VAGALLY INDUCED GASTRIC MOTILITY FACILITATION AND INHIBITION IN ANESTHETIZED RABBITS

Thesis for the Degree of M. S.

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Thomas D. Burns

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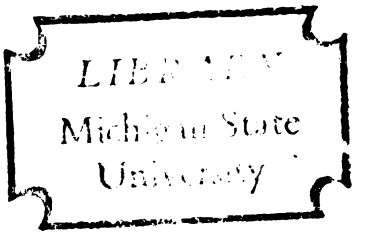

ERRATA

Page	Line	Correction
Abstract	5	ILP increase correlated with simultaneous gastric antral facilitation (r=0.98) and gastric body relaxation (r=0.99).
ii	9	assistance
	12	expresses
	13	assistance
		The author failed to acknowledge Mrs. Jackie Hellmann for her assistance in preparing this final manuscript, and especially for her willingness to type into the morning hours so a deadline could be met.
18	2	procedure
47	10	Accummulation
48	12	gastric blood flow
49	23	Alvarez
51	6	Ambache
53	6	inhibition

ABSTRACT

VAGALLY INDUCED GASTRIC MOTILITY
FACILITATION AND INHIBITION
IN ANESTHETIZED RABBITS

by Thomas D. Burns

Gastric antral and body contractile activities recorded by extraluminal force transducers were correlated with intraluminal pressure (ILP) responses elicited by electrical stimulation of the decentralized vagus nerves of anesthetized, fasted rabbits weighing 2-4 kg. ($r = 0.98$) and gastric body relaxation ($r = 0.99$), having a consistent frequency dependent pattern with maximum at 16-32 cps. Correlations with $r > 0.90$ suggest that the amplitude of the ILP post-facilitation inhibition is related to the amplitude of the immediately preceding ILP facilitation. Atropine (1 mg/kg, i.v.) converted the vagally induced ILP facilitation to inhibition but failed to abolish the subsequent after-contractions. Barium chloride (4 mg/kg, i.v.) increased pre-stimulation ILP in the atropinized animal, thereby allowing an augmented vagally induced ILP and antral inhibition.

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By

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INTRODUCTION

Bayliss and Starling (5) reported that the vagus (parasympathetic) and splanchnic (sympathetic) nerves exert reciprocal control on gastrointestinal motility. Although vagal parasympathetic efferents, which comprise less than 10% of the total cervical vagal trunk fibers (1), primarily facilitate, they also inhibit gastric motility (5,14,43,44,47). It has been suggested that gastric receptive relaxation, (13,37) occurring primarily in the proximal portion of the stomach, results from excitation of vagal inhibitory fibers (13,14,40). Vagally induced gastric inhibition is apparently noncholinergic (5,32,43), and contingent on gastric tonus (pre-stimulatory contractile state) (12,32,40,50).

The following review of vagally induced gastric inhibition presents the methods, results, and conclusions of previous investigators.

Methods for Study of Vagally Induced Gastric Inhibition

The existence of vagal inhibitory fibers innervating the alimentary canal was first reported by Openchowski in 1883. Since Openchowski's work, many investigators of gastrointestinal motility have observed vagally induced gastric inhibition (39,44,47,48).

Openchowski (51) studied esophageal-cardia motility in anesthetized rabbit, dog and cat, by inserting into the cardiac opening a small elastic bag connected to a Marey's tambour, recording intraluminal pressure changes on a smoked kymograph drum. Dilation of the cardiac orifice was obtained by stimulating a nerve on the lower esophagus which he called "nervus dilator cardiac". Openchowski reported a relationship between stimulating impulse frequency and magnitude

of excitation and inhibition with increasing frequency depressing excitation and augmenting inhibition.

Langley (40) inserted an open tip rubber tube into the rabbit esophagus (positioning the tip at the lower thoracic level) monitoring intraluminal pressure changes by a fluid filled graduated verticle column. In the atropinized rabbit (15-20 mg) vagal stimulation, either electrically or mechanically, produced cardia dilation allowing the fluid to enter the stomach. Direct visual observation showed relaxation of the esophageal cardia region. According to Langley, passage of fluid from the esophagus to the stomach was the result of cardiac dilation. When minimal effective induction shocks were applied, initial low amplitude esophageal-cardia contractions were recorded which rapidly decreased in amplitude with prolonged stimulation. Increased stimulating impulse intensity produced a brief rise in pressure followed by a rapid fall. To Langley, this indicated a struggle between excitatory and inhibitory fibers. The same effects were observed whether the vagi were stimulated supra- or sub-diaphragmatically. The most constant visible effect of vagal stimulation was an after-contraction of the cardiac-fundic region upon cessation of stimulation. After-contractions (contractions occurring upon cessation of stimulation) were conspicuous even when relaxation was not observed, with the magnitude of these contractions decreasing with prolonged stimulation.

