Effect of Pilocarpine and Pancreatectomy on Amylase and Lipase Levels of Serum and Hepatic Lymph* (33733)

HIRA SINGH AND HUBERT A. APPERT
(Introduced by M. John Boyd)
Departments of Surgery and Physiology, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania 19102

Pilocarpine (1) and other secretory stimulants (2–4) are known to increase the enzyme content of the blood and lymph. Numerous other investigations have indicated that these enzymes originate from the pancreas and enter the blood to a large extent by means of the lymphatic pathway (5–8). Recently, it has been shown that rat liver synthesized amylase in isolated perfusion experiments (9, 10). Relatively little information, however, is available on the contribution of hepatic lymph to the increased enzyme levels observed in the thoracic duct lymph after the administration of various secretory stimulants. In this series of experiments, enzyme levels were studied in normal dogs as well as in pancreatectomized dogs in order to evaluate the effect of pilocarpine on the production of serum enzymes by the liver as well as the pancreas. In addition, the enzyme levels of the hepatic and portal blood were studied in order to determine the relative importance of the venous versus the lymphatic pathways.

Materials and Methods. Two series of experiments were performed. Mongrel dogs of either sex, weighing 15–20 kg, were used in these experiments. Six experiments were done in the first control series. The animals were subjected to laparotomy under pentobarbital anesthesia and controlled endotracheal ventilation. Lymphatic vessels were identified in the hilum of the liver after injecting Evan's blue dye subcapsularly in the liver. The efferent lymphatics from the hepatic lymph node were stripped of the surrounding tissue, and the largest one was incised. A polyethylene tube was inserted toward the liver, being held in place by ligatures.

The remaining efferent lymphatics from the hepatic lymph node, as well as those coming towards it from the direction of the pancreas and duodenum, were ligated (11). The right side of the thorax was then opened along the ninth intercostal space. A polyethylene tube was introduced into the inferior vena cava and passed down into the hepatic vein. This tube was available for sampling of hepatic venous blood.

Cannulation for sampling of the portal vein was accomplished by passing a similar tube through the superior mesenteric vein into the portal vein. Peripheral blood was sampled from the femoral vein.

Two control samples of heparinized lymph and blood were taken simultaneously at 20-min intervals from each of the catheters. Pilocarpine (20 mg diluted in 200 ml of normal saline) solution was administered intravenously by drip over a period of 40 min. Four samples of lymph and blood, each simultaneously at 20-min intervals, were taken after pilocarpine infusion (Table IA and B). The lymph samples were collected continuously throughout each 20-min interval.

In the second series of four experiments, hepatic lymphatic cannulation was done immediately after subjecting the animal to total pancreatectomy. Three samples of hepatic lymph and peripheral venous blood, each obtained at 15-min intervals, were taken simultaneously. Pilocarpine (20 mg diluted in 200 ml of normal saline) was administered intravenously by drip over a 40-min period. Three samples of lymph and blood, each at 15-min intervals, were taken simultaneously after pilocarpine infusion.

Amylase and lipase activities were determined by a modification of Somogyi-Nelson and Cherry-Crandall methods, respectively (12, 13).

Results. Effect of pilocarpine on lymph flows. There was no consistent or significant

* Supported by Grants AM-02723, HE-04182 from the National Institutes of Health.

TABLE I A. Amylase Concentration before and after Pilocarpine Infusion (Somogyi units/100 ml: mean ± SE).

Source	No. of expts.	Minutes prestimulation		Minutes after pilocarpine stimulation			
		40–20	20–0	0–20	20–40	40–60	60–80
Femoral blood[a]	6	4890 (±372)	3980 (±322)	5566 (±1188)	7376 (±1684)	10,211 (±3184)	13,075 (±4604)
Portal blood[a]	6	4500 (±161)	4500 (±530)	6900 (±1500)	7700 (±2200)	12,750 (±3500)	18,575 (±4300)
Hepatic blood[a]	6	5000 (±340)	4280 (±300)	5570 (±1200)	6200 (±1400)	9280 (±2700)	14,600 (±3600)
Hepatic lymph	6	7600 (±1200)	3800 (±1800)	17,400 (±10,800)	18,600 (±4900)	34,750 (±9700)	47,033 (±11,319)

[a] Serum analyzed.

TABLE I B. Lipase Concentration before and after Pilocarpine Infusion (Cherry-Crandall units/ml).

