Differential Response to Various Stimulants in the Body and Antrum of the Canine Stomach

J. J. Anderson, M.D., R. J. Bolt, M.D.,* B. M. Ullman, M.P.H., and P. Bass, Ph.D.

THE MAJORITY of studies on gastric motility have been performed with intraluminal devices,[1-3] yet the experimental procedures may in themselves influence gastric contractility by initiating mucosal reflexes. In acute animal studies, the anesthesia and surgical trauma depress contractility. In 1944 Brody and Quigley,[4] recognizing such disadvantages, developed an extraluminal gastric recording system. Recently, an extraluminal force transducer became available and was found to be useful in the quantitative evaluation of contractile force of either circular or longitudinal smooth muscle in unanesthetized dogs.[5] These sensor units are relatively small, and, being extraluminal, do not interfere with the flow of contents.

Using extraluminal force transducers, we undertook this study to evaluate various stimulants on both body and antral contractility and to quantify the differential effects beween muscle layers in these two areas.

METHODS

Sensor Units

The current method of constructing extraluminal force transducers has been described.[6] Each transducer consisted of 2 0.250 × 0.090 in. foil strain gages which are bonded to the convex and concave surfaces of a beryllium-copper clip. The clip measured 12 × 4 × 0.5 mm., was formed to a $\frac{7}{16}$-in. radius of curvature, and had 2 0.030-in. holes at each end for suturing purposes. The units were waterproofed with epoxy cement and encapsulated in silicone rubber. The terminal lead wires were soldered to a 15-contact Cannon connector and sealed in an epoxy button similar to the technic described by Blouin and Potoczak.[7] The transducers were calibrated by hanging gram weights from the suturing holes and noting the height of deflections on the polygraph. All forces recorded in these experiments were in the linear range of calibration. The entire unit was cold-sterilized.

From the University of Michigan Medical Center and Parke, Davis & Company Research Laboratories, Ann Arbor, Mich.

*Present address: Department of Internal Medicine, University of California School of Medicine, Davis, Calif.

Animal Preparations

Using aseptic surgical technics, we implanted 4 extraluminal force transducers in each of 5 healthy beagle dogs (8.5–11 kg.). The surgical procedure was performed in two stages. An incision was made between he scapulas, and a trocar containing the sensor units was passed subcutaneously down the back and brought through a left-flank incision. The epoxy button with the Cannon connector was placed subcutaneously between the scapulas. The animal was then placd on his back and a midline incision was made, with the sensor units brought into the peritoneal cavity through a stab wound in the left rectus muscle.

Two transducers were sutured perpendicular to each other on the serosal surface of both the gastric body and the gastric antrum. The sensor units were perpendicularly oriented to measure contractile activity of either longitudinal or circular muscle. The antral transducers were located 4 cm. and the body units 14 cm., respectively, from the gastroduodenal junction (see Fig. 1, on p. 158). Ten days were allowed for recovery from operation. At the time of recording, an electrical connection was made between the Cannon terminal in the back of the unanesthetized dog and an Offner Type R dynograph recorder. (The animals, used also for other experiments, were laboratory dogs with a Thiry loop.)

Experimental Procedure and Analysis of Data

The animals were studied in digestive and interdigestive states. For the digestive state, 200 gm. of horse meat was fed to the dogs. In the interdigestive state, the following gastric stimulants were infused for a 30-min. period: (1) acetylcholine (ACH) in 2 concentrations, 50 and 200 μg./kg./min. I.V., respectively; and (2) 5-hydroxytryptophane (5-HTP) in a concentration of 600 μg./kg./min. A control (interdigestive state) was obtained by infusing saline alone. ACH was always infused into the brachial vein; 5-HTP was administered into the saphenous vein. All experimental procedures were randomized and duplicated, leading to 10 treatments for each of the 5 animals. In our laboratories dogs are routinely fed their daily standard ration at 3 P.M. and deprived of water from 6 A.M. to 8:10 A.M. All experiments in this study were performed between 8:00 and 10 A.M. This routine assured us that the dogs had received no food for at least 17 hr. and no water for at least 2 hr.

