Studies on the exocrine ducts of the pancreas and the liver in reindeer *(Rangifer tarandus tarandus L).*

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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.

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

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.

Generalreview 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 compaet 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 granules 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).

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.

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.

Results

1. Topography of the pancreas (I)

The pancreas of the reindeer is a bilobulated gland. The lobus sinister (= tail, processus caudalis) 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 goblet 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.

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 differenees 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/mm^2 in formolsaline specimens, but 268 cells/mm^2 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, 1973). 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 & Mo-

rales, 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).

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 epithelialis. 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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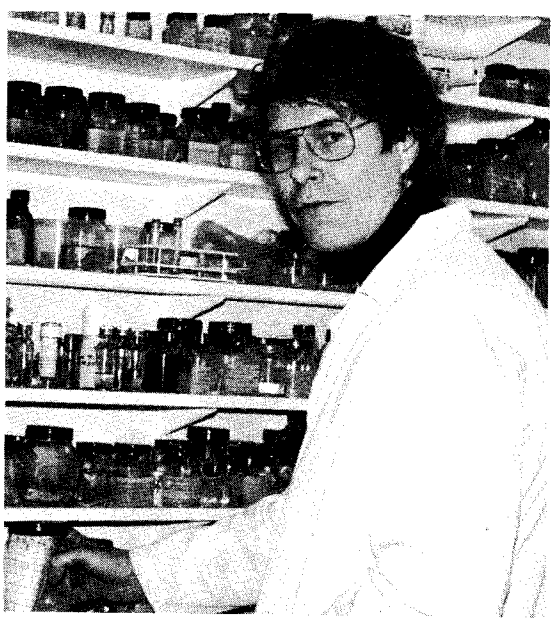
Dissertation



Sven Nikander defended his D.Med.Vet. thesis «Studies on the exocrine ducts of the pancreas and the liver in reindeer (*Rangifer tarandus tarandus* L)» at the College of Veterinary Medicine, Helsinki, Finland on 10 June 1991.

He was born 24 November 1936 in Åbo in Finland but grew up on the Åland islands. He studied biology at the Åbo Academy (M. A. 1969; D. Lic.Vet. 1979.) He is currently Assistant professor at the College of Veterinary Medicine, Helsinki. Special interests: Parasites, especially in reindeer.

The study showed that the pancreas in reindeer consisted of two lobes, the right lobe situated in the first loop of duodenum and the left one near the medial wall of the rumen. The pancreatic ducts unite to form a common duct leading into the common bile duct which has a thin, elastic wall surrounded by fat and pancreatic tissue. An anatomic curiosity was the occurrence of pancreatic tissue in the wall of the common bile duct under the mucosal layer. This mucosal layer consisted of covering- and glandular ciliated epithelial cells.

Large cells with cytoplasmatic granules sometimes so big that they deformed the cell nuclei occurred within the epithelium. These globular leukocytes differed distinctly from the small, granulated mast cells which were observed under the mucosa and the connective tissue.

Neutral-, carboxy- and sulphomucines were detected in the mucus secreted from goblet and glandular cells in the epithelium and in the globular leukocytes. Sulphomucines were seen in the cytoplasmatic granules of the mast cells.