

# Pressure, volume and the pancreas

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**SUMMARY** The effects of injection volume and pressure on the rat pancreas have been investigated. An experimental model using transduodenal cannulation of the rat bile-pancreatic duct was used. Injection volumes of 100  $\mu$ l or above produced gross ductal extravasation regardless of pressure. With a 50  $\mu$ l volume leakage from the ducts occurred *via* intercellular clefts at a pressure of 20 cmH<sub>2</sub>O and *via* duct ruptures at 50 cmH<sub>2</sub>O. Survival experiments (24 hours) were carried out using the 50  $\mu$ l volume. Infusion of 50  $\mu$ l saline at increasing pressures produced rises in amylase concentrations, pancreatic gland weights and water content of the gland at pressures of 20 cmH<sub>2</sub>O or above. These changes were maximal when 50 cmH<sub>2</sub>O of pressure was maintained for 60 minutes. The changes correlated with extravasation shown by Indian ink. Histological oedema related closely to pressure ( $r=0.92$ ), and was the most pronounced histological change observed. In experiments using intraduct injection into the rat pancreas a volume of 50  $\mu$ l or less should be used with careful consideration given to pressure. Unless these prerequisites are followed the results of experimental investigation cannot be extrapolated to acute gall stone pancreatitis in man.

Although the pathogenesis of acute gall stone pancreatitis remains an enigma it appears likely that reflux of bile and/or duodenal contents into the pancreatic ductal system is important. Such reflux occurs as the result of passage of a gall stone through the ampulla of Vater,<sup>1 2</sup> with the amount of reflux being dependent on pressure differentials between the pancreatic and bile ducts and duodenum. It has been postulated<sup>3</sup> that an increase in pressure within the pancreatic duct might be an aetiological mechanism in the genesis of acute gall stone pancreatitis; an increase in pressure leading to rupture of ducts<sup>4</sup> and escape of ductal contents into the interstitium.<sup>5</sup> Most experiments investigating acute gall stone pancreatitis have used intraductal injection of various solutions without attention to the volume or pressure of injection.<sup>6-8</sup> As the rat<sup>6-8</sup> is now the most commonly used experimental animal we have investigated the effect of injection volume and pressure on the rat pancreas as a prelude to developing a physiological model of acute gall stone pancreatitis.

## Methods

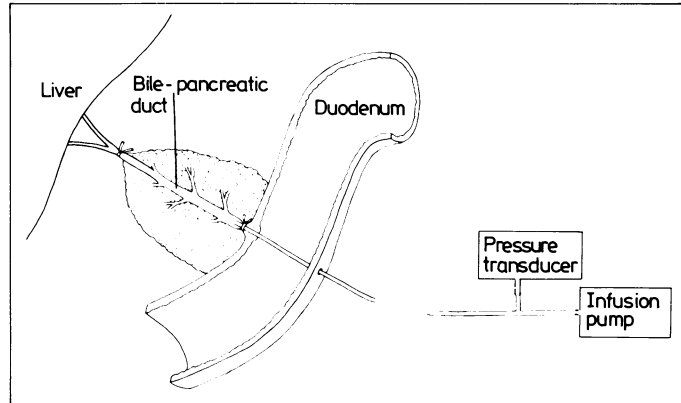
Male adult Sprague-Dawley rats (250-300g), fasted

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for eight hours, were carefully weighed and anaesthetised with 1.2 mg intraperitoneal sodium pentobarbitone, and maintained at a constant temperature of 37°C. Through a midline laparotomy wound the duodenum and bile-pancreatic duct were identified. In the rat multiple pancreatic ducts drain into a common bile-pancreatic duct which empties into the second part of the duodenum.<sup>9 10</sup> The greater part of the bile-pancreatic duct is enveloped in pancreas but at each end there is an exposed segment 1-2 mm long allowing free access to the duct. The common bile duct was ligated close to the liver and the animals left for 30 minutes to enable the bile-pancreatic duct to clear of bile. A preliminary experiment on 10 animals showed that bile duct ligation did not affect pancreatic structure or function over a 24 hour period. Through a small duodenotomy a thin Portex polyethylene cannula (id 0.4mm, od 0.8mm) was inserted through the ampulla into the bile-pancreatic duct and tied in place (Fig. 1) with care taken to avoid any pancreatic damage. The cannula was connected to a three way tap; one arm attached to a constant infusion pump (Slow infusion apparatus, Scientific and Research Instruments Ltd, Croydon, England); the other arm to a Statham pressure transducer with a mingograph 81 recorder (Elema-Schönander, Sweden) to allow pressure recordings within the system. Pressure measurements were checked in 10

Fig. 1 *Experimental preparation. Transduodenal cannulation of the rat bile pancreatic duct (BPD) allows infusion into the pancreatic duct system.*



animals with a Bell and Howell (Basingstoke, England) (type 4/422) transducer and Lectromed (St Peter, Jersey, Channel Islands) recorder and showed  $\pm 5\%$  concordance.

