External pancreatic response to food and its relation to the maximal secretory capacity in dogs¹

F. W. HENRIKSEN AND H. WORNING

From the Institute for Experimental Research in Surgery, University of Copenhagen, the Central Laboratory, Blegdamshospitalet, Copenhagen, and the Medical Department P, Rigshospitalet, Copenhagen, Denmark

Few attempts have been made to investigate the normal pancreatic response to the stimulus of a meal (Annis and Hallenbeck, 1951; Cooke, Nahrwold, and Grossman, 1967). This may depend upon difficulties in obtaining physiological conditions during the investigation (Gregory, 1962). A comparison between the physiological pancreatic response to food and to exogenous stimuli has, to our knowledge, not been carried out. In the present investigation we have tried to study the pattern of the pancreatic response to food during conditions as close as possible to normal physiological conditions. The secretory rates thus obtained were compared with the maximal secretory capacity of the gland, determined as recently described (Henriksen, 1968).

The secretory rates were compared with the secretion rates following stimulation with secretin or pancreozymin in an attempt to estimate the endogenous release of secretin and pancreozymin during digestion of the meal.

MATERIAL AND METHODS

Four mongrel dogs were used (body weight 19 to 20 kg). In three of the dogs (nos. 64-208, 64-224, and 65-231) a modified Thomas cannula (1941) was inserted into the duodenum exactly opposite the main pancreatic duct. The last dog (no. 65-201) was operated upon by the technique described by Preshaw and Grossman (1965), allowing for collection of the pancreatic juice from a duodenal pouch. The accessory pancreatic duct was ligated and a Thomas cannula was inserted into the stomach at the most dependent part in all the dogs. The dogs were used in experiments after a recovery period of at least three weeks.

The dogs were kept alive for 11 to 47 months. They all remained in a good nutritional state, without loss of weight. At the end of the investigations, the dogs were sacrificed. A pancreatograph through the main pan-

creatic duct showed that the ductal system was normal in all dogs, as was the pancreas.

During the experiments the dogs were standing in a sling harness. The gastric cannula was opened in all basal periods, and in the experiments using exogenous stimulation, to prevent gastric juice from entering the duodenum. In the three dogs with Thomas fistulas a glass catheter connected to a glass syringe by a short polyvinylchloride tube was inserted into the main pancreatic duct through the duodenal Thomas cannula. The dependent plunger of the syringe exerted a slightly negative pressure. The dead space of the collecting system was 0.4 ml. The pancreatic juice from the dog with the duodenal pouch was collected through a metal cannula. The dead space of this collecting system was 0.7 ml, the volume of the pouch not included.

During the feeding experiments the duodenal cannula was closed by a rubber diaphragm through which the glass catheter and a polyethylene tube were led, through which a bicarbonate solution (120 m-equiv/l.) was infused into the duodenum opposite the opening of the pancreatic duct, synchronously with and equal to the amount of bicarbonate secreted by the pancreas. In dog no. 64-208 four experiments were performed without simultaneous infusion of bicarbonate into the duodenum.

Except for small amounts of water, the dogs were fasted for 18 hours before the experiments, and the fasting (basal) secretion was collected for at least two periods of 15 minutes each. After the basal period the dogs were given a meal composed of 400 g of boiled pork hearts, cut in lumps and freed from all visible fat. The meal was eaten in one to two minutes. The pancreatic juice was collected in 10-minute periods during the first hour, and in 10 or 20-minute periods during the subsequent hours. The total period of collection was ordinarily between two-and-a-half and three hours. In some experiments the pancreatic catheter slid out, but usually it was replaced within a few minutes. The mean secretion rate in such periods was calculated from the amounts secreted in the rest of the period. If the catheter had been removed for more than a few minutes the result for that period was discarded.

In the three dogs with Thomas fistulas the maximal secretory capacity of the pancreatic gland was determined by combined stimulation with secretin² and pan-

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²Boots Pure Drug Co., Nottingham, England.

creozymin¹ (Henriksen, 1968) as single rapid injections into a peripheral vein. Secretin was given as 6 Crick, Harper, and Raper units/kg body weight (CHR-U/kg body weight) in all dogs, and was combined with pancreozymin, varying in doses from 6 to 12 Ivy dog units/kg body weight (ID-U/kg body weight). Two to four observations were made at each dose level to determine the maximal secretory capacity. In dogs nos. 64-208, 64-224, and 65-231 the entire dose-response curves for secretin and pancreozymin respectively were determined.

The volume of the pancreatic secretion and the concentration of bicarbonate and protein in the samples were measured. The total concentration of CO₂ was determined according to Van Slyke's manometric method (Van Slyke and Neill, 1924), and is referred to as 'bicarbonate'. The concentration of protein was estimated spectrophotometrically at 280 nm after appropriate dilution of the samples with Sørensen phosphate buffer pH 6.8. The extinction values were converted to concentrations of protein by means of a factor empirically determined through Kjeldahl analysis on pooled canine pancreatic juice.

