Effect of Excluding Pancreatic Juice from Duodenum on Secretory Response of Pancreas to a Meal. (18785)

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In experiments in which all pancreatic juice is collected and thereby prevented from reaching its physiologic destination, an abnormal situation is created which could alter the natural pancreatic secretory process. We have carried out the following experiments to ascertain whether the postprandial secretory activity of the pancreas is modified when the juice is excluded from the duodenum.

Methods. Pancreatic juice was obtained from 4 trained dogs by Scott and associates' (1) modification of the method of Thomas and Crider(2). A metal cannula was installed in the duodenum directly opposite the opening of the major pancreatic duct. At the time of this surgical procedure, the minor pancreatic duct was ligated and divided. During experiments the major pancreatic duct was cannulated, via the larger metal cannula, with a fine glass cannula. Between experiments the glass cannula was removed, the metal cannula was capped, and pancreatic juice flowed into the duodenum in a normal manner. Pancreatic secretion was stimulated by the feeding of 200 g of lightly cooked horse meat. Juice was collected for 3 hours after feeding. Four series of experiments were performed. In the first, all pancreatic juice was collected and withheld from the animals. In the second, pancreatic juice was collected, its volume was measured, and it was placed in the duodenum at intervals of 5 minutes. Thus, the animals retained the pancreatic juice they secreted, though they received it in bursts and from 0 to 5 minutes later than would have been the case under normal circumstances. In the third, all juice was collected and withheld, but equivalent volumes of isotonic saline solution were placed into the duodenum, at intervals of 5 minutes. In the fourth, all juice was collected and withheld, and a tenth normal solution of sodium bicarbonate was continuously instilled into the duodenum through the Thomas cannula during the experiment by means of a continuous drip apparatus of the type commonly used for intravenous fluid therapy. Fifteen centimeters of sodium bicarbonate were instilled during the 15 minute period preceding feeding, 120 cc per hour for the first 1½ hours after feeding and 40 to 60 cc per hour for the second 1½ hours after feeding. Thus the quantity of bicarbonate solution reaching the duodenum was from 2 to 4 or more times the quantity reaching it in the form of pancreatic juice during normal digestion. The volume of juice secreted was measured in all instances. Except when all juice was instilled into the duodenum, measurements were also made of alkali content by the method of Van Slyke(3), and of total nitrogen content by the micro-Kjeldahl technic.

Results. Data regarding the volume, bicarbonate content and total nitrogen in pancreatic juice secreted during the first 3 hours after feeding are presented in Table I. Considerable variation occurred in the results obtained. When all juice was replaced into the duodenum, the volume of juice secreted by the 4 dogs in 3 hours averaged 40.7, 42.1, 55.2, and 73% of comparable figures obtained when all juice was removed. Only in dog 3 did the ranges of determinations overlap. We interpret the differences as being significant. Because all juice was replaced in this series of experiments, no values for bicarbonate or nitrogen content were obtained.

When isotonic saline solution was substituted for pancreatic juice in the duodenum, volumes secreted during the 3 hour period averaged 75, 92.8, 74.1, and 113.1% of the mean values obtained when all juice was re-

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3. Van Slyke, D. D., *J. Biol. Chem.*, 1922, v52, 495.

TABLE I. Total Volume, Bicarbonate and Nitrogen in Canine Pancreatic Juice Secreted During 3 Hr After Ingestion of a Meal of Meat.

Exp.	Dog	No. of exp.	Vol., ml Mean	Vol., ml Range	Vol., ml %*	Bicarbonate content, mEq Mean	Bicarbonate content, mEq Range	Bicarbonate content, mEq %*	N content, mg Mean	N content, mg Range	N content, mg %*
All juice removed	1	5	51.9	41.6– 64	100	6.5	4.9– 8.6	100	126	111–139	100
	2	4	76	63.8– 85.1	100	10.5	8.4–12.1	100	200	180–236	100
	3	7	148.5	91.7–176	100	21.9	13.1–26.9	100	206	156–256	100
	4	4	97.9	81.3–116.2	100	13.1	10.8–15.9	100	185	150–218	100
All juice replaced	1	4	21.1	12.2– 26.5	40.7	—	—	—	—	—	—
	2	3	32	27.3– 38	42.1	—	—	—	—	—	—
	3	7	81.9	45.2–112.4	55.2	—	—	—	—	—	—
	4	4	71.5	56.4– 81.1	73	—	—	—	—	—	—
Juice replaced by isotonic saline sol.	1	3	38.9	30.5– 52.1	75	5	3.7– 7.4	76.9	124	108–140	98.4
	2	3	70.5	65.6– 72.9	92.8	9.8	9.4–10.1	93.3	196	139–240	98
	3	4	110.1	80.6–130.7	74.1	15.8	11.3–19.3	72.1	190	171–212	92.2
	4	3	110.7	87.7–144.9	113.1	15.8	11.8–22.1	120.6	176	148–216	95.1
HCO_3 sol. instilled into duodenum	1	3	21	15 – 27.9	40.5	2.3	2.1– 2.4	35.4	93	65–136	73.8
	2	3	27.2	23.5– 32.3	35.8	3	2.7– 3.6	28.6	127	104–157	63.5
	3	3	84.1	73 – 94	56.6	11	9.1–12.8	50.2	175	152–202	85
	4	2	51	38.9– 63.2	52.1	6.4	4.2– 8.6	48.9	149	122–176	80.5

* Mean values expressed as % of comparable values obtained when all juice was removed.

moved and nothing was replaced into the duodenum. Ranges overlapped in all 4 dogs and we do not consider the differences to be significant. Likewise, replacement of juice by isotonic saline solution did not significantly alter the output of bicarbonate or nitrogen from that which occurred when nothing was substituted for the pancreatic juice.