Source	No. of expts.	Minutes prestimulation		Minutes after pilocarpine stimulation			
		40–20	20–0	0–20	20–40	40–60	60–80
Femoral blood[a]	6	1.1 (±0.03)	1.1 (±0.07)	3.4 (±0.29)	4.2 (±0.3)	5.2 (±0.3)	5.7 (±0.4)
Portal blood[a]	6	1.2 (±0.2)	1.2 (±0.09)	3.7 (±0.4)	4.9 (±0.6)	6.0 (±1.1)	8.0 (±0.8)
Hepatic blood[a]	6	1.2 (±0.09)	1.5 (±0.3)	3.2 (±0.5)	4.3 (±0.6)	5.4 (±0.7)	6.6 (±0.5)
Hepatic lymph	6	2.8 (±0.3)	2.9 (±0.1)	7.9 (±2.0)	14.2 (±4.2)	14.2 (±2.5)	14.6 (±3.4)

[a] Serum analyzed.

change in volume in hepatic lymph flow with the dose of pilocarpine administered. Mean average lymph flow before pilocarpine administration was 0.30 ml/min, and after pilocarpine, 0.29 ml/min. This flow rate did not change significantly after pancreatectomy.

Serum and hepatic lymph enzymes. The concentration of amylase and lipase in serum from femoral, protal, and hepatic venous blood and hepatic lymph, measured before and after pilocarpine stimulation in the non-pancreatectomized series of dogs, is shown in Table IA and B. The mean serum and lymph enzyme concentrations for each of the two prestimulation periods and 4 individual post-stimulation periods are given in Table IA (amylase) and B (lipase), along with the standard error of each mean.

Table IIA and B, shows the effects of pancreatectomy and pilocarpine on the amylase and lipase activity of serum and hepatic lymph.

The significant rise in lymph and serum enzymes, following pilocarpine administration, shown in the first series of experiments (Table IA and B), was no longer seen when pilocarpine was administered after pancreatectomy (Table IIA and B). The administration of intravenous pilocarpine failed to increase the amylase and lipase concentration in lymph and serum following pancreatectomy.

TABLE II A. Amylase Activity before and after Pilocarpine (pancreatectomized dogs: Somogyi units/100 ml: Mean ± SE).

Source	No. of expts.	Minutes prestimulation			Minutes after pilocarpine stimulation		
		45–30	30–15	15–0	0–15	15–30	30–45
Peripheral blood (serum)	4	4275 ± 400	3850 ± 350	3900 ± 370	4125 ± 600	3700 ± 550	3500 ± 460
Hepatic lymph	4	2500 ± 500	2850 ± 580	2800 ± 520	1800 ± 47	2000 ± 48	2020 ± 90

TABLE II B. Lipase Concentration before and after Pilocarpine (pancreatectomized dogs).

Source	No. of expts.	Minutes prestimulation			Minutes after pilocarpine stimulation		
		45–30	30–15	15–0	0–15	15–30	30–45
Peripheral blood (serum)	4	1.1 ± 0.2	1.2 ± 0.1	1.2 ± 0.2	1.2 ± 0.3	1.0 ± 0.2	1.2 ± 0.5
Hepatic lymph	4	2.0 ± 0.2	1.4 ± 0.3	1.5 ± 0.2	1.5 ± 0.3	1.4 ± 0.3	1.8 ± 0.5

Discussion. In the present studies, the first series of experiments performed on dogs not subjected to pancreatectomy showed a significant rise in amylase and lipase concentration in portal lymph and serum following pilocarpine infusion. After pancreatectomy, no such response was seen. This indicates that the extrapancreatic sources of serum and lymph amylase and lipase after pancreatectomy were not appreciably affected by pilocarpine.

The conclusion that pilocarpine exerts its effects on serum and lymph enzymes because of its action on the pancreas is supported by observations made by other investigators(4–16). The volume and amylase concentration of pancreatic juice were shown to increase independently of bicarbonate ion concentration following pilocarpine infusion in sheep (14). Acetyl choline, administered alone or simultaneously with secretin in dogs, caused a significant increase in amylase concentration of blood (2, 15). Subcutaneous injection of acetyl-β-methylcholine chloride with eserine sulfate in normal dogs caused a prompt rise of amylase concentration in serum; but, depancreatized dogs lacked such response when similary treated(16).

