAS).

Acid mucosubstances were investigated by staining with alcian blue at pH 2.5 and pH 1.0 (AB 2.5; AB 1.0) and counterstained with nuclear fast red and with aldehyde fuchsin at pH 1.7 (AF).

Sialomucins were studied with a sialidase digestion of the sections prior to AB 2.5 staining (sialidase-AB). The sialidase digestion was carried out in 0.1 M acetate buffer containing 0.04 M calcium chloride and 0.1–4.5 U/ml sialidase (Neuraminidase, Sigma). The sections were digested at 40°C for 1 hour. Control sections were treated with buffer without the enzyme.

In order to differentiate acid (blue or blue purple) from neutral (red) mucosubstances, the sequence AB 2.5 followed by PAS (AB 2.5-PAS) was employed.

Sulphated mucosubstances were studied with alcian blue staining at pH 1.0 followed by PAS (AB 1.0 -PAS) to differentiate sulphomucins (blue or purple) from neutral mucins (red). Aldehyde fuchsin staining at pH 1.7 followed by alcian blue at pH 2.5 (AF-AB 2.5) was further used to differentiate sulphomucins (purple) from nonsulphated (blue) acidic mucins.

MC and GL were studied by staining selected sections with amidoblack for identification of GL, with Kossa's staining for calcium, with oil red O for neutral fats, and with Fontana-Masson staining for melanin and iron-containing pigments. Subsequently, the cells were stained with alcian blue at pH 0.3 followed by safranin at pH 1.0 (AB-S) to differentiate heparin (red) from other highly sulphated mucosubstances (blue), and with toluidine blue at pH 4.0 and pH 0.5 (TB 4.0, TB 0.5) to study the metachromatic properties of the granules in MC and of the globules in GL.

2.4. *Transmission electron microscopy (TEM)*

The blocks were stained and processed by the commonly used methods for TEM. The thin sections cut with an ultramicrotome were stained with 3 % uranyl acetate and 3 % lead citrate (Kay, 1965) and examined with a JEOL JEM 100 S electron microscope in the Laboratory of Electron Microscopy, College of Veterinary Medicine.

2.5. *Scanning electron microscopy (SEM)*

A piece of the common bile duct with the ostium pancreaticum was removed and fixed. After dehydration and drying in alcohol, the duct wall was golded. A JEOL JSM-820 scanning electron microscope was used for the study of the surface of the bile and pancreatic ducts and especially of the opening of the pancreatic duct. SEM-studies were performed in the Laboratory of Electron Microscopy, University of Helsinki.

V. RESULTS

1. Topography of the pancreas (I)

The pancreas of the reindeer is a bilobulated gland. The lobus sinister (= tail, processus caudahs) extends in a ventro-dorsal direction and ends in an almost horizontal triangular part. The edge of the left lateral side is attached to the edge of the spleen. At the posterior edge of the triangular part is a cavity for the left adrenal gland. Anterolaterally the rumen and jejunum touch the tail, and the main part of the lateral side is in contact with the colon transversum.

The middle part of the pancreas surrounds anteroventrally the vena porta hepatis and ventrally touches the abomasum. The lobus dexter (= caput, processus duodenalis) extends in an anterior and posterior direction from the body in the loop between the pars descendens and pars ascendens of the duodenum. The cross-section of the body is triangular. Its lateral surface touches the liver, the dorsal part the right kidney, and the median surface the ansa distalis of the colon. In the anterior end of the lobus dexter, close to the vena porta hepatis, there is a groove for the arteria mesenterica cranialis.

2. Macroscopical structure of the duct systems (I, II)

2.1. *The pancreatic duct*

Branched ducts, analogous to the ductus pancreaticus major (Wirsung) in lobus sinister and the ductus pancreaticus minor (Santorini) in lobus dexter, run through the middle of both lobes. They join and have a 2-3 cm long duct in common (ductus pancreaticus) which opens into the common bile duct (ductus hepaticus communis).

The ductus pancreaticus is not visible because it is surrounded and covered by pancreatic tissue. The end of the ductus pancreaticus runs parallel to the bile duct and penetrates the mucosa in a transverse fold forming the ostium pancreaticum about 2 cm before the duodenal opening. The ostium is located about half a centimeter before the longitudinal folds of the bile duct mucosa, which extend about one centimeter from the duodenal opening.

2.2. *The common bile duct*

The common bile duct is a 3-5 cm long, 2-3 mm wide, white, thinwalled, elastic tube between the liver and the oral part of duodenum. The duct is partly surrounded by fat and pancreatic tissues. No papilla is observed in connection with its duodenal opening. The mucosa of the sectioned duct appears to be yellow in color near the hilus of the liver.

3. Light microscopical structure of the common bile duct (II, III)

The wall of the common bile duct measured on the slides for light microscopy is about 1 mm thick. The outer layer consists of circular connective tissue fibers, surrounded by the serosa. Smooth muscle layers are seen in sections of the duct near the duodenum. Certain areas of the wall contain epithelial structures interpreted as pancreatic acini.

The inner layers of the wall of the common bile duct consist of a folded mucosal layer. The regular epithelium is supported by proprial tissues. The epithelium is composed of the surface epithelial cells (tall columnar cells with basal rounded nuclei and glands) interspersed with go-

blet cells. A few goblet cells are located also within the superficial epithelium.

Separated granulocytes and lymphocytes are present in the lamina propria. Prominent accumulations of lymphatic cells may also be seen.

The superficial and glandular epithelium contains numerous basally located GL. They are identified on the basis of having a few large, eosinophilic, rounded globules. The chromatin-rich nuclei are rounded, with indentations caused by the pressure of the globules. Generally the nuclei of GL are quite similar in appearance to those in the lymphocytes. Eosinophilic granulocytes with small granules in the cytoplasm are easy to detect in proprial tissues, while MC with small and faintly-stained granules are difficult to observe in HE-stained sections.

4. Electron microscopical studies of the common bile duct (IV, V)

4.1. *Epithelial cells*

The ultrastructural features of different organelles in the superficial epithelial cells are fairly regular. The basal nuclei are round, showing mainly marginal chromatin. The nuclear membrane is smooth. Nucleoli are not distinct. The cytoplasm of the cells shows the typical structures of epithelial cells. The Golgi complex is prominent usually in the supranuclear area. There are numerous small mitochondria, profiles of granular endoplasmic reticulum, and free ribosomes. The general density of the cytoplasmic matrix varies between different cells. Cells with a great density and dark cytoplasmic matrix contain an abundance of microfilaments. In some areas the microfilaments are concentrated into small bundles supporting the microvilli. Microfilaments are abundant also in areas adjoining the intercellular connections.

The intercellular spaces appear empty. The cell membranes form villous projections into them. The basal cell membrane is smooth, resting on the basal lamina.

The lumen of the bile ducts is either empty or contains fine-granular or amorphous substances particularly in areas adjoining the walls.

4.2. *Mast cells and globule leukocytes*

MC and GL are identifiable on the basis of the characteristics described for the cells in general. CTMC are rare in the tissue samples while MMC occur frequently in subepithelial areas. The numerous intracytoplasmatic granules of MMC are usually electron dense with rounded profiles. MMC display mostly diffusely electron-dense granula but also a few fine-granular matrices are seen.

The intraepithelial GL are easily distinguished from the subepithelial MMC on the basis of their location. The globules of GL vary in size and structure, the largest being even 6–7 microns in diameter. They are few in number and distinctly larger than the granules of MC. The globules are surrounded by smooth membranes. They mostly show diffusely electron-dense cores, but also fine-granular or dissoluting matrices are observable. The nucleus of GL is often deeply indented by the large globules. GL are closely apposed to the epithelial cells. Intercellular connections, however, are not observed. Parts of GL with several intact globules and mitochondria are also seen freely as free entities in the lumen.

4.3. *Other granular cells*

Enterochromaffin cells appear as intraepithelial cells with numerous small electron-lucent intracytoplasmatic granules. The cells are located only intraepithelially. No other types of intraepithelial granular cells (e.g. eosinophilic granulocytes) were studied.

5. Histochemical study of the common bile duct (III)

5.1. *Epithelial cells*

The cytoplasm of the epithelial cells is pale in HE-stained sections, especially in the deep glands. In mucous glands the epithelial and goblet cells are ballooned by mucus. Free mucus also is present in the lumina of the glands.

Neutral, carboxy- and sulphomucins are detected on the basis of their color reactions in the bile duct epithelium and lumina. Neutral mucins stain red with PAS and in the sequence of PAS with AB at pH 2.5 and pH 1.0 when acidic groups of mucins are not present. Carboxymucins are purple or blue when stained with AB 2.5 with or without PAS staining, respectively. The sulphomucins stain blue with AB 2.5 and 1.0. When AB is followed by PAS at pH 2.5 or 1.0 the positive color reaction of sulphomucins is blue or purple.

In superficial mucins the reaction of neutral mucins is the most intense compared with that of carboxymucins. Sulphomucins are demonstrable as a thin layer covering the surface epithelium in the goblet cells and in the deep glands. The mucins are blue or purple with AB 1.0-PAS staining of goblet cells and deep glands, but red in superficial mucins: AB 2.5-PAS stains most superficial mucosubstances predominantly blue and the mucins of the other areas purple. The carboxymucins do not show digestibility by sialidase. Glycogen is not identifiable in the bile duct epithelial cell. The reactions for carbohydrate-rich compounds are most intense in the goblet cells and deep glands.

5.2. *Mast cells and globule leukocytes*

MC-granules stain blue with AB at pH 2.5 and 1.0 also if the sequences are followed by PAS. The color reaction with AB-S and AF-AB is blue. Toluidine blue staining reveals metachromacy in the MC-granules.

The granules of MC give either no reaction or a weak blue color with AF-AB 2.5 and AB-S.

GL, with the AB 2.5 staining of Bouin-fixation, have the cores of their globules stained red with nuclear fast red counterstain being surrounded by a blue cortical zone.

VI. GENERAL DISCUSSION

1. Material and methods

According to the available literature on the reindeer, the present study is the first attempt to describe the topography of the pancreas and the structure and histochemical composition of the common bile duct and to elucidate the properties of different granular cells of the bile duct mucosa. After the closing of the present investigations it was found that Akaevskij in 1939 had reported notes on the excretory duct systems of the pancreas and liver of reindeer.

One reason for the sparse literature on the macroscopic and microscopic morphology of all organs in reindeer may be the difficulties in achieving proper tissue samples because of the hard climatic condition in winter when the animals normally are slaughtered. The specimens appeared suitable for structural studies although the MC probably were affected by the postmortem circumstances.

Proper fixation and staining techniques of different tissues are necessary and especially important with regards to the MC and GL. Methodological differences explain the contradictory results in the published results on human MC. For example, biopsy specimens of human jejunum used for determining the frequency of MC have been fixed in Carnoy's, Baker's and Bouin's fluids and in isotonic formol-acetic-acid, 10 % neutral buffered formalin, formol sublimate, and formol saline. Paraffin-embedded sections have been stained with either astra-blue and safranin at pH 0.3 or with toluidine blue at pH 0.5. With astra-blue/safranin staining, the mean number of MC was only 40 cells/mm² in formolsaline specimens, but 268 cells/mm² in Carnoy's-fixed specimens. However, MC could be stained with toluidine blue in all fixatives reported (Strobel et al., 1981). All the fixatives used in the present study preserved the material sufficiently for microscopical studies. Bouin's solution and paraformaldehyde appear to preserve the tissues of reindeer particularly well.

2. Topography of the pancreas and the duct systems

The pancreas develops from a dorsal and two ventral buds or primordia. In the reindeer, the growing pancreas extends between the abdominal organs, filling the spaces between them. This results, according to the present findings, in a flat, triangular cross-section for the pancreas body and lobes. The bases of the lobes join cranially where the gland empties into the common bile duct in reindeer.

In most domestic mammals, the pancreatic and hepatic ducts are separated. (Schmidt & Ivy, 1937, Elias & Sherrick, 1969, Dellman & Brown, 1976). The present study showed only one duct in reindeer, which joins the pancreas with the common bile duct. There also is only one common opening in the duodenum for the ducts from the liver and the pancreas.

3. Morphology of the common bile duct

In reindeer the morphological characteristics of the wall of the common bile duct are generally similar to those in other mammalian species (McMinn & Kugler, 1961, Dellman & Brown, 1976, Rahko, 1971). The duct is short, elastic and thin-walled. Mucosal folds consist of epithelial and glandular structures supported by the propria. According to Mann et al. (1920) there is no relation between the dimensions of the common bile duct and also the distance of its

entrance into the duodenum from the pylorus and the presence of absence of a gall bladder. Thus the relative capacity of the extrahepatic biliary system of the deer, which has no gall bladder, is not greater than that of sheep or goats which do have one (Mann et al., 1920).

The exocrine excretory system of the pancreas is reduced to a single duct i many species. It enters the duodenum aborally compared with the entry of the bile duct. The reason for this obviously is that the ventral lobe loses its direct connection to the gut during the development of the embryo. The entering of a single ductus pancreaticus into the common bile duct forming the ductus communis is typical for small ruminants such as sheep and goats (Dellman & Brown, 1976, Dyce et al., 1987). In reindeer the anatomy of the pancreas is comparable to that found in small ruminants and particularly in deer (Mann et al., 1920).

The bile duct epithelium of reindeer consists of two types of epithelial cells; absorptive superficial and secretory glandular cells. The presence of abundant microvilli on the luminal surface indicates the absorptive function of the surface epithelium. The mucin of the glands is mixed with the products of goblet cells. Analogous features for absorptive and secretory functions have been described in the rat (Yamada, 1969, 1970), the mouse (Yamada, 1969, 1970, Rahko, 1971) and cattle (Rahko, 1975). In the rat the superficial absorptive epithelium also shows signs of pinocytosis (Yamada, 1969, 1970).

Numerous intraepithelial GL and subepithelial MC have been found in the wall of the common bile duct. In the present study, MC were located both in subepithelial and connective tissues while GL displayed a strictly intraepithelial occurrence in reindeer, as has also been seen in other species (Gregory, 1979).

GL have not previously been identified in reindeer, although they have been reported within different epithelial tissues of many other species including man (Greogry, 1979, Nikander, 1991). The frequency of GL is usually correlated with the occurrence of parasitic infections. It is noteworthy that the reindeer studied had been treated with anthelmintica late in the preceding autumn and were thus considered to be parasite-free.

Microscopic examination revealed subepithelial pancreatic acini in the common bileduct wall of reindeer. This was an unexpected finding not described in previous reports of ruminants. The significance of this peculiar anatomical feature is unclear. Further studies with serial sectioning are needed to elucidate whether the pancreatic acini in the common bile duct wall of the reindeer represent an ectopic pancreas.

The mucins covering the epithelium were histochemically positive with stainings for neutral and acidic mucins. Electron microscopy showed fine granular or amorphous material covering the luminal surface of the epithelium. It is obvious that in reindeer the bile duct mucins are qualitatively analogous to those in other mammals (McMinn & Kugler, 1961, Rahko, 1971). Superficial epithelial cells displayed either light or dark cytoplasm by electron microscopy. In reindeer the richness of microfilaments and ribosomes contributes to the electron density of the dark epithelial cells. Similar differences in cytoplasmic contrast of the cells have been reported in the mouse and the rat where the darkness of epithelial cytoplasm is also determined by the abundance of microfilaments (Yamada, 1969, 1970). Microfilaments are exceptionally abundant in the «brush cells» of the bile duct walls of the mouse and the rat (Luciano & Reale, 1969, Luciano, 1972).

4. Properties of mast cells and globule leukocytes

MC and GL are obviously migratory cells (Gregory, 1979, Morales & al., 1980, Toledo & Morales, 1981, Morales, 1983, Nikander, 1991). In the present material, GL did not form desmosomes. The cells were usually located in basal areas of the epithelium which is typical for GL in general (Gregory, 1979). However, in the present study it was found that lumen of the common bile duct contained intact globules of GL and even parts of cytoplasm with globules and other cell organelles. The presence of GL in the lumen of the bile ducts may be an implication of the migratory capacity of the cells, but the possibility cannot be eliminated that their presence was only an artificial phenomenon caused by trauma of the lamina epithelialis during the process of slaughter and the sampling of the tissue blocks.

According to present findings, the globules of GL in reindeer show different sizes and ultrastructural appearances. Some giant globules were also observed, as has been found in the

goat (Rahko, 1972). The inner matrix of the globules appeared electron-dense and also fine-granular similar to that described in the rat (Baert, 1989).

