

## The Effect of Secretin and Pancreozymin on Pancreatico-Duodenal Lymph Flow and Lipase Activity in Normal Dogs and on Thoracic Duct Lymph Flow and Lipase Activity in Rats with Chronic Pancreatitis

M. Papp, S. Ormai, E. J. Horváth, I. Fodor\*

Institute of Experimental Medicine Hungarian Academy of Sciences,  
and Department of Pathology, National Institute of Rheumatology  
and Physiotherapy\*, Budapest

Lymphology 3 (1971), 67-73

*Bainbridge* (1) was the first to describe that the intravenous injection of secretin enhanced the rate of secretion of pancreatic juice and thoracic duct lymph flow; he found a close correlation between pancreatic secretion and the enhancement of lymph flow. *Razin, Feldman and Dreiling* (2), also working on dogs, confirmed that secretin augmented pancreatic secretion and lymph flow from the thoracic duct, and observed that intravenous pancreozymin too increased lymph flow, however, neither secretin nor pancreozymin augmented the activity of amylase in the lymph. Furthermore, lymph flow was increased by secretin also in pancreatectomized dogs while it was not affected after removal of the small intestine. From these observations it has been concluded that enhanced lymph flow from the thoracic duct in response to secretin is derived from the small intestine and not the pancreas.

In healthy human subjects, secretin was found to augment thoracic duct lymph flow and amylase and lipase activity in the lymph, whereas it failed to affect either lymph flow or lipase activity in patients with chronic pancreatitis (3). On the other hand, *Bartos, Brzek, Groh and Keller* (4) obtained no change in thoracic duct lymph flow in response to secretin either in healthy individuals or in patients with chronic pancreatitis (5), however, secretin increased amylase activity in the lymph both in healthy subjects and patients with chronic pancreatitis while having no appreciable effect on lipase activity. Pancreozymin augmented thoracic duct lymph flow in healthy individuals and, similarly to secretin, increased the activity of amylase in the lymph.

Since there is much controversy about the data pertaining to this question it seemed worth to investigate whether

1. the flow of pancreaticoduodenal lymph and lipase activity in the lymph of the normal pancreas are affected alone by secretin or only in combination with pancreozymin;
2. whether there is a difference in the secretin and pancreozymin-induced changes of thoracic duct lymph flow and lipase activity in chronic pancreatitis as compared to the normal pancreas.

### Material and Methods

1. Twenty-three mongrel dogs of either sex, weighing 16 kg on the average, were anaesthetized with chloralose. Lymph was collected from the lymphatics draining the pancreas and the duodenum. The technique of lymph collection and withdrawal of pancreaticoduodenal venous blood has been described in a previous paper (6). Polyethylene cannulas were inserted into the duct of Santorini and the duodenal branch of the superior pancreaticoduodenal artery; the cannula left the blood flow in the artery unaffected. Pancreatic juice was collected through the cannula inserted into Santorini's duct. Through the arterial cannula, physiological saline, or hormones stimulating pancreatic secretion were infused by a constant-rate pump over periods of 45 min. In 8 dogs, the effect of physiological saline infused into the superior pancreaticoduodenal artery at a rate of 1 ml/min for 45 min was studied on the amount of pancreaticoduodenal lymph collected during this period from the intact pancreas. In 9 dogs with untreated pancreas, the effect of 0.1 U/kg b.w./min of secretin infused in physiological saline at a rate of 1 ml/min was tested using Sinbio (Nice, France) and Boots (Nottingham, England) preparations in 5 and 4 animals, respectively. The effect of the above dose of secretin and 0.1 U/kg b.w./min of pancreozymin (Boots, Nottingham, England) dissolved in physiological saline and infused at a rate of 1 ml/min was also investigated in 6 dogs after treatment with 1 ml/min of physiological saline infused into the superior pancreaticoduodenal artery. Pancreaticoduodenal lymph was collected for 45 min periods in heparinized tubes kept in an icy bath. At the end of each lymph collection period blood was withdrawn into heparinized tubes from the superior pancreaticoduodenal vein. The portion of the pancreas supplied by the superior pancreaticoduodenal artery and the lymph vessels draining this portion were visualised at the end of the second lymph collection period by injecting 1% Evans blue solution into the artery.