The recorded tracings obtained from each muscle layer were analyzed for frequency and amplitude of contraction. Frequency was determined by counting the deflections for a 30-min. period and expressed as total frequency. The amplitude of each contraction was measured in millimeters and converted to grams of force on the basis of the calibration for each transducer. Amplitude determinations were summed in 3 preset sampling periods: 3–6, 13–16, and 23–26 min. from the start of the 30-min. record.

Analysis of variance was applied to the frequency and amplitude data for comparison between muscle layers, gastric areas, stimulants, and dog population. This is a technic in which measurements depending on several factors are analyzed to estimate the effect of each, under the assumption that the total effect is the sum of those for the individual factors and for interactions between them. One is able to test whether there is a significant difference in the mean values of a measurement at different levels of the same factor. In the present instance, significance will be taken to be at the 5% level. Where significant difference between the means was detected, the Duncan's multiple range test was applied to identify the levels at which their differences occurred. In the frequency measurements, the four factors are: stimulants, areas, animals, and replications. In the amplitude analysis a fifth factor denoted "time" accounts for the sampling periods.

Because of the complex nature of the experimental design, exact statistical tests could not be done in the evaluation of some effects, and in these cases, approximate methods were used.

RESULTS

General Effects

The over-all mean frequency of contractions (Table 1) for all areas of the stomach under each of the conditions studied was analyzed with the Duncan's multiple range test. Food and 5-HTP significantly increased the number of contractions; both doses of ACH did not significantly alter the number of contractions when compared to the control. The over-all average of amplitudes (Table 2) for the whole stomach (as analyzed by Duncan's test) shows that 5-HTP induced a significant increase in amplitude; 200 μg. of ACH had no effect, while food and 50 μg. of ACH significantly induced lower-amplitude contractions.

A similar analysis was performed on the frequencies of the circular and longitudinal axes for both body and antrum (Table 1). The frequency of contractions in the body was significantly higher in the circular axis (119.9) than in the longitudinal axis (80.0). In the antrum both axes had similar

TABLE 1. MEAN TOTAL NUMBER OF CONTRACTIONS (FREQUENCY) IN A 30-MIN. PERIOD, AS INFLUENCED BY VARIOUS STIMULANTS

	Body		*Antrum*		
Stimulants	*Circular axis*	*Long. axis*	*Circular axis*	*Long. axis*	*Over-all mean*
Interdigestive	115.7	52.3*	76.4	77.1	81.1
Digestive	151.2*	121.4	136.4	121.9	132.3
ACH (50 μg./kg.)	107.4	58.2*	61.5	64.2	73.2
ACH (200 μg./kg.)	72.9	37.0	85.0	83.4	69.6
5-HTP (600 μg./kg.)	155.2	131.2*	134.0	137.6	139.7
Over-all mean	119.9	80.0	98.7	96.8	—

*Mean of 9 observations; other entries are the means of 10 observations.

TABLE 2. MEAN AMPLITUDE OF CONTRACTIONS EXPRESSED AS GRAMS OF FORCE IN BOTH BODY AND ANTRUM DURING THE 3 SAMPLING PERIODS

	Body						*Antrum*						
	Circular axis			*Longitudinal axis*			*Circular axis*			*Longitudinal axis*			
Stimulants	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*Over-all mean*
Interdigestive	136.7	147.3	102.9	25.8*	25.9	25.5	163.0	217.8	128.4	183.2	185.2	135.9	124.0
Digestive	95.5	100.0	91.2*	48.4	56.9*	49.7	109.1	76.6	64.5	91.4	70.7	71.9	77.2
ACH (50 μg./kg.)	117.3	65.6	55.1*	36.2	18.0	22.0	203.8	53.3	48.2	112.7	51.3	32.0	68.1
ACH (2002 μg./kg.)	108.4	16.0	17.5	51.7	9.0	8.8	458.1	62.2	232.1	281.8	56.9	134.6	119.8
5-HTP (600 μg./kg.)	191.6	290.6	228.3	74.0	110.6	88.5	301.7	444.5	326.7	318.5	493.9	387.5	271.4
Over-all mean	129.9	123.9	100.0	47.7	43.8	38.9	247.1	170.9	160.0	197.5	171.6	152.4	132.4
Over-all mean	—	117.9	—	—	43.5	—	—	192.6	—	—	173.8	—	—

