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Comparative Morphohistological Study of Pancreatic Ducts in Rabbits and Guinea Pigs

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THE study aims to determine the morphology and histology of the duct system in pancreases ofrabbits and guinea pigs as two important experimental models in diverse medical investigations. To perform this aim, eight adult animals of each species have been collected and used. Animals were euthanized and subsequent dissection was implemented to cut and collect the pancreases. Samples from these organs were fixed by using 10% neutral buffered formalin which then processed routinely to prepare sections stained by either hematoxylin-eosin or Masson's trichrome stains. Gross findings of the rabbit pancreas revealed an absence of major pancreatic duct and the persistence of only minor pancreatic duct as well as an accessory small pancreatic duct, whereas, in guinea pigs, the only minor pancreatic duct was detected. Microscopically, similar branched ducts were found but with some differences between the two species. In both, it was started with centroacinar cells, then connected to intercalated ducts that were lined with cuboidal cells (in the guinea pig, ducts were surrounded with myoepithelial cells). The latter converged to form interlobular ducts lined with columnar cells (in guinea pig, ducts showed goblet cells and subepithelial mucous glands). The larger interlobular ducts were converged to form a minor pancreatic duct that was lined with tall columnar epithelium with goblet cells surrounded by connective tissue (in guinea pigs, also mucous glands were found invested in the surrounding fibrous tissue with the presence of circularly arranged smooth muscle fibers).

Keywords: Rabbit, Guinea pigs, Pancreas, Exocrine glandular portion, Pancreatic duct.

Introduction

It is well known that experiments are conducted variability on laboratory animals which provides at the varying extent the knowledge to be applied to humans and domestic animals [1]. Both rabbits and guinea pigs wereconsidered very interested laboratory animals usually selectedto conducts biomedical researches.In studies of the human immune system, the immunological genes of guinea pigs were found more similar to those of humans than those from mice., they were acceptable in molecular and cellular biology model for studying the human immune system because its immunological genes appeared similar to those of human genes than of the mice [2]. In the same aspect, rabbits were also considered an ideal experimental animal model in different researches of various morphological anomalies and diseases in both humans and animals. They have been selected in many studies concerned toxicology, pharmacology and surgery at several universities [3].Exocrine and endocrine portions of the pancreas have imperative roles to secret many important digestive enzymes into the duodenum as well as some important hormones into blood stream that play a role in the metabolism of the

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blood sugar. Consequently, disorders that afflict the pancreas can occur in both portions so that researches are required on the histomorphology of the pancreas to provide much insight into the etiology of many of the related diseases that may be established in such an important organ [4]. Pancreatic duct morphology is very important for endoscopic and surgical practice in both human and certainly small and laboratory animal species[5].

Up to date, there is a paucity of work in the kind of literatures to study and compare the morphology and histology of the pancreases in rabbits and guinea pigs. The previous works of literatures focused on the pathological aspect and concerning diseases of the pancreas such as duct obstructions and tumour development. The obtained data will provide a good animal model for both public healths in human and the veterinary fields in animals.

Material and Methods

Ethical approval

The experimental procedures were approved by the Institutional Animal Ethics Committee. The present investigation was carried out at the Baghdad University, Veterinary Medicine College, Iraq.

Clinically healthy eight adult local rabbits (*Lepus cuniculus*) and eight adult guinea pigs (*Cavia porcellus*) were bought from local farms from Baghdad province and they were caged at the animal house till their euthanasia and subsequent dissection to obtain their pancreases. Each animal was euthanized before its dissection by intravenous injection of overdose of 200 mg/kg of sodium Phenobarbital (Euthasol; Delmarva Laboratories, Midlothian, VA). The animals were dissected on a dissecting board and then their abdominal wall was opened to view the abdominal viscera, the pancreases were collected and photographed *in situ*. Fine dissection by dissecting microscope was performed to identify the pancreatic ducts grossly. For histological techniques, specimens of pancreases were immersed in10% neutral buffered formalin for 72 hrs . These specimens were dehydrated through an ascending series of ethyl alcohol (70%, 80%, 90% and 100%) each for 60 minutes for two changes and then cleared with xylene for 15 minutes for two changes too. The processed specimens were infiltrated with paraffin wax with 58 ºC then embedded with new paraffin wax to obtain blocks of paraffin. Sections of 6 µm were obtained by using a rotary microtome. The sections were selected from all portions of each pancreas. The sections were stained with hematoxylin and eosin which was conducted to describe the general histological features. To identify the collagenous connective tissue fibers, Masson trichrome stain was implemented. The tissue sections were examinedand photographed by using Olympus light microscope provided by an eye piece camera (Scope Image 9.0).