RESULTS

A different technique for obtaining pancreatic juice was used in dog no. 65-201, and results were compared with the results from the other three dogs. If no significant difference was observed, a mean value common to all dogs was calculated.

The effect of a synchronous infusion of bicarbonate into the duodenum on the pancreatic response after ingestion of a meal is illustrated in Table I. The secretion rates for fluid and bicarbonate were significantly higher when no bicarbonate was instilled. The protein secretion was independent of the infusion.

¹Cholecystokinin-pancreozymin was obtained from Professor E. Jorpes and Dr V. Mutt, Stockholm, Sweden.

TABLE I

PANCREATIC SECRETION RATE AFTER INGESTION OF A MEAT MEAL BY DOG NO. 64-208

		A ¹	B	p ²
Volume (ml/min)	m ²	0.68	1.12	<0.05
	n	7	4	
Bicarbonate (μ-equiv/min)	m	81	139	<0.05
	n	7	4	
Protein (mg/min)	m	8.6	8.6	n.s.
	n	7	4	

¹Group A: sodium bicarbonate solution (120 m-equiv/l) infused into the duodenum synchronously with the juice secreted. Group B: no bicarbonate infusion

²m = mean of observations in the period 30-180 min.

n = number of observations

³p = probability of difference between secretion rates

The secretion rates for fluid, bicarbonate, and protein in different periods after ingestion of the meal are shown in Table II. The results for two representative experiments are shown in Figs. 1 and 2, where it can be seen that the secretion of all components increased above basal level within five to 10 minutes after the ingestion of the meal.

The secretion rates for fluid and bicarbonate were significantly lower in the first 30 minutes than in the subsequent 150 minutes, when they usually remained at a constant level (Table II). Between 180 and 240 minutes the secretion rate decreased slightly.

The secretion rate for protein was significantly higher in dog no. 65-201 ($p < 0.01$) than in the remaining dogs (Table II). The highest secretion rates in all dogs were observed in the period 0-30 minutes, after which they remained at a lower level (Table II), but apart from this period, the rate was remarkably constant (Figs. 1 and 2).

In the individual dog, the pancreatic response varied considerably from experiment to experiment (Table III), but the pancreatic secretion was main.

TABLE II

PANCREATIC SECRETION RATES OF FLUID, BICARBONATE, AND PROTEIN IN THE PERIOD 0-180 MINUTES AFTER INGESTION OF A MEAT MEAL

Dog No.	Minutes	Volume (ml/min)				Bicarbonate (μ-equiv/min)				Protein (mg/min)			
		0-30	30-60	60-120	120-180	0-30	30-60	60-120	120-180	0-30	30-60	60-120	120-180
64-208	m ¹	0.39	0.64	0.73	0.66	37	76	87	75	9.5	8.6	9.1	8.5
	n	6	7	5	4	6	7	5	4	4	6	5	4
64-224	m	0.53	0.89	1.00	1.06	62	119	136	143	10.3	9.3	9.6	10.0
	n	7	7	5	4	7	7	5	4	5	7	5	4
65-201	m	0.63	0.84	0.76	0.77	71	105	92	98	14.1	10.9	8.7	9.5
	n	8	8	2	1	8	8	2	1	4	4	2	1
65-231	m	0.51	0.71	0.57	0.43	61	93	72	54	10.7	7.9	7.3	5.9
	n	5	6	5	3	5	6	5	3	5	6	4	1
All (weighted mean)		0.52	0.77	0.77	0.75	59	99	98	94	10.2 ²	8.6 ²	8.8 ²	8.9 ²

¹m = mean

n = number of observations

²Dog no. 65-201 not included

FIG. 1.

FIG. 2.

FIGS. 1-2. The pattern of pancreatic secretion of fluid, bicarbonate, and protein in response to ingestion of a meat meal.

tained at a relatively constant level. The secretion rate for protein showed smaller variations than did those for fluid and bicarbonate (Table III). The concentration of bicarbonate in the pancreatic juice was highly reproducible (Table III).

The peak secretion rates for fluid and bicarbonate, *ie*, the highest secretion rate obtained in any 10-minute period occurred at different periods after ingestion of a meal. In 12% of the experiments the peak occurred in the first 30 minutes, in 75% between 30 and 180 minutes. The peak secretion of protein was, however, observed in the first 10 minutes after the ingestion of the meal in all experiments.

The individual peak secretion rates and the mean secretion rates between 30 and 180 minutes following the intake of food were compared to the maximal secretion rates after combined exogenous stimulation (Table IV). On average, the mean secretion rates for fluid and bicarbonate amounted to 37% of the maximal rate and the protein secretion rate to 21% of the maximal secretion rate. The peak secretion rate was roughly double the mean secretion rate.