Two series of experiments were performed to investigate volume (part 1) and pressure (part 2).

#### PART 1

Indian ink solution was passed through a membrane filter of 5 microns pore size and had an osmolality of 300 mOsm/kg. Volumes of Indian ink (50–1000  $\mu$ l) were infused at 50  $\mu$ l/minute into the pancreatic duct over a 20 minute period and the pressures measured. At each volume five animals were studied. The volumes produced by the constant infusion pump were checked by weighing (1ml=1g) with a  $\pm 2\%$  concordance. Comparison of these volumes with the equivalent volume in the human pancreas is given by the ratio 1 : 100 (pancreas of rat=1g, human=100g). At 20 minutes the animals were killed and the pancreas immediately removed. Macroscopic assessment of the degree of blackness of both the head and tail portions of the gland was made by two independent observers and scored 0–3. (0 – no blackness, 1 – mild, 2 – moderate, 3 – severe). Portions of the head and tail of the pancreas were fixed in formalin and fine plastic (4  $\mu$  thick) sections of each cut and stained with haematoxylin and eosin. Microscopic assessment of ductal extravasation was made by two independent observers and graded 0–4 (Indian ink; 0 – all in ducts, 1 – little outwith ducts but no duct ruptures, 2 – few duct ruptures, 3 – moderate extravasation with multiple duct ruptures, 4 – severe extravasation with multiple duct ruptures).

#### PART 2

On the basis of the part 1 experiments a volume of 50  $\mu$ l sterile 0.9% saline was chosen for the study of

pressure effects using groups of 10 rats. Measurement of the infusion volume at each pressure showed a mean  $\pm$ SD volume of  $51 \pm 2 \mu$ l. Two groups of rats were used for comparative purposes at each occlusion time – laparotomy only (sham); cannulation only (control). Infusion of 50  $\mu$ l saline was performed at maximum pressures of 10, 15, 20, 25 and 50 cmH<sub>2</sub>O. The lower pressures required a longer period of infusion. After delivery of 50  $\mu$ l the infusion was discontinued and the cannula left *in situ* for either 5 or 60 minutes. After the set occlusion time the cannula and ligature were removed to allow free pancreatic drainage. The duodenotomy was closed with a single suture of 7/0 prolene and the abdomen in layers. The animals made a full recovery with free access to food and water until at 24 hours the animals were re-anaesthetised with ether and the laparotomy wound reopened. Peritoneal fluid was carefully collected, blood was taken from the inferior vena cava before manipulation of the pancreas and the animal was then killed. The pancreas was removed, dissected free of fat and carefully weighed. Representative portions were fixed in formalin for plastic sectioning and haematoxylin and eosin staining.

To overcome the disparity in animal body weights the pancreatic gland wet weight was combined with the total body weight to produce a ratio of gland weight: body weight (mg/100g). Quantitation of the degree of oedema or water content of the gland was carried out by the method of Aho.<sup>11</sup> The wet weight of each gland was measured. For the measurement of dry weight each pancreas was fixed in ethanol for 10 hours and then dried to a constant weight of 60°C. The degree of oedema was expressed as the water content of pancreatic tissue (% of wet weight). Amylase estimations were performed using the Phadebas technique.

Light microscopy was undertaken by two

independent observers. Five histological features were assessed using a modification of the criteria of Nevalainen:<sup>12</sup> oedema, haemorrhage, acinar necrosis, duct changes and inflammatory infiltrate. Each was scored 0–3 (0 – normal, 1 – mild, 2 – moderate, 3 – severe changes). Thus the pancreas of each animal had a histology score of 0–15 and each group of 10 animals had a score of 0–150.

Statistical analysis between groups was performed using Student's *t* test for unpaired samples and the Mann-Whitney U test where appropriate. Linear regression was used to obtain relationships between histology and pressure, gland weight ratios and oedema, and the macroscopic and histological assessments of the two independent observers.

## Results

There was close correlation between the two independent observers for all histological and macroscopic assessments ( $r=0.92$ ,  $p<0.001$ ).

### PART 1

The macroscopic and histological changes in the head and tail of the pancreas at each volume are summarised in the Table. Volumes of 100  $\mu$ l and above produced gross ductal extravasation in all pancreatic glands examined. With volumes of 150  $\mu$ l or less extravasation was invariably more severe in the head of the pancreas. Volumes of 150  $\mu$ l or more were associated with a steady rise in pressure until an abrupt decline which was taken to indicate gross rupture of the duct system.