The volume of the pancreaticoduodenal lymph samples was measured. Lipase activity was determined in the lymph, blood plasma and pancreatic juice by the method of *Weber* (7). The effect of the various procedures was analysed by Student's *t*-test, while the effect of the various treatments was compared by analysis of variance.

2. In the second set of experiments, male albino rats of the CFE strain, weighing 200 to 250 g, were anaesthetized with sodium pentobarbital (4 mg/100 g b.w.) given intraperitoneally. Pancreatitis was induced by sunflower oil or 2.5 mg of trypsin (*Richter*, Budapest). These substances were injected into the pancreas in a volume of 0.2 ml, according to the technique of *Heinkel* (8). Six rats treated with trypsin and 9 sunflower oil treated rats which survived by the 14th to 18th day, and 8 intact CFE male rats weighing 200 to 250 g were anaesthetized with sodium pentobarbital. Pancreatic juice was collected by a polyethylene cannula inserted into the common bile duct via the duodenum. The common bile duct was dissected in the liver porta, and the duodenal stump was ligated; bile was drained through the central stump of the common bile duct. Lymph was collected through a polyethylene tubing introduced into the thoracic duct by the method of *Bollman, Cain and Grindlay* (9). The femoral vein was also cannulated, and 4 U/kg b.w. of secretin and pancreozymin (Boots, Nottingham, England) dissolved in 1 ml of physiological saline were infused at a constant rate within 45 min. Pancreatic juice and thoracic duct lymph were collected continuously into tubes kept in an icy bath

over a period of 45 min. At the end of the collection period, blood was withdrawn from the femoral artery, then the animals were killed and their pancreas removed and fixated in 6% formalin. Lipase activity was determined in the lymph, blood plasma and pancreatic juice using the method of *Weber* (7). The removed pancreas was embedded in paraffin; the 5- $\mu$  sections were stained with haematoxylin-eosin and examined under the light microscope.

### Results

1. Both physiological saline and secretin enhanced the rate of pancreaticoduodenal lymph flow in the untreated pancreas in dogs ( $p < 0.05$ ); there was no substantial difference between the efficiency of treatments (Fig. 1). Secretin and pancreozymin, when given together, significantly increased the rate of pancreaticoduodenal lymph flow from the pancreas pretreated with physiological saline ( $p < 0.05$ ). Secretin and pancreozymin failed to produce a greater effect on pancreaticoduodenal lymph flow than either physiological saline or secretin given alone (Fig. 1).

Lipase activity in pancreaticoduodenal lymph and in superior pancreaticoduodenal venous blood was increased only when secretin and pancreozymin were given simultaneously ( $p < 0.01$ ; Fig. 2).

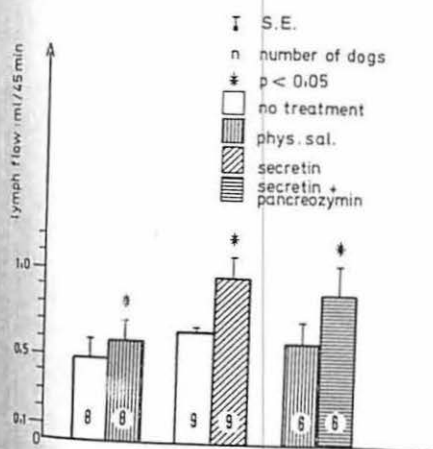


Fig. 1 Rate of pancreaticoduodenal lymph flow prior to and after the infusion into the superior pancreaticoduodenal artery of physiological saline (1 ml/min), 0.1 U/kg b.w./min of secretin in 1 ml/min of physiological saline, and 0.1 U/kg b.w./min of secretin and 0.1 U/kg b.w./min of pancreozymin in 1 ml/min of physiological saline in dogs.

