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Pancreatic Duct Obstruction in the Pig: Light Microscopy of Chronic Pancreatitis

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Rahko, T., T. Kalima and H. Saloniemi: Pancreatic duct obstruction the pig: Light microscopy of chronic pancreatitis. Acta vet. scand. 1987, 28. 285-289. - Morphological changes of pancreatic tissue in young pigs caused by surgical ligation of the main pancreatic duct are described. Nineteen animals from 6 to 7 weeks in age were operated on and necropsied 3 or 6 to 8 weeks later. Twelve pigs developed a pronounced chronic pancreatitis with complete exocrine insufficiency. Of the 7 animals failing to develop ectasia of pancreatic ducts, 2 died due to surgical complications. In addition, 3 pigs were sham-operated and served as controls.
In macroscopical studies it was observed that in the pronounced pancreatitis cases the ligated duct was greatly dilated by a clear watery fluid. Only remnants of pale and firm grandular tissues were seen around the ectatic ducts. Microscopically, typical changes of chronic pancreatitis were noted. Complete disappearance of acini was followed by ductular cell proliferations. Glandular tissues were divided into lobuli by fibrotic tissues and fat cells. The wall of the main pancreatic duct was greatly thickened and fibrotic, presenting intensely proliferating ductular cells and round cell infiltrates. Furthermore, enlarged endocrine islets surrounded by connective tissue fibres were seen.

pancreatic duct ligation; exocrine pancreatic insufficiency; experimental chronic pancreatitis; porcine pancreas diseases.

Introduction

In the literature there is extensive data on experimental pancreatitis in small laboratory animals (*Walters et al.* 1969, *Boquist & Edström* 1970, *Zeligs et al.* 1975) and in the dog (*Idezuki et al.* 1969, *Churg & Richter* 1971, *Horaguchi et al.* 1983). On the other hand, the results of studies performed on the pig only deal with the acute phase of the disease (*Schröder et al.* 1981). Thus exocrine insufficiency and morphological changes associated with chronic pancreatitis have not previously been elucidated in this animal. Of all animal species, only the digestive system of the pig closely resembles that of man (*Capella & Solcia* 1972), therefore the development of an omnivorous animal model seemed apropriate. From the medicoclinical standpoint it especially appeared important to experimentally obtain relevant data on the effectiveness of pancreatic enzyme preparations used in the treatment of this disease in humans (*Rahko et al.* 1985).

In order to study insufficiency in animals surviving long enough the authors developed a surgical method for producing chronic pancreatitis in the pig by ligating the pancreatic duct (*Rahko et al.* 1985). This report deals with the macroscopical and light microscopical alterations of pancreatic tissues caused by the ligation.

Materials and methods

Yorkshire pigs (females and castrated males) aged from 6 to 7 weeks ranging in weight from 11.1 to 16.1 kg, were used in the experiment. The 19 experimental animals were laparotomized, then the pancreatic duct was ligated near duodenum and the serosal connection between the right branch of pancreas and duodenum was cut. Three control animals were sham-operated by being subjected only to laparotomy and killed 6 to 8 weeks after the operation. The pigs were fed commercial pig food ad libitum. At the end of the experiment (Table 1) the animals were sacrificed by stunning with a captive-bolt pistol, followed by bleeding and immediate necropsy.

At necropsy the pancreas was examined macroscopically. To avoid traumatic and cadaverous processes, no attention was paid to examination of the ligature, and tissue samples for histopathology were immediately taken from 3 parts of the pancreas: the corpus and the left and right branches. The blocks were fixed in 10% neutral formaldehyde in phosphate-buffered saline and in Mota's and Bouin's solutions, then dehydrated in alcohol and xylen, embedded in paraffin and sectioned at 4-5 μm. Mayer's haematoxylin-eosin (HE) was used for histopathology.