Results

Gross findings

Rabbit pancreas

Gross findings showed two different types of the pancreas. It was a diffused type in rabbit, it differently appeared in guinea pigs. In the rabbit pancreas, findings revealed an absence of a major pancreatic duct and the persistence of only a minor pancreatic duct as well as an accessory small pancreatic duct. The two ducts drain the pancreatic secretion from all parts of the pancreas and run towards the ascending duodenum. The minor pancreatic duct opened into the ascending duodenal lumen before the latter reflected towards the left side of the abdominal cavity and its subsequent joining with the jejunum (Fig. 1A). The minor duct empties into the ascending duodenum approximately 45-55 cm distal to the pyloric-duodenum junction. The duct traversed the wall of the duodenum vertically at site before it winded to form a third duodenal flexure and its subsequent joining to the jejunum. This entrance of the duct was directly into the duodenal lumen which will be well described in the histological findings later on. Moreover, the entrance of the minor pancreatic duct was always found covered by the descending colon. The second smaller accessorypancreatic duct was very thin and short emptied into the lumen of the ascending duodenum at its junction with the transverse portion of the duodenum. In fact, a rabbit poses a very long and U-shaped duodenum. As its name implies, it was formed from descending, transverse and ascending portions.It closely embraces most of the pancreas which was of diffused type in the rabbit (Fig. 1B).

Guinea pig pancreas

Gross findings revealed the presence of only a minor pancreatic ducts and the absence of the major pancreatic duct in the pancreas of studied guinea pigs. This duct was created in the body and directed toward the last portion of the ascending duodenum. The body lobe appeared as the site of

a collection of the larger interlobular ducts of left (splenic) and right (duodenal) pancreatic lobes. Markedly, the pancreas of guinea pigs showed a connecting portion between the body lobe and the ascending duodenum in which both minor pancreatic and portal vein were running together (Fig. 1C & 1D). In fact, both connecting portion of pancreas and portal vein are considered good indictor to find out the minor duct. Unlike rabbits, the duodenum was very short and V-shaped and the entrance of minor pancreatic duct into the last portion of the ascending duodenum about 7.5 cm to 8.5 cm away from the first duodenal flexure. Similarly to the rabbit pancreas, the major pancreatic duct was absent in the guinea pigs. Unlike the pancreas of rabbits, the second small accessory pancreatic duct was absent. the duodenal lobe of the pancreas in the guinea pig was very small and was adherent to the descending duodenum, whereas, it was larger and adherent to the ascending duodenum in the rabbit, so it empties its secretion via a small accessory duct into the ascending duodenum in the rabbits. Unlike to the rabbits ,the pancreas of the guinea pig was a compact type and only a small part of it was embraced by the V-shaped duodenum.

Microscopic findings

Histology of rabbit's pancreas

A large number of intralobular and large interlobular ducts was characteristically developed at the left pancreatic lobe. They were lined with cuboidal and columnar epithelium, respectively. The minor pancreatic duct which was formed by the joining of several large interlobular ducts traversed duodenum wall. It collects the exocrine secretions from the tail (splenic) body and duodenal lobes and drains into the lumen of the ascending duodenum.