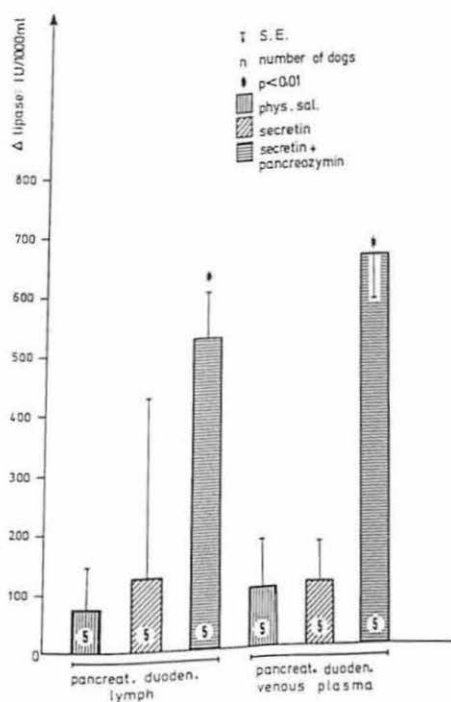


Fig. 2 Changes in lipase activity of the pancreaticoduodenal lymph in response to the infusion into the superior pancreaticoduodenal artery of physiological saline (1 ml/min), 0.1 U/kg b.w./min of secretin in 1 ml/min of physiological saline, and 0.1 U/kg b.w./min of secretin and 0.1 U/kg b.w./min pancreozymin in 1 ml/min of physiological saline in dogs.



Fig. 3a Histological appearance of the pancreas in rat 14 days after the intraductal injection of sunflower oil. The structure of the gland is hardly recognizable. The excretory ducts are dilated, the remaining acini are dedifferentiated. Interlobular and intralobular connective tissue shows proliferation and inflammatory reaction. The islets of Langerhans are normal. (Haematoxylin-eosin;  $\times 52$ .)

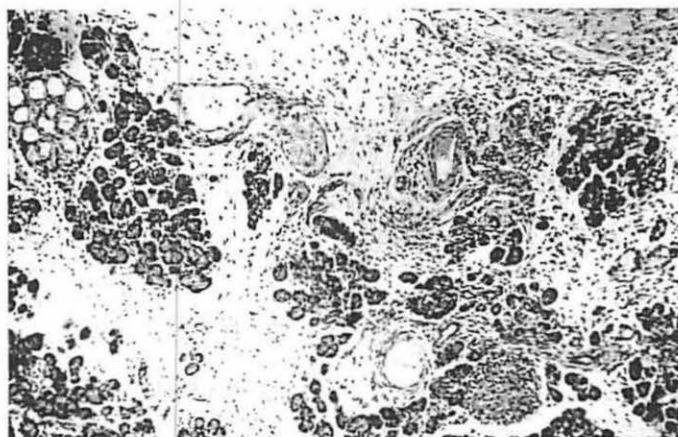


Fig. 3b Histological appearance of the pancreas in rat 14 days after the intralobular injection of trypsin. Focal damage of the gland is well recognizable, intact acini are seen also. Proliferation of intralobular and interlobular connective tissue. Marked inflammatory reaction. The islets of Langerhans are normal. (Haematoxylin-eosin;  $\times 52$ .)

The amount of pancreatic juice and its lipase activity were according to expectation, significantly augmented by secretin alone and in association with pancreozymin.

2. Sunflower oil as well as trypsin, when injected into the pancreas of rats, gave rise within 14 to 18 days to histological alterations that are characteristic to chronic pancreatitis. The structure of the pancreas treated with sunflower oil was hardly recognizable, groups of dedifferentiated acini with a broad lumen and lined with cubic epithelium

predominating the microscopic picture, with proliferation of intralobular and particularly of interlobular connective tissue which was invaded by lymphocytes, monocytes and eosinophil leucocytes (Fig. 3 a). In the pancreas treated with trypsin, completely damaged areas were seen besides relatively intact regions. Besides the obstructed acini lined with flattened epithelium there were also groups of lobules with a preserved structure and morphologically normal acini. In the proliferating intralobular and interlobular connective tissue there was a marked inflammatory reaction (Fig. 3 b). The histological finding was similar to that seen in chronic pancreatitis of the human. Remarkably enough, the islets of Langerhans were apparently intact, despite the severe damage seen in the acini.



Fig. 3 c Histological appearance of the pancreas and that of an islet of Langerhans in untreated rat. (Haematoxylin-eosin;  $\times 52$ .)

Lipase activity in the pancreatic juice following stimulation with secretin and pancreozymin was about thousand times higher in the normal controls than in the rats treated with sunflower oil, and approximately ten times higher than in the trypsin-treated animals (Table 1). In thoracic duct lymph, lipase activity attained measurable levels only when the secretion of the intact pancreas was stimulated. The amount of lymph collected from the thoracic duct, however, was practically the same irrespective of whether the normal or the sunflower oil treated or trypsin treated pancreas was stimulated by secretin and pancreomyzin. Lipase activity was undetectably low in the blood plasma of the rats previously treated with sunflower oil and stimulated by secretin and pancreozymin.