A, B, and C indicate samplings during 3–6, 13–16, and 23–26 min., respectively, from the start of the 30-min. recording period.
*Mean of 49 readings; other entries are the means of 50 readings.

frequencies of contraction (98.7 and 96.8). The average combined frequencies of the 2 axes of the body and antrum did not differ (100 vs. 97). The amplitude of contractions (Table 2) varied as follows: longitudinal body (43.5) < circular body (117.9) < longitudinal antrum (173.8) < circular antrum (192.6). In the body, the amplitude of the longitudinal axis was significantly lower than that of the circular axis. There were no significant differences between the axes of the antrum. The combined amplitude of the two areas indicates that the antrum has significantly more powerful contractions. The values of the analysis of variance for the data in Tables 1 and 2 are presented in Tables 3 and 4.

TABLE 3. ANALYSIS OF VARIANCE FOR FREQUENCY MEASUREMENTS

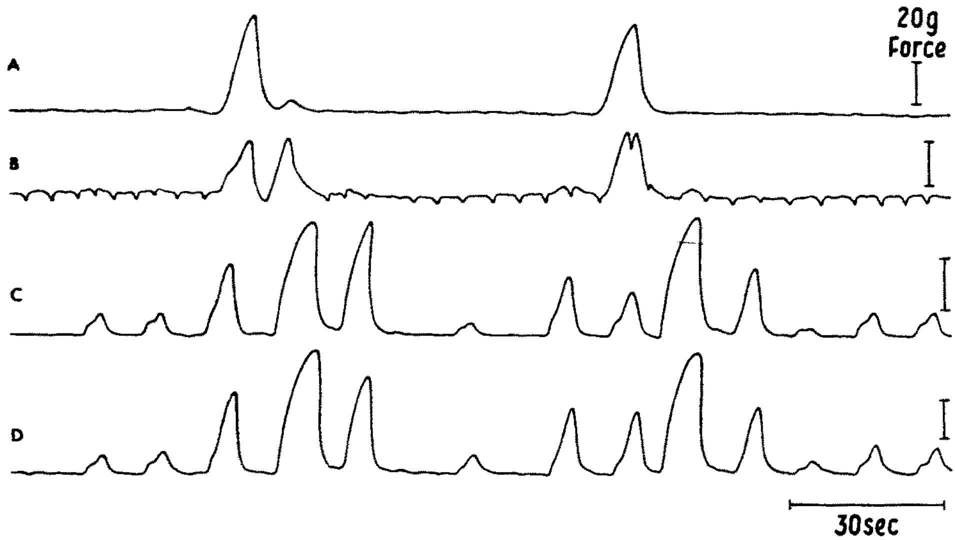
Source	M.S. (10^4)	f value	Degrees of freedom n_1	n_2	p value
A (Sites)	1.06	5.58	3	12	<.01
B (Animals)	.48	12.6	4	44	<.01
C (Stimulants)	4.54	14.2	4	16	<.01
D (Repetitions)	.32	1.28	1	4	>.10
INTERACTIONS					
AB	.188	5.00	12	44	<.01
AC	.166	2.39	12	48	<.05
AD	.01	<1	—	—	>.10
BC	.32	8.4	16	44	<.01
BD	.25	6.6	4	44	<.01
CD	.038	<1	—	—	>.10