Bayliss and Starling (5) recorded gastrointestinal motility on a float-type piston recorder, using the enterograph connected to a tambour, simultaneously recording intraluminal pressure changes via intraluminal balloons. Vagally induced gastrointestinal motility in the dog, resulted in both excitation and inhibition suggesting two function-

ally different groups of fibers.

Cannon and Lieb in 1911 (13) investigated receptive relaxation using thin rubber balloons positioned in the cat's gastric fundus. A cylinder (5.5 cm internal diameter) type float recorder, monitored motility, a glass cannula positioned in the cardiac sphincter resisted any cardiac pressure. Gastric intraluminal changes were transferred to the cylinder through a connecting catheter and recorded by a writing lever secured to a floating cork. The relatively large, fluid surface area in the cylinder permitted the recording of volume changes with little change in intraballoon pressure. Cannon and Lieb observed a relaxation in the gastric fundus 2-3 sec. after the rise of the larynx in deglutition. Even though the food bolus was expelled through an esophageal fistula, relaxation occurred at the time the bolus would have reached the cardia.

Bercovitz and Rogers (6) recorded turtle gastric motility kymographically, by inserting through the esophagus intraluminal balloons connected to a water-float manometer with a writing lever. Vagal stimulation elicited gastric relaxation in the turtle.

Carlson et al. (14) studying gastric motility in the ether anesthetized cat, positioned a small condom balloon (4-6 cm in length) in the gastric cardia. Intraballoon pressure was monitored by two separated water manometers, one through an esophageal fistula and the other through a gastric fistula allowing the measurement of cardiac tone by recording respiratory excursion through the cardiac end of the balloon. A larger condom (10-15 cm) simultaneously recorded gastric motility. Vagal stimulation with high frequency impulses produced esophageal-cardia excitation when tonus was low and inhibition during conditions of high tonus, which varied at different anesthesia levels. Carlson

suggested that the vagus and splanchnics contain both facilitory and inhibitory fibers innervating the esophagus and gastric cardia.

Veach (59) investigated vagal inhibition to the cat lower esophagus, cardia and gastric body, using intraluminal balloons, observing two distinct responses: 1) excitatory motor responses when low intensities of electrical stimulation were applied, and 2) inhibitory responses when the intensity was increased above a critical frequency level. Initial contractions occurred more rapidly as the frequency or intensity increased with inhibition becoming more pronounced as intensity or frequency increased above that yielding maximum contractions. The duration and magnitude of the after-contractions which were observed on cessation of stimulation, seemed to be related to duration of the stimulation period. Inhibition induced by one vagus could be nullified by an excitatory effect of the other.

McSwiney and Wadge (50) investigating tone-inhibition relationships recorded gastric motility with an open tip catheter connected to a float manometer. Anesthetized cats laparotomized with a midline incision, the duodenum ligated and the abdominal walls retracted to form a warm saline bath. Stimulation of either vagal trunk produced inhibition, but a greater effect occurred when both were stimulated simultaneously, with initial atony requiring a greater stimulating intensity to produce excitation or inhibition. The results obtained did not show a relationship between reaction and intensity or frequency of stimuli as suggested by Veach, for low or high frequency or intensity produced inhibition when tone was high.

McSwiney and Spurrell (49) working with anesthetized cats inserted an open tip catheter through the esophagus, ligated the pylorus and

monitored motility with a fluid manometer. McSwiney and Spurrell (49) reported inhibition depending upon the intensity of stimulation, gastric tonus and animal's postsurgical condition. Intracranial (vagal trigone region of medulla) vagal stimulation was as effective in producing inhibition as intrathoracic vagal stimulation. Harrison working with McSwiney (32), isolated vagally innervated strips from the gastric fundus of cats, suspending them in a bath (125 cc) of oxygenated Ringer-Locke solution maintained at 37^{o}C. Harrison and McSwiney (32) reported that gastric excitation and gastric inhibition were tone dependent.