Differences between MC granules and GL globules were seen in their ultrastructure and their pattern of staining reactions. In MC granules the carbohydrate-rich compounds were more acidic than those in the globules of GL. Granules of MC in reindeer appeared to contain sulphomucins while globules of GL stained for neutral, carboxy- and sulhpomucins. Heparin was not demonstrable by the present metods. It is noteworthy that previously mentioned postmorten changes caused difficulties in the interpretations of locations and shades of stainable material in MC and GL. Stainable material has been demonstrated to diffuse from GL in the bile duct of goats (Rahko, 1972) and in the urinary bladder of rats (Ahlqvist & Kohonen, 1959).

VII. SUMMARY AND CONCLUSIONS

The anatomy and topography of the pancreas in the reindeer is analogous to that of small ruminants. It consists of two triangular flat lobes. The right lobe (head) is located within the loop of duodenum. The left lobe (tail) is situated close to the rumen. The ductus pancreaticus major (Wirsung) and minor (Santorini) join forming a common duct (ductus pancreaticus). It opens via the ostium pancreaticum into the common bile duct (ductus hepaticus communis).

The common bile duct is a thin elastic tube about 3 to 5 cm long. It is surrounded by fat and pancreatic tissues. An anatomic peculiarity is the presence of pancreatic acini in the wall of the common bile duct, even in the propria. The wall of the common bile duct is about 1 mm thick. It displays a folded mucosa surrounded by connective tissues and distally also muscular fibers, being covered by a serosal layer. The mucosa contains surface and glandular epithelial cells with mucous secretion. Numerous intraepithelial globule leukocytes are identifiable in the lamina epithehalis. Mucosal mast cells are present mainly at subepithelial sites.

Ultrastructurally, the surface and glandular epithelium carry villous structures on the luminal surfaces, and their parietal cytoplasmic membranes are connected with intercellular desmosomes. The intraepithelial globule leukocytes do not show desmosomes with other cells. The goblet and deep glandular cells contain numerous large mucin granules. Their secretions are more abundant than those in the superficial epithelial cells which obviously are absorptive. The lumen of the bile ducts is almost empty of mucus but may in exceptional cases contain free globule leukocytes. The granules of connective tissue and subepithelial mast cells are small and usually diffusely electron-dense but fine granular matrices are also present. The electron-dense globules of intraepithelial globule leukocytes are fewer in number and distinctly larger than the granules of the mast cell. Also intraepithelial granulated cells appearing similar to the neuroendocrinic cells are present within epithelium.

Histochemically, both neutral and carboxy- and sulphomucins are identifiable in the mucous secretions of epithelial cells and in the globule leukocytes. The mucosubstances of the mast cell contain sulphate groups indicative of sulphomucins showing predominantly a lower degree of sulphation degree than heparin.

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APPENDIX
Papers I–V

I

On the anatomy and topography of the pancreas and the pancreatic duct in reindeer (*Rangifer tarandus tarandus* L)

On the anatomy and topography of the pancreas and the pancreatic duct in reindeer (*Rangifer tarandus tarandus* L)

Sven Nikander
Laboratory of Parasitology and Department of Pathology, College of Veterinary Medicine, Postbox 6, SF-00581 Helsingfors, Finland

Summary: The complex development of the pancreas accounts for the differences in its morphology among various animal species. According to the present study, the anatomy of the pancreas in the reindeer is quite similar to that in small ruminants. It consists of two lobes, the left one (tail) extending in a ventrodorsal direction is in contact with the rumen, spleen, and the left adrenal gland. The right lobe (head) lies within the curve of the duodenum. Ducts analogous to the *ductus pancreaticus major* (Wirsungi) and *minor* (Santorini) join in a common pancreatic duct (*ductus pancreaticus*) which opens into the common bile duct (*ductus hepaticus communis*).

Key words: bile duct.

Rangifer, 10 (1): 25—29

Nikander, Sven. 1990. Haiman ja haimakäytävän anatomia ja topografia porolla.

Yhteenveto: Haiman kehittyminen on monimutkaista, mikä aiheuttaa sen, että haiman rakenne vaihtelee eri eläinlajeilla. Tämän tutkimuksen mukaan haiman rakenne porolla on hyvin samanlainen kuin pienillä märehtijöillä. Haimassa on kaksi lohkoa. Vasen lohko (häntä) on ventrodorsaalisessa suunnassa ja koskettaa pötsiä, pernaa ja vasenta lisämunuaista. Oikea lohko (pää) sijaitsee pohjukaissuolen mutkassa. Ductus pancreaticus majoria (Wirsungi) ja minoria (Santorini) vastaavat haimakäytävät yhtyvät muodostaen ductus pancreaticuksen, joka avautuu yhteiseen sappikäytävään (*ductus hepaticus communis*).

Rangifer, 10 (1): 25—29

Nikander, Sven. 1990. Pankreas och ductus pancreaticus anatomi och topografi hos ren.

Sammandrag: Pankreas ontogenes är invecklad, detta medför morfologiska variationer hos de olika djurarterna. Enligt denna undersökning påminner pankreas anatomi hos renen om de små idisslarnas. Pankreas består av två lober. Den vänstra loben (svansen) sträcker sig i ventrodorsal riktning och gränsar till våmmen, mjälten och den vänstra binjuren. Den högra loben (huvudet) är i en slinga av tolvfingertarmen. Analoga gångar till *ducuts pancreaticus major* (Wirsungi) och *minor* (Santorini) förenas till *ductus pancreaticus* som mynnar ut i gallgången (*ductus hepaticus communis*).

Rangifer, 10 (1): 25—29

Introduction

In lower vertebrates such as amphioxus no separate pancreas exists. Instead, groups of cells lie within the wall of the intestine. In the lamprey, the pancreatic cells form a cluster of separate glands around the gut near the opening of the bile duct.

The development of the pancreas shows that it is not a single structure but a compound one. At the dorsal edge of the gut tube an upward folding of pancreatic cells occurs. However, a portion of this tissue becomes involved in the opening of the future bile duct, and in the wall of that tube either one or a pair of pancreatic outgrowths are formed. These tend to grow upward and fuse with the dorsal pancreas. These three structures do not always persist in the adult, but usually the dorsal and at least one of the ventral pair contribute to the adult pancreas.

Each part may retain a separate duct. The dorsal part may drain separately into the intestine but usually the ducts fuse and a single outlet, dorsal or ventral, may serve the whole pancreas (Romer, 1959).

There is scanty information about the gross anatomy of the pancreas and the ducts of the pancreas in the reindeer (Akaevskij, 1939, Engebretsen, 1975).

Materials and methods

The pancreas of 13 adult male and 2 adult female reindeer and 2 fully developed newborn calves were studied.

The opening of the pancreatic duct was examined by injecting black latex into the major duct (duct of Wirsung). The opening into the common bile duct (*ductus hepaticus communis*) was studied by a scanning electron microscope (SEM).

The ducts of Wirsung and Santorini (minor duct) were injected with a contrast medium consisting of a mixture of silicon and red lead pasta and then visualized by x-ray.

The two calves were fixed in paraformaldehyde and dissected for topographic studies of the developing pancreas.

Results

The pancreas of the reindeer calves was a slightly yellow, lobulated and soft gland. It consisted principally of two lobes; the left (tail) or *lobus sinister* and the right (head) *lobus dexter* (Figures 1 and 2). The *lobus sinister* extended in a ventrodorsal direction. It ended in an almost horizontal triangular part. One edge of the triangle was attached to the edge of the spleen. The left adrenal gland made a depression in the posterior part of the triangle. Anterolaterally the rumen and jejunum touched the tail but the main part of the lateral wall was in contact with the colon.

The body of the pancreas surrounded the hepatic portal vein and ventrally touched the abomasum. The right lobe extended in an anteroposterior direction from the corpus (body) between the *pars descendens* and *pars ascendens* of the duodenum. The cross section of this lobe was triangular. The lateral surface touched the liver, the dorsal the right kidney and the median surface of the colon. At the anterior end of the left lobe there was a groove close to the hepatic portal vein for the mesenteric artery (*arteria mesenterica cranialis*).

Ducts analogous to the *ductus pancreaticus major* (Wirsungi) and the *ductus pancreaticus minor* (Santorini) ran through the middle of the left and right lobes, respectively. They joined to form a common pancreatic duct (*ductus pancreaticus*) 2–3 cm long which opened into the common bile duct (*ductus hepaticus communis*). The *ductus pancreaticus* ran parallel to the bile duct and penetrated the mucosa in a transverse fold. The opening of the *ductus pancreaticus* was located about half a centimeter before the longitudinal folds of the bile duct mucosa (Figures 3 and 4). These folds extended one centimeter from the *papilla duodeni*.

Discussion

In reindeer, the pancreas is a flat organ extending between many of the abdominal organs. It consists of two lobes that join cranially where the gland empties into the bile duct. The pancreas has only one opening into the bile duct. In the duodenum there is a common opening for the liver and the pancreas.

In ruminants, the pancreas consists principally of a left and a right lobe developed from the dorsal and ventral primordia. The excretory system in cattle is usually reduced to a single accessory duct which enters the descending duodenum some 20 to 25 cm past the entry of the

bile duct. The ventral lobe loses its direct connection to the gut.

In small ruminants a single duct, the ventral duct, opens jointly with the bile duct into the duodenum usually by means of a common trunk (Dellman and Brown, 1976, Dyce et al., 1987). It appears that the anatomy of the pancreas in the reindeer is more like that found in small ruminants than that in large ruminants.

Previous microscopic studies of the common bile ductal wall in the reindeer revealed the occurrence of pancreatic tissues even in the mucosal layer. (Rahko and Nikander, 1990). Fairly normal exocrinic acini were observed to be present in the wall of the bile duct. Electron microscopy showed e.g., zymogen granula in the cells. However, serial sectioning has not been performed to study whether the acini observed are ectopic tissues or normal extensions of the corpus pancreaticus.

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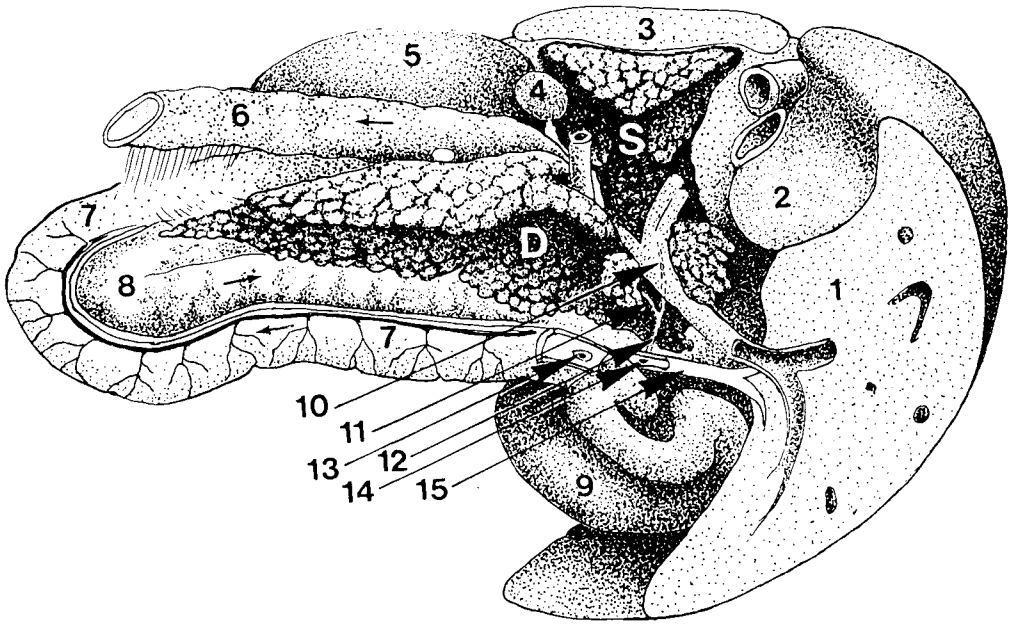

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Fig. 1. Topography of the pancreas of a young reindeer.
1 liver, 2 right renal impression, 3 spleen, 4 adrenal gland, 5 left kidney, 6 and 8 colon, 7 duodenum, 9 abomasum, 10 *ductus pancreaticus major* (Wirsung), 11 *ductus pancreaticus minor* (Santorini), 12 *ductus pancreaticus*, 13 papilla duodeni, 14 *ductus pancreaticus*, opening into common bile duct, 15 common bile duct (*ductus hepaticus communis*), D-right, S-left.

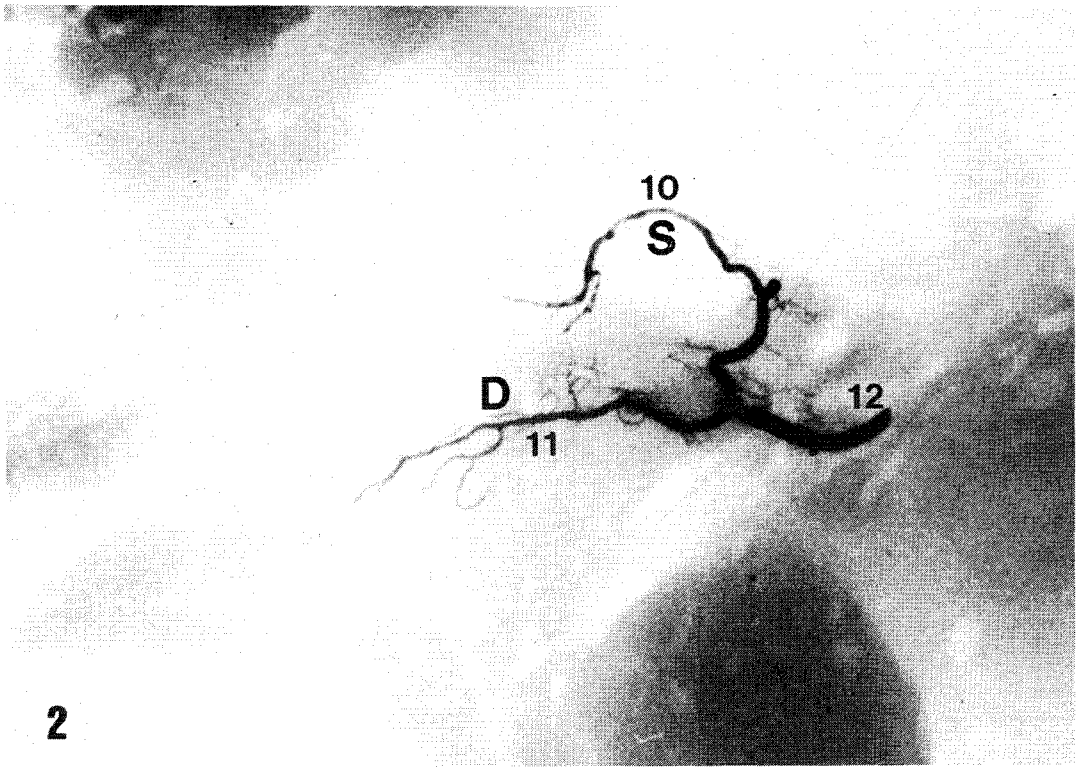

Fig. 2. X-ray of the pancreatic ducts injected with contrast medium. 10 *ductus pancreaticus major* (Wirsung), 11 *ductus pancreaticus minor* (Santorini), 12 *ductus pancreaticus*, D-right, S-left.

Fig. 3–4. SEM pictures. Opening (marked with arrows) of the *ductus pancreaticus* in the common bile duct. The fixed point is marked with asterisk (bar = 0.1 mm).