TABLE 4. ANALYSIS OF VARIANCE FOR AMPLITUDE MEASUREMENTS

Source	M.S. (10^4)	f value	Degrees of freedom n_1	n_2	p value
A (Sites)	61.86	6.33	3	13	<.01
B (Animals)	30.22	20.28	4	8	<.01
C (Stimulants)	80.19	3.76	4	17	<.05
D (Times)	12.01	8.06	4	8	<.05
E (Repetitions)	40.70	3.11	1	4	>.10
INTERACTIONS					
AB	8.95	13.9	12	24	>.10
AC	10.33	2.63	12	45	<.05
AD	1.46	2.27	6	24	>.05
AE	3.66	.87	—	—	>.10
BC	10.24	7.53	16	32	<.01
BD	1.49	1.79	8	92	>.05
BE	12.24	9.13	4	8	<.01
CD	12.42	9.13	8	32	<.01
CE	2.89	3.18	4	1	>.10
DE	2.19	1.63	2	8	>.10

INTERDIGESTIVE STATE

Animals in this condition demonstrate different levels of contractile activity between the body and the antrum. When the stomach is relatively quiet (basal state), continuous low-amplitude contractions are frequently seen on the circular axis of the body. This may, in part, account for the higher frequency in this axis vs. the longitudinal axis of the same area (115.7 vs. 52.3; Table 1).



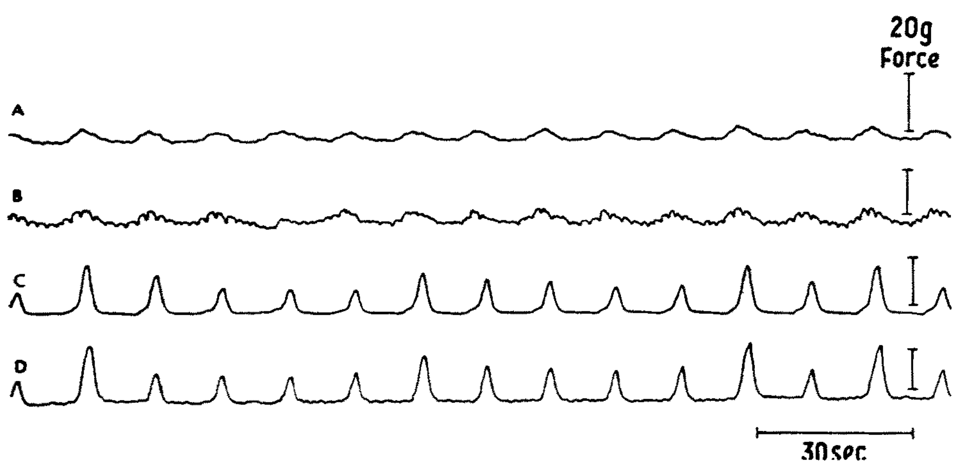


Fig. 1 (*top*). Interdigestive (control) state. Note 1:1 ratio between muscle layers in given area, but no consistent ratio between body and antrum. KEY: *top line,* circular body; *second line,* longitudinal body; *third line,* circular antrum; *bottom line,* longitudinal antrum. The time bar (*horizontal*) indicates 30 sec.; the force bar (*vertical*), 20 gm. **Fig. 2** (*bottom*). Digestive (200 gm. food) state. Note persistent cyclic activity in body and antrum with higher amplitude in the antrum. (See key to Fig. 1.)

When a burst of activity (high-amplitude contractions) occurs in the body and antrum, the two axes of a given area will usually have simultaneous contractions—but there is no consistent one-to-one relationship between the body and antrum (Fig. 1). This lack of relation suggests that each contraction originating in the body is not propagated over the remainder of the stomach. In the body, the amplitude of contraction in the longitudinal axis is lower than in the circular axis. The amplitude of contraction in the antrum in both axes is characteristically higher than that of the body (Table 2).