### Discussion

1. Since the rate of pancreaticoduodenal lymph flow in the dog varies between 0.01 and 0.03 ml/min as shown in the present and previous experiments (6) the 50% increase caused by secretin cannot appreciably affect the rate of lymph flow in the thoracic duct which has been found 0.2 to 0.6 ml/min (10, 11). Similar conclusions were drawn by *Razin, Feldman and Dreiling* (2) who suggested that the increased thoracic duct lymph

Table 1 The effect of secretin and pancreozymin on lipase activity in blood plasma, thoracic duct lymph and pancreatic juice, and on thoracic duct lymph flow in rats with chronic pancreatitis. In parentheses, the number of rats.

	Blood plasma Lipase activity IU/1,000 ml	Thoracic duct lymph Flow rate ml/45 min	Thoracic duct lymph Lipase activity IU/1,000 ml	Pancreatic juice Lipase activity IU/1,000 ml
Intact pancreas (8)				
Mean $\pm$ S.E.	232 $\pm$ 76.9	0.60 $\pm$ 0.11	551 $\pm$ 306	5.05 $\times$ 10 <sup>5</sup> $\pm$ 1.14 $\times$ 10 <sup>5</sup>
Range	0 - 563	0.20 - 1.10	0 - 2,400	2.10 $\times$ 10 <sup>5</sup> - 9.50 $\times$ 10 <sup>5</sup>
Pancreatitis induced by sunflower oil (9)				
Mean $\pm$ S.E.	0	0.44 $\pm$ 0.06		2.22 $\times$ 10 <sup>2</sup> $\pm$ 1.78 $\times$ 10 <sup>2</sup>
Range		0.10 - 0.70	0	0 - 16.0 $\times$ 10 <sup>2</sup>
Pancreatitis induced by trypsin (6)				
Mean $\pm$ S.E.	38 $\pm$ 19	0.48 $\pm$ 0.11		2.46 $\times$ 10 <sup>4</sup> $\pm$ 1.04 $\times$ 10 <sup>4</sup>
Range	0 - 104	0.20 - 0.90	0	2.60 $\times$ 10 <sup>3</sup> - 7.40 $\times$ 10 <sup>4</sup>

flow in response to secretin originated in the small intestine and not the pancreas. The present findings unequivocally demonstrate that neither secretin nor secretin in combination with pancreozymin produce a significantly greater effect on pancreaticoduodenal lymph flow than physiological saline alone. Lipase activity of the pancreaticoduodenal lymph was augmented by secretin only when given in association with pancreozymin. It is likely that during "maximal stimulation" of the pancreas some lipase passes from the excretory ducts into the interstitial space and then into the lymph of the pancreas, presumably by the mechanism suggested by Duprez, Godart, Plattenborse and Dupont (12).

2. Bernard (13), when failing to remove surgically the pancreas, attempted to "dissolve" the gland by injecting edible oil into the gland of dogs. The small amount of tallow when injected into the pancreas of dogs, in fact caused atrophy of the exocrine portion of the gland. In the present experiments edible oil caused parenchymal atrophy of the exocrine pancreas in rats, while trypsin gave rise to histological changes closely resembling those seen in chronic pancreatitis of man. The activity of lipase in the pancreatic juice of rats following the administration of secretin and pancreozymin was less by orders of magnitude than the activity found in the similarly stimulated normal pancreas. During "maximal stimulation" of the previously trypsin or oil treated pancreas in rats there was no detectable lipase activity in the lymph draining from the thoracic duct. This observation indicates that, in chronic pancreatitis, the transport of lipase from the pancreas is impeded by obstruction of the lymph vessels in and around the pancreas. Similar conclusions can be drawn from the observations made by Sarles (14) and Reynolds (15) in chronic human pancreatitis.

The present experiments show that the "maximal stimulation" of the pancreatic secretion failed to enhance thoracic duct flow either in intact rats or in rats with chronic pancreatitis. The rate of thoracic duct lymph flow in rats used in the present experiments are corresponding to the published data (9, 16).