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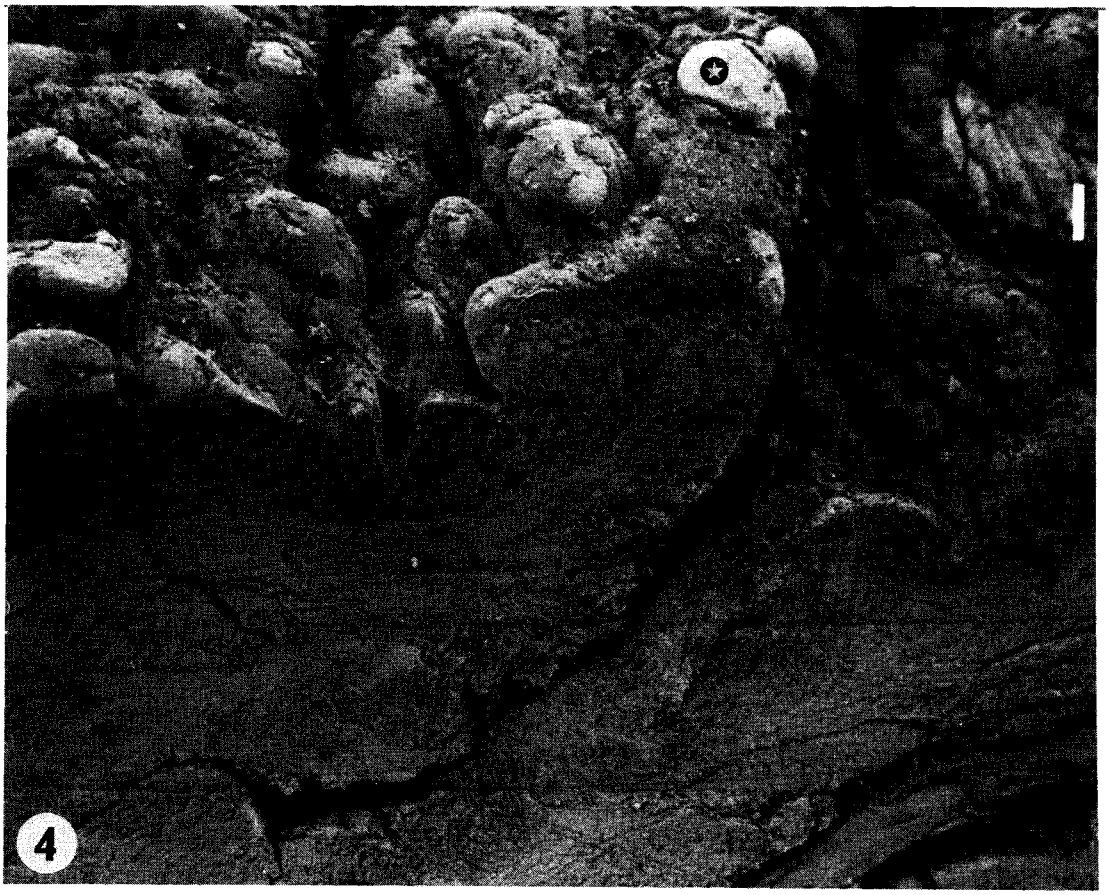
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II

Macroscopical and microscopical studies of the common bile duct in reindeer (*Rangifer tarandus tarandus* L)

Macroscopical and microscopical studies of the common bile duct in reindeer (*Rangifer tarandus tarandus* L)

Timo Rahko and **Sven Nikander**
College of Veterinary Medicine, Laboratory of Parasitology and Department of Pathology, Postbox 6, SF-00581 Helsinki, Finland

Summary: The histological structure and secretory function of the common bile duct (*ductus hepaticus communis*) has not been previously described in reindeer. Macroscopical studies were thus performed in 25 reindeer to reveal the morphology and topography of the *ductus hepaticus communis* and adjoining organs. Histologic structure of the common bile duct was investigated in 20 animals. Our studies showed that the *ductus hepaticus communis* and pancreaticus join about 2 cm before the duodenal opening to form the common duct.

The common bile duct is an elastic tube about 3 to 5 cm long and 2 to 3 mm thick partly surrounded by fat and pancreatic tissues. The wall of the duct, being about 1 mm thick by light microscopy, consisted of folded mucosa surrounded by connective tissue fibres and a serosal layer. Distally, also muscular bands were seen. In some areas separate leucocytes and even lymphatic nodules were present. Surprisingly pancreatic acini occurred in certain areas of the wall, even in close contact to subepithelial tissues. Mucosal epithelium consisted of surface and glandular epithelial cells with mucous secretion. Numerous intraepithelial globule leucocytes were identifiable within the lamina epithelialis.

Key words: anatomy, histology, ductus pancreaticus, globule leucocyte, mast cell.

Rangifer, 10 (1): 3—8

Rahko, T. ja **Nikander, S.** 1990. Tutkimus yhteisen sappikäytävän rakenteesta porolla.

Yhteenveto: Yhteisen sappikäytävän (*ductus hepaticus communis*) histologista rakennetta ja eritystoimintaa ei ole aikaisemmin kuvattu porolla. Makroskooppisia tutkimuksia suoritettiin 25 porolla yhteisen sappikäytävän rakenteen ja topografian selvittämiseksi. Seinämän histologinen rakenne selvitettiin 20 porolla. Tutkimukset osoittivat, että porolla *ductus hepaticus communis* ja *ductus pancreaticus* yhtyvät noin 2 cm ennen ohutsuolta muodostaakseen yhteisen tiehyeen.

Ductus hepaticus communis on noin 3–5 cm pitkä ja 2–3 mm:n läpimittainen käytävä. Se on elastinen ja osittain rasva- ja haimakudoksen ympäröimä. Seinämä on mikroskooppisesti noin 1 mm paksu. Sisäosan muodostaa poimuuntunut limakalvo. Limakalvoa ympäröivät sidekudossäikeet ja serosa, sappikäytävän loppuosassa myös lihassäikeistö. Seinämässä havaittiin yksittäisiä valkosoluja ja imusolukasautumia. Poikkeuksellisena anatomisena piirteenä voidaan pitää haimasaarekkeiden esiintymistä sappikäytäväseinämän kudoksissa jopa läheisessä kosketuksessa pintaepiteeliin. Limakalvon epiteelikudos on sekä pinta- että rauhassolukkoa, joka erittää limaa. Epiteelissä tunnistettiin lukuisia kerässoluja huolimatta siitä, että poroille oli suoritettu loishäätö edellisenä syksynä.

Rangifer, 10 (1): 3—8

Rahko, T. och **Nikander, S.** 1990. Studier av gallgången (*ductus hepaticus communis*) hos ren.

Sammandrag: Den gemensamma gallgångens histologi och sekretoriska funktion hos renen har inte tidigare beskrivits. För att klarlägga den makroskopiska byggnaden och topografin av den gemensamma gallgången (*ductus hepaticus communis*) undersöktes dessa i 25 renar. Väggen i 20 gallgånger granskades histologiskt. Undersökningarna visade att *ductus pancreaticus* mynnar ut i ductus hepaticus communis bildande en gemensam utförselgång till duodenum. *Ductus hepaticus communis* är c. 2–3 mm i diameter och 3–5 cm lång. Den elastiska gångens vägg är c. 1 mm tjock delvis omgiven av fett- och pankreasvävnad. Gångens vägg består av serosa, bindvävsfibrer och nära mynningen ses muskelfibrer. Insidan av gången är beklädd med en veckad slemhinna. Lymfatisk vävnad och enstaka lymfocyter observeras. Anmärkningsvärt är att pankreasvävnad förekommer i gallgångens vägg och under slemhinnan. Slemhinnans epitel består av yt- och körtelepitel som avsöndrar slem. De globulära leukocyterna var talrika i epitelet trots att renarna avmaskats på hösten.

Rangifer, 10 (1): 3—8

Introduction

The anatomy of the liver and main bile ducts varies among different animal species (Schache, 1907, Bevandic & al., 1967, Elias & Sherric, 1969, Dellman and Brown, 1976). The reindeer belongs to the family Cervidae, which do not possess a gall bladder. In the reindeer, bile secreted by the liver is thus directly conducted to the duodenum through the ductus hepaticus communis, the common bile duct (Akaevskij, 1939). The secretory function of the duct bile wall is not yet known.

In bovines, on the other hand, bile is stored in a gall bladder, the wall of which is rich in serous and mucous glands (Dellman and Brown, 1976). In cattle the original bile is thus concentrated and mixed by mucous fluids of the gall bladder and the wall of main bile duct is profoundly glandular (Rahko, 1971, 1973).

This paper describes the structure of the wall of the common bile duct in reindeer.

Materials and methods

Macroscopical studies were performed in 24 reindeer. One animal was selected for more thorough dissection of the inner organs to reveal the topography of the liver, pancreas and adjoining organs.

The tissue specimens of *ductus hepaticus communis* used in the light microscopical studies originate from 20 reindeer. Eight animals were slaughtered at the Reindeer Research Institute in Kaamanen outdoors at -30°C. 12 reindeer were slaughtered in a slaughterhouse located in Savukoski in eastern Lappland.

Approximately 5–10 mm long specimens of *ductus hepaticus communis*, surrounded by fat and pancreatic tissues, were cut into small tissue blocks, which were soon transferred into the different fixatives. Care was taken to prevent freezing of the tissue specimens.

Six specimens were fixed in diluted formaldehyde solution (25 %) buffered at pH 7 by phosphate buffer (fixation time 4 days). Additional six specimens were fixed in Bouin's solution for one day, then transferred into 70 % alcohol. Furthermore, 14 specimens were fixed during 1 to 4 months in 4 % paraformaldehyde in 0.1 M phosphate buffer containing 0.1 % sodium azide (NaN_3) and 0.05–0.1 % glutaraldehyde.

The material was embedded in paraffin in the usual manner, sectioned at 4 microns and routinely stained with haematoxylin and eosin (HE).

Results

Macroscopical studies

The common bile duct appeared as a 2–3 mm thick elastic tube between the liver and oral part of the duodenum. It was 3–5 cm long, white and thin-walled. The duct was partly surrounded by fat and pancreatic tissues. No papilla was observed around its duodenal opening. At slaughter when the duct was cut the mucosa of the stump appeared to be yellow in colour near the hilus of the liver.

The *ductus pancreaticus* connected with the common bile duct at about 2 cm before the duodenal opening.

Microscopical studies

The wall of the common bile duct appeared to be about 1 mm thick by microscopy. The outer layers were surrounded by a serosal coat and

consisted of circular connective tissue fibres. Smooth muscle layers were detected in only distal sections of the duct near the duodenum. In some areas pancreatic tissues were observed in the wall of the duct (Fig. 1).

The inner layers of the wall consisted of folded mucosal tissues with epithelial surface and glandular structures surrounded by a loose connective tissue network of *lamina propria* (Fig. 2).

In the *lamina propria*, dispersed granulocytes and lymphocytes were present and also prominent accumulations of lymphatic cells were observed (Fig. 3 and 4).

The epithelium was composed of tall columnar epithelial cells with basal, rounded and regular nuclei (Fig. 5 to 7). A few goblet cells were located within *lamina epithelialis*. The cytoplasm of the epithelial cells was pale in HE-stained sections, especially in the deep glands. In mucous glands the cytoplasm of epithelial and goblet cells was ballooned by mucous secretions. Mucus also was present freely in the lumina of the glands.

Numerous globule leucocytes were present in superficial and glandular epithelium. The cells were located basally within the epithelium. They were identified on the basis of large eosinophilic rounded globules. The nuclei were chromatin-rich and rounded being quite similar in appearance to those of the lymphocytes. Eosinophilic granulocytes were easily identifiable in proprial tissues (Fig. 8) while mast cells were difficult to detect in HE-stained sections. Mast cell granules appeared small and faintly-stained.

All the fixatives used were suitable for histologic studies. However, our impression was that Bouin's solution and paraformaldehyde preserved the tissues particularly well.

Discussion

The results of the present study showed that morphological characteristics of the wall of the common bile duct in Finnish reindeer are largely similar to those in other animal species (e.g. McMinn and Kugler, 1961, Dellman and Brown, 1976, Rahko 1971, 1973). The duct is short, elastic and thin-walled with smooth mucosal folds consisting of epithelial and glandular structures. Connective tissue fibres of *lamina propria* are loose while the outer layers of the duct contain circular mesenchymal elements with smooth muscle fibres in distal parts of the duct.

The entering of the *ductus pancreaticus* into bile duct to form the *ductus communis* was observed as previously described by Akaevskij (1939). This is typical in sheep and goats, while in most domestic mammals the pancreatic and hepatic ducts are separate anatomic structures (Schmidt and Ivy, 1937, Elias and Sherrick, 1969, Dellman and Brown, 1976).

A new finding not previously described is the extension of pancreatic acini into proprial tissues of the bile duct (Figure 1). The significance of this peculiar anatomical feature is unclear to the authors. Further studies are needed to elucidate if the pancreatic acini described are ectopic pancreatic tissues or normal projections in the form of *caput biliaris* in additon to the previously described *capiti duodenalis* and *omentalis*. Electron microscopic studies showed that the ultrastructural features of pan-creatic acini of bile ductal walls appear similar to those in normal acini of mammalian animals (Rahko and Nikander, 1990).

Lamina epithelialis contains numerous globule leucocytes (Gregory, 1979, Nikander and Rahko, 1990). The cell has not previously been identified in reindeer. The globule leucocyte is known to occur within different epithelial tissues of many other animal species and in man. The frequency with which the cell appears, however, is usually associated with parasites e.g. Gregory, 1979. The animals in the present study were treated with anthelmintica in the preceding autumn and were thus considered to be free from clinical helminthosis (Holmström & al., 1989). Further studies are to be performed in order to elucidate the frequency of globule leucocytes in Finnish reindeer.

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Fig. 1. Transverse section of *ductus hepaticus communis.* The dark-stained areas of the mucosa consist of pancreatic acini (arrows). HE, x 32.

Fig. 2. Mucosal layer of *ductus hepaticus communis*, containing surface epithelium (to the left) and glands with pale cytoplasm (arrow). HE, x 400.

Fig. 3. The cytoplasm of epithelial cells in the glands is filled with mucous. Round cells and eosinophilic granulocytes are present in submucosal areas (arrows). HE, x 256.

Fig. 4. Cross section through the wall of common bile duct, showing a prominent lymphatic nodule. HE, x 256.

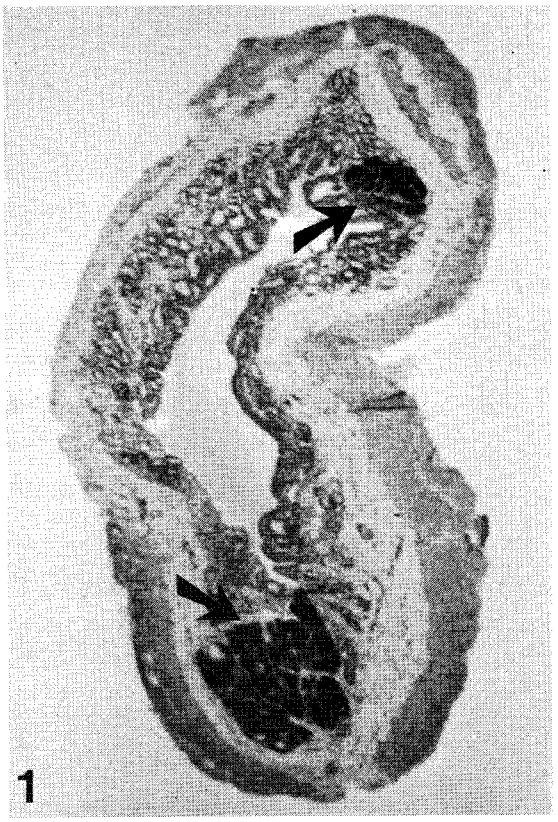
Figures 5–8, see page 8

Fig. 5. The cytoplasm of epithelial cells is pale in the deep glands. Note the structural difference between intraepithelial globule leucocyte (GL) and mast cell (MC) located in the *lamina propria.* HE, x 1040.

Fig. 6. Globule leucocytes (arrows) are frequent in superficial and glandular epithelium. HE, x 1040.

Fig. 7. Typical location and appearance of intraepithelial globule leucocytes (arrows). HE, x 1040.