The maximal secretory capacity was not attained in any experiment.

In an attempt to calculate the endogenous release of secretin and pancreozymin in the postprandial period, the mean secretion rates for fluid, bicarbonate, and protein after ingestion of the meal were compared with the secretion of fluid and bicarbonate in the first 10 minutes after isolated stimulation with secretin, and to the secretion of protein in the 10 minutes after isolated stimulation with pancreozymin. The figures in Table V are calculated by interpolation on the dose-response curves for secretin and pancreozymin. As evaluated from the secretion rates, the endogenous release of secretin and of pancreozymin was equal to 0.3-1.0 CHR-U of secretin/kg body weight and to 0.1-0.6 ID-U of pancreozymin/kg body weight (Table V).

DISCUSSION

Essential in the present investigation is whether the secretory pattern observed equals the actual secre-

TABLE III

VARIATION IN PANCREATIC MEAN SECRETION RATES AND MEAN BICARBONATE CONCENTRATION IN THE PERIOD 30-180 MINUTES AFTER INGESTION OF FOOD

Dog No.		Secretion Rates			Bicarbonate Concentration
		Volume	Bicarbonate	Protein	
64-208	v ¹	32.1	40.6	13.6	13.2
	n	7	7	7	7
64-224	v	14.1	15.1	10.6	2.3
	n	7	7	7	7
65-201	v	40.1	47.5	12.3	12.3
	n	8	8	4	8
65-231	v	35.6	41.0	24.3	7.6
	n	6	6	6	6

¹v = coefficient of variation (SD × 100/mean)

n = number of observations

TABLE IV

PANCREATIC SECRETION RATES IN THE 30-180 MINUTES AFTER INGESTION OF A MEAT MEAL AND THE PEAK SECRETION RATES COMPARED WITH THE MAXIMAL SECRETORY CAPACITY

Dog No.		Volume (ml/min)	Bicarbonate (μ -equiv/min)	Protein (mg/min)
64-208	m ¹	0.67 (29%) ^a	79 (26%)	8.7 (20%)
	p	1.20 (51%)	160 (52%)	13.3 (30%)
	c	2.34 (100%)	306 (100%)	43.8 (100%)
64-224	m	0.97 (46%)	130 (52%)	9.6 (24%)
	p	1.35 (64%)	187 (74%)	20.1 (50%)
	c	2.11 (100%)	252 (100%)	40.2 (100%)
65-201	m	0.82	102	10.1
	p	1.50	198	24.8
	c	—	—	—
65-231	m	0.66 (36%)	77 (33%)	7.5 (18%)
	p	1.27 (69%)	177 (77%)	23.4 (57%)
	c	1.85 (100%)	231 (100%)	41.3 (100%)
All ^a	m	—	— (37%)	— (21%)
	p	—	— (68%)	— (46%)

¹m = mean of all observations in the period 30-180 minutes

p = peak secretory rate (10 minutes)

c = maximal secretory capacity

^am and p calculated as a percentage of c

^bDog no. 65-201 not included

TABLE V

CALCULATED ENDOGENOUS RELEASE OF SECRETIN AND PANCREOZYMIN AFTER INGESTION OF A MEAT MEAL

Dog No.	Secretin ¹		Pancreozymin ²
	Fluid Secretion	Bicarbonate Secretion	Protein Secretion
64-208	0.5	0.5	0.4
64-224	0.8	1.0	0.6
65-231	0.3	0.3	0.1
Mean	0.5	0.6	0.4

¹Crick, Harper, Raper units/kg body weight

²Ivy dog units/kg body weight

tion under normal physiological conditions. The surgical procedures did not damage the pancreatic gland as proven by the postmortem investigation. Interductal anastomosis are present in the dog between the accessory and the main duct system (Nielsen and Bishop, 1954). When the accessory duct is ligated, as in the present investigation, the secretion of juice through the main duct will be quantitative if the interductal anastomoses are sufficient and no other accessory ducts are present. Postmortem pancreatography excluded the existence of other accessory ducts. The normal appearance of the pancreas indicated that the interductal anastomoses were sufficient to prevent fibrosis, secondary to the ligation of the accessory duct. The collection of pancreatic juice through the catheter in the main pancreatic duct was considered quantitative, as this was the case in the investigations with exogenous stimulation of the pancreas.

Diversion of the pancreatic juice from the duodenum after ingestion of a meal induces increased secretion of fluid and bicarbonate from the pancreas

but has no influence on the secretion of protein (Annis and Hallenbeck, 1951; Cooke *et al*, 1967, Table I). The secretion obtained when the secreted pancreatic juice (Annis and Hallenbeck, 1951; Cooke *et al*, 1967) or an equivalent amount of bicarbonate is instilled into the duodenum must be considered normal, as the instillation secures a normal pH and PCO₂ in the duodenal lumen.