### Summary

Pancreaticoduodenal lymph flow in anaesthetized normal dogs was enhanced to the same extent by the infusion over 45 min into the superior pancreaticoduodenal artery of physiological saline (1 ml/min), 0.1 U/kg b.w./min of secretin dissolved in physiological saline (1 ml/min) and after pretreatment with physiological saline (1 ml/min) 0.1 U/kg b.w./min of secretin and 0.1 U/kg b.w./min of pancreozymin dissolved in physiological saline and infused at a rate of 1 ml/min. Lipase activity in the lymph was augmented only by the combined administration of secretin and pancreozymin.

Sunflower oil injected into the pancreas of rats caused severe damage to the acinar parenchyma, while the injection of 2.5 mg trypsin gave rise to changes characteristic to human chronic pancreatitis, 14 to 18 days after the intraductal injection of these substances. Lipase activity in the pancreatic juice following the intravenous injection of 4 U/kg b.w. of secretin and 4 U/kg b.w. of pancreozymin dissolved in 1 ml of saline and infused over a period of 45 min was less by orders of magnitude in the rats with histologically feasible damage to the pancreas. Secretin and pancreozymin failed to affect the rate of thoracic duct lymph flow, however, lipase activity in thoracic duct lymph could not be detected during stimulation of the histologically damaged pancreas.

The authors are indebted to Mr. G. Folly for the performance of mathematical-statistical analysis.

### References

- Bainbridge, F. A.: The lymph-flow from the pancreas. *J. Physiol. (Lond.)* 32 (1905), 1-7
- Razin, E., M. G. Feldman, D. A. Dreiling: The hormonal regulation of thoracic ductal lymph flow. The effect of secretin and related hormones on the thoracic ductal flow and composition in dogs. *J. Surg. Res.* 2 (1962), 320-331
- Dumont, A. E., H. Doubilet, J. H. Mulholland: Lymphatic pathway of pancreatic secretion in man. *Ann. Surg.* 152 (1960), 403-409
- Bartos, V., V. Brzek, J. Groh: Alterations in human thoracic duct lymph in relation to the function of the pancreas. *Amer. J. Med. Sci.* 252 (1966), 31-38
- Bartos, V., V. Brzek, J. Groh: Thoracic duct lymph amylase in patients with chronic pancreatic disease after administration of secretin. *Amer. J. med. Sci.* 252 (1966), 660-667
- Papp, M., P. Németh, J. Horváth: Pancreaticoduodenal lymph flow and lipase activity in acute experimental pancreatitis. *Lymphology* 4 (1971), 48-53
- Weber, H.: Mikromethode zur Bestimmung der Pankreaslipase im Serum. *Dtsch. med. Wschr.* 90 (1965), 1170-1174
- Heinkel, K.: Die Ratte als Versuchstier in der experimentellen Pankreasdiagnostik. *Klin. Wschr.* 31 (1953), 815
- Bollman, J. L., J. C. Cain, J. H. Grindlay: Techniques for the collection of lymph from the liver, small intestine or thoracic duct of the rat. *J. Lab. clin. Med.* 33 (1948), 1349-1352
- Rusznayk, I., M. Földi, Gy. Szabó: *Lymphologie Physiologie und Pathologie der Lymphgefäße und des Lymphkreislaufes.* Akadémiai Kiadó (1969), Budapest
- Dreiling, D. A.: The lymphatics, pancreatic ascites, and pancreatic inflammatory disease. *Amer. J. Gastroent.* 53 (1970), 119-131
- Duprez, A., S. Godart, R. Plattenborse, M. Dupont: Interstitial and lymphatic pathway of the external secretion of the pancreas. In: Heinkel, K., K. Schön, Pathogenese, Diagnostik, Klinik und Therapie der Erkrankungen des exokrinen Pankreas. Schattauer, Stuttgart 1964
- Bernard, M., Cl.: Mémoire sur le pancréas et sur le rôle du suc pancréatique dans les phénomènes digestifs particulièrement dans la digestion des matières grasses neutres. Baillière, Paris 1856
- Sarles, J. C.: Lymphographische Befunde bei der chronischen Pankreatitis. *Radiologie* 8 (1968), 167-169
- Reynolds, B. M.: Observations of subcapsular lymphatics in normal and diseased human pancreas. *Ann. Surg.* 171 (1970), 559-566
- Schooley, J.: Lymphocyte output and lymph flow of thoracic and right lymphatic ducts of anaesthetized rats. *Proc. Soc. exp. Biol. (N. Y.)* 99 (1958), 511-513

M. Papp, M.D., Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest 9, Ungarn, P. O. Box 67