In the 50  $\mu$ l infusion experiment extravasation through intercellular clefts into a periacinar space was demonstrated at a pressure of 20 cmH<sub>2</sub>O and gross duct rupture at a pressure of 50 cmH<sub>2</sub>O. The amount of extravasation increased with pressure; none was visible below 15 cmH<sub>2</sub>O (Fig. 2 A–C).

Table Indian ink: Ductal extravasation with varying volumes (mean values with range) ( $n=5$  for each volume)

Volume ( $\mu$ l)	Highest pressure (cmH <sub>2</sub> O)	Macroscopic score (0–3)		Histology score (0–4)	
		Head	Tail	Head	Tail
50	15	0.3(0–1)	0.2(0–1)	1.1(0–2)	0.2(0–1)
100	43	2.1(2–3)	1.2(1–2)	2.3(2–3)	1.7(0–2)
150	62	2.9(2–3)	2.4(2–3)	3.1(2–4)	2.8(2–4)
200	81	3.0	3.0	4.0	4.0
500	90	3.0	3.0	4.0	4.0
1000	93	3.0	3.0	4.0	4.0

### PART 2

All animals survived to 24 hours.

#### Pancreatic gland weight ratios

These weight ratios significantly correlated with the histological degree of oedema ( $r=0.75$ ,  $p<0.001$ ). The PGWRs were higher for 60 minutes occlusion than five minutes at most pressures (Fig. 3). Maximum values were observed with a pressure of 50 cmH<sub>2</sub>O ( $p<0.01$ ).

#### Water content (Fig. 4).

The water content in the normal gland (sham) was 68% and 70% for the five and 60 minute occlusion times respectively, rising to a value of above 80% with 50 cmH<sub>2</sub>O of pressure. These increases were significant at or above 25 cmH<sub>2</sub>O for five minutes occlusion and at 15cmH<sub>2</sub>O for 60 minutes occlusion. The longer occlusion time produced a significantly higher water content at most pressures than the short five minutes occlusion.

#### Serum amylase

These levels were very variable (Fig. 5) and the normal level in these rats was  $1250 \pm 500$  u/l. Cannulation of the duct alone (control) caused rises of the serum amylase. Significant rises ( $p<0.01$ ) in serum amylase were seen for five minutes occlusion at or above 15cmH<sub>2</sub>O. For both occlusion times maximum rise of serum amylase occurred at a pressure of 50cmH<sub>2</sub>O ( $p<0.01$ ).

#### Peritoneal fluid amylase

This could not be detected in those animals undergoing laparotomy only (sham). For both occlusion times there was a progressive rise in peritoneal fluid amylase with pressure. A significant rise was noted at or above 15 cmH<sub>2</sub>O ( $p<0.01$ ) (Fig. 6).

### HISTOLOGY

Five minutes occlusion was associated with a steady rise in the histology score with increased pressure ( $r=0.93$ ,  $p<0.01$ ) (Fig. 7). The most important histological feature was oedema. The maximum score of 15/150 was observed at 50cmH<sub>2</sub>O.

Sixty minutes occlusion was also associated with a steady rise in histology score with increased pressure ( $r=0.98$ ,  $p<0.01$ ) (Fig. 8); the values being significantly higher than for five minutes occlusion ( $p<0.01$ ). Oedema was the most noticeable feature (Fig. 9 a,b) with mild duct changes observed at higher pressures. The maximum score was 30/150 at 50cmH<sub>2</sub>O.