Fig. 8. Infiltration of eosinophilic granulocytes (arrows) surrounded by connective tissue elements. Compare the appearance of nucleus and intracytoplasmic granules of eosinophilic granulocytes to those of the globule leucocytes illustrated by Figures 5 to 7. HE, x 1040.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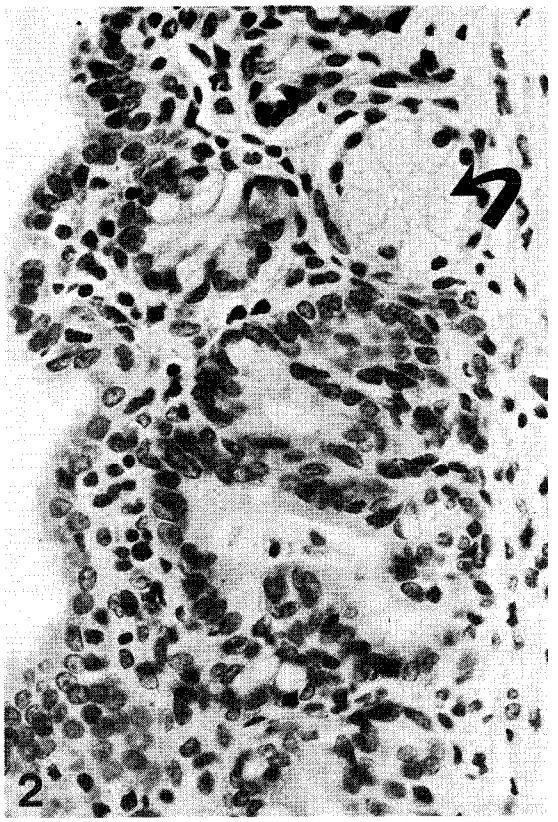
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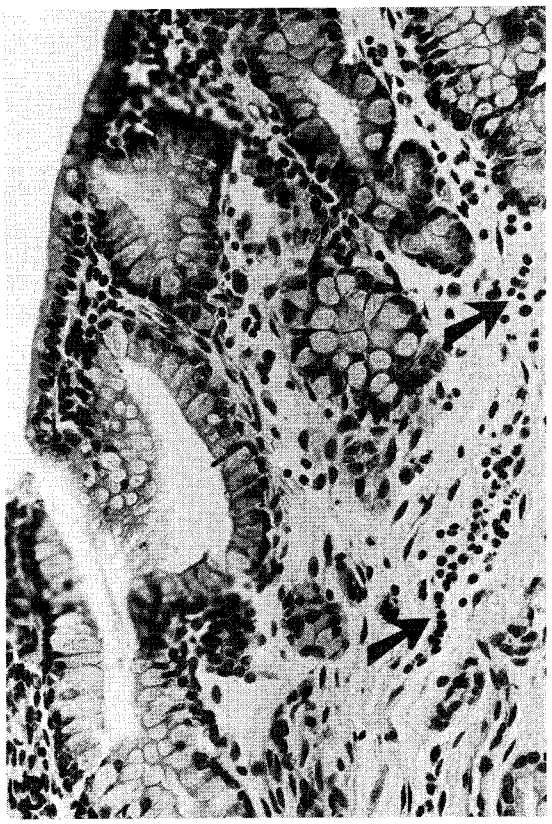

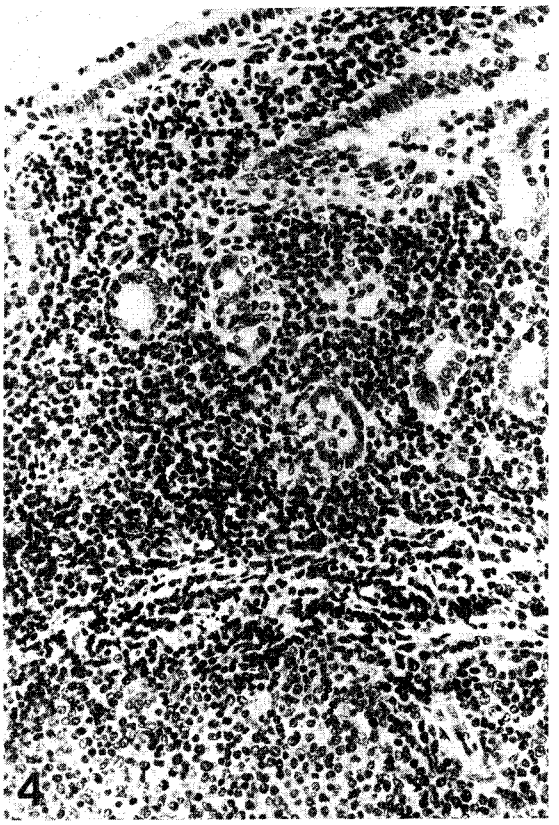
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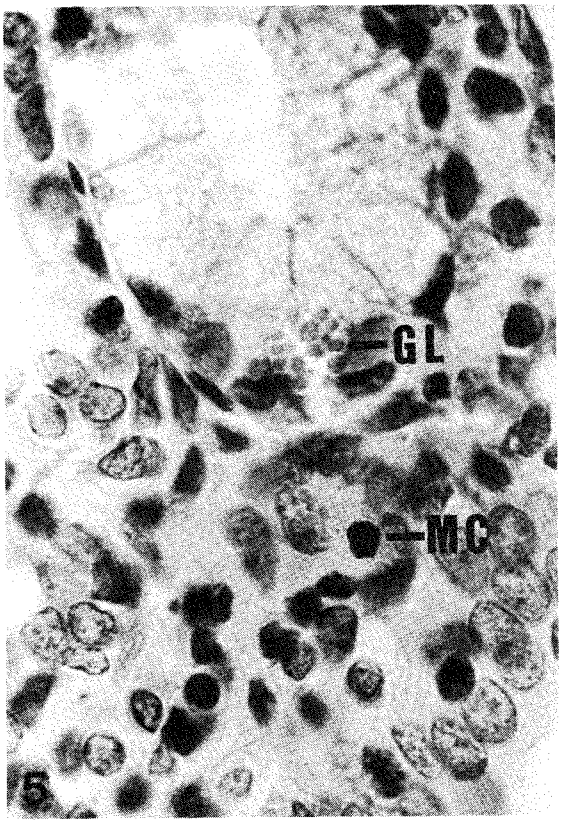
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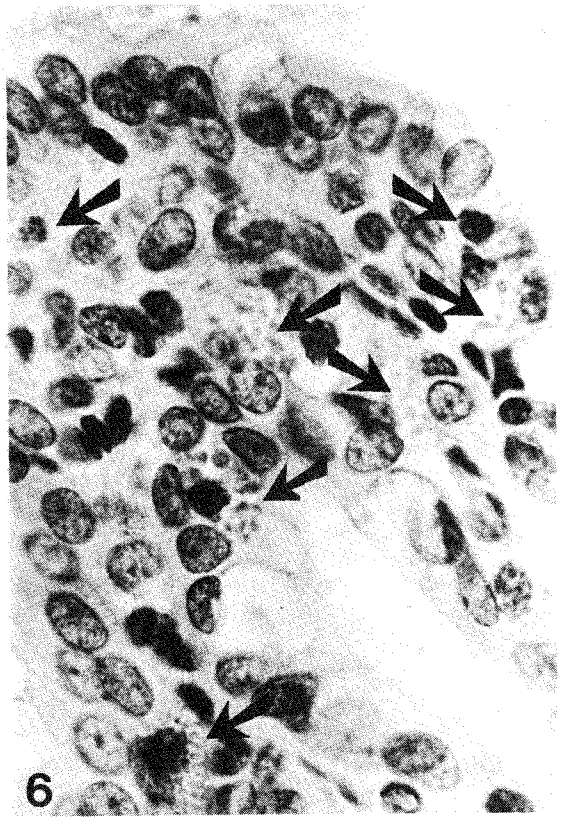
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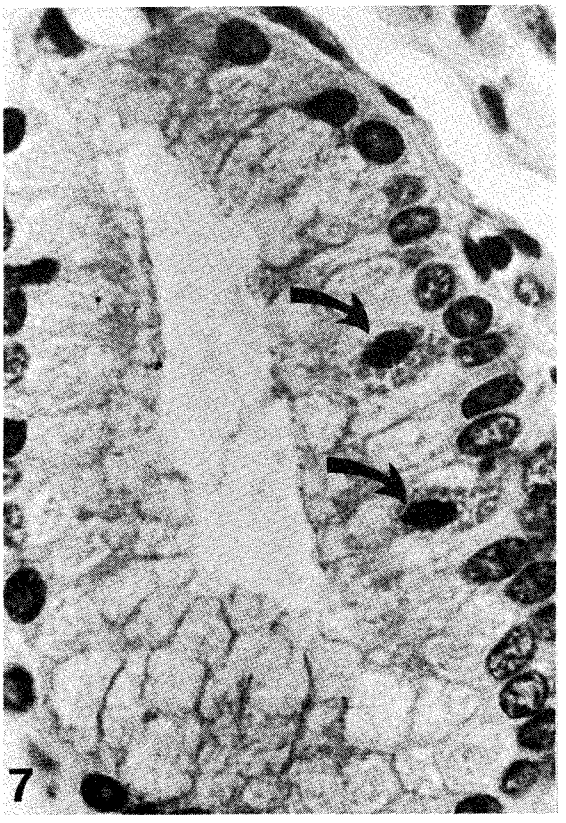
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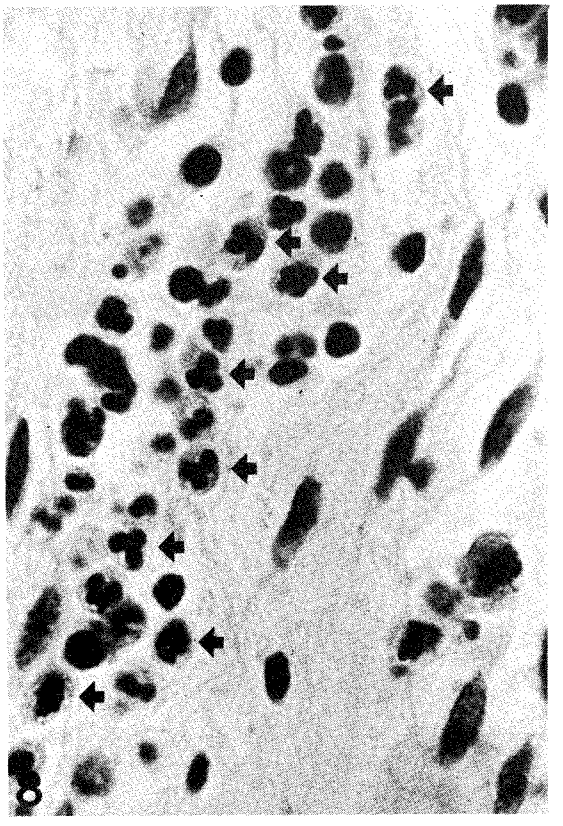
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III

Histochemical studies of the common bile duct in reindeer

Histochemical studies of the common bile duct in reindeer

Timo Rahko and **Sven Nikander**
College of Veterinary Medicine, Laboratory of Parasitology and Department of Pathology, Postbox 6, SF-00581 Helsinki, Finland

Summary: Histochemical characteristics of bile duct mucosubstances, mast cells and globule leucocytes have not previously been described in the reindeer. Therefore various staining methods were applied on 1 to 6 specimens cut from formaline or Bouin-fixed histological blocks of the *ductus hepaticus communis* from 20 reindeer. The present study showed that bile duct mucins include neutral, carboxy- and sulphomucins located chiefly in goblet cells and in the deep glands and as a thin superficial layer covering the surface epithelium. PAS-reactivity was diastase resistant, indicating that glycogen was not demonstrable in the epithelial layer of reindeer, contrary to previous studies e.g., on carnivores. Furthermore, carboxymucins were sialidase-resistant, as sialic acid could not be identified in the present material. Certain differences were noted in the appearance and composition of intracytoplasmic granules and globules of mast cells and globule leucocytes, respectively. The mucosubstances of the mast cell contained sulphate groups indicative of sulphomucins while both neutral, carboxy- and sulphomucins were identifiable in globule leucocytes. However, due to the sensitivity of mast cells and globule leucocytes to postmortal changes the above interpretations need to be confirmed by further studies.

Key words: anatomy, histology, histochemistry, mucosubstances, globule leucocyte, mast cell.

Rangifer, 10 (1): 9—15

Rahko, T. ja **Nikander, S.** 1990. Histokemiallinen tutkimus yhteisen sappikäytävän rakenteesta porolla.

Yhteenveto: Yhteisen sappikäytävän (*ductus hepaticus communis*) seinämän epiteelisolukon ja syöttö- ja kerässolujen lima-aineiden koostumusta porolla ei ole aikaisemmin kuvattu. Sen vuoksi katsottiin aiheelliseksi suorittaa histokemiallinen tutkimus formaliini- ja Bouin-kovetetusta histologisesta aineistosta, jonka kirjoittajat ovat julkaisseet aikaisemmin. Aineisto on peräisin 20 porosta. Erilaisia histokemiallisia värjäyksiä sovellettiin 1–6:een valikoituun leikkeeseen tarkoituksella analysoida nimenomaan lima-aineiden koostumusta.

Tutkimuksissa ilmeni, että sappikäytävien erittämä lima koostuu neutraaleista, karboksy- ja sulfomusiienista. Eritys tapahtuu pääasiassa pikarisoluista ja seinämien syvistä rauhasista. Myös pintasolukkoa peittää ohut limakerros. Glykogeeniä ja sialiinihappoa ei todettu. Syöttö- ja kerässolujen soluliman jyvästen koostumuksessa todettiin tiettyjä eroavaisuuksia. Syöttösolujen lima-aineet ovat sulfomusiineja, mutta kerässolujen jyväsissä on sulfomusiinien lisäksi myös neutraaleja- ja karboksymusiineja. Syöttö- ja kerässolujen todettiin olevan herkkiä kuolemanjälkeisille muutoksille, mikä vaikeutti varmojen johtopäätösten tekemistä.

Rangifer, 10 (1): 9—15

Rahko, T. och Nikander, S. 1990. Histokemiska studier av gallgången hos ren.

Sammandrag: Mucinernas sammasättning i globulära leukocyter, mast- och epitelceller i gallgångarna på ren har inte beskrivits tidigare. Det ansågs motiverat att histokemiskt undersöka det formalin- och Bouin fixerade material som tidigare publiserats av undertecknade. Materialet härstammar från 20 renar. Olika histokemiska färgningar utfördes på 1–6 utvalda preparat i avsikt att analysera muchinernas sammansättningen. Det framgick att slemmet i gallgången innehåller neutrala-, karboxyl- och sulfomuciner. Exkretionen sker i huvudsak från bägarceller och körtlar i gallgångväggen. Ytepitelet täckes också av et tunnt slemskit. Glykogen och sialinsyra påvisades icke. Det konstaterades skillnader i mucinsammansättningen hos mastceller och globulära leukocyter. I mastcellernas granulae påvisades sulfomuciner och de globulära leukocyterna innehöll förutom sulfomuciner, neutrala- och karboxymuciner. Konklusionerna bör verifieras medan mastcellerna och de globulära leukocyterna föreföll att var känsliga för postmortala förändringer.

Rangifer, 10 (1): 9—15

Introduction

In the available literature there are several histochemical studies on extrahepatic bile ducts of e.g., small laboratory rodents (McMinn and Kugler, 1961, Rahko, 1971), dogs (Seeliger, 1937, McMinn and Kugler, 1961), goats (Rahko, 1972a, b, Cheema and Hooshmand-Rad, 1985) and cattle (Rahko, 1971) while corresponding information is lacking in reindeer.

In a previous report, the authors have described the histologic structure of the common bile duct the reindeer (Rahko and Nikander, 1990). The present paper describes some histochemical characteristics of bile duct mucosubstances, mast cells and globule leucocytes.

Materials and methods

Staining methods were applied on 1 to 6 specimens cut from formalin- or Bouin-fixed material of 20 reindeer included in the histological material of our previous report (Rahko and Nikander, 1990). Stainings were performed according to the instructions described in the manual of the AFIP (1968) and Pearse (1968) and by Rahko (1971).

Mesenchymal tissues were studied by staining the sections with Herovic's staining and Ladewig's modification of Masson's trichromstaining.

Carbohydrate rich and mucinous compounds were analyzed by the following procedures:
Periodic acid-Schiff with and without diastase (d-PAS, PAS) for glycogen and neutral glycoproteins. Acid mucosubstances were investigated by variously staining with alcian blue at pH 2.5 and pH 1.0 (AB 2.5, AB 1.0), nuclear fast red counterstain and with aldehyde fuchsin at pH 1.7.

The presence of sialomucins was studied with a sialidase digestion of the sections prior to AB 2.5 staining (sialidase-AB). The sialidase digestion was carried out in 0.1 M acetate buffer pH 8.9, containing 0.04 M calcium chloride and 0.1–4.5 U/ml sialidase (Neuraminidase, Sigma). The sections were digested at 40°C for 1 hour. Control sections were treated with buffer without the enzyme.

In order to differentiate acid (blue or blue purple) from neutral (red) mucosubstances, the sequence AB 2.5 followed by PAS (AB 2.5-PAS) was employed.

Sulphated mucosubstances were studied with alcian blue staining at pH 1.0 followed by PAS (AB 1.0 -PAS) to differentiate sulphomucins (blue or purple) from neutral mucins (red). Aldehyde fuchsin staining at pH. 1.7 followed by alcian blue at pH 2.5 (AF-AB 2.5) was further used to differentiate sulphomucins (purple) from nonsulphated (blue) acidic mucins.

Mast cells and globule leucocytes were studied by staining selected sections with amidoblack for identification of globule leucocytes, with v. Kossa's staining for calcium, with oil red O for neutral fats, with Fontana-Masson staining for melanin and iron-containing pigments, but no positive staining was observed. Furthermore, the cells were stained with alcian blue at pH 0.3 followed by safranin at pH 1.0 (AB-S) to differentiate heparine (red) from other highly sulphated mucosubstances (blue), with toluidine blue at pH 4.0 and pH 0.5 (TB 4.0, TB 0.5) to study the metachromatic properties of the granules in mast cells and the globules in globule leucocytes.