Celander (15), using a rubber intraluminal balloon technique, recorded intestinal motility in exteriorized intestinal loops filled with Tyrode-solution. A polyethylene tube from the cut end of the loop was connected to a reservoir with a relatively large cross-sectional area maintaining pressure constant even when volume changed markedly. He reported that vagally induced intestinal relaxation differed characteristically from the splanchnically induced inhibitory effect. Splanchnic stimulation at impulse frequencies less than 10 cps had little effect on motility, but at supraphysiological frequencies (greater than 10 cps) vasoconstriction was prompt with intestinal relaxation occurring after a considerable latency. However, vagal stimulation produced prompt gastrointestinal relaxation at 1-10 cps. Celander suggested that intestinal relaxation following splanchnic stimulation was the result of vasoconstriction coupled with a slight overflow of transmitter, known to occur at high frequencies.

Martinson and Muren (45, 46) introduced latex balloons (inflated with 40 cc of air), connected to a water manometer, into the cat

stomach *via* the esophagus, recording gastric motility elicited by cervical vagal stimulation. Jansson, working with Martinson (37) using chloralose (60-80 mg/kg) anesthetized cats recorded gastric motility through an open tip pyloric cannula connected to an isotonic saline filled volume reservoir. The reservoir was arranged in such a way that intragastric pressure could be set at a desired level. To ascertain an exact transmural pressure across the gastric musculature, the open abdominal cavity was filled with Tyrodes solution. The transmural pressure was equal to the difference between the fluid height in the reservoir and that in the abdominal cavity.

Martinson in reviewing vagally induced gastric inhibition (44), described two basic methods:

1. pressure recording system - using fluid or air filled intraluminal balloons connected to a water manometer.
2. volume recording system - using low intragastric pressure (2-4 cm H_2O).

Martinson *et al.* (43,44,46) reported inhibition superimposed on excitation. Vagal stimulation (4 cps and 10 v) produced a reduction in the gastric excitatory response, an interruption in the initial rising phase of excitation and a prolonged depression of basal tone when duration was as low as 0.02 msec. Gastric inhibition had a higher threshold than gastric excitation, which in turn had a higher threshold than cardio-inhibition. Their results did not confirm those of McSwiney and Wadge (50) who considered inhibition to be related to tone. The intensity-response data obtained implies Veach's hypothesis of frequency dependent, not tone dependent responses.

Kewenter (39), investigating the vagal control of gastrointestinal

motility in the urethane-chloralose anesthetized cat, recorded motility kymographically *via* a gastric intraluminal balloon (30-40 cm) and intestinal open tip catheters. Kewenter reported absence of cervical vagal inhibitory fibers to the small intestine, with inhibition induced by sub-diaphragmatic vagal stimulation resulting from activation of high-threshold fibers. Kewenter suggested that the simultaneous occurrance of intestinal inhibition, decreased intestinal blood flow and increased blood pressure, are the consequence of antidromic activation of thin afferent fibers exciting the intestino-intestinal inhibitory reflex arc.

Campbell (12), studying some pharmacological aspects of vagally induced gastric inhibition, used an *in vitro* preparation of the guinea pig stomach. The stomach, first few centimeters of duodenum, and vagal trunks were placed in a 100 ml bath of Krebs solution bubbled with 95% oxygen and 5% carbon dioxide maintained at $37^{o}C$. Gastric responses were recorded *via* a pyloric cannula connected to either a float recorder or a wide bore saline manometer. Using this preparation, Campbell studied the effects of amphetamine, atropine, bretylium and hyoscine on vagally induced gastric inhibition. He observed a more effective vagally induced relaxation than splanchnically induced relaxation at frequencies less than 10 cps. Vagally induced relaxation was a more rapid response (0.8 sec latent period) than the sympathetically induced relaxation (greater than 1.0 sec latent period).

Bulbring and Gershon (9) extensively studied the effect of drug administration on vagally induced gastric vagal inhibition using the same *in vitro* technique used by Campbell (12). Their results are presented in a following section.