In all glands examined the most striking

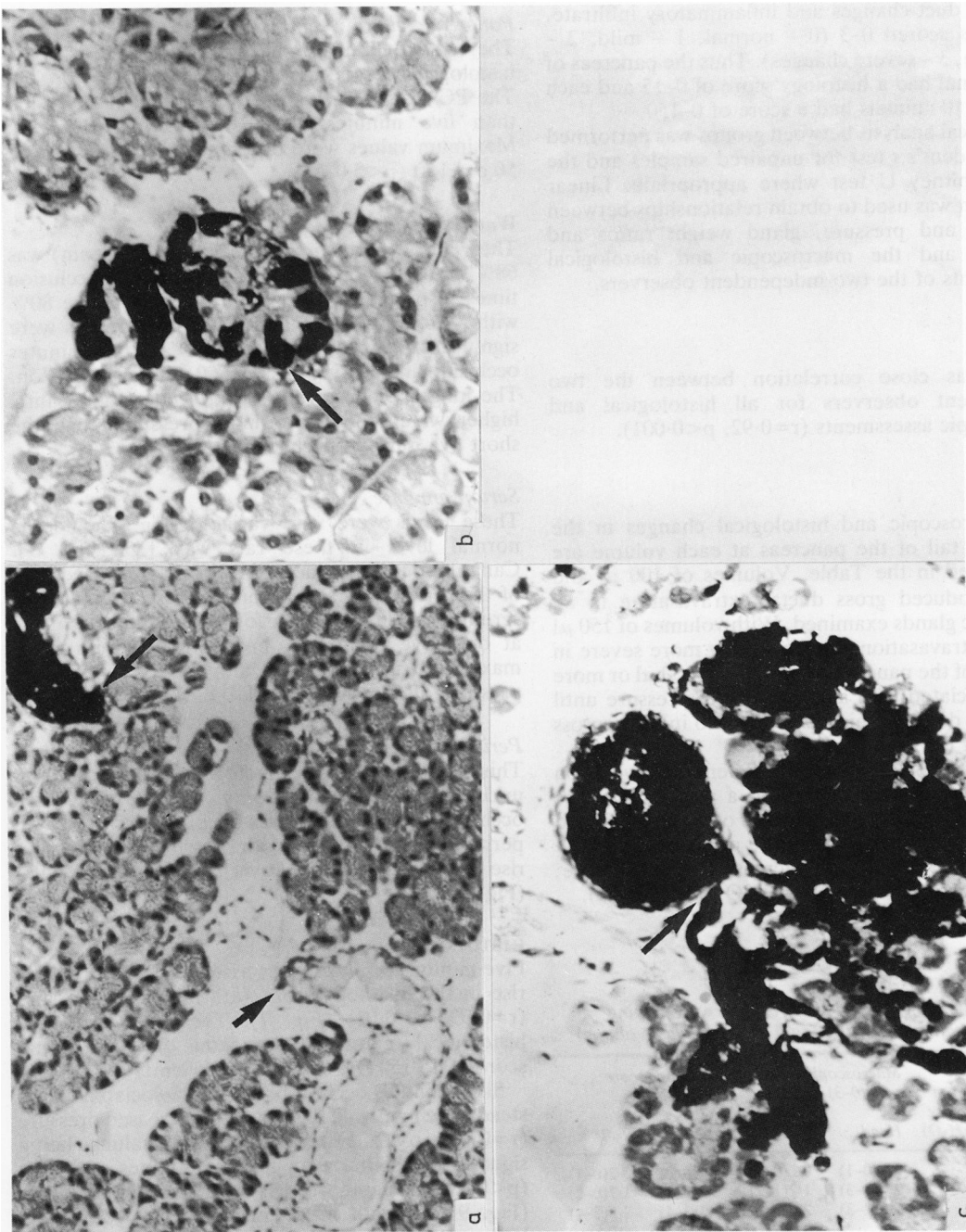


Fig. 2 Histological sections of rat pancreas after infusion of 50  $\mu$ l Indian ink. Haematoxylin-eosin (HE), (a) pressure 15 cmH<sub>2</sub>O; ink all within ducts (arrowed) (HE $\times$ 200). (b) pressure 25 cmH<sub>2</sub>O; extravasation of ink through inter-cellular clefts (arrowed) into periductal space (HE $\times$ 250). (c) pressure 50 cmH<sub>2</sub>O; ductal rupture with gross extravasation of ink (arrowed) (HE $\times$ 250).

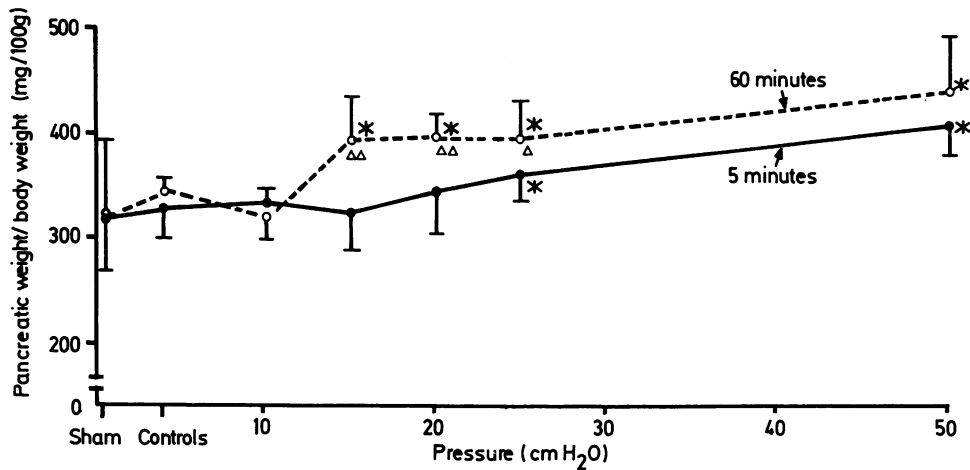


Fig. 3 Pancreatic gland weight ratios v pressure,  $n=10$  at each pressure. (● 5 minutes, ○ 60 minutes) (mean  $\pm$  SD).  $\star p < 0.01$  v controls,  $\Delta p < 0.02$  v 5 minutes,  $\Delta\Delta p < 0.001$  v 5 minutes.

histological feature was oedema. In no gland was there evidence of acute pancreatitis.