Results

Control animals

The pancreata of the controls appeared normal in macroscopic and microscopic studies (Figs. 1 and 2). The exocrine parenchymal cells were arranged in the regular acinar pattern (Fig. 3). The nuclei of the cells were round, locating basally. The basal cytoplasmic areas stained more intensely basophilic with HE-staining than apically. In some specimes pycnotic nuclei were seen in areas accidentally traumatized during autopsy. Slight interlobular oedema was observed between acini but no inflammatory cells were present. The pancreatic ducts and endocrine islets appeared normal, presenting no signs of fibrosis.

Experimental animals

Two animals died after the operation. One died a week after the laparotomy in cholemia caused by accidental ligation of the main bile duct near duodenum. The other died 2 weeks after the laparotomy as a results of duodenal obstruction caused by postoperative adhesions. Due to cadaverous processes no closer microscopical examinations were performed on these animals.

In 5 euthanized animals the pancreata appeared macroscopically normal (Table 1).

Table 1. Macroscopical and microscopical autopsy findings of the pancreatic tissues in control pigs and in pigs killed 3 (group I) or 6 to 8 weeks (group II) after the ligation of ductus pancreaticus.

Number of animals		Macroscopically normal ductus pancreaticus		Macroscopically ectatic ductus pancreaticus
		Normal histology	Mild chronical pancreatitis	Pronounced chronical pancreatitis
Control pigs	3	3	-	-
Group I	10	-	2	8
Group II	9*	-	3	4

*Two animals died 1 and 2 weeks after the operation.