Vagally Induced Gastric Motility Following Drug Administration

Langley (40) was the first to report the augmentation of vagally induced inhibition of the esophagus and gastric cardia following the administration of atropine. That inhibition was recorded if the dose of atropine was large enough to block excitatory cholinergic responses. Very large doses weakened the stimulation induced effects of both motor and inhibitory fibers.

Anticholinergic drugs (atropine or scopolamine) block the muscarinic responses of the stomach (52). It has been shown by use of tetrodotoxin which abolishes nerve mediated responses, that catecholamines like epinephrine act directly on the smooth muscle (9). Harrison and McSwiney suggested in 1936 (32) that the high threshold gastric inhibitory responses were adrenergically mediated (atropine abolished the excitatory responses but only slightly suppressed the inhibitory response). However, Campbell (12) observed little change in vagally induced gastric inhibition following both atropine administration and adrenergic blockade by bretylium (10^{-6} - $3x10^{-6}$g/ml) while gastric inhibition induced by perivascular nerve stimulation was completely abolished. At high concentrations, bretylium ($3x10^{-5}$g/ml) was able to reduce vagally induced gastric inhibitory responses markedly confirming Greeff et al. (27). Amphetamine (10^{-6}-10^{-5}g/ml) failed to antagonize high concentrations of bretylium, therefore indicating some action of bretylium other than the specific blockade reported by Day (18). Burnstock et al. (10) suggested that bretylium was acting in some other way than specific neuron blockade, possibly a ganglionic action. Martinson (44) was able to show this same noncholinergic, nonadrenergic response in atropinized animals by adrenergic blockade with quanethidine (4.5 mg/kg) or pronethalol (5.0 mg/kg)

and phenoxybenzamine (0.5, 0.8, 1.0 mg/kg).

Greeff et al. (27) reported that vagally induced inhibitory responses were mediated by some adrenergic mechanism. However, Martinson (44) abolished sympathetic inhibition with pronethalol, a beta adrenergic blocking drug which only slightly suppressed the vagal response. It has been argued that vagal inhibitory fibers were adrenergic fibers which were resistant to pharmacological blockade in much the same way as some cholinergic fibers were resistant to atropine blockade (34).

Bulbring and Gershon (9) reported that vagal electrical stimulation (supramaximal voltage, 0.1 msec, and 2-6 cps) or 5-hydroxytryptamine (5-HT) administration (10^{-5}g/ml), after the administration of scopolamine produced relaxation of the isolated guinea-pig stomach. With increasing concentrations of 5-HT (10^{-9}-10^{-4}g/ml) the gastric excitation was depressed, only relaxation was observed following 10^{-4}g/ml of 5-HT. 5-HT, failing to antagonize the acetylcholine (10^{-7}g/ml) response, appeared to produce a prolonged stimulation of the gastric inhibitory neurons. From these data it was concluded that there are two types of postsynaptic receptors: one sensitive to acetylcholine, and the other sensitive to 5-HT. The inhibitory responses were completely abolished only when the competitive block of acetylcholine receptors by scopolamine was combined with desensitization of 5-HT receptors by biguanides or quaternary derivatives of 5-HT ($2x10^{-6}$g/ml). After the depletion of 5-HT stores by reserpine, 5-HT (10^{-5}g/ml) resulted in a greater vagally induced gastric inhibitory response.

Postulated Neuromechanisms for Vagally Induced Gastric Inhibition

Langley (40) in his explanation of gastric vagal inhibition, acknowledged that vagal inhibitory fibers exist and may predominate during

vagal stimulation.

Bayliss and Starling (5) suggested that gastrointestinal inhibition and excitation are regulated by a local nervous system (Auerbach's plexus) containing long inhibitory pathways and short augmenting pathways which carry impulses from one intramural neuron to another.

Cannon and Lieb (13)observinga relaxation of the cardia and fundus as a result of deglutition mentioned that receptive relaxation was vagally controlled. The lowest intragastric pressure was observed at the time the expelled bolus would have been delivered to the stomach indicating a possible correlation with the cephalic phase of digestion.