**Discussion**

Acute gall stone pancreatitis is a common disease with a mortality rate of 10–15%.<sup>13</sup> Despite intensive experimental research its pathogenesis remains uncertain. The finding of gall stones in the faeces of 90% of patients after an attack of acute gall stone pancreatitis<sup>1 2</sup> suggests that passage of a gall stone through the ampulla of Vater with reflux of bile or duodenal contents into the pancreatic duct is

important.

As fluid flows down hydrostatic pressure gradients a knowledge of pressure relationships within the pancreatic and biliary ducts and duodenum is important. These pressures have been closely studied in different species (man, dog, rat, cat) by several investigators;<sup>14–17</sup> median values with the ranges during basal fasting conditions are bile duct 8cmH<sub>2</sub>O, [4–17], pancreatic duct 12cmH<sub>2</sub>O [4–29] and duodenum 8cmH<sub>2</sub>O [4–12]. Although there is considerable variation between animals of the same species and even the same animal tested on different days, the pressure in the pancreatic ducts is, in most instances, greater than that in the biliary system.<sup>15–17</sup>

More important than static measurements are the dynamics of pancreatico-biliary-duodenal pressure gradients, which may well be important for a complete understanding of pancreatic disease.<sup>13 18</sup> Pancreatic ductal pressures themselves vary considerably and are related to feeding, secretin release and autonomic stimulation.<sup>3</sup> The relationship of duodenal and pancreatic pressures has been closely studied by DiMagno and colleagues<sup>19 20</sup> who showed that duodenal pressures were greater than those in the pancreatic ducts after feeding and definite duodeno-pancreatic duct reflux occurred. Bile duct pressure may increase above than in the pancreatic duct after obstruction of the common channel,<sup>9 14</sup> after cholecystectomy,<sup>14</sup> administration of cholecystokinin<sup>21</sup> and occasionally after feeding.<sup>15 16</sup> Definite bile reflux into the pancreatic duct has been shown by Becker and

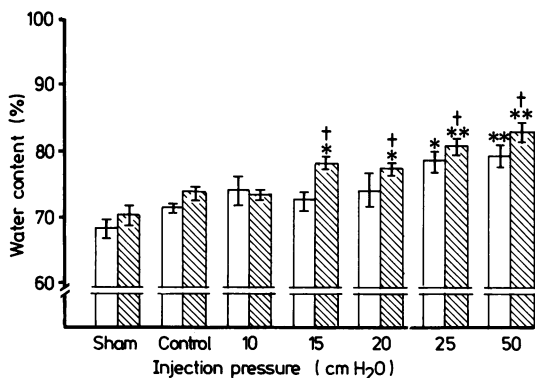


Fig. 4 Water content (%) of gland v pressure,  $N=10$  at each pressure □ 5 minutes, ▨ 60 minutes) mean  $\pm$  SD.  $\star p < 0.01$  v controls,  $\star\star p < 0.001$  v controls.  $\dagger p < 0.01$  v 5 minutes.

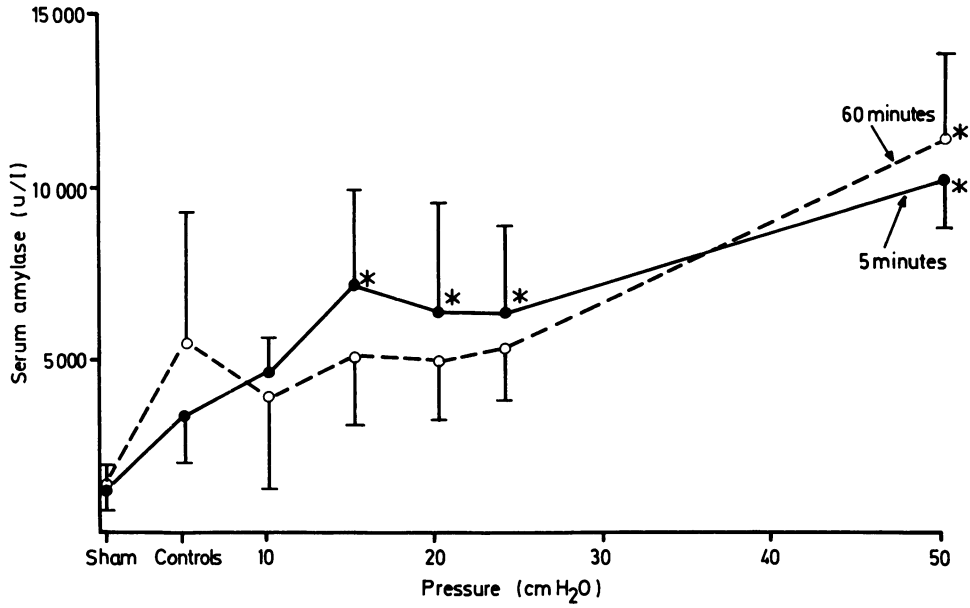


Fig. 5 Serum amylase (u/l) v pressure, n = 10 at each pressure (● 5 minutes, ○ 60 minutes) (mean±SD) ★ p<0.01 v controls.