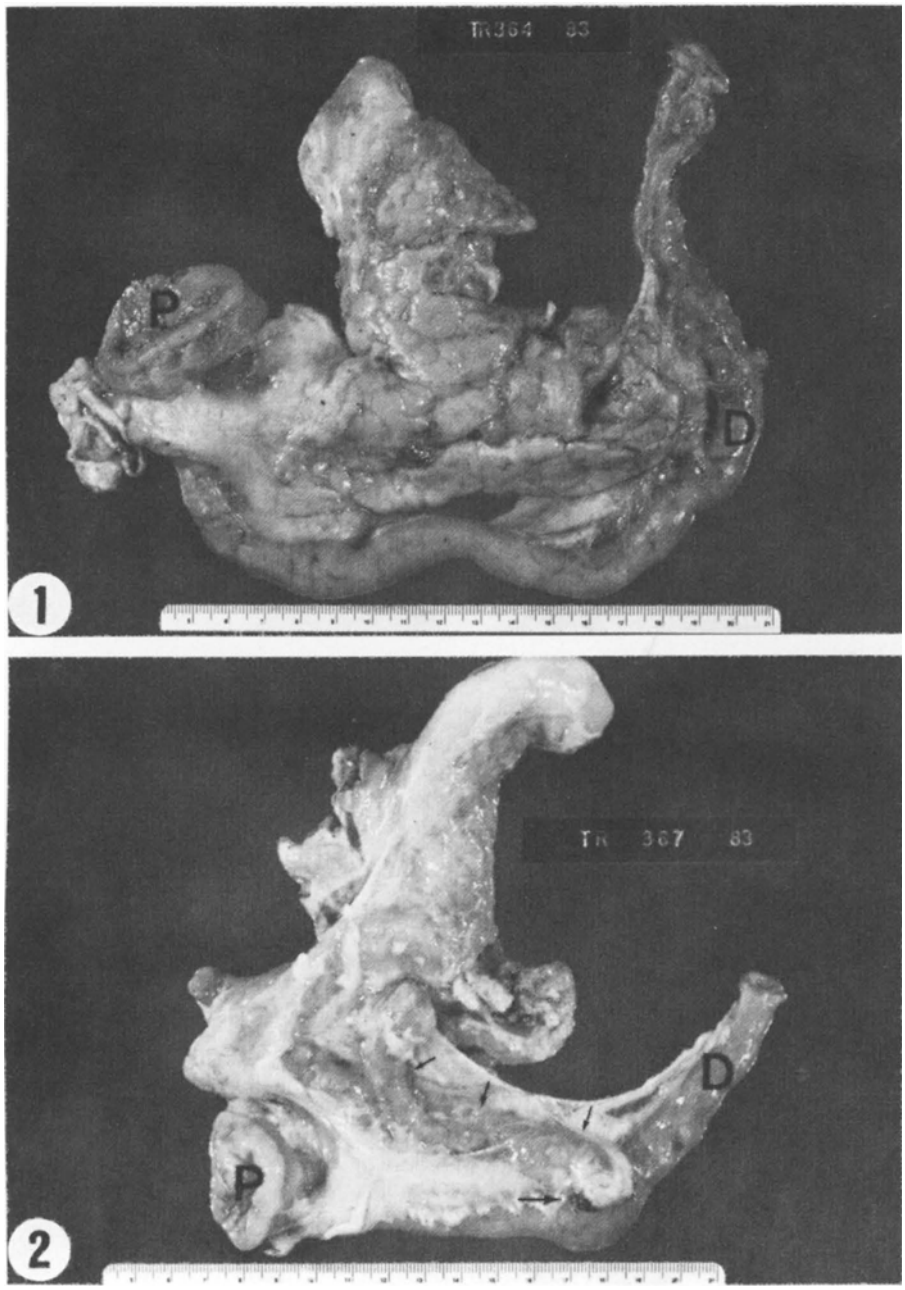


Figure 1. Normal pancreas. Photomicrograph of pancreas from a shamoperated control animal killed 6-8 weeks after the operation. P = pylorus, D = duodenum.

Figure 2. Experimental pancreatitis 6-8 weeks postligation. Macroscopical appearance of chronic pancreatitis produced by ligation of the pancreatic duct. Ligature is indicated by a thick arrow. Thin arrows show ectasia of the main pancreatic duct. P = pylorus, D = duodenum.