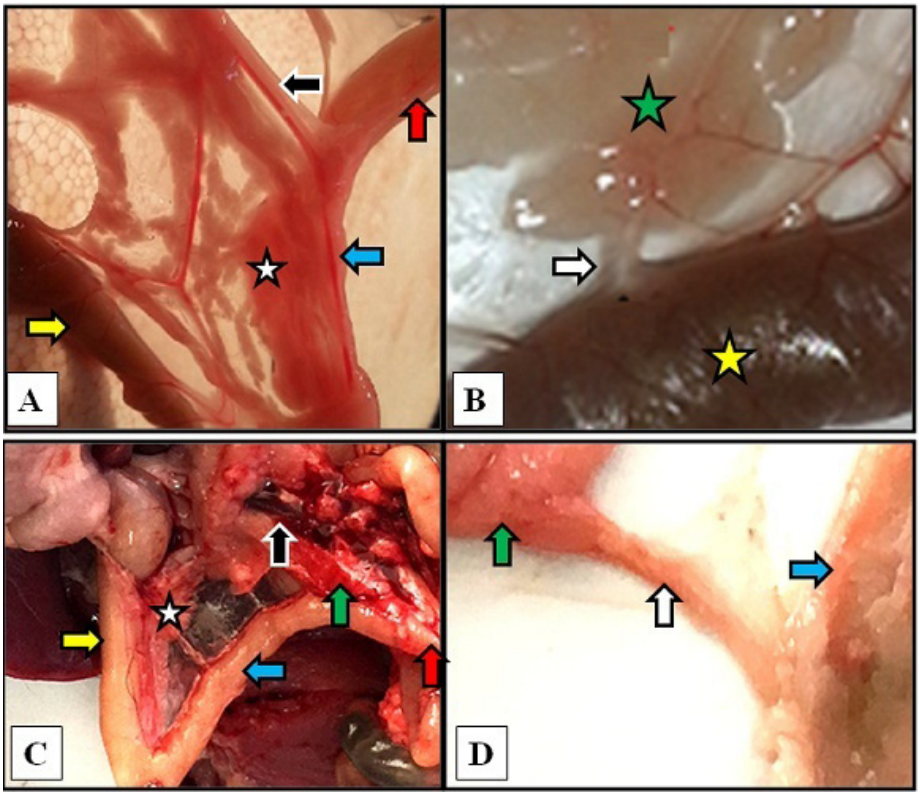

Fig. 1. Upper panel showed pancreas of rabbit. A: duodenal lobe (white star) embraced by descending (yellow arrow) and ascending duodenum (blue arrow), minor pancreatic duct (black arrow) and jejunum (red arrow). B: second accessory pancreatic duct (white arrow) that conveys the secretion from the duodenal lobe (green star) into the ascending duodenum (yellow star).

Lower panel showed pancreas of guinea pig. C & D: It showed V-shaped duodenum formed by descending (yellow arrow) and ascending (blue arrow) limbs, duodenal lobe (white star), connecting part (green arrow), portal vein (black arrow), jejunum (red arrow), minor pancreatic duct (white arrow).

Histological sections of different pancreatic lobes showeda tree-like series of ductsinvested between the pancreatic acini. which responsible to covey secretions toward the duodenal lumen to complete the process of digestion. Microscopic examination revealed that the duct system begins with numerous centroacinar cells that were found as terminal end duct cells. They wererounded or polyhedral in shape with prominent centrally located nuclei (Fig. 2A). They were interfaced with the lining pyramidal cellsof the acini, the exocrine glandular portion of the pancreas.The centroacinar cells (2 to 4 cells) were occupied the lumina of acinithat convey the secretion to the next duct called intercalated duct (Fig. 2B). The latter second typeof duct system was considered intralobular duct lined with typical flattened cuboidal epithelium distributed within the parenchyma of pancreatic lobules. Gathering of many intercalated ducts will create a larger intralobular duct lined with simple cuboidal epithelium. These ducts run out of the lobules and were intervening within the interlobular connective tissue septae. These now called interlobular ducts that were varied considerably in their sizes(Fig. 2B). The smaller ones were lined with simple cuboidal epithelium, whereas, the larger ducts were lined with simple columnar epithelial cells. Interlobular ducts have formed the bulk of the duct system and they have transmitted secretions from different intralobular ducts to the minor pancreatic duct. Interlobular ducts were mostly filled with secretory materials. The minor pancreatic duct was lined with simple tall columnar cells with many prominent goblet cells (Fig. 2C).The section at the site of duct entrance in the duodenal lumen appeared stellate in shape, lined with tall columnar epithelium surrounded with mainly collagenous fibrous connective tissue (Fig. 2D).