Results and discussion

Bile duct epithelium

Neutral, carboxy- and sulphomucins were iden-

Table 1. Identification of neutral, carboxy- and sulphomucins.

Staining method	Neutral mucins	Carboxymucins	Sulphomucins
PAS	red	–*	–
d-PAS	red	–	–
AB 2.5	–	blue	blue
sialidase-AB	–	blue**	blue
AB 2.5-PAS	red	blue/purple	blue/purple
AB 1.0	–	–	blue
AF	–	–	purple
AB 1.0-PAS	red	–	blue/purple
AB-S	–	–	purple/red***

* no staining reaction
** sialomucins -
*** heparin

Table 2. Histochemical characteristics of mucosubstances in the granules of mast cells and the globules of globule leucocytes.

	Mast cells	Globule leucocytes
PAS	0	0
d-PAS	0	0
TB 4.0	purple or blue 1+	blue 1+ or 0
TB 0.5	purple or blue 1+	0
AB 2.5	blue 2+	*blue 1+**
AB 1.0	blue 2+	*blue 1+**
AB 2.5-PAS	blue 2+	*blue or red 1+
AB 1.0-PAS	blue 1+	*blue or red 1+ or 0
AF-AB 2.5	blue 2+	*blue 1+ or 0
AB-S	blue 1+	*blue 1+ or 0

0: no reaction;
1+: weak positive reaction;
2+: moderate or strong positive reaction.

* blue staining appeared as intracytoplasmic precipitates between the globules or
** as cortical zones in the globules (in Bouin-fixed sections).

tified on the basis of different histochemical reactions presented in Table I.

The reactions for carbohydrate rich compounds were the most intense in the goblet cells and in the deep glands (Figures 1 to 5). Furthermore, a thin layer of mucins covered the surface epithelium.

Sulphomucins were demonstrable only in the goblet cells and in the deep glands. Carboxymucins, on the other hand, were present also in superficial mucins where the reaction of neutral mucins was also the most intense. Mucins were thus blue or purple with AB 1.0-PAS staining of the deep glands and goblet cells but red in superficial mucins, while AB 2.5-PAS stained superficial mucosubstances blue and mucins of the other areas purple. The carboxymucins did not show digestibility by sialidase, indicating

that sialomucins were not demonstrable in the present material. Otherwise, it appears that the bile duct mucins of reindeer are largely similar to those in cattle, goats and mice (McMinn and Kugler, 1961, Rahko 1971, 1972a and b). Glycogen was not identified in the bile duct epithelial cell as described by Seeliger (1937) in dogs.

Mast cells and globule leucocytes

The histochemical characteristics of carbohydrate rich compounds detected in mast cells and globule leucocytes are presented in Table II and Figures 5 to 8. It is, however, noteworthy to comment that the conclusions made on the staining properties of the intracytoplasmic granules and globules are based on observations of only a restricted number of cells detectable by light microscopy. Difficulties were also encountered due to the diffusion of stainable substances both in Bouin- and formalin-fixed material. Furthermore, when some, but not all, of the granules and globules were stained, the reaction was nevertheless considered positive.

In the AB 2.5 staining of Bouin-fixed material the cores of globules in the globule leucocytes stained red with nuclear fast red counterstain being surrounded by a blue cortical zone. In the Bouin-fixed material globule leucocytes did not show AF 1.7 stainable material.

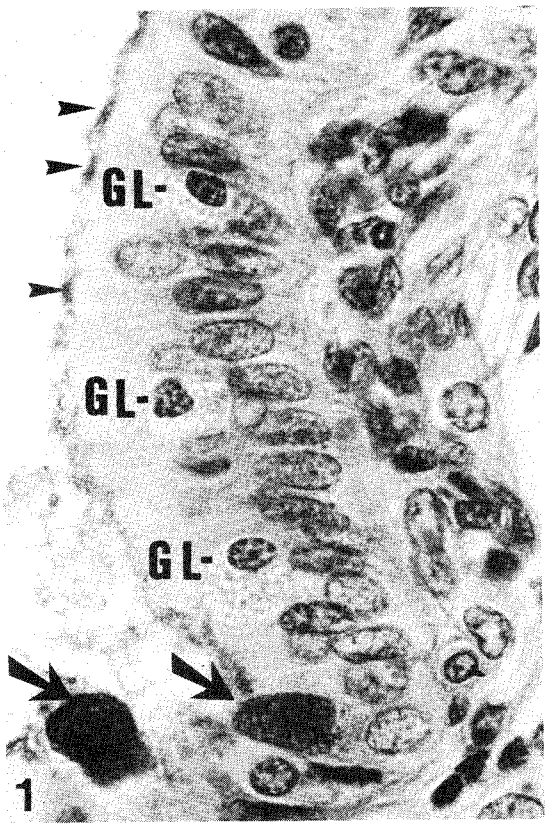
Mast cell granula and globule leucocyte globules displayed a somewhat different pattern in the staining reactions applied. The carbohydrate rich compounds of mast cell granules were more acidic than those in the globules of globule leucocytes. It is obvious that mast cell granula contain sulphomucins while globules of globule leucocytes show the staining reactions of neutral, carboxy- and sulphomucins. However, difficulties were produced by postmortal changes in interpretations of locations and shades of stainable material in mast cells and globule leucocytes. This phenomenon is analogous to that previously described in globule leucocytes in the bile ducts of goats (Rahko, 1972 b) and in the urinary bladder of rats (Ahlqvist and Kohonen, 1959). Also the mast cells of reindeer appeared to be sensitive to artificial changes so that the presence of heparin was not demonstrable by the present methods in the cells.

Fig. 1. Periodate-reactive neutral mucins are present in abundance in goblet cells (arrows) and cover the surface epithelium (arrowheads, GL = globule leucocyte) PAS, x 1040.

Fig. 2. Pancreatic acini are seen as dark-stained islets within mucosa. Arrowheads point to mast cells. TB 4.0, x 256.

Fig. 3. Dark-stained areas of surface and glandular epithelium contain vicinal groups of neutral and carboxymucins. Compare with Figure 4. AB 2.5-PAS, x 400.

Fig. 4. Reaction for hydroxyl and sulphate groups gives a weaker contrast than that for neutral and carboxymucins shown in Figure 3. Compare the figures and note that the stain in Figure 4 is weaker especially in the deep glands. AB 1.0-PAS, x 400.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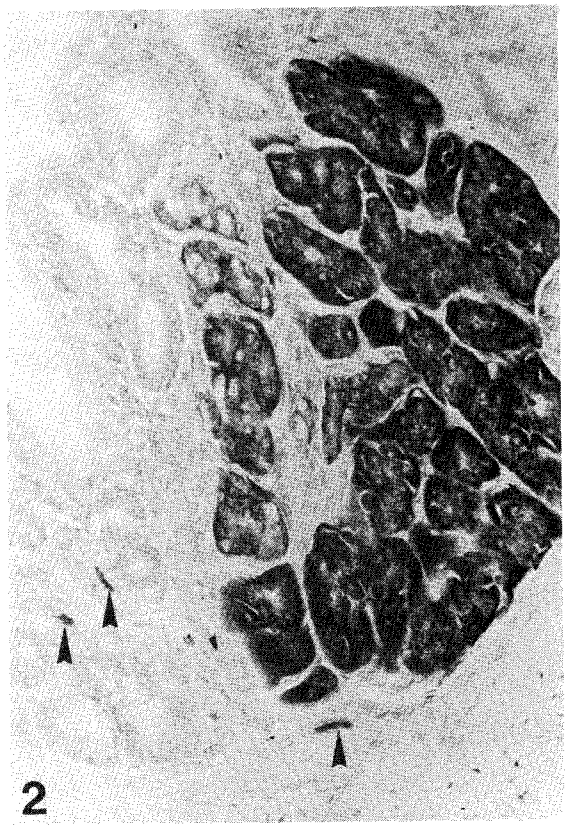
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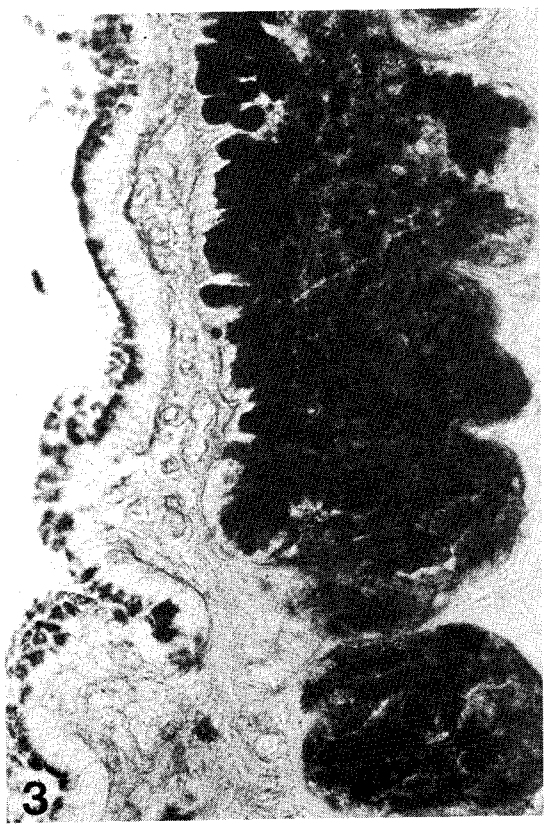
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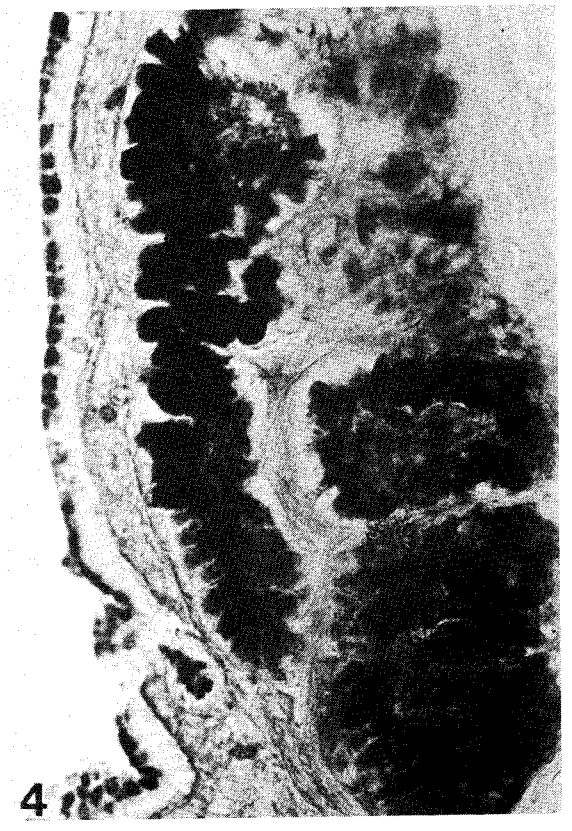
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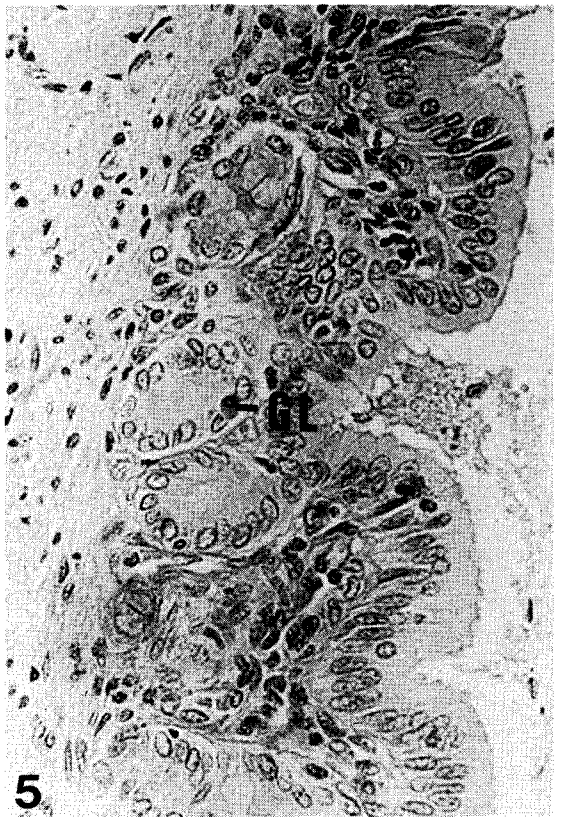
Manuscript delivered 12 December, 1989

Fig. 5. A weak blue (dark) staining reaction indicative of sulphomucins is present in connective tissue matrix and in epithelial surfactant and in globules of globule leucocyte (GL). AB 1.0, X 400.

Fig. 6. Blue (dark) staining in globules of globule leucocytes (GL) and weakly purple metachromasy in granules of mast cells (MC) differentiate the sulphomucins of the globules and granules. Arrows indicate to pancreatic tissues within the mucosal layer. TB 4.0, x 256.

Fig. 7. An abundance of blue (dark)-stained mast cells and globule leucocytes (e.g. arrowheads) in the wall of the common bile duct. AB-S, x 100.

Fig. 8. Globule leucocytes (arrows) show a blue (dark) stain indicative of sulphomucins concentrated in the cortex of the globules. AB 1.0, x 1040.



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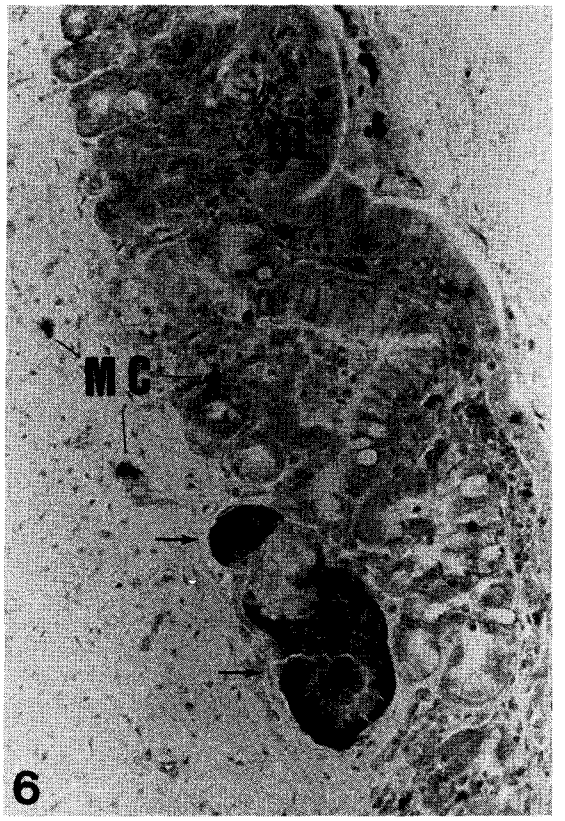

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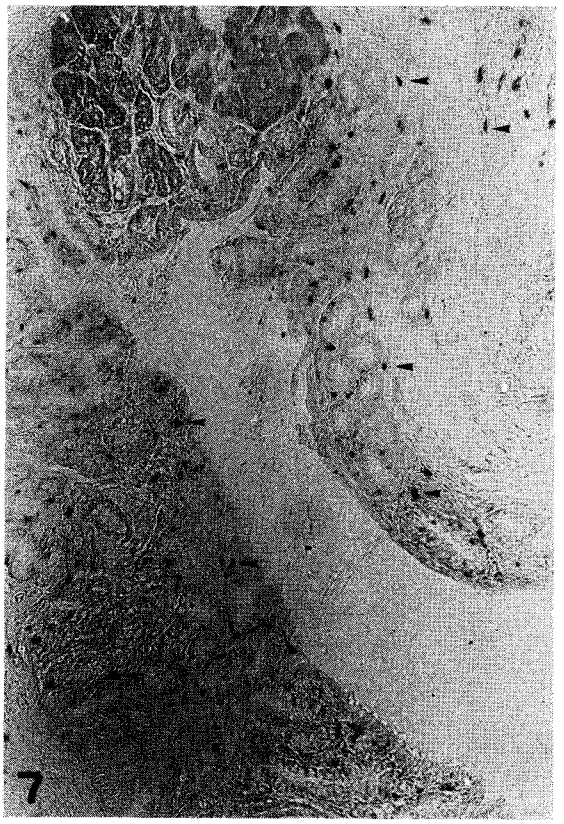

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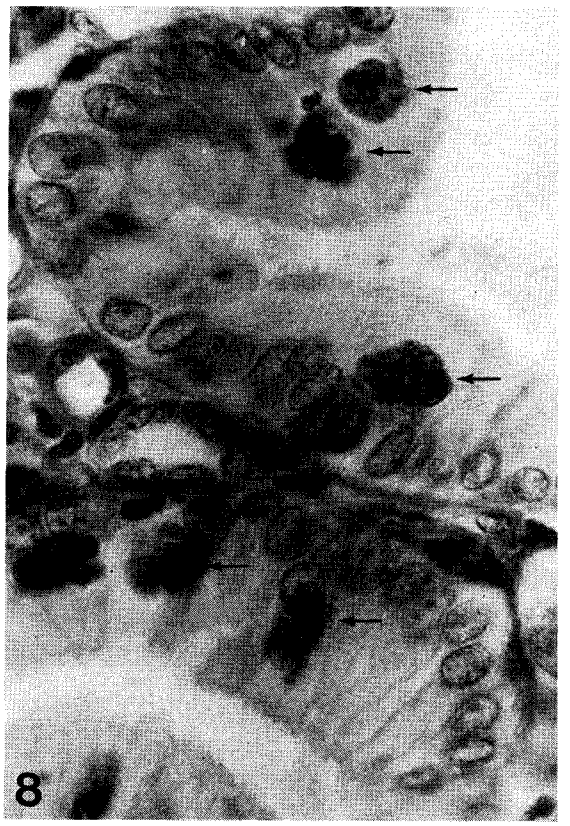

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IV

Electron microscopical studies of the common bile duct in reindeer

Electron microscopical studies of the common bile duct in reindeer

Timo Rahko and **Sven Nikander**
College of Veterinary Medicine, Laboratories of Parasitology and Electron Microscopy and Department of Pathology, Postbox 6, SF-00581 Helsinki, Finland.