Digestive State

The ingestion of food produces a continuous motor pattern of 4 to 5 contractions per minute in both the body and antrum (Fig. 2). At any given time the longitudinal axis of the body has a lower amplitude of contraction than does the circular axis (Table 2). The antrum has a higher amplitude of contraction than the body, with no difference between longitudinal and circular axes. The rise time of the contraction (time interval from base line to peak) is longer in the body than in the antrum (Fig. 2).

Acetylcholine

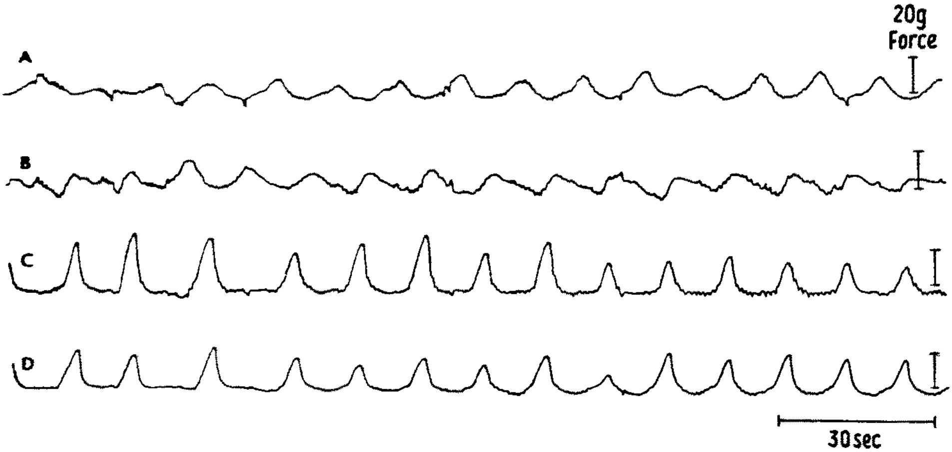
ACH in doses of 50 and 200 μg./kg./min. was given intravenously to fasted unanesthetized dogs. Continuous muscle contractility was maintained only for the first 6–10 min. of infusion (Fig. 3). The over-all effect of ACH showed a tendency to decrease frequency and amplitude when compared to values in the controls. As in the previous states, the amplitude of contractions of the body was lower than in the antrum (Table 2 and Fig. 3). The expected side effects to a cholinergic drug (salivation, occasional vomiting, or defecation) were observed during the 30 min. of infusion.

5-Hydroxytryptophane

The most potent gastric stimulant used was 5-HTP (Fig. 4). Continuous contractions at a rate of 4–5/min. were seen in both body and antrum. The amplitude of contractions in response to 5-HTP was higher in each area than that seen with the use of any other stimulant. As observed in the other conditions studied, the amplitude of contractions in the longitudinal axis of the body $<$ circular axis of the body $<$ circular axis of the antrum $\cong$ longitudinal axis of the antrum. At the dose administered 5-HTP produced midriasis, conjunctival congestion, and increased restlessness.

DISCUSSION

Three types of activity have been described for the gastrointestinal tract: basal, burst, and intermediate motor patterns.[8] The presence of different motor patterns for the two physiologic states (digestive and interdigestive) have been described.[8] We have confirmed the presence of these conditions and have attempted to quantify the motor pattern seen in these two states and also while the animal was under the influence of ACH and 5-HTP. A characteristic finding, particularly during the interdigestive state, was that the frequency and the amplitude of the contractions were unrelated between the body and the antrum. The classic "peristaltic" wave between the body and antrum was not recorded. In general, during burst activity more and higher amplitude of contraction was seen in the antrum than in the body. This antral activity has been postulated as mobilizing mucus secretion. During cinefluorography ob-