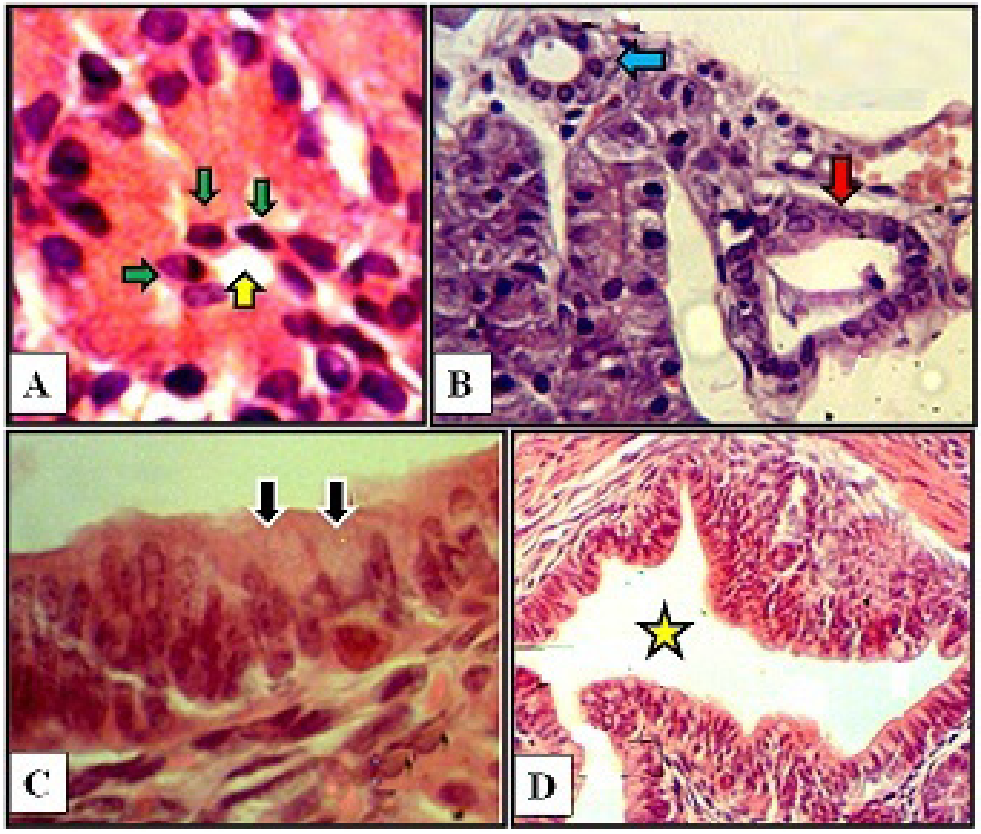

Fig.2. Pancreas of the rabbit.Upper panel: A. showed centroacinar cells (green arrows) surrounded the lumen of an acinus (yellow arrow). B. Intralobular (blue arrow) and interlobular ducts (red arrow) lined with simple cuboidal epithelium.

Lower panel: C. Lining epithelium with goblet cells (black arrows) of the minor pancreatic duct. D. Stellate –shaped opening of the minor duct when it precise the wall of duodenum and inter into its lumen.

Histology of guinea pig's pancreas

Light microscopic examination of the pancreas of guinea pigs showed well developed duct system. Similar to the rabbit pancreas, the duct was begun by the appearance of centroacinar cells around the lumen of each pancreatic acini(Fig. 3A). These cells subsequently joined the small second type intercalated ducts which were lined with cuboidal epithelial cells. The latter ducts were surrounded by myoepithelial cells which were not identified in the case of rabbit pancreas (Fig. 3B). Many of intercalated ducts were connected to creates the intralobular duct lined with simple cuboidal epithelium. These intralobular ducts left the pancreatic lobules to leave lobules directed toward the interlobular septae where they were still lined with simple cuboidal epithelium. Subsequently, many of these interlobular ducts were converged together to constructs large interlobular ducts lined with simple columnar epithelium with goblet cells (Fig. 3C). Markedly, the connection of many of these large interlobular ducts will form the larger interlobular ducts characterized by the simple columnar epithelium with goblet cells accompanied with subepithelial connective tissue filled with mucous glands (Fig. 3D).

The larger interlobular ducts of both right and left lobes were directed toward the body where they were converged forming duct, the minor pancreatic duct. It was characterized by a thick wall, lined by simple columnar epithelium with goblet cells. It showed subepithelial mucous glands with the presence of circularly arranged smooth muscle bundles (Fig. 3D).The duct was established in the body and directed toward the