Summary: In a previous publication the authors have described some ultrastructural characteristics of granulated cells in the common bile duct of the reindeer. On the basis of the same material, electron microscopic observations on other tissue elements of bile duct wall are now reported.

The surface and glandular epithelium were composed of tall columnar epithelial cells with villous structures on the luminal surfaces. The parietal cytoplasmic membranes of epithelial cells were equipped with intercellular desmosomes while intraepithelial globule leucocytes did not form any junctional complex with other cells. Apical cytoplasmic areas of superficial epithelial cells showed electron-dense small bodies possibly consisting of mucinous substances. The goblet and deep glandular cells, on the other hand, contained numerous large mucin granules with less electron-dense matrices. It appears that their secretions are more abundant than those in superficial epithelial cells which obviously are absorptive as their main function. The nuclei and other cytoplasmic organelles showed profiles similar to those in epithelial cells generally. The lumen of the bile ducts was usually empty or contained fine-granular or amorphous material. An unusual feature was the presence of parts of globule leucocytes or even almost whole cells occurring freely in ductal secretions.

Key words: anatomy, globule leucocyte, mast cell, ultrastructure.

Rangifer, 10 (1): 17—23

Rahko, Timo ja **Nikander, Sven.** 1990. Elektronimikroskooppinen tutkimus yhteisen sappikäytävän rakenteesta porolla.

Yhteenveto: Aikaisemmassa julkaisussa tekijät kuvasivat poron yhteisen sappikäytävän (ductus hepaticus communis) seinämän jyväsellisten solujen hienorakennetta. Tässä artikkelissa selostetaan saman aineiston perusteella (6 tervettä teurasporoa) elektronimikroskooppisia havaintoja sappikäytäväseinämän muista kudosrakenteista.

Sappikäytäväseinämän pinta- ja rauhasepiteeli koostuu korkeista epiteelisoluista. Pinnallisia epiteelisoluja kattavat säännölliset mikrovillukset, ja niillä on vain vähän ilmeisesti limaa sisältäviä jyväsiä solulimassaan. Rauhas- ja pikarisoluissa säännölliset mikrovillukset sen sijaan puuttuvat. Niiden sytoplasman täyttävät runsaat limapalloset, joita solut muodostavat hyvin kehittyneessä Golgin laitteessaan erittäen limaa sappikäytävän onteloon. Erite näkyy hienojyväisenä tai tasa-aineisena seinämiä reunustavana aineena. Poikkeuksellisena havaintona voidaan pitää kerässolujen kerästen tai lähes kokonaisten kerässolujen esiintymistä sappikäytäväonteloissa mahdollisesti osoituksena solujen vaelluskyvystä. Vaikka epiteelisolut muodostavat lujia solukalvosidoksia toisiinsa, kerässolut eivät kiinnittyneet muihin soluihin. Ilmeistä on, että pinnallisten epiteelisolujen toiminta on pääasiassa absorptiivista, mutta rauhasepiteelisolut ovat erikoistuneet eritystoimintaan.

Rangifer, 10 (1): 17—23

Timo Rahko och Sven Nikander 1990. En elektronmikroskopisk studie av gallgången hos ren.

Sammandrag: Gallgångarnas yt- och körtelepitel bestod av höga epitelceller beklädda med ett regelbundet villusskikt. Intercellulära desmosomer sågs i epitelcellernas parietala cytoplasmamembraner. De intraepiteliala globulära leukocyterna saknade desmosomer eller andra bindningar med närliggande celler. I de superficiala epitelcellernas apikala cytoplasma fanns elektrontäta små kroppar antagligen bestående av mucin. Bägar- och de djupare belägna körtelcellerna innehöll rikligt med stora mucin granulor med ett mindre elektrontätt matrix. Det föreföll som om dessa celler skulle vara sekrerande och de superficiala epitelcellerna absorberande. Kärnen och andra cytoplasmatiska organeller hade egenskaper jämförbara med epitelceller i allmänhet. Gallgången var oftast tom men ibland sågs ett finkornigt amorft material i den. Som en anmärkningsvärd observation ansees förekomsten av delar och t.o.m. hela globulära leukocyter i gallgången.

Rangifer, 10 (1): 17—23

Introduction

Our understanding of the function of different organs is based on a thorough knowledge of structural features. This also applies to the liver and bile ducts. The relative size and structure of the liver varies among different animal species. Some species such as the rat and the reindeer do not possess a gall bladder.

The ultrastructure of the common bile duct differs between rats and mice (Yamada, 1969, 1970, Luciano, 1972). These differences were attributed to the presence or absence of a gall bladder. To clarify if the reindeer shows some special ultrastructure in the wall of the common bile duct, this was in this study examined by electron microscopy.

Materials and methods

The same tissue blocks, from six healthy reindeer, were used in this study. The ultrastructure of granulated cells of bile ducts in reindeer have been described elsewhere (Nikander & Rahko, 1990).

Results

The general architecture of the mucosal layer of the common bile duct showed a regular epithelium supported by proprial tissues. The epithelium was of two types; the surface epithelial cells and glands with goblet cells (Figures 1 to 6).

Superficial epithelial cells were characterized by abundant and regular villous projections (Fig. 3). Their secretory granules appeared small and electron dense. The glands, on the other hand, did not carry regular microvilli and appeared to be secretory in nature (Figs. 1 and 2). Their cytoplasm showed and abundance of large electron opaque granules and globules of obviously mucinaceus composition. Golgi complexes appeared prominent in the glandular cells without profound mucin contents (Fig. 1).

The mucosal layer was surrounded by connective tissue elements containing blood capillaries and different types of migratory cells such as mast cells.

Ultrastructural features of cytoplasmic organelles in the superficial epithelial cells were fairly regular (Figs. 3 to 5). The cells were tall columnar with regular oval or elongated basal nuclei showing mainly marginal chromatin and a smooth nuclear envelope. Nucleoli were not distinct. The cytoplasm of the cells showed typical structures of epithelial cells such as prominent Golgi complex usually in the supranuclear area, numerous small mitochondriae, short prophiles of granular endoplasmic reticulum and free ribosomes. No glycogen particles were identified in the cytoplasmic matrix, which was rich in microfilaments (Figs. 3 and 4). The general contrast of the cytoplasmic matrix varied between different cells. Those with great contrast and dark cytoplasmic matrix contained an abundance of microfilaments (Fig. 5). In basal areas of apical villous projections the microfilaments were concentrated in small bundles supporting the microvilli. Also in areas adjoining the intercellular connections the microfilaments were abundant (Figs. 3 and 4).

The intercellular spaces between the cells were empty. The cellular membranes formed villous projections also into the intercellular spaces (Figs. 4 and 5) but the basal cell membrane was smooth, resting on the basal lamina (Fig. 2).

The lumen of the bile ducts was either empty or contained fine granular or amorphous substances particularly in areas adjoining the walls (Figs. 7 and 8). Additionally, parts of globule leucocytes with several intact globules and mitochondriae were seen to occur freely in the lumen, indicating a possible migratory capacity of the cells into the lumen (Figs. 7 and 8).

Certain areas of the walls contained epithelial structures interpreted as pancreatic acini. These epithelial cells contained numerous granules similar to the zymogen granules in the exocrinic pancreas and an abundance of granular endoplasmic membranes.

The globules in the globule leucocytes varied in structure and size, the largest being even 6–7 microns in diameter. The globules were surrounded by smooth double membranes. They mostly showed diffusely electron dense, but some contained fine granular or dissoluting matrices (Fig. 6). The cells were migratory in nature and though they were closely apposed to the epithelial cells, intercellular connections were never observed.

Discussion

The bile duct epithelium of the reindeer is composed of two types of cells; absorptive superficial and secretory glandular cells. The presence of microvilli on the luminal surface is thought to indicate the absorptive function of the superficial cells. Obviously, the mucin granules and globules and mucin products of ordinary goblet cells in the deep glands are secreted into the ducts. Similar evidence for absorptive and secretory function has previously been presented in the rat (Yamada, 1969, 1970), the mouse (Yamada, 1969, 1970, Rahko, 1971) and cattle (Rahko, 1973). Also the rat displayed intervillous depressions in superficial epithelium as signs for pinocytosis (Yamada, 1969, 1970).

In a previous histological and histochemical study the authors showed the presence of mucin on the surface epithelium (Rahko & Nikander, 1990 a, b). It appears that the surfactant, which was histochemically positive by stainings for neutral and weakly acidic mucins, is seen in the present material by electron microscopy as fine granular or amorphous material on the luminal surface of the *lamina epithelialis.*

Superficial epithelial cells appeared as either light or dark in electron micrographs. Also the mouse and the rat show differences in cytoplasmic contrast of the cells (Yamada, 1969, 1970). Yamada proved that in small rodents the darkness of cytoplasm in certain cells is produced by an abundance of microfilaments in the cells. An especially large amount of microfilaments has been described in the so-called brush cells of the bile duct walls of the mouse and rat (Luciano & Reale, 1969, Luciano, 1972). According to the present findings in the reindeer, obviously not only the richness of microfilaments but also profuse amounts of free and attached ribosomes contribute to the electron density of the dark epithelial cells. It remains uncertain whether the differences in the cellular ultrastructure indicate different functional status.

Globule leucocytes and mast cells are considered to be migratory cells (Gregory, 1979, Morales & al., 1980, Toledo & Morales, 1981, Morales, 1983). In the present material globule leucocytes never carried desmosomes on their cytomembranes. The cells were usually located in basal areas of the epithelium. However, in the present study it was shown that luminal contents of the common bile duct may show intact globules of globule leucocytes and even cytoplasmic areas with globules and mitochondriae (see Figs. 7 and 8). The presence of parts of globule leucocytes in the lumen of the ducts may be an indication of the migratory capacity of the cells. However, in this kind of study it is impossible to determine if the presence of such cells in the lumen is only an artificial phenomenon following a possible mechanical rupture of the *lamina epithelialis* during the process of slaughter and sampling of the tissue specimens. Freely migrating globule leucocytes have not previously been described by electron microscopy in luminal sites of other mucosal tissues.

In this study, the globules of the globule leucocytes showed different size and ultrastructural appearances. Also some giant globules even 6 to 7 microns in diameter were observed. The inner matrix of the large globules was faintly electron-dense and fine-granular as also Baert (1989) has described in the rat.

The authors have tried to study by immunohistochemical methods the intracytoplasmic antigenicity of mast cells and globule leucocytes of the reindeer but the commercial antisera so far applied have failed to show positive immunoreactivity (Nikander and Rahko, unpublished data).

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Manuscript delivered 26 March, 1990.

Fig. 1. Electron micrograph, showing glandular epithelial cells devoid of regular microvilli. The Golgi (G) lamellae and vesicles of the cells are prominent. A goblet cell filled with mucin globules is marked with an arrow. (GER = granular endoplasmic reticulum). x 4.600.

Fig. 2. General appearance of glandular epithelial cells containing numerous mucinaceous granules in the cytoplasm (BM = basal membrane). x 4.600.

Figures 3–5, see page 22

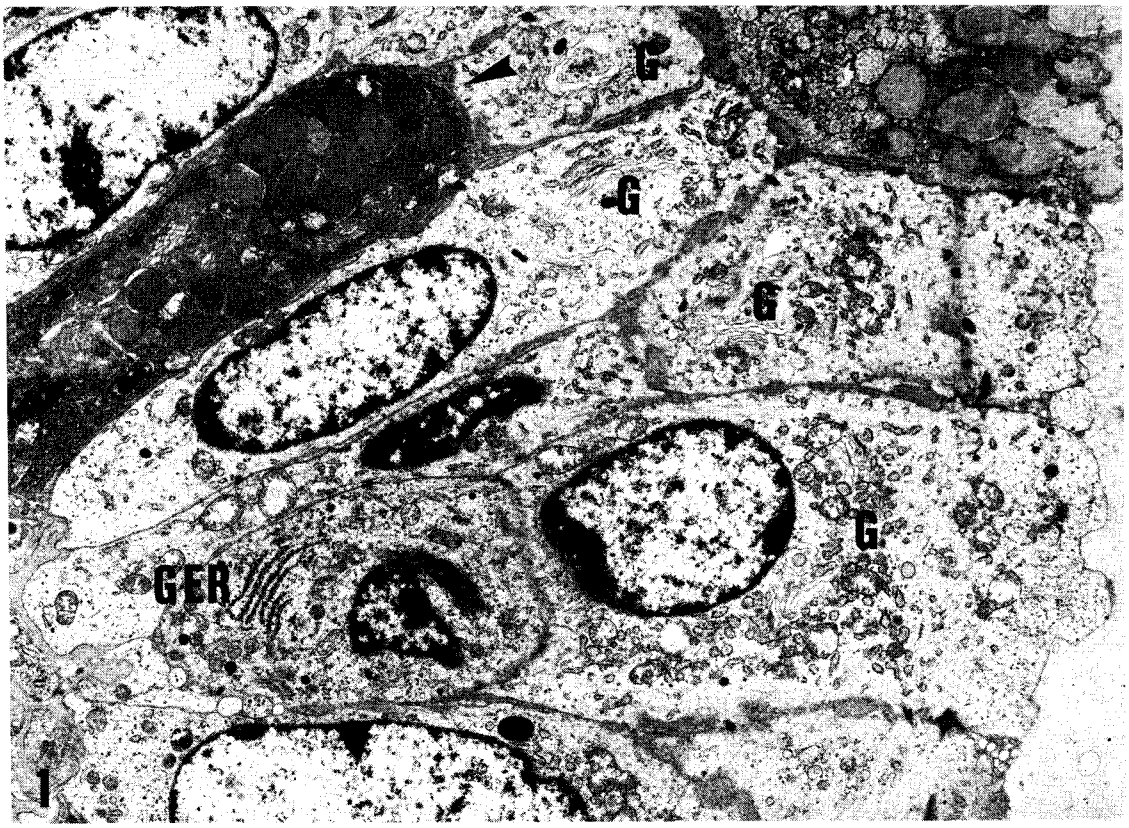
Fig. 3. and 4 are electron micrographs of superficial epithelial cells carrying microvilli on the luminal surface and showing projections of cytoplasmic membranes into the intercellular spaces (arrows). Note the abundance of microfilaments and dense desmosomes (D). Fig. 3 x 60.000, Fig. 4 x 30.000.

Fig. 5. The cytoplasmic matrix of superficial epithelial cells is either light (on the left) or dark (on the right). Both contain small rounded granules (e.g. arrows) in apical cytoplasmic areas but the electron density of the dark cell is mainly produced by more abundant microfilamentous structures. The dark cell also contains numerous free or attached ribosomos. (D = desmosomes). x 18.400.

Figures 6–7, see page 23

Fig. 6. Two globule leucocytes (GL) showing either regular globules of usual size and structure or some giant globules with fine-granular matrices (arrows). x 4.600.