In this series of experiments, an attempt was also made to determine the relative importance of the lymphatic versus the venous pathway in the entry of enzymes into the blood. In order to do this the enzyme levels of the portal lymph were compared with those of serum from the femoral, portal, and hepatic veins. Since the enzyme levels in the portal blood were usually higher than those of the femoral vein, it appears that, in addition to the lymphatic route, the venous route accounts for the absorption of a large amount of the enzymes. The enzyme levels of the hepatic venous blood, however, were usually slightly lower than those of the portal vein. As a result, the enzyme levels of the hepatic vein were nearly equal to the enzyme levels of the femoral vein. These findings, although preliminary in nature, suggest that, in addition to the dilutional effect of hepatic arterial blood, the liver may remove, from the serum, part of the enzymes which are liberated into the portal circulation by the abdominal organs.

In this series of experiments, an attempt was also made to determine the relative importance of the lymphatic versus the venous pathway in the entry of enzymes into the blood. In order to do this the enzyme levels of the portal lymph were compared with those of serum from the femoral, portal, and hepatic veins. Since the enzyme levels in the portal blood were usually higher than those of the femoral vein, it appears that, in addition to the lymphatic route, the venous route accounts for the absorption of a large amount of the enzymes. The enzyme levels of the

hepatic venous blood, however, were usually slightly lower than those of the portal vein. As a result, the enzyme levels of the hepatic vein were nearly equal to the enzyme levels of the femoral vein. These findings, although preliminary in nature, suggest that, in addition to the dilutional effect of hepatic artierial blood, the liver may remove, from the serum, part of the enzymes which are liberated into the portal circulation by the abdominal organs.

These findings indicate that in studies on enzyme levels of amylase and lipase in lymph collected from the portal area, it is important to exclude enzymes of pancreatic origin. In spite of ligating the lymphatic vessels in the hepatoduodenal ligament coming from the pancreas, complete exclusion of pancreatic enzymes without pancreatectomy could not be accomplished in this study.

Summary. Pilocarpine (20 mg diluted in 200 ml of normal saline), infused in 6 dogs for 40-min durations, caused a significant rise of amylase and lipase concentration in the serum and hepatic lymph collected in the portal area. In four pancreatectomized dogs, however, similarly treated with pilocarpine, there was no significant change in the amylase and lipase concentration of either the hepatic lymph or serum. Pilocarpine infusion did not cause any significant change in the hepatic lymph flow.

Since pilocarpine does not stimulate the secretion of amylase and lipase in the absence of the pancreas, it appears that pilocarpine enhances the secretion of these enzymes by its action on the pancreas alone. The higher enzyme concentrations after pilocarpine stimulation in portal venous blood in comparison with hepatic and femoral venous blood, suggests that a portion of the enzymes passing into the portal blood is taken up by the liver.

1. Osato, S., Tohoku J. Exptl. Med. **2**, 514 (1921).

2. Antopol, W., Schifrin, A., and Tuchman, L., Proc. Soc. Exptl. Biol. Med. **32**, 383 (1934).

3. Roe, J. H. and Goldstein, N. P., J. Lab. Clin. Med. **28**, 1334 (1943).

4. Burton, P., Hammond, E. M., Harper, A. A., Howat, H. T., Scott, J. E., and Varley, H., Gut **1**, 125 (1960).

5. Zucker, T. F., Newburger, P. G., and Berg, B. N., Am. J. Phys. **102**, 209 (1932).

6. Dumont, A. E., Doubilet, H., and Mulholland, J. H., Ann. Surg. **152**, 403 (1960).

7. Bartos, V., Brzek, V., and Groh, J., Am. J. Med. Sci. **252**, 31 (1966).

8. Vega, R. E., Appert, H. E., and Howard, J. M., Ann. Surg. **166**, 995 (1967).

9. Rutter, W. J., Arnold, M., Brosemer, R. W., and Miller, J. A., J. Biol. Chem. **236**, 1259 (1961).

10. Brighenti, L., Ottolenghi, C., Passerini, L., and Barnabei, O., Biochim. Biophys. Acta **130**, 272 (1966).

11. Cain, J. C., Grindlay, J. H., Bollman, J. L., Flock, E. V., and Mann, F. C., Surg. Gynecol. Obstet. **85**, 559 (1947).

12. Somogyi, M., J. Biol. Chem. **125**, 399 (1938).

13. Cherry, I. S. and Crandall, L. A., Jr., Am. J. Physiol. **100**, 266 (1932).

14. Magee, D. F., J. Physiol., (London) **158**, 132 (1961).

15. Lin, T. M. and Ivy, A. C., Am. J. Physiol. **189**, 361 (1957).

16. Friedman, I. and Thompson, W. R., Ann. Surg. **104**, 388 (1936).

Received Nov. 6, 1968. P.S.E.B.M., 1969, Vol. 130.