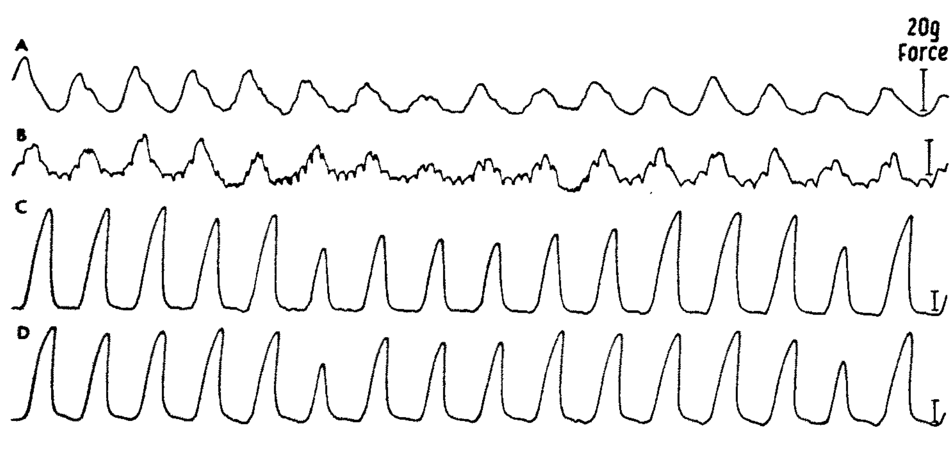


Fig. 3 (*top*). Effects of acetylcholine infusion (200 µg./kg./min.) on gastric motility. Continuous activity was present only for initial 6–10 min. of infusion. (See key to Fig. 1.) **Fig. 4** (*bottom*). Response to 5-hydroxytryptophane (600 µg./kg./min.). Note greater amplitude of contractions particularly in antrum in comparison with readings in other illustrations. (See key to Fig. 1.)

servations, retropulsion has been described.[9] This would suggest that the high level of activity seen in the antrum tends to move material from an area of high muscular contractility to that of an area of low muscular contractility. The ability of the colon to propel its material more readily to areas of low activity has been shown in conditions of diarrhea.[10] Similarly, in the digestive state, the higher amplitude of contractions seen in the antrum compared to that recorded from the body would tend to pump material in a retrograde direction. Thus, we suggest that antral motor function is mainly that of mixing gastric contents—allowing only a "minimal" amount of material to pass into the duodenum.

Of the 4 sites monitored, the longitudinal axis of the body had the lowest amplitude of contraction—regardless of the stimulant used. As the transducers are sensitive to unidirectional force only, one would assume that the anterior midbody portion of the stomach contains a minimal amount of longitudinal fibers. This physiologic observation confirms the anatomic description. The ability to record similar amounts of force from both axes of the antrum indicates that both muscle layers are present and active. Regardless of the condition or the stimuli, the highest number of contractions in either body or antrum was 5/min.

The various stimulants had different effects. ACH at both concentrations produced persistent and high contractility for the first 6–10 min. This was followed by quiescence or mild activity for the duration of the infusion, while the side effects persisted. The decreased activity to ACH infusion could be caused by the development of tachyphylaxis, rapid cholinesterase inactivation of ACH, or development of sympathetic dominance. Martinson[11] reported that the vagus nerve of the cat contained two types of efferent nerve fibers. The nerve fibers responding to low threshold stimuli produced a lowering of intragastric pressure, owing to stomach relaxation. Possibly the ACH infusion activated this relaxing system in the stomach while other organs under cholinergic control (e.g., salivary glands) persisted in responding to ACH.

Because of tachyphylaxis, 5-hydroxytryptamine (5-HT) will not persistently stimulate intestinal[12] or gastric[13] motility. As 5-HTP can provide a sustained stimulus to intestinal motility in man,[12] the substance was used in this study. This differential response between 5-HTP and 5-HT is thought to occur when the former penetrates the cells and is decarboxylated to 5-HT, which in turn has an elevated turnover rate at the neuroeffector sites within the intestinal wall. In contrast, 5-HT, when administered exogenously, remains in the extracellular spaces and quickly saturates the serotonergic receptors. Regardless of the mechanism, 5-HTP proved to be the most persistent stimulant—with high-amplitude contractions in both areas of the stomach. The ability of 5-HTP to stimulate the gastric stomach of dog is in contrast to the results reported by Schmid *et al.*,[14] who noted that 5-HTP inhibited spontaneous stomach contractions in man.