Fig. 7. and 8 are electron micrographs of the lumen of the bile duct, showing cytoplasmic structures of globule leucocytes (GL) surrounded by different luminal secretions of fine-granular composition. Fig. 7 x 15.000, Fig. 8 x 9.000.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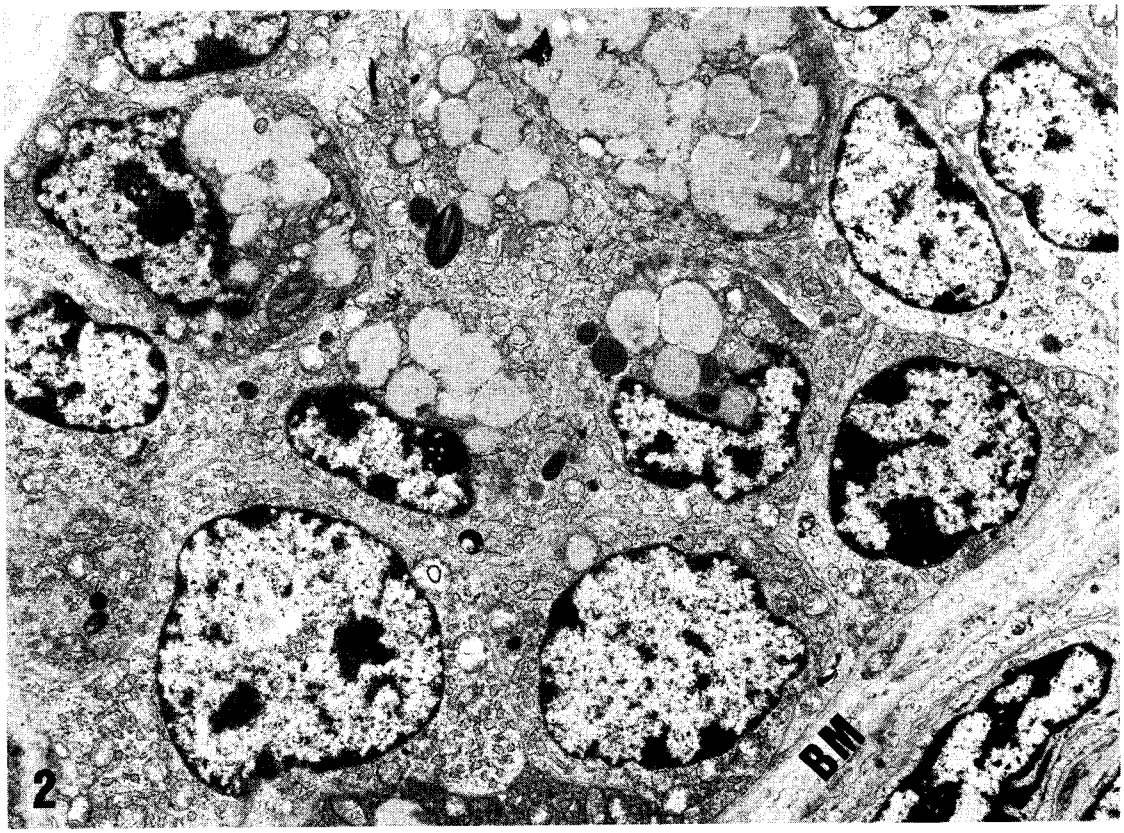
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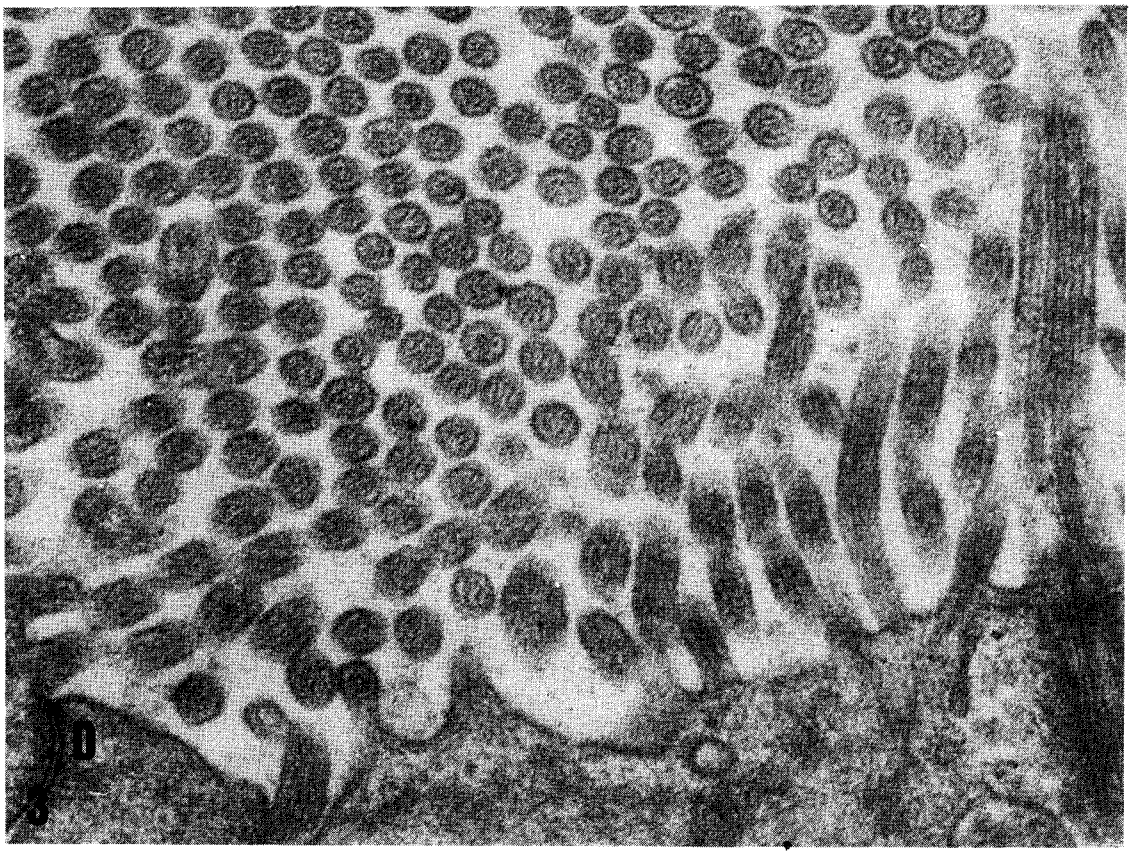
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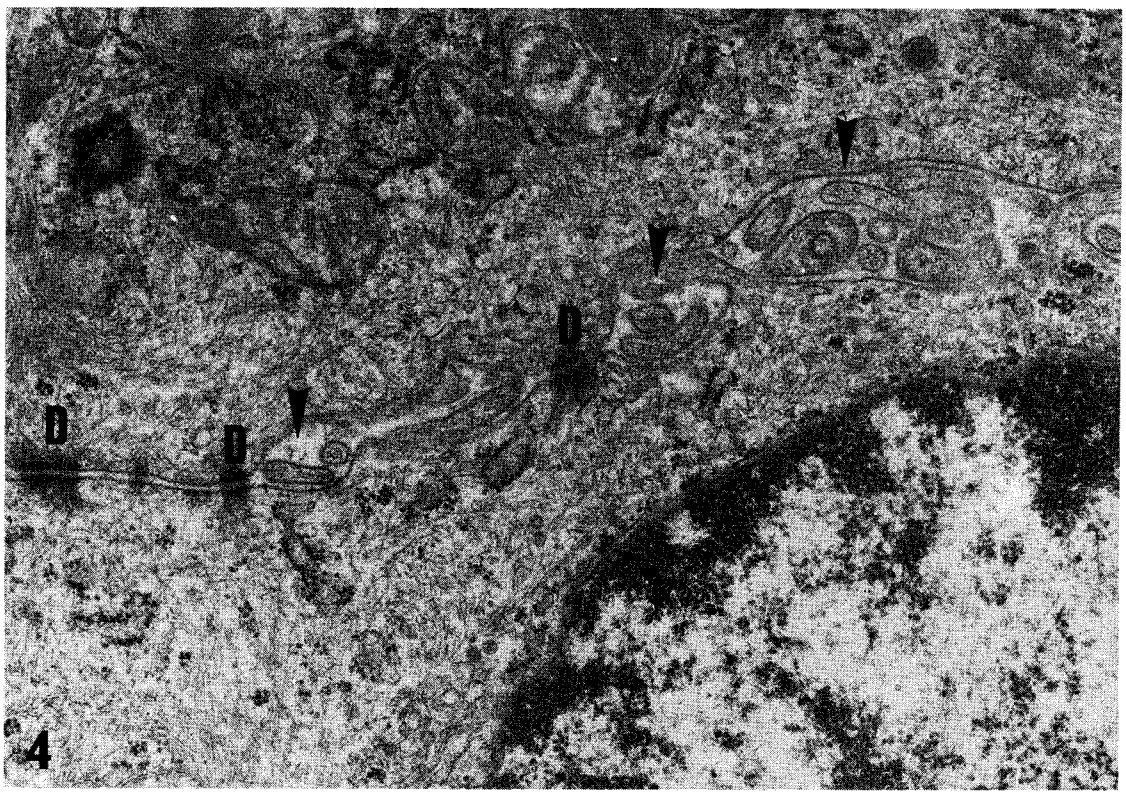
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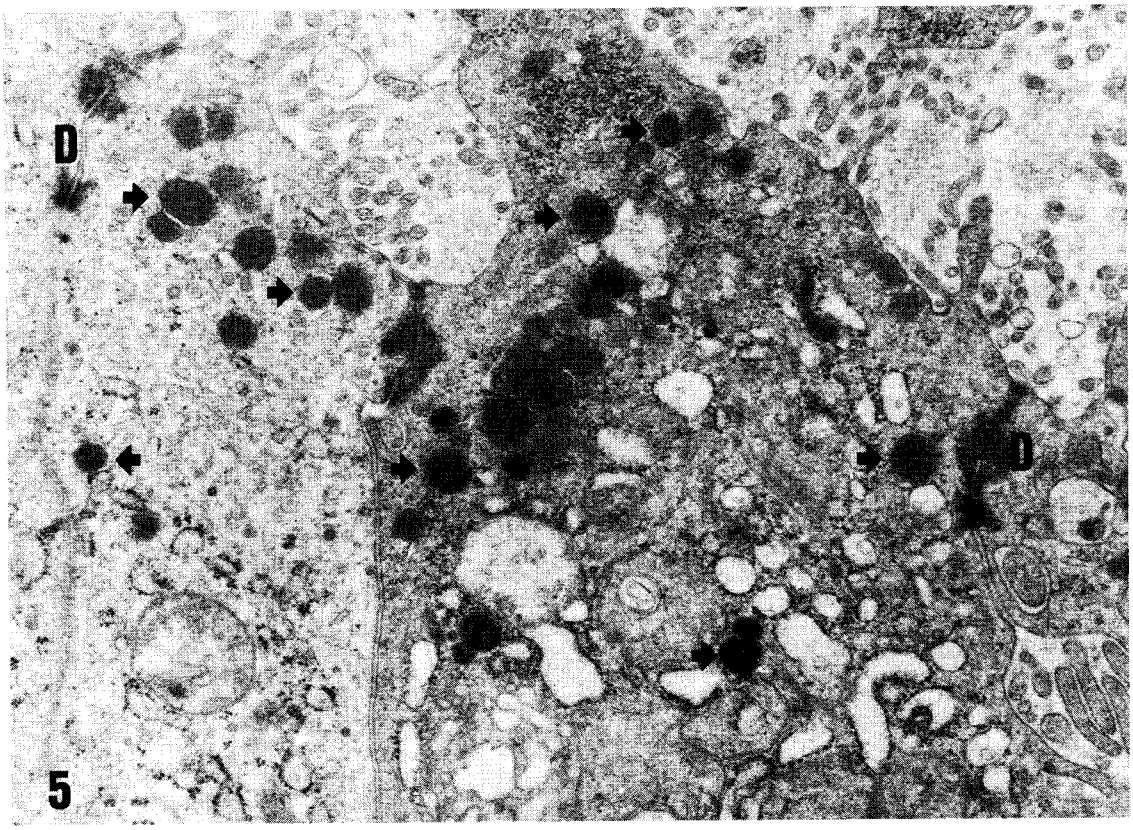
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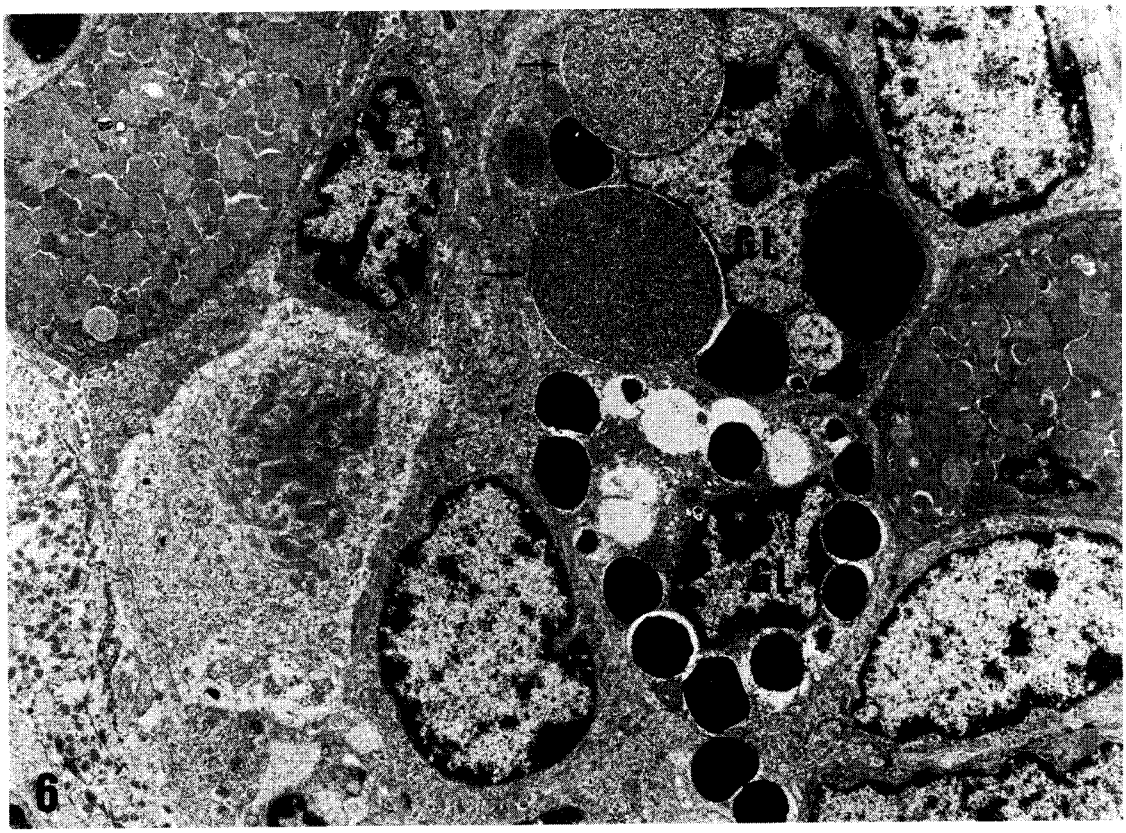
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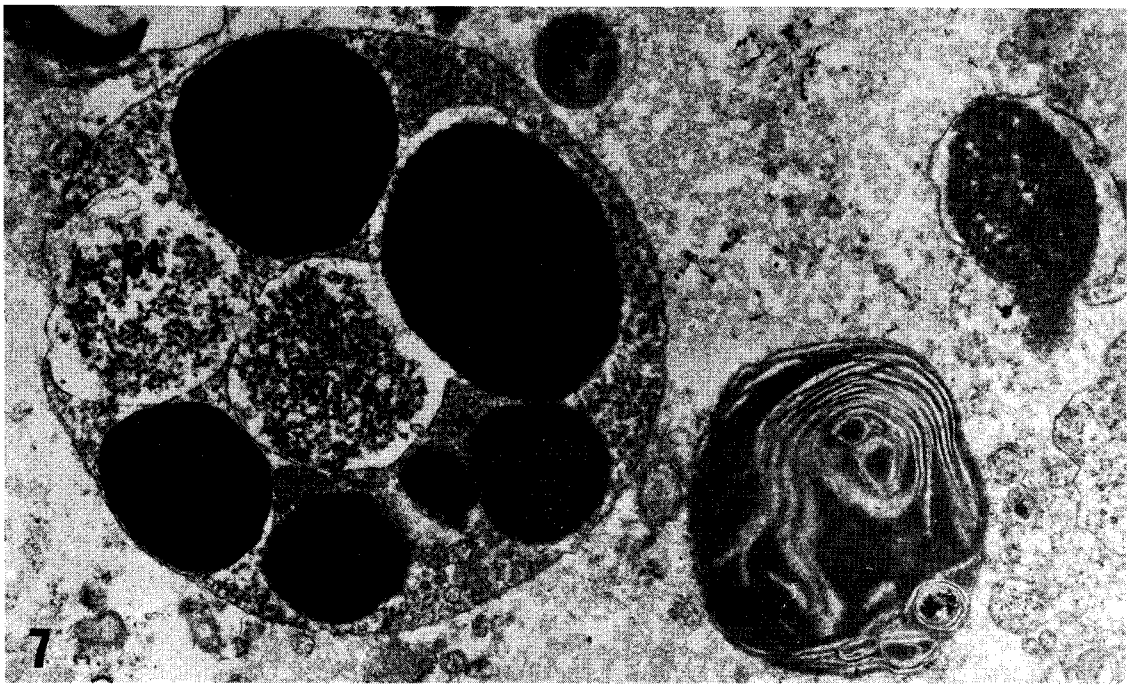
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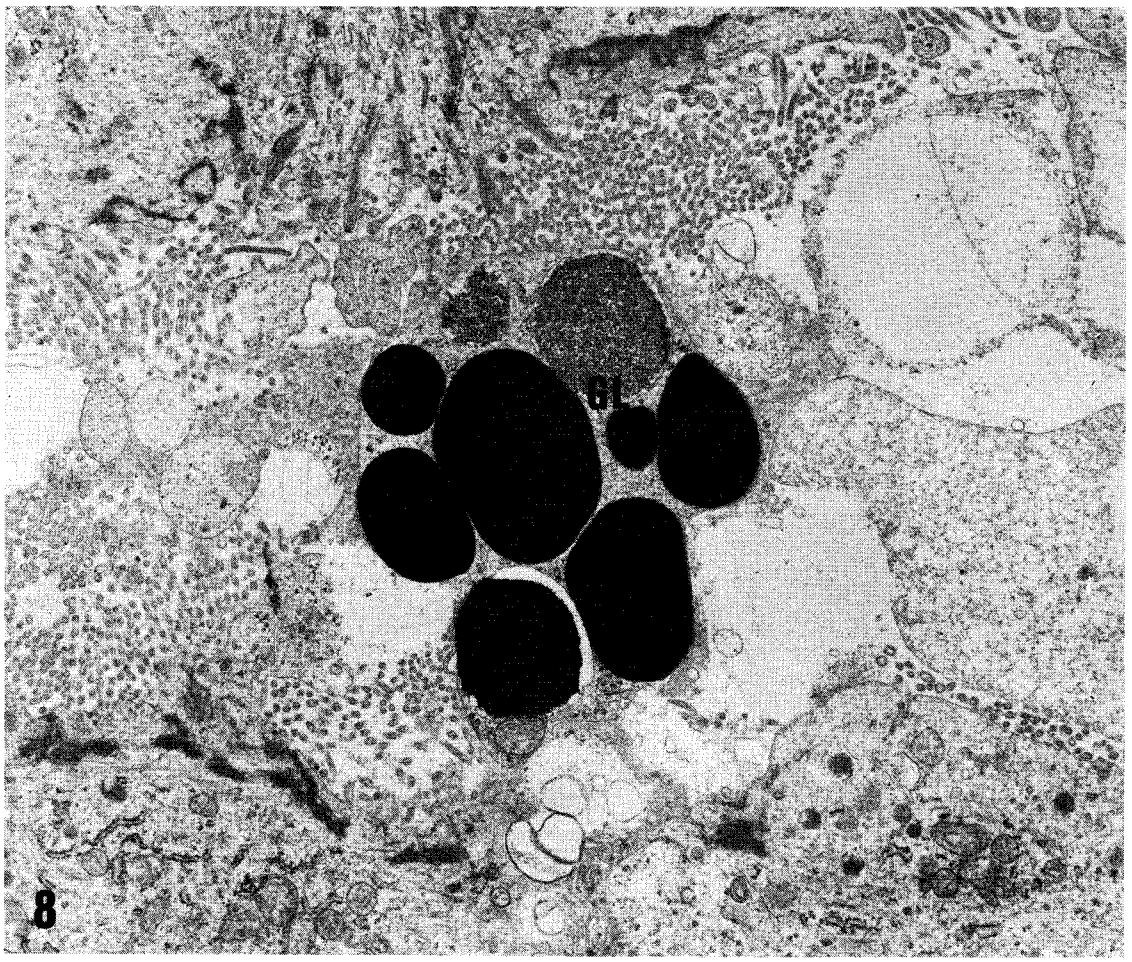
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Ultrastructure of granulated cells in the bile duct of the reindeer