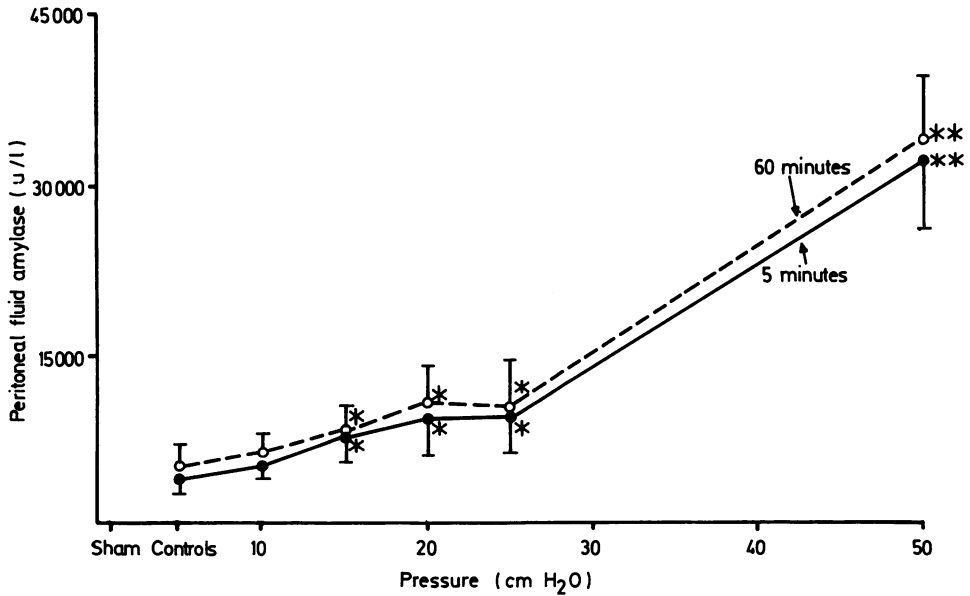


Fig. 6 Peritoneal fluid amylase (u/l) v pressure, n = 10 at each pressure (● 5 minutes, ○ 60 minutes) (mean±SD). ★ p<0.01 v controls, ★★ p<0.001 v controls.

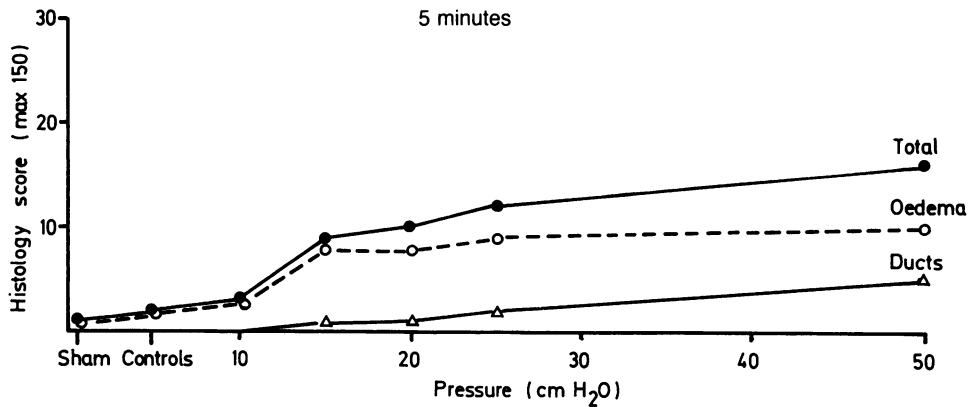


Fig. 7 Histology score v pressure for 60 minutes occlusion ( $r=0.93$ ,  $p<0.01$ ) (● total, ○ oedema, △ duct changes).

associates<sup>22</sup> in a normal pressure system. The increased incidence of pancreatic duct reflux on operative cholangiograms of patients with acute gall stone pancreatitis further evidences the importance of reflux.<sup>2 23</sup> In addition, it is important to emphasise that duct and duodenal pressures may be modified by pressures in the subhepatic space as values of 12cmH<sub>2</sub>O are reached on deep breathing and 60cmH<sub>2</sub>O on coughing.<sup>17 24</sup> Nevertheless, despite these observations, uncertainty exists regarding both the pathological significance of pancreatic reflux of bile and duodenal juice and its frequency and quantity under physiological conditions.<sup>22 25</sup>