Introduction of the stated quantities of sodium bicarbonate solution into the duodenum, however, reduced the mean volume of pancreatic juice secreted to 40.5, 35.8, 56.6, and 52.1% of values observed when no replacement was made, values essentially similar to those obtained when juice was returned to the duodenum. Again, ranges of determinations overlapped only in dog 3, in which the secretory response seemed more variable than in the other dogs. The total bicarbonate secreted was also markedly less when sodium bicarbonate was instilled into the duodenum. The total nitrogen, though less on the average than that observed when no bicarbonate solution was placed in the duodenum, again showed overlapping of the ranges of values in 3 of the 4 dogs and the difference is of doubtful significance.

When data for successive samples collected during periods of 15 minutes were plotted against time, it was noted that when pancreatic juice was replaced into the duodenum the general shape of the secretory curve remained unchanged though it was quantitatively depressed.

Comment. Of the 4 series of experiments in the present study, that in which pancreatic juice was returned to the duodenum most closely approximates the normal state. A similar quantity of juice was secreted when large amounts of sodium bicarbonate were substituted for pancreatic juice. A larger quantity of juice was secreted when pancreatic juice was excluded from the duodenum both with and without the intraduodenal instillation of a nonalkaline solution (isotonic saline solution). These events can be explained by assuming that the alkalinizing effect of pancreatic juice normally secreted in response to a meal is sufficient to minimize the number of instances in which the pH of duodenal contents becomes low enough to activate the secretin mechanism, and that this alkalinizing effect does not occur when all pancreatic juice is excluded from the duodenum. If the acid-secretin mechanism played a dominant role in stimulation of postprandial pancreatic secretion one would expect instillation of an excess of alkali into the duodenum to result in a still lower volume of juice than that secreted when normally secreted pancreatic juice alone was replaced. Since this was not the case, the data suggest that the acid-secretin mechanism does not play a major role in the response of the canine pan-

creas to a meal of meat under normal circumstances. Studies of the importance of products of digestion of protein and fat as pancreatic secretagogues and the discovery of pancreozymin have demonstrated other physiologic mechanisms capable of stimulating the pancreas during normal digestion. The protective role of the acid-secretin mechanism in instances of gastric hypersecretion seems unquestionable. Data concerning this concept have been reviewed recently by Thomas(4).

Conclusions. 1. When canine pancreatic juice secreted in response to a meal was permanently removed by the method used in these experiments, the total volume of juice secreted was significantly greater than was the case when the juice was replaced in the duodenum. 2. A similar reduction in the volume of postprandial pancreatic secretion occurred when tenth normal sodium bicarbonate solution was instilled into the duodenum in place of pancreatic juice. 3. No similar reduction in secretion occurred when equivalent volumes of isotonic saline solution were substituted for pancreatic juice.

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Probable Mechanism of Alizarin Inhibition of Calcification. (18786)

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Alizarin red S has been found to prevent calcification in embryonic metatarsals without interference with normal cartilage and fibrous connective tissue growth(1). This paper describes our attempts to determine the mechanism of the inhibition by observing the effects of alizarin on the enzyme, phosphatase, as well as on the calcium and phosphate ions.

Experimental. Alizarin and bone phosphatase. Because of the diversity of materials and methods employed, these will be presented along with our experimental results. The effect of alizarin on phosphatase production by many paired metatarsals and fibulas from 10-day-old chick embryos was studied first. One member of each pair was placed on the surface of a hanging drop consisting of equal parts of blood plasma and embryonic extract; the other member was treated similarly save that the medium contained one drop of a 1% Seitz-filtered aqueous alizarin solution to 120 drops of medium. At this dye concentration, calcification is inhibited. After 4 days of cultivation both members of live and fixed (95% alcohol) preparations were placed either in calcium nitrate as controls or in glycerophosphate substrates for phosphatase visualization. The preparations were then treated with silver nitrate, washed with distilled water and mounted in glycerine gelatin. Phosphatase was found abundantly distributed in the paired bones from both the alizarin-containing and alizarin-free media. Thus, no indication of bone phosphatase inhibition by alizarin in cultures was observed. The *in vitro* effect of alizarin on phosphatase was examined next. To chick alkaline bone phosphatase(2), assaying 46.8 Bodansky units per 100 ml(3), alizarin red S was added in an amount (0.6 mg per ml) approximately 6 times that required to inhibit calcification in tissue culture. There was no significant reduction in phosphatase activity (45.2 Bodansky units per 100 ml). Since bone phosphatase is not inactivated by alizarin either

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