SUMMARY

This study was initiated to evaluate gastric motility with the use of extraluminal force transducers in unanesthetized dogs. The contractile activity of the circular and longitudinal muscle in both antrum and body of the canine stomach was monitored in the interdigestive state and under the influence of food, acetylcholine, and 5-hydroxytryptophane. The latter two chemicals were infused for a 30-min. period. Each of the two muscle layers in antrum and body had a maximum frequency of 5 contractions per minute. This activity was continuous during 30 min. only under the influence of food and 5-hydroxytryptophane. Acetylcholine stimulant effects did not persist beyond the initial 10 min. of infusion. Amplitude of contractions under spontaneous or induced activity varied. The height of these contractions were circular antrum > longitudinal antrum > circular body > longitudinal body. This method of recording muscle contractility is believed to be useful for quantitatively assaying gastric activity without compromise of physiologic environment.

P. B.

Department of Experimental Therapeutics
Parke, Davis & Company Research Laboratories
2800 Plymouth Rd.
Ann Arbor, Mich. 48106

REFERENCES

1. HIGHTOWER, N. C., CODE, C. F., and MAHER, F. T. A method for the study of gastrointestinal motor activity in human beings. *Proc Mayo Clin 24*:453, 1949.
2. TEXTER, E. C. Fluorocinematography. *Amer J Dig Dis 6*:983, 1961.
3. WATSON, B. W., ROSS, B., and KAY, A. W. Telemetering from within the body using a pressure-sensitive radio pill. *Gut 3*:181, 1962.
4. BRODY, D. A., and QUIGLEY, J. P. Application of the "inductograph" to the registration of movements, particularly of body structures such as the pyloric sphincter. *J Lab Clin Med 29*:863, 1944.
5. JACOBY, H. I., BASS, P., and BENNETT, D. R. In vivo extraluminal contractile force transducer for gastrointestinal muscle. *J Appl Physiol 18*:658, 1963.
6. LUDWICK, J. R., and BASS, P. Contractile and electric activity of the extrahepatic biliary tract and duodenum. *Surg Gynec Obstet 124*:534, 1967.
7. BLOUIN, L. T., and POTOCZAK, R. A simple technique to prevent aortic rupture following implant of chronic flow probes. *IEEE Trans Biomed Engin 14*:56, 1967.
8. REINKE, D. A., ROSENBAUM, A. H., and BENNETT, D. R. Patterns of dog gastrointestinal contractile activity monitored in vivo with extraluminal force transducers. *Amer J Dig Dis 12*:113, 1967.
9. CARLSON, H. C., CODE, C. F., and NELSON, R. A. Motor action of the canine gastroduodenal junction: A cineradiographic, pressure, and electric study. *Amer J Dig Dis 11*:155, 1966.
10. CONNELL, A. M. The motility of the pelvic colon: II. Paradoxical motility in diarrhoea and constipation. *Gut 3*:342, 1962.
11. MARTINSON, J. Studies on the efferent vagal control of the stomach. *Acta Physiol Scand 65* (Suppl. 255) :5, 1965.
12. HAVERBACK, B. J., and WIRTSCHAFTER, S. K. "The Gastrointestinal Tract and Naturally Occurring Pharmacologically Active Amines." In *Advances in Pharmacology* (Vol. 1), Garattini, S., and Shore, P. A., Eds. Acad. Press, New York, 1962, pp. 309–348.
13. MISIEWICZ, J. J., WALLER, S. L., and EISNER, M. Motor responses of human gastrointestinal tract to 5-hydroxytryptamine in vivo and in vitro. *Gut 7*:208, 1966.
14. SCHMID, E., ZICHA, L., and SCHIFFFARTH, F. Über die Wirkung von 5-Hydroxytryptophan auf den Magendarmtrakt des Menschen. *Med Exp (Basel) 2*:266, 1960.