Most experimentation regarding acute gall stone pancreatitis has used retrograde injection of varying substances into the pancreatic duct of various species, with the rat now the most commonly used

animal. Analysis of recently published experimental data on retrograde injection into the rat pancreas demonstrates the variability of the volumes which range from 100  $\mu$ l<sup>6</sup> through 200  $\mu$ l,<sup>7</sup> 600  $\mu$ l<sup>8</sup> to 1000  $\mu$ l<sup>26</sup> and above. To produce a meaningful experiment the volume injected must be representative of the amount of reflux likely in man and comparison of these experimental volumes with their human equivalents showed them to be grossly in excess of possible reflux volume. Whereas all volumes above 100  $\mu$ l produced gross ductal rupture, with a volume of 50  $\mu$ l the amount of extravasation was closely related to pressure. These observations suggest that projection of the results of other experiments<sup>6-8</sup> to the pathophysiology of acute gall stone pancreatitis in man should be treated with caution owing to unphysiological duct rupture.

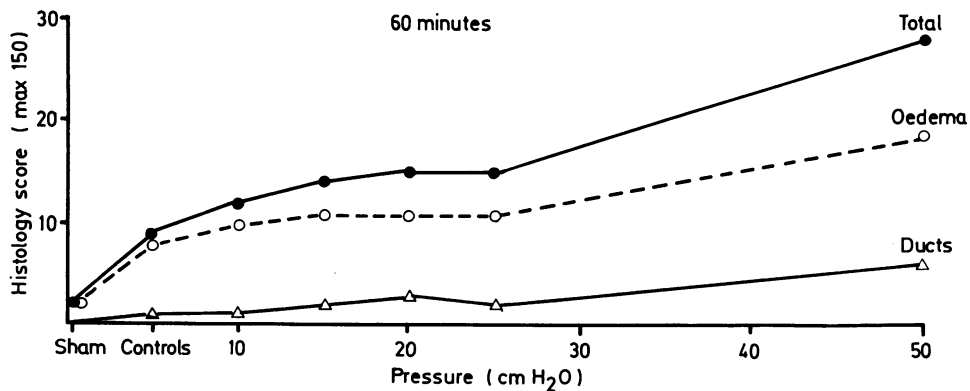


Fig. 8 Histology score v pressure for 60 minutes occlusion ( $r=0.98$ ,  $p<0.01$ ) (● total, ○ oedema, △ duct changes).