The decrease in proteolytic digestion in the duodenum by excluding the pancreatic enzymes from the duodenum may involve a decreased stimulation of the protein secretion (Wang and Grossman, 1951). However, Cooke *et al* (1967) demonstrated that the protein secretion following ingestion of a meal was independent of whether the pancreatic juice was replaced in the duodenal lumen or not.

It seems justified therefore to conclude that the secretory pattern obtained was very close to the actual secretion under normal physiological conditions.

No difference was observed in the secretion rates of fluid and bicarbonate in the dog operated on after the method described by Preshaw and Grossman (1965) and those supplied with a Thomas cannula. However, the secretion rate of protein was significantly higher in the dog supplied with a duodenal pouch (no. 65-201). This may be explained by the finding of Preshaw and Knauf (1966) who reported the duodenum to secrete a proteolytic enzyme with pepsin-like activity in response to the stimulus of food.

Cooke *et al* (1967) recently reported on the pancreatic response to food in dogs with duodenal pouches. In spite of a different technique and different amounts of food, the pattern and the secretion rates

of fluid and bicarbonate in the present investigation were almost identical to those observed by Cooke *et al* (1967). However, the mean secretion rate of protein was about three times as high in the material of Cooke *et al* as in the present investigation. This difference cannot be explained by the additional protein contained in the duodenal juice (Table II, dog no. 65-201). It is due to a different standardization of the analytical methods. In both investigations the concentration of protein was estimated spectrophotometrically at wavelength 280 nm. In the present material a standard solution of pooled canine juice was used, whereas Cooke *et al* employed bovine serum albumin as a standard. An estimate of the extinction values at 280 nm for solutions with identical concentrations of protein-N showed the extinction coefficient for canine pancreatic juice to be three times that for bovine serum albumin. By using this substance as standard, the protein concentration in canine pancreatic juice will be estimated to three times the actual concentration.

The secretion rates for fluid and bicarbonate were significantly lower in the initial 30 minutes than in the subsequent 150 minutes. This is in accord with results obtained with continuous stimulation with secretin (Baron, Perrier, Janowitz, and Dreiling, 1963; Preshaw, Cooke, and Grossman, 1966a) where steady state secretion was reached in about 15 to 60 minutes after stimulation was started.

The protein secretion reached the highest values in the initial 30 minutes in all the dogs (Table II). The same applied to secretion after sham feeding (Preshaw, Cooke, and Grossman, 1966b) and after stimulation with secretin (Delcourt, 1961; Preshaw *et al*, 1966a). The fact is explicable as a 'washout' phenomenon. In the subsequent period, the secretion rate for protein was remarkably constant, and to a certain degree independent of the secretion rates for fluid and bicarbonate (Figs. 1 and 2). The gastric secretion of acid after ingestion of a meal of the type applied here is equal to the maximal secretion obtainable with histamine (Rune and Henriksen, 1967). The results presented here clearly indicate that pancreatic secretion after ingestion of a meal is far from maximal (Table IV). The mean secretion rates for fluid and bicarbonate were about one third of the maximal obtainable, whereas that for protein amounted to about 20% of the maximal level. The secretion rate varied considerably from day to day in the individual dog (Table III), but not even the individual peak secretion rates attained the maximal secretion level (Table IV).

The figures for calculated endogenous release of secretin and pancreaticoimin after ingestion of a meal (Table IV) must be taken with some reservations. Single injections and not a continuous infusion of

secretin and pancreaticoimin were used for the comparison, and the potentiation between secretin and pancreaticoimin (Henriksen and Worning, 1967) was not taken into account in the calculations.

SUMMARY

In four dogs the pancreatic secretion of fluid, bicarbonate, and protein after ingestion of a meat meal has been studied under conditions closely similar to those occurring in the normal postprandial period.

The secretion rates for fluid and bicarbonate were lower in the initial 30 minutes than in the subsequent two-and-a-half hours, in which they usually remained at a constant level. The secretion rate for protein was highest in the initial 30 minutes, probably on account of a 'washout' phenomenon, and remained at a constant, but lower level in the subsequent two-and-a-half hours. Secretion varied considerably from day to day in the individual dog, with the least variation in the bicarbonate concentration. The secretion rate for protein showed smaller variations than did the secretion rates for fluid and bicarbonate.

The mean secretion rates for fluid and bicarbonate were about one third of the maximal secretion rates obtainable with combined stimulation with secretin and pancreaticoimin. The mean secretion rate for protein attained one fifth of the maximal rate.

A guarded estimate of the endogenous release of secretin and pancreaticoimin was attempted.

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