10. Zampa, G. A., Geminiani, G. D., and Odifreddi, M. T., Res. on Steroids, Rome, p. 323 (1965).
11. Watanabe, M. and Baril, E. F., J. Cell Biol. 35, A139 (1967).
12. Feigin, R. D., Klainer, A. S., and Beisel, W. R., Nature 215, 512 (1967).

Received June 3, 1968. P.S.E.B.M., 1968, Vol. 129.

Canine Pancreatic Secretion in Response to Acceptable and Aversive Taste Stimuli* (33317)

HAROLD R. BEHRMAN[1,2] AND MORLEY R. KARE[3]
Sensory Physiology Laboratory, North Carolina State University, Raleigh, North Carolina 27607

In lower vertebrates, the chemical senses have been reported to serve as distance and directional sensors, and also as detectors of such critical environmental factors as salinity (1). In higher animals, the physiological role of these senses may be more complex. Taste encourages eating, influences the volume and character of saliva flow, and has been implicated in the selection of nutrients (2) and in intake regulation (3). Since the oral cavity is the primary route by which the body receives food, it seems reasonable to consider that taste may affect gastrointestinal function in some controlling or initiating capacity. For example, Pavlov (4) demonstrated that sham feeding in dogs results in a transient increase in the rate of pancreatic secretion: Preshaw *et al.* (5) confirmed this finding. No cephalic phase of gastric secretion occurred when the dog chewed neutral-tasting subtances, whereas appealing foods resulted in copious gastric secretion (4). Crittenden and Ivy (6) reported an increased rate of pancreatic secretion in enterectomized dogs when they were fed meat broth, but water or milk were without effect. Thus, it is possible that the sensory qualities of the food influence gastrointestinal secretions.

In the present study, interest was centered on the effects of palatable and aversive food stimuli on pancreatic secretion in the dog. The stimuli consisted of water and aqueous solutions of sucrose, citric acid, and quinine mixed with a portion of the basal diet. Both flow rate and protein content of the pancreatic juice were studied.

Materials and Methods. An adult male and a spayed female dog weighing 20 and 25 kg, respectively, were fitted with gastric and intestinal cannulae. The intestinal cannula permitted intubation by the major pancreatic duct according to the method outlined by Thomas (7). This permitted collection, from conscious animals, of pancreatic juice uncontaminated with intestinal contents. At least 6 months elapsed between operation and experimentation. The animals were maintained in a temperature-controlled room (23°). They were allowed to run free in a compound (6× 6 ft) except when individually caged during feeding. Between test days, a commercial diet (Speak)[4] was regularly fed once daily (20 g/kg) at 9 a.m. On test days, pancreatic collections and test feeding occurred 24–30 hours after the last regular meal.

The animals were suspended in a canvas sling during the collection of pancreatic juice; they passively accepted this physical restriction after several preliminary trials. Before making the test feedings and collections, the gastric and intestinal cannulae were

* This investigation was supported in part by NIH Grant NB-03896 and FR-0011-05.

[1] Present address: Endocrinology Research Laboratories, Harvard Medical School, Shields Warren Radiation Building, 50 Binney Street, Boston, Massachusetts 02115

[2] Recipient of grant-in-aid from General Foods Corporation.

[3] Present address: Monell Chemical Senses Center, University City Science Center, University of Pennsylvania, Philadelphia, Pennsylvania 19104.

[4] Kindly supplied by General Mills Corporation.

opened, the accumulated contents drained, and the pancreatic duct was intubated. During the test, the gastric cannula was left open to permit continuous drainage of gastric contents. Pancreatic secretions were collected in a 24-ml graduated cylinder surrounded with ice. Basal secretion was measured for 15 min just prior to test. A second collection was then made for 15 min after the first oral stimulus. The food stimuli were administered as 8-g suspensions at 3-min intervals. A preliminary study, using a water-dye mixture, showed that this amount and frequency of presentation allowed complete drainage from the gastric cannula with no overflow into the duodenum. Flow rate was read directly at the conclusion of the basal and treatment collections. The protein content of the pancreatic juice was estimated using the biuret reaction (8) with bovine serum albumin as the standard.

The taste stimuli were prepared immediately before use by suspending 10 g of ground basal diet in 30 ml of distilled water, or of 10% sucrose solution (w/v), 0.001 *M* quinine hydrochloride or 0.1 *M* citric acid. It has been reported (6) that administration of water alone elicits no pancreatic response. Some preliminary experiments showed, however, that mixing a portion of basal diet with water would markedly stimulate pancreatic flow. It was felt that the stimuli could be better compared under the conditions of high flow occurring when some basal diet was included than under the probable low flow expected if presenting the solutions alone. Further, inclusion of the basal diet would render the test conditions more "natural."