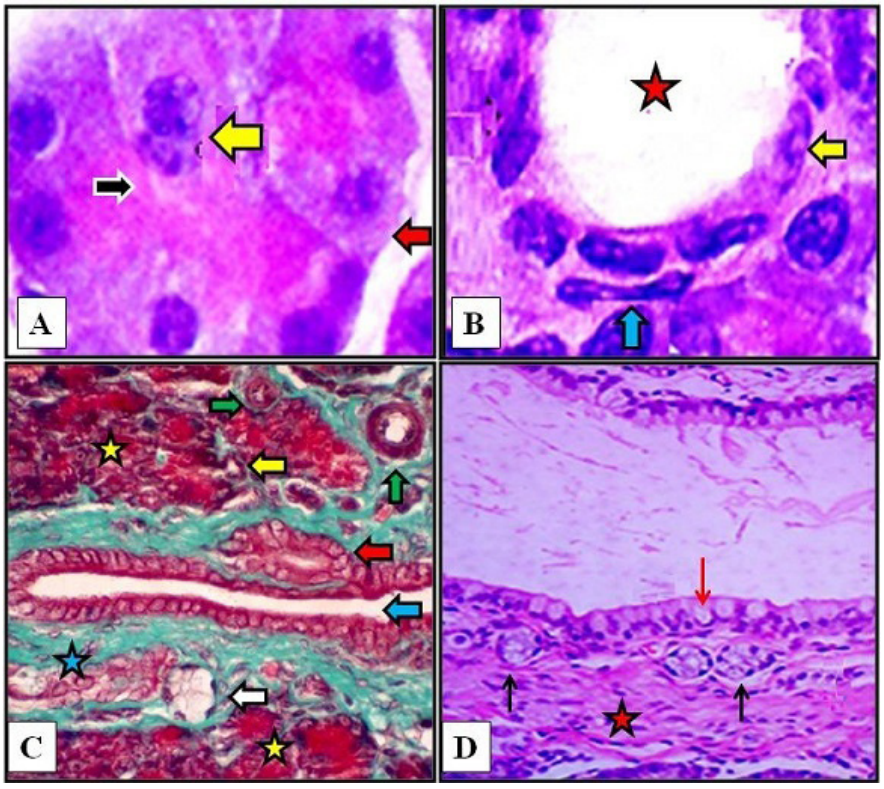

Fig. 3. Pancreas of the guinea pig.

Upper panel: A. showed centroacinar cells (yellow arrow) surrounded the lumen (black arrow) of an acinus (red arrow). B. Intercalated duct(red star) lined with simple cuboidal epithelium, surrounded (yellow arrow) lined by myoepithelial cell (blue arrow)..

Lower panel: C. Intralobular duct (yellow arrow) opens into interlobular duct (red arrow) which subsequently opens into the large interlobular duct (blue arrow) invested in the interlobular connective tissue (blue star), mucous gland (white arrow), pancreatic acini (yellow stars) and blood vessels (green arrows). D. Minor pancreatic duct showed simple columnar epithelium with goblet cells (red arrow), subepithelial mucous glands (black arrows) and circularly arranged smooth muscle bundles (red star).

last part of the ascending duodenum passing through the tissue of the connecting part of the body. It was characteristically traversed the wall of the ascending duodenum obliquely. It précised and pass through the duodenal wall in between the external and internal layers of tunica muscularis and subsequently opened into the lumen of the ascending duodenum.

Discussion

The presence of minor pancreatic duct and absence of the major one in both studied rabbits and guinea pigs was in agreement with what was found in the pancreas of pigs and Ox [6]. Whereas differently, in some small and laboratory animal species such as mice [7], hamster [8] and cat [9], dogs [10,11], the major pancreatic duct was present as the only and the main pancreatic duct andabsence totally minor pancreatic duct or even the second accessory duct. In the rat, the duct system of the pancreas had a great variation because in the past decade, [12] observed anterior and posterior pancreatic ducts instead of the major pancreatic ducts. They were empty their secretion just 17.96 mm and 2 to 8 mm away from the duodenal papilla, respectively.

The site of empty of minor pancreatic duct into duodenum in rabbit's pancreas away from pyloric-duodenum junctionwas comparable to those found in the adult white New Zealand rabbit in which this duct is opened into the duodenum 30-50 cm away from the pylorus [13] whereas it was so short in guinea pig approximately 7.5 cm to 8.5 cm away from the first duodenal flexure.

The existence of the second accessory pancreatic duct only in the pancreas of rabbits confirmed the previous records of Davies and Davies [14] in the pancreas of the rabbit too. Also the latter reference was recorded the empty of the second duct into the duodenum at the point of junction of transverse duodenum with the ascending limb. This duct was not documented in the pancreas of the studied guinea pigs and it has not been recorded in other similar rodent species up to date.