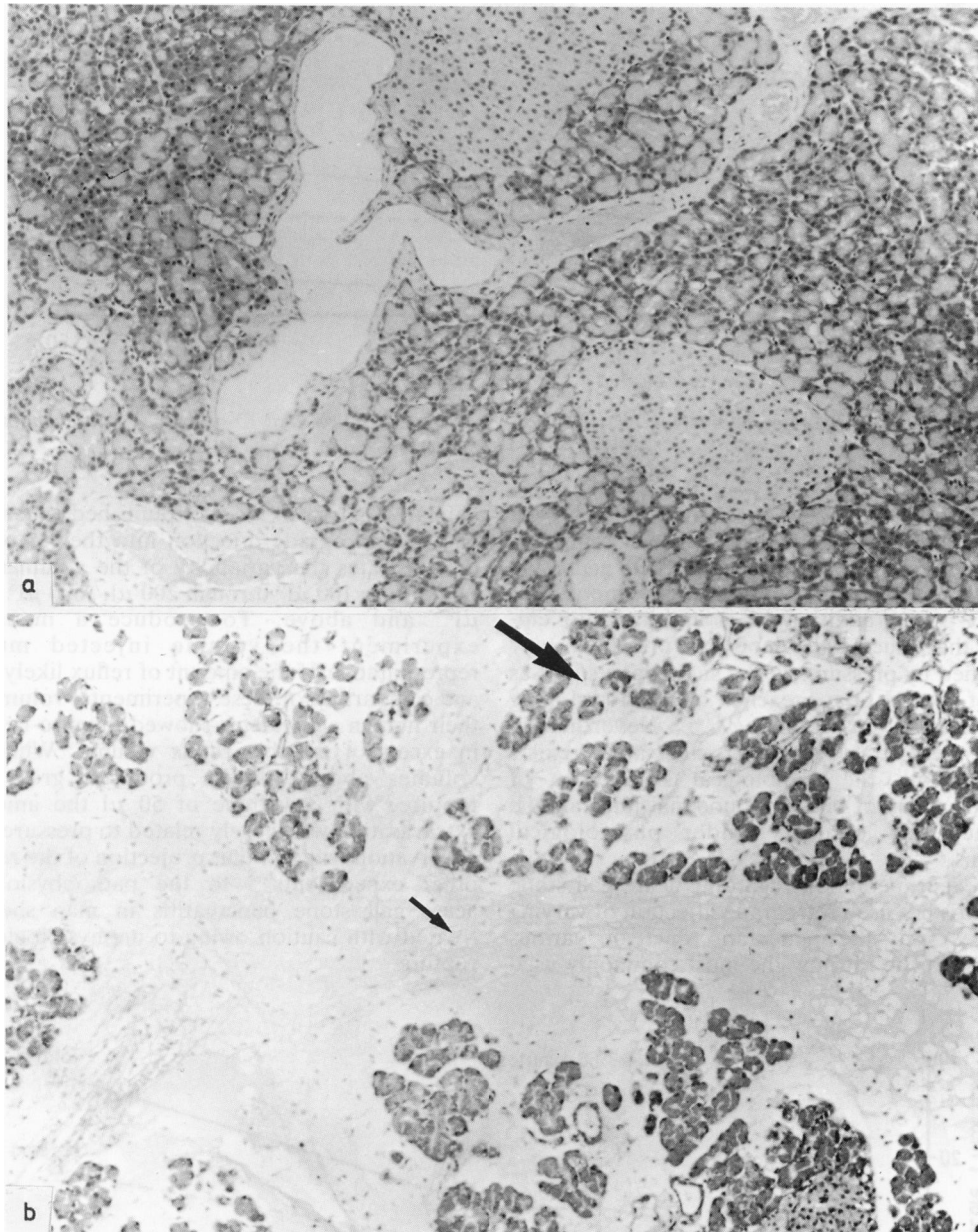


Fig. 9 Histological sections of rat pancreas. (a) Normal pancreas (HE×140). (b) Pancreas following injection at a pressure of 50 cmH<sub>2</sub>O. Note marked interlobular (small arrow) and intralobular (large arrow) oedema. (HE×120).



Previous investigators have evaluated effects of pressure on the pancreatic ductal system. Studies using Indian ink<sup>4 27 28</sup> and fluorescein<sup>29</sup> showed passage between acinar cells into the interstitial space with a close relationship between pressure and extravasation as extravasation was only obvious at or above 30cmH<sub>2</sub>O.<sup>4</sup> Herriott<sup>9</sup> showed that Indian ink extravasation could occur as a result of biliary pressure alone and was associated with evidence of possible small duct ruptures. Further evidence of passage through intercellular clefts into a periacinar space at moderate pressures was produced by Bockman and associates<sup>30</sup> using ferritin, who also described minute disruptions of the cellular lining at the ducto-acinar junction and gross ductal rupture with high pressures. The results of our study parallel these observations to a large degree. At low ( $\leq 15$ cmH<sub>2</sub>O) intraduct pressures no extravasation occurs; at moderate pressures (20–25 cmH<sub>2</sub>O) there is passage through intercellular clefts into a periacinar space and at high pressures (50cmH<sub>2</sub>O) duct ruptures occur. It appears that extravasated saline or Indian ink passes into the periacinar and interstitial spaces before being rapidly cleared by the capillary and lymphatic system.<sup>31</sup>

This study investigated the pancreatic effects of pressure on an *in vivo* rat model using the 50  $\mu$ l volume of saline with a view to the subsequent study of the effects of more toxic substances. The animal preparation described produced reproducible results. The pancreatic damage resultant on pressure was minimal but significant in the context of ductal extravasation. This damage was associated with raised amylase concentrations, gland weights and both chemical and histological oedema, with both pressure and occlusion time important. The pancreatic damage seen was closely related to the known duct extravasation studied earlier with Indian ink. No pancreatic damage occurred at low pressures without ductal extravasation; moderate damage was associated with extravasation through intercellular clefts; more marked damage appeared with known duct rupture at high pressures.

Reflux into the pancreatic ducts may cause extravasation of the contained duct contents with the amount of reflux and extravasation being dependent on pressure. As several constituents of bile and duodenal contents have been shown to increase pancreatic duct permeability,<sup>32</sup> extravasation might well occur at lower pressures than those mentioned. The extravasation of these toxic substances into the periacinar and interstitial spaces might therefore be the initiating step in acute gall stone pancreatitis. The relationship of pressure and bile to acute pancreatitis is important when considering reflux and pressure as initiators of

inflammation. Whereas bile at low pressures produces no pancreatic damage,<sup>33</sup> the same bile at increased intraductal pressure will induce acute pancreatitis.<sup>34</sup> These observations, while requiring further elucidation, show a definite relationship between pressure, bile and pancreatitis.

Experiments using retrograde injection into the rat pancreatic duct must carefully control both the volume of injectate to equate with that likely to reflux in man, and the pressure of injection. This study has stimulated us to investigate the pathophysiology of acute gall stone pancreatitis using a volume of 50  $\mu$ l infused at low pressures into the rat pancreas. Reflux into the pancreatic duct under pressure may be associated with extravasation and thus be an important factor in the initiation of acute pancreatitis.

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