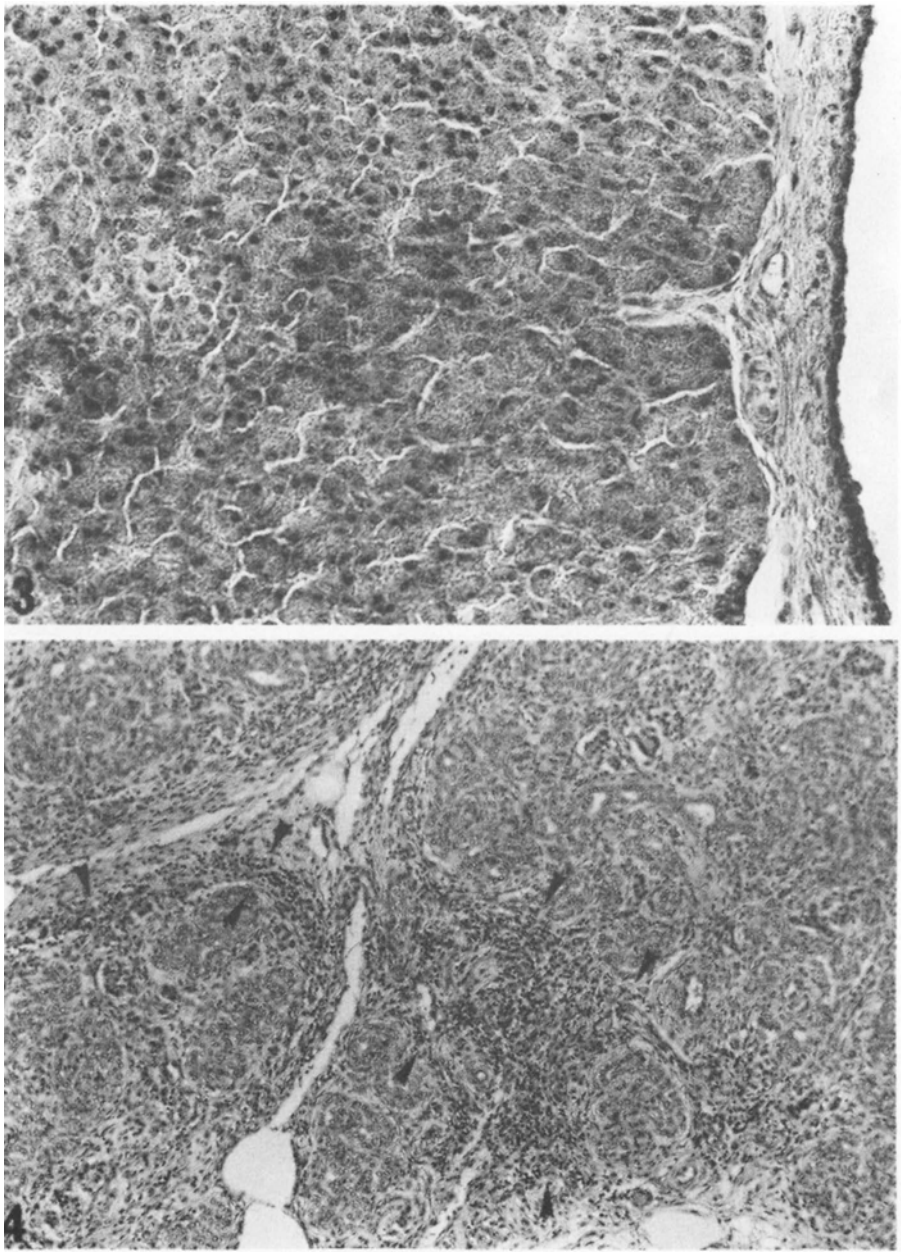
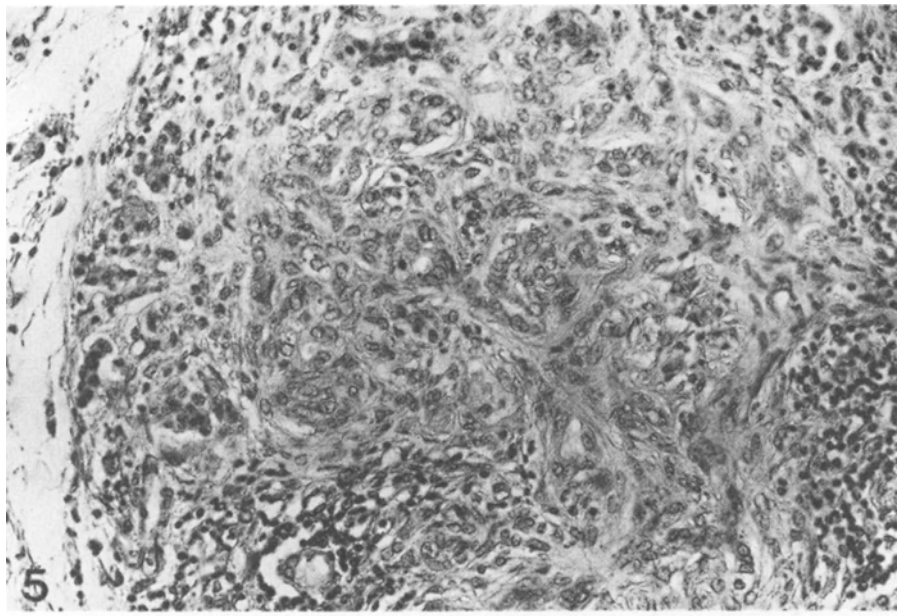


Figure 3. Normal pancreas from a sham-operated control animal killed 6-8 weeks after the operation. Section through exocrine tissues surrounding a large pancreatic duct (to the right). HE × 225.

Figure 4. Experimental pancreatitis 6-8 weeks postligation. All the acini are replaced by proliferating ductular cells. Inflammatory response (dark areas, marked with arrows) is seen in interlobular tissues. HE × 90.