Ultrastructure of granulated cells in the bile duct of reindeer

Sven Nikander and **Timo Rahko**

College of Veterinary Medicine, Laboratory of Parasitology and Department of Pathology, Helsinki, Finland.

Abstract: The presence and ultrastructural characteristics of mast cells and globule leucocytes in bile ducts of the reindeer have not been previously documentated. Tissue blocks of *ductus hepaticus communis* from six reindeer were processed by commonly used methods for TEM and examined with a JEOL JEM 100 S electron micrsocope. The present material originates from reindeer without clinical signs of parasites. However, several types of granulated cells were identifiable. The granules of connective tissue and subepithelial mast cells were small and mostly diffusely electron dense but also fine granular matrices were shown. The globules of intraepithelial globule leucocytes were fewer in number and distinctly larger than the granules of the mast cell. In addition there were noted intraepithelial granulated cells appearing similar to the neuroendocrinic cells reported in bovine bile ducts.

Key words: anatomy, bile ducts, mast cells, globule leucocytes

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Introduction

The presence of numerous mast cells and globule leucocytes in tissues of animals and man has been associated with parasitic infections (Befus and Bienenstock 1982). The function of these cells is, however, far from clear (Befus et al. 1986).

It has been established that the properties of mast cells depend on the animal species and even on the tissue location (Barrett and Metcalf 1984). Since no reports on mast cells and globule leucocytes in bile ducts of the reindeer are available, this study seemed appropriate.

Material and methods

The tissue samples originated from six reindeer slaughtered in the winter at the Reindeer Research Station at Kaamanen, in Lapland of Finland. Small tissue blocks of common bile duct wall were taken and as soon as possible transferred into cold 2.5 % glutaraldehyde in 0.1 M phosphate buffer and fixed for 24 hours. Then the blocks were processed by commonly used methods for TEM and examined with a JEOL JEM 100 S electron microscope.

Results and discussion

Mast cells and globule leucocytes were identified according to the characteristics described for these cells in general (Rahko 1971). Connective tissue mast cells were rare in the tissue samples (Figs. 1 to 3). On the other hand, the subepithelial mast cells occured frequently. The numerous intracytoplasmatic granules of the mast cells were usually electron dense with rounded profiles.

The intraepithelial globule leucocytes were easily distinguished from the subepithelial mast cells (Figs. 4 to 6). The globules of the globule leucocytes were few in number and distincly larger than the granules of the mast cell. The nucleus of the globule leucocyte was often deeply indented by the large globules. All the globules were usually electron dense while some cells possessed globules with apparently dissolving matrices (Figs. 7 and 8). The mast cells, on the contrary, displayed mostly electron dense granula but also fine-granular matrices in a few granula.

In addition to these granulated cell, there were intraepithelial cells with numerous small

electron-lucent intracytoplasmatic granules (Fig. 9). The intraepithelial location and ultrastructural appearance of these cells was similar to the neuroendocrinic cells present in bile ducts of cattle (Morales 1983) and in intestine of some other species of animals (Dellman and Brown 1976).

The present material originates from reindeer without clinical signs of parasites. However, numerous globule leucocytes and subepithelial mast cells were identified in the wall of the common bile duct. Mast cells located both in subepithelial and connective tissues while globule leucocyte displayed a strictly intraepithelial occurrence. To contribute to the clarifying of functional significance of the mast cells and globule leucocytes future studies by the present authors will be concentrated on these cell types in other mucosal tissues of reindeer.

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Fig. 1. A transverse section of the wall of the common bile duct. x 2.400.

Fig. 2. Ultrastructure of granules of a connective tissue mast cell. x 4.800.

Fig. 3. Detail of exceptionally small mast cell granules. x 24.000.

Fig. 4. A subepithelial mast cell. The granules appear homogenic. x 3.600.

Fig. 5. Ultrastructural appearance of a subepithelial mast cell and a globule leucocyte (GL). x 2.400.

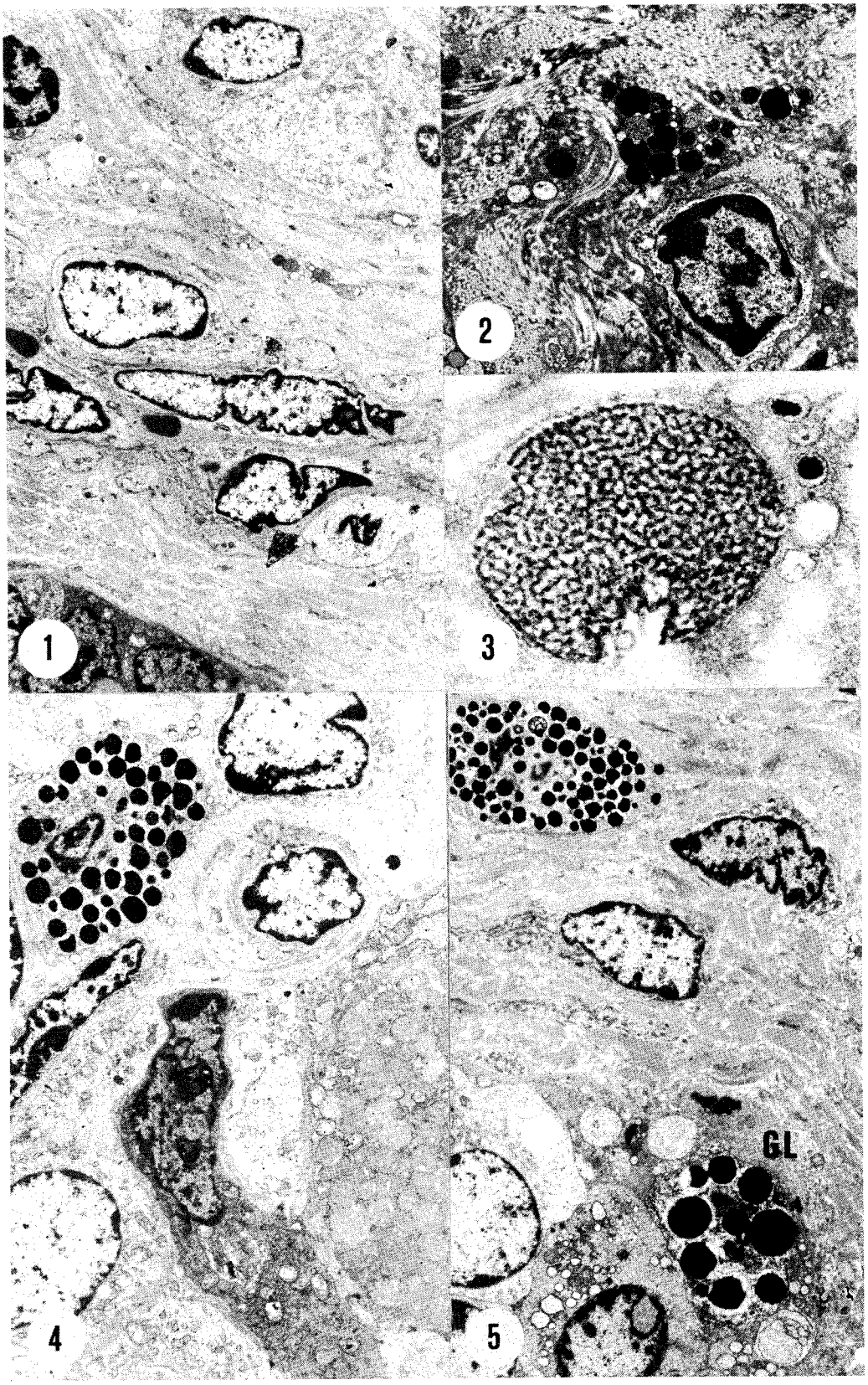
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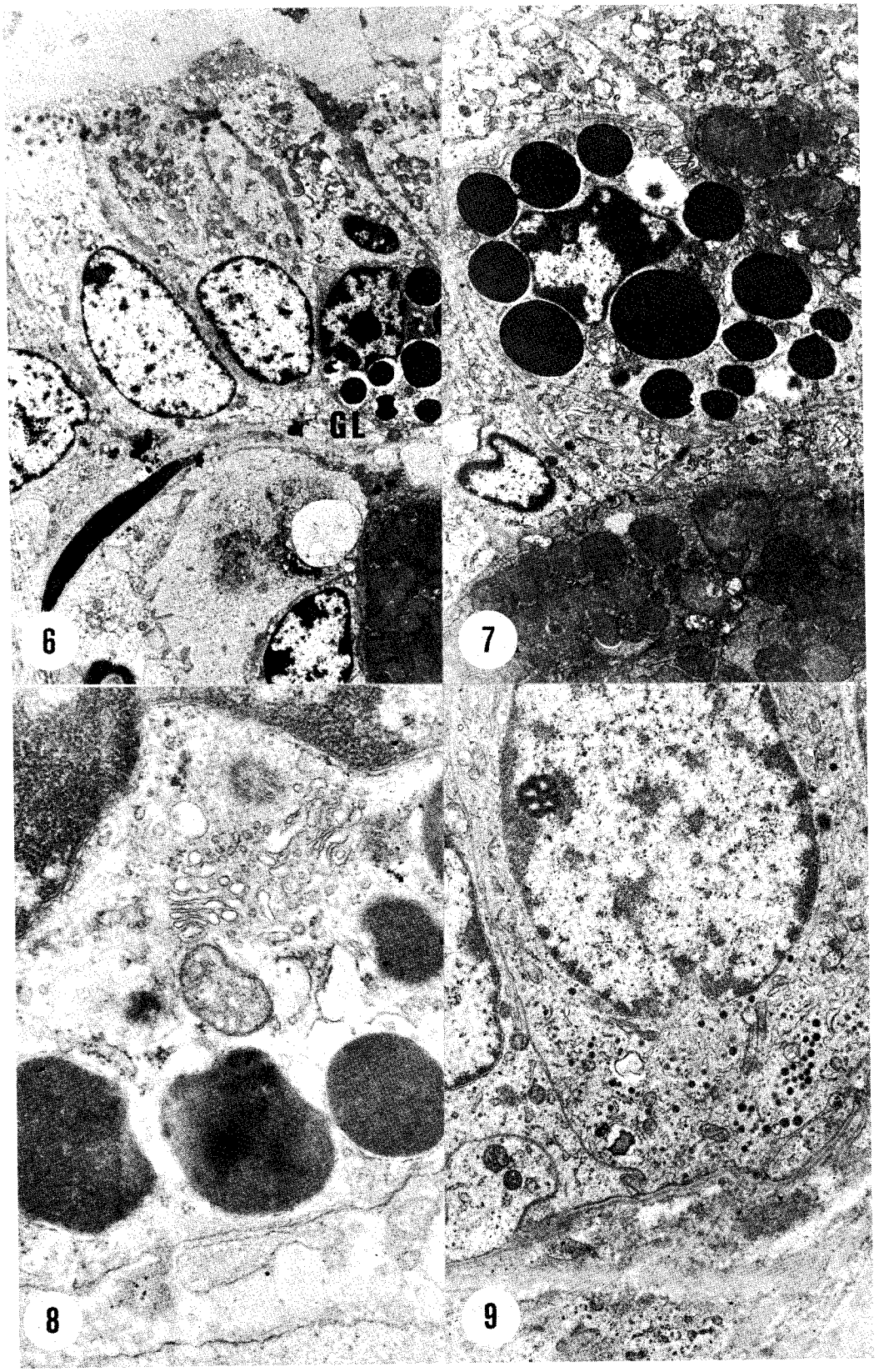
Fig. 6. Typical location of a globule leucocyte (GL). x 2.400.

Fig. 7. Typical appearance of the nucleus and globules of a globule leucocyte. x 4.800.

Fig. 8. Ultrastructure of different organelles in a globule leucocyte. x 24.000.

Fig. 9. A detail of an apparently endocrinic cell with small basally locating granules. x 7.200.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