Rabbits were found different from rodents and that's why they were classified in separate order since 1912[15]. Many of their organs are found different structurally and functionally than the same organs in rodents. Recent researches were published which confirmed some of these differences by many investigators [16, 17, 18, 19 and 20] in the intestine, ovary, reproductive tract, respectively.

The organization of the duct system and its branching design in the studied rabbit's pancreas was in a good agreement with the description of recent reference [21]. The ductal branches are very important to convey the acinar enzymes toward the lumen of the duodenum. Moreover, the ductal epithelial cells were active to produce the bicarbonate-rich fluid to neutralize the acidity in the duodenum resulted from the conveyed gastric juice [22].

In the pancreas of guinea pigs, the lining epithelium of duct branches was so clearly different than that recorded in the case of the rabbit. The intercalated ducts were surrounded peripherally by myoepithelial cells that were missed in the same ducts of the rabbit's pancreas. The simple columnar epithelial lining of the interlobular ducts wase well provided with goblet cells. These ducts again showed subepithelial mucous glands which were increased in the larger interlobular ducts. Such features were not recorded in the case of the rabbits. The final portion of the duct system, i. e the minor pancreatic duct was characterized by the presence of muscular bundles in its wall which was not observed in the minor pancreatic duct of rabbit pancreas. The presence of mucous glands in the ducts and muscularity of the wall minor duct in guinea pig was quite different from those of rabbit indicated different requirements to complete the digestion in the duodenum. , guinea pigs reflected parallel histological similarities in their duct structures and functions to the other rodents but showed grossly one minor pancreatic duct instead of a major pancreatic duct similar somewhat to rabbit's pancreas.

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Conflict of interest

The authors have declared that no conflict of interest exists.

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دراسة مظهريه نسيجيه مقارنة للقنوات البنكرياسيه في الأرانب والخنازيرالغينية

مسرات سوادي المياحي[١] ، علاء عبد الخالق سواد[٢] و اكتفاء شاكر خيون[١]
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الهدف من الدراسه للتعرف ومقارنة عيانيه ونسجيه للنظام القنوي في البنكرياس للأرانب وخنازير غينيا كنموذجين مهمين في التجارب الطبية المتنوعة. ولتحقيق هذا الهدف ، تم جمع واستخدام ثمانية حيوانات بالغة من كل نوع. تم قتل الحيوانات بطريقة القتل الرحيم وتم إجراء التشريح لاحقا لقطع وجمع عينات من البنكرياس.

تم تثبيت العينات من هذه الأعضاء باستخدام محلول الفورمالين المحايد المحايد بنسبة ١٠٪ أو محلول بوين الذي تم معالجته بعد ذلك بشكل روتيني لإعداد المقاطع المصبوغه إما بصبغة الهيماتوكسيلين إيوسين أو صبغة ماسون ثلاثية الكروم.

أظهرت النتائج العيانيه للبنكرياس في الأرانب عدم وجود قناة البنكرياس الرئيسية ووجود القناة البنكرياسية الصغيرة فقط بالإضافة إلى القناة البنكرياسية الصغيرة الملحقة ، بينما في خنازير غينيا ، تم الكشف عن القناة البنكرياسية الصغيرة فقط.

اظهر الفحص المجهري وجود قنوات متفرعة متشابهة ولكن مع بعض الاختلافات بين النوعين. في كليهما ، بدأ بالخلايا المركزية ، ثم تم توصيله بالقنوات المقحمة التي كانت مبطنة بخلايا مكعبة (في خنزير غينيا ، كانت القنوات محاطة بخلايا الظهارة العضلية). تقاربت القنوات الأخيرة لتشكيل قنوات بين الفصوص مبطنة بخلايا عمودية (في خنزير غينيا ، أظهرت القنوات خلايا كاسيه وغدد مخاطية تحت الظهارة). تم تقارب القنوات الكبيرة بين الفصوص لتشكيل قناة بنكرياسية صغيرة مبطنة بظهارة عمودية طويلة مع خلايا كاسيه محاطة بنسيج ضام (في خنازير غينيا ، تم العثور أيضًا على الغدد المخاطية مندمجه في الأنسجة الليفية المحيطة مع وجود ألياف عضلية ملساء مرتبة دائريًا).