It was Veach (59) who first attempted to explain the vagal-gastric inhibitory effect. The nullifying effect of stimulating one vagus with low intensity impulses on gastric inhibition produced by simultaneous high intensity stimulation of the contralateral vagus, suggested a postganglionic inhibitory site. Finding that the excitatory and inhibitory responses depended on the intensity or frequency of the applied stimulus, Veach suggested that the response was related to the frequency of impulses over the postganglionic fibers. Therefore, vagally induced gastric inhibition was the result of Wedensky inhibition (60) in which the frequency of propagated impulses is such that each impulse travels in a refractory phase of the previous one, resulting in a decreased conduction within the neuromuscular complex. He suggested that tonus level was the result of propagated disturbances over the peripheral part of the neuromuscular mechanism. Vagal stimulation increased the postganglionic frequency first into the excitatory range. If the increase in frequency is continued further, it enters into the inhibitory range. When tone was high the propagated disturbances would be at a greater frequency, therefore vagal stimulation

would result in a more rapid inhibition. Veach suggested that the after-contraction was due to a decrease in impulse frequency over the conduction mechanism from a high inhibitory level, passing through the excitatory range at a lower frequency.

McSwiney et al. (32,49) stated that possibly the vagus contained sympathetic fibers originating in the superior cervical ganglia. Failure of atropine to abolish gastric vagal inhibition caused them to conclude that inhibition was adrenergically mediated. However, this was contradicted later when adrenergic blocking agents failed to abolish inhibition (12). Burnstock et al. (10) suggested that the postganglionic fibers in the vagal inhibitory pathway were the intramural inhibitory nerves.

Martinson and Muren (46) suggested that the vagus contained two distinctly different groups of fibers, with the low threshold excitatory responses mediated cholinergically and the high threshold responses mediated by some unknown transmitter.

Bulbring and Gershon (9) were able to show a possible role of 5-hydroxytryptamine in the inhibitory innervation of the stomach. They concluded that 5-HT and acetylcholine function as neurotransmitters in the inhibitory pathway. Both function preganglionically indicating either two different types of vagal fibers or two functionally different post-ganglionic receptors. Bulbring and Gershon concluded that cholinergic and 5-hydroxytryptaminergic fibers work synergistically on the same cells. The release of 5-HT during gastric vagal inhibition and the resistance of both 5-HT release and gastric inhibition to anticholinergic drugs supports their hypothesis that 5-HT participates as a neurotransmitter.

Statement of Problem

A recently developed method, the extraluminal contractile force transducer (ECFT) quantitatively samples discrete contractile activity of the longitudinal or circular muscle with uniaxial sensitivity. Jacoby et al. (36), reporting the reliability of the extraluminal force transducer, correlated dog duodenal electrical activity, intraluminal pressure change, and extraluminal contractile force. Reinke et al. (53,54) modified the transducer and recorded dog gastrointestinal control patterns of the circular and longitudinal muscle layers during the digestive and interdigestive periods. Rosenbaum et al. (55) more extensively studied the in vivo force, frequency and velocity of dog gastrointestinal contractile activity. The ECFT has also been used to record uterine contractile activity in unanesthetized rabbits by Dominic and Reinke (20) and Callantine et al. (11) and in dogs by Bass and Callantine (4).

The problem of this thesis was the study of vagally induced gastric facilitation and inhibition in the atropinized and non-atropinized rabbit.

Vagally induced gastric inhibition has been studied using methods which record only mean gastric motility changes. Due to the limitations of these methods in studying gastric antral and body motility, antral and body ECFT monitored contractile activities were correlated with intraluminal pressure responses elicited by electrical stimulation of the decentralized vagus nerves in anesthetized rabbits.

EXPERIMENTAL METHODS

Extraluminal Contractile Force Transducers

ECFT's were fabricated according to the method described by Reinke et al. (53,54) and Dominic (19,20). Two etched-foil strain gages[1] were bonded, one to the convex and the other to the concave surface of a metal shim stock (12 mm, 5 mm, 0.2 mm)[2], in which four suturing holes (1 mm diameter) were drilled. The ECFT and adjacent soldered portions of the lead wires were encapsulated with Dacron mesh reinforced raw silicone elastimer[3], then heat-cured at 220°F for 24 hours. Recordings were made on a curvilinear ink-writing oscillograph (Dynograph Type R)[4]. ECFT's were calibrated as described by Dominic (19,20) by hanging equal weights on sutures tied one to each end of the supported transducer.

Preparation of Animal

New Zealand White rabbits (N=9) of either sex, weighing from 2-3 kg, were fasted with water for 18-24 hours in a restraining stock to prevent coprophagia. The animals were anesthetized with urethane-allobarbital[5] (.7-1.0 ml/kg) i.p. A pyloric open-tip cannula was connected to a venous pressure transducer (Statham P23-BC)[6]. The stomach was washed

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[1]Model ED-DY-090-DG-350, Micro-Measurements Inc., Romulus, Michigan.

[2]Berylco 25, The Beryllium Corp., Reading, Pennsylvania.

[3]Silastic 372, Compliments of Dow Corning Corp., Midland, Michigan.

[4]Spinco Division, Beckman Instruments, Lincolnwood, Illinois.

[5][Dial, Allobarbital (100 mg/ml)-urethane (400 mg/ml)], Veterinary Sales Division, CIBA Pharmaceutical Corp., Summit, New Jersey.

with five 20 cc aliquots of 37°C saline, and then filled with 30 cc of 37° saline. Ligatures were tied around the pylorus and cervical esophagus to prevent loss of fluid. ECFT's were oriented transversely on the greater curvatures of the gastric antrum and body (Fig. 1) with two Mersiline-000[7] sutures tied snugly through the suturing holes in the shim stock. The cervical vagi were isolated and cut, the adjacent skin was formed into a well containing mineral oil which served as an electrical insulator. Respiration was maintained artificially at 40 breaths/min.

Nerve Stimulation and Drug Administration

The distal ends of the cut vagus nerve trunks were placed on hooked, dipolar, silver electrodes positioned within the oil-filled well. Electrical impulses were delivered to the electrodes by a square-wave stimulator (Grass S-5)[8]. Impulse trains (15 sec duration) were applied at 1 or 2 minute intervals at supramaximal intensity (2 msec duration, 30 volts) while increasing the frequency from 2-128 cps in progressive multiples of 2.

Atropine and barium chloride solutions were administered through a femoral venous cannula.

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[6]Statham Instrument Inc., Oxnard, California.

[7]Ethicon Inc., Somerville, New Jersey.

[8]Grass Medical Instruments, Quincy, Massachusetts.

Integrating Intraluminal Pressure (ILP) Recordings

Facilitory responses to vagal stimulation were recorded at low oscillograph sensitivity (0.2-1.0 mv/cm) and inhibitory responses at high recorder sensitivity (0.05 mv/cm). A composite ILP tracing is shown in Fig. 2.

Figure 1 -

Positioning of the antral (A) and body (B) contractile force transducers on the gastric serosa in relation to the intraluminal pressure cannula (I) inserted through the ligated pylorus (L_2), with the esophagus (E) ligated in the cervical region (L_1). W_1 and W_2 represent three-conductor lead wires to the transducers.

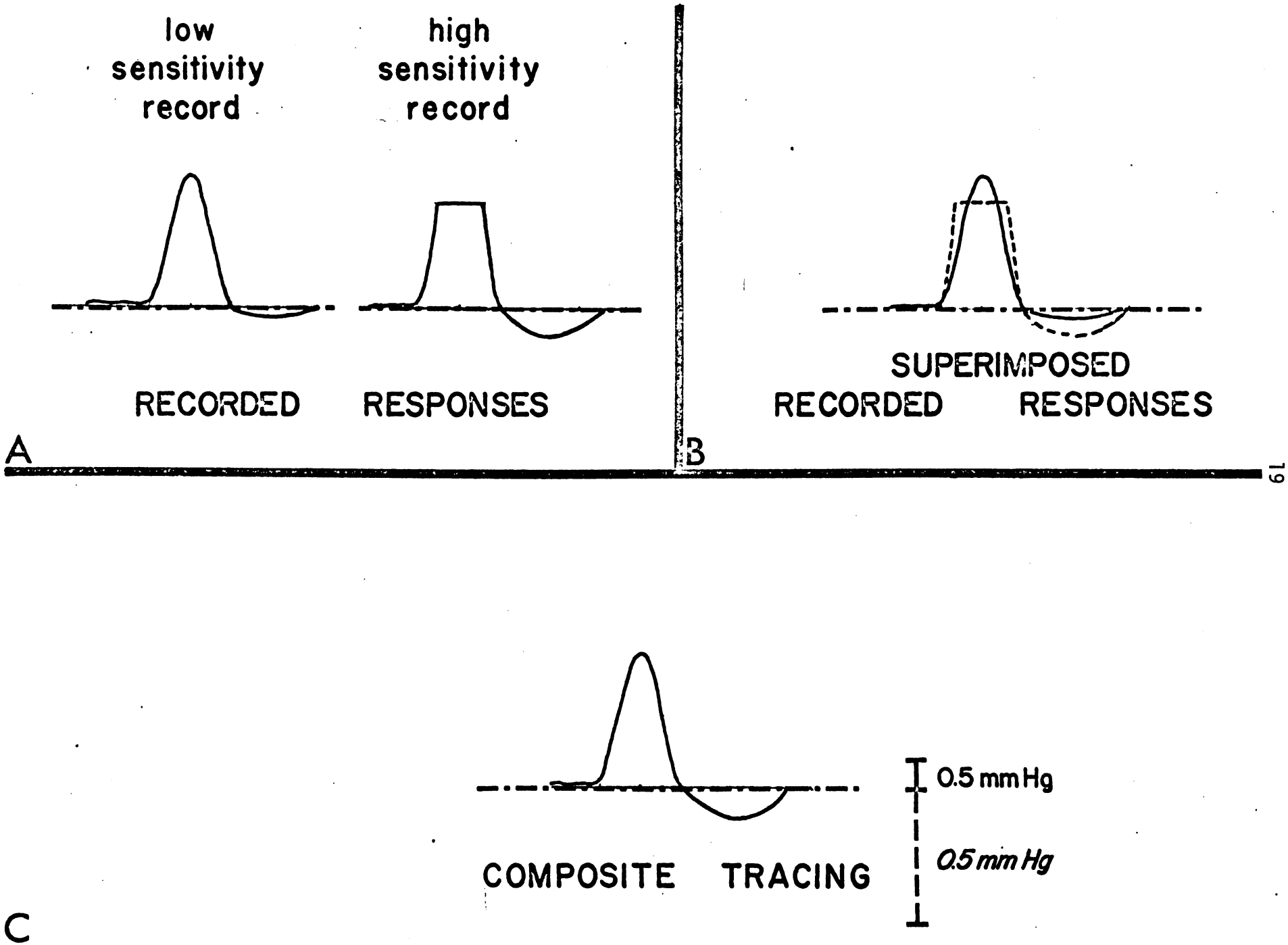
Figure 2 -

Diagrammatical representation of the precedure used to combine facilitory ILP responses (recorded at low sensitivity, indicated by solid vertical calibration bars) and ILP poststimulatory inhibitory responses (recorded at high sensitivity, indicated by dashed vertical calibration bars and slanted numerals).

A. Responses recorded separately in the same animals at two different recording sensitivities (0.05 and 1.0 mv/cm).

B. Superimposed tracings of low sensitivity (solid tracing) and high sensitivity (dashed tracing) records.

C. Composite tracing with vagally induced facilitation followed by poststimulation inhibition.


low
sensitivity
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high
sensitivity
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A
B
SUPERIMPOSED
RECORDED
RESPONSES
19
COMPOSITE
TRACING
0.5 mm Hg
0.5 mm Hg
C

Effects of Vagal Stimulation on Rabbit Gastric Motility

Intraluminal pressure (ILP) responses to vagal stimulation with impulses of supramaximal intensity (30 volts, 2 msec duration) and varied frequency (2-128 cps) have a characteristic pattern, (Figs. 3,4) increasing in magnitude with increasing impulse frequency, with maximum ILP increase recorded at 16-32 cps (Figs 5,6). Stimulation of both vagi resulted in a greater absolute ILP change than stimulation of the left vagus; however, the change expressed as a percent of maximum revealed no statistical difference, therefore maintaining the characteristic pattern (Fig. 5).

Upon cessation of stimulation, ILP increased transiently (3-5 sec) then fell to a level below control ILP (Figs. 4,8). Although it is not conspicuous in Figs. 3 or 4, the absolute level of the post-stimulation ILP increase appeared to be dependent on the stimulating impulse intensity and the gastric contractile state at cessation of stimulation. Post-facilitation inhibition (PFI) increased as facilitation increased (Figs. 3,4). PFI duration ranged from 30-65 seconds and increased as frequency increased with maxima at 16-32