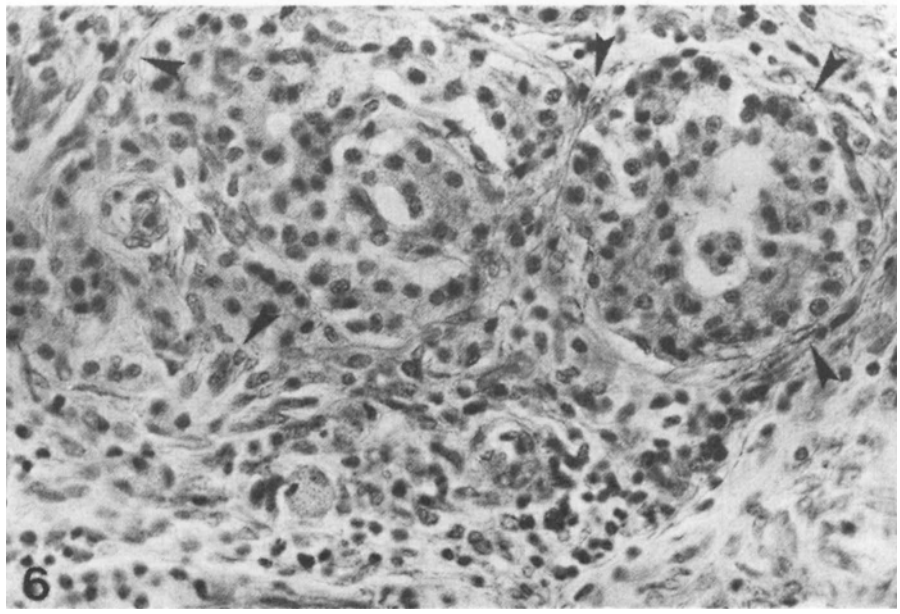


Figure 5. Experimental pancreatitis 6-8 weeks postligation. Duct cell proliferations surrounded by inflammatory cells. HE × 225.

Figure 6. Experimental pancreatitis 6-8 weeks postligation. Section through proliferating endocrine tissues with peri-insular fibrosis (arrows) HE × 350.

However, in microscopical investigations a mild chronical pancreatitis with slight proliferations of tubular cells and thickening of the wall of the main duct were observed. Acini were mostly normal but in some areas the acinar cells were smaller with chromatin-rich nuclei. No apparent destruction of acini was seen. Furthermore, in some sections inconsiderable thickening of the septa, fat cell infiltrations and inflammatory cell response was observed. No intra- or peri-insular fibrosis was seen and endocrine cells appeared normal.

Macroscopical studies revealed pronounced alterations in the pancreata of the 12 animals (Table 1, Fig. 2). Only remnants of parenchymal tissue were present around the cystic main duct. The glandular tissues were paler and firmer than normal, presenting fibrosis. The duct was greatly dilated with thickened walls and contained clear watery fluid. Light microscopy of exocrine pancreas showed the characteristic features of pronounced chronic pancreatitis in all of the animals (Figs. 4 and 5). Only the severity of lesions varied among different individuals, increasing according to the length of the experiment. In the mildest cases of pigs killed 3 weeks postoperatively some acini survived the acute phase of the disease without destruction. These acini appeared smaller than usual. The cytoplasm of the cells was scanty and stained intensely basophilic by the HE-technique. Proliferating ductular cells were arranged irregularly among the remaining acini. Connective tissue with some fat cells divided parenchyma into lobuli, scarring the intralobular tissues also. In the most advanced cases all the acini were replaced by duct cell proliferations arranged in tubuli-like structures. The enormously thickened and fibrotic wall of the main duct presented intensely proliferating ductular cells and slight infiltrations of macrophages and cells of the lymphocytic series. Endocrine pancreas revealed changes correlating to the degree of alterations in the exocrinic tissues (Fig. 6). In mild cases the only noticeable alteration was peri-insular fibrosis. In the most advanced cases, originating from pigs killed from 6 to 8 weeks after surgery, the size and number of islets increased. Furthermore, pronounced peri-insular fibrosis was accompanied by intrainsular fibrosis in several islets.

Discussion

In the present experiments performed on the pig chronic pancreatitis was produced by ligating the pancreatic duct. In man, analogous chronical changes occur spontaneously due to excretory inhibition of pancreatic fluid associated with obstructive or compressive stenosis or mucoviscoidosis (*Eder & Gedick* 1974). In previous animal experiments production of the chronic phase of the disease has also succeeded in such small laboratory animals as the rat (*Boquist & Edström* 1970) and the guinea pig (*Zelings & al.* 1975), as well as in carnivores (*Horaguchi & al.* 1983).

In developing the surgical technique, some difficulties were encountered at the beginning in 7 animals. Firstly, many of the animals operated on failed to show changes typical of exocrine insufficiency. The deaths of 2 animals were caused by technical failure, due either to impairment of the flow of duodenal contents, or that of bile. In the other 5 animals the observed morphological changes may implicate a possible loosening of the initially complete ligature around the pancreatic duct. It is noteworthy that to avoid manipulation and consequent artificial morphological changes, the tightness of the ligature was not examined. These animals had some thickening of the wall of the main pancreatic duct and proliferations of ductal cells, but no actual destrution of aci-

nar tissues. The morphological changes noticed may also imply that the flow of pancreatic secretions had improved by the opening of accessory ducts. In the (available) literature accessory pancreatic ducts have not been described in the pig. This fact was ascertained from the pancreas preparates of 10 pigs originating from a slaughterhouse, using a technique by which coloured fluid was forcibly applied directly into the ligated pancreatic duct (*Grönlund & Nikander* 1984).

In the 12 pigs whose surgery was successful, fully developed chronic pancreatitis was demonstrated in the present study. In the most pronounced cases all of the acini had disappeared during the acute phase of the disease. The glandular paranchyma had regenerated by proliferations of immature ductular epithelial cells surrounded by scarred stroma. The morphological features observed are typical of exocrine pancreatic insufficiency (*Rahko et al.* 1985).

The present study showed that it takes longer for the pig to develop chronic pancreatitis than for small laboratory animals and the dog. *Churg & Richter* (1971) have reported that some acini were still left 7 days after ligation of the pancreatic duct in the dog, whereas in the rat acinar parenchyma is destroyed within 1 week (*Boquist et al.* 1970). The few acini observed in the pigs sacrificed 3 weeks after the operation were apparently degenerating. The observations made by the present authors are in accordance with the previous results of studies elucidating the pathogenesis of chronic pancreatitis.

In the pigs killed from 6 to 8 weeks postoperatively, neoformation of endocrine islets was noted. The animals possessed an increased number of large islets with fibrosis. It has been shown in the dog that neoformation is produced by ductuloinsular proliferations (*Horaguchi et al.* 1983).

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Sammanfattning

Obstruktion av ductus pancreaticus hos svin: Ljusmikroskopiska undersökningar av kronisk pankreatit.

Morfologiska alterationer av pankreasvävnaderna rapporteras hos växande svin opererade med ligation av huvudpancreasgången. 19 djur blev opererade i 6-7 veckors ålder och obducerades 3 eller 6-8 veckor se-

nare. 12 svin utvecklade den kroniska fasen av sjukdomen med komplett exocrin insuffisiens. Av 7 djur, som inte uppvisade kronisk pankreatit,dog 2 i komplikationer som tillstötte efter operationen. Dessutom blev 3 svin shamopererade och använda som kontrolldjur.

Vid makroskopiska studier av kroniska pankreatiter kunde man observera att den ligerade gången var mäktigt utvidgad med klar serös vätska. Bara små områden med ljus och fast körtelvävnad kunde ses omkring de förstorade gångarna. Vid mikroskopiska undersökningar observerades alterationer typiska för kronisk pankreatit. Acinära vävnader hade fördärvats och var kompletterade med duktcellproliferationer. Körtelvävnaderna var separerade i lobuli med fibrotiska vävnader och fettceller. Huvudpankreasgångens vägg var mycket förtjockad och fibrotisk med rikliga proliferationer av duktceller och rundcelleinfiltrationer. Vidare obseverades förstorade endokrina öar omgivna av bindvävsfibrer.

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