Both dogs were tested with all four treatments. The order of treatment was determined randomly for each dog. After all four treatments had been administered once to each dog the experiment was repeated to provide two replicates. Responses to treatment were computed as the ratios, test volume/basal volume, and test protein secretion/basal protein secretion. These ratios were then subjected to analysis of variance.

Results and Discussion. The average ratios of test to basal for both flow and protein secretion are shown in Fig. 1.

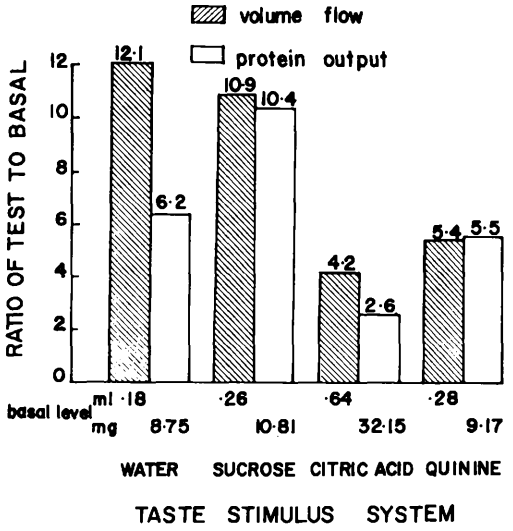

FIG. 1. Mean ratio of test to basal for pancreatic volume flow and protein output in response to presentation of food stimuli.

The analysis of variance for the flow and protein response are given in Table I.

Treatment effects on flow were significant. Partitioning the treatment sum of squares and study of Fig. 1 show that water and sucrose yielded similar responses that were significantly different ($p<.01$) from those for citric acid and quinine; the latter two were in turn similar. The average flow responses for water and sucrose were 12.1 and 10.9 respectively, whereas those for citric acid and quinine were 4.2 and 5.4 respectively. During the tests, the animals eagerly accepted the water and sucrose preparations but were reluctant to ingest the citric acid and quinine preparations. Correlation between the pancreatic flow responses and the behavioral responses is striking and no doubt reflect the survival value of a close association between sensory reactions and internal bodily processes.

The treatment effects on protein response were not as clearly significant ($p< .10$) as those for flow. This was associated with a higher coefficient of variation (Table I). Partitioning the sum of squares for treatments and Fig. 1 show that sucrose and water tended to give higher responses than did citric acid and quinine, but the water response was considerably lower than that for sucrose. Re-

TABLE I. Analyses of Variance for Responses in Pancreatic Flow and Protein Secretion.

Source of variation	Degrees of freedom	Pancreatic flow		Protein secretion	
		Mean square	F-ratio	Mean square	F-ratio
Dogs	1	90.2	8.20[a]	3.6	<1
Replications	1	4.4	<1	.2	<1
Replications by dogs	1	34.8	3.16	4.6	<1
Treatments	3	61.8	5.62[a]	40.8	2.83[b]
H_2O + sucrose vs citric + quinine	1	179.6	16.33[c]	72.2	5.01[b]
H_2O vs sucrose	1	2.9	<1	34.0	2.36
Citric vs quinine	1	3.1	<1	16.0	1.11
Error	9	11.0		14.4	
General mean		8.1		6.2	
Coefficient of variation (%)		41		62	

[a] Significant at $p < .05$.
[b] Significant at $p < .10$.
[c] Significant at $p < .01$.

sponses for water, citric acid, and quinine were 6.2, 2.6, and 5.5 respectively, whereas that for sucrose was 10.4.

Sucrose increased pancreatic flow, and protein secretion in a parallel fashion. In contrast, water increased the flow about as did sucrose but it increased protein secretion little more than quinine. It is thus suggested that pancreatic flow and protein secretion are affected by the oral stimulus but may operate independently. Further observations on this point are needed.

The initial event in pancreatic secretion excited by oral stimulation has been reported to be neural, operating via the vagal cholinergic fibers (9). Grossman (10) speculated that the vagal effects may be due to acetylcholine release within the pancreatic tissue. Recent evidence indicates that a gastric phase of pancreatic secretion exists that is mediated by gastrin (11). Stimulation of gastrin secretion by vagal activity has been reported to occur in the dog during sham feeding (12), and subsequent evidence indicates that a part of the pancreatic response to sham feeding arises as a result of gastrin release from the pyloric gland area of the stomach (5). In the present study the food stimuli were drained by a gastric cannula and did not enter the duodenum. Preshaw *et al.* (5) have reported that gastric juice will not induce a secretin response if it does not enter the duodenum. This is supported by the work of Crittenden and Ivy (6) who reported that they were unable to stimulate pancreatic secretion by introduction of food into the stomach. Thus it appears safe to assume that the pancreatic responses observed in the present study can be attributed to oral sensory mechanisms.

Summary. The present study, with conscious dogs, indicates that both pancreatic flow and pancreatic protein output can be affected by the nature of the taste stimuli. Water and a sucrose solution mixed with a basal diet produced a similar and significantly greater stimulation of flow than did citric acid or quinine, which in turn were similar. Although it appeared that protein secretion was more strongly enhanced by sucrose solution than by water and solutions of citric acid and quinine, it was not clearly established that sucrose solution and water were really different. The flow responses for the four taste stimuli were highly correlated with the readiness with which the various food mixtures were accepted by the dogs.

Appreciation is expressed to Drs. A. C. Linnerud and H. L. Lucas for computational help and aid in interpretation of the statistical results.

1. Bardach, J. E., *in* "The Chemical Senses and Nutrition" (M. R. Kare, and O. Maller, eds.) p. 19. Johns Hopkins Press, Baltimore, Maryland (1967).

2. Richter, C. P., *in* "Gestation" (C. A. Villee,

ed.), p. 11. Josiah Macy Jr. Foundation Publications, New York (1956).

3. Maller, O., Kare, M. R., Welt, M., and Behrman, H. R., Nature 213, 713 (1967).

4. Pavlov, I. P., "The Work of the Digestive Glands" (W. H. Thompson, ed.) Griffin, (1910).

5. Preshaw, R. M., Cooke, A. R., and Grossman, M. I., Gastroenterology 50, 171 (1966).

6. Crittenden, P. J. and Ivy, A. C., Am. J. Physiol. 119, 724 (1937).

7. Thomas, J. E., "The External Secretion of the Pancreas." Thomas, Springfield, Illinois (1950).

8. Gornall, A. G., Bardawill, C. J., and David, M. M., J. Biol. Chem. 177, 751 (1949).

9. Gregory, R. A., "Secretory Mechanisms of the Gastrointestinal Tract." Arnold, London (1962).

10. Grossman, M. I., *in* "Ciba Foundation Symposium on the Exocrine Pancreas" (A. V. S. DeReuck and M. P. Cameron eds., p. 208. Little, Brown, Boston, Massachusetts (1961).

11. Gregory, R. A. and Tracy, H. J., Gut 5, 103 (1964).

12. Pethein, M. and Schofield, B., J. Physiol. 148, 291 (1959).

Received June 10, 1968. P.S.E.B.M., 1968, Vol. 129.

Effect of Connective Tissue and Collagen on Platelet Lactate Production: Role of Acid Mucopolysaccharides* (33318)

ELENA PUSZKIN[1] AND ZOHARA JERUSHALMY[2]

Department of Pathology and American National Red Cross Research Laboratory, New York University Medical Center, New York, New York 10016

Glycolysis, a major source of energy for platelets (1, 2), is responsible to some extent for serotonin uptake (3), potassium transport (4), and clot retraction (5, 6). Other investigators have already studied the effect on glycolysis of substances such as thrombin (5, 7–9), epinephrine (8), and ADP (8) which may be involved in the formation of an *in vivo* platelet plug. The first stage in the formation of a thrombus or a hemostatic plug is probably the interaction of platelets with the collagen of connective tissue (10, 11). The present report describes the different effects of connective tissue and collagen on the rate of formation of lactate, the end product of glycolysis, and establishes that platelet glycolysis is inhibited by the mucopolysaccharides of connective tissue.

Materials and Methods. Concentrated platelet suspension. Blood was collected from normal human volunteers in polyethylene centrifuge bottles containing one-quarter vol of acid-citrate-dextrose (ACD) (12) anticoagulant. It was centrifuged at 100 *g* for 20 min. Platelet-rich plasma (PRP) was collected in 50-ml polycarbonate tubes and spun at 1465 *g* for 15 min. All centrifugations were carried out at 4° to minimize metabolic activity. The platelet-poor plasma (PPP) was removed, and the pH was adjusted to 6.7 at 18° with either 0.1 *N* HCl or 0.1 *N* NaOH. The sedimented platelets were resuspended in a small volume of PPP. The volume of packed platelets was measured in a microhematocrit tube; it comprised 5–7.2% of the suspension, equivalent to 0.75 to 1.1 $\times$ 10^{10} platelets/ml suspension respectively.

Imidazole buffered saline (IMB). One part of 0.2 *M* imidazole buffer, pH 7.2, was diluted with 10 parts of isotonic saline.

Connective tissue suspension. Human connective tissue (CT) was obtained from regions of radical mastectomy specimens with no gross evidence of tumor. It was stored frozen, and prepared as described by Spaet and Zucker (13) except that the particles were washed twice in IMB instead of four times. Each milliliter of suspension was de-

* This study was partly supported by USPHS Grants 05003-08 and 08537-02 from the National Heart Institute and Grant U-1359 from the Health Research Council of the City of New York.

[1] Present address: Dept. of Hematology, Mt. Sinai School of Medicine, 100th St. and Fifth Ave., New York, N. Y. 10029. Please send reprint requests here.

[2] Present address: Rogoff Medical Research Institute, Tel-Aviv University, and Beilinson Hospital, Petah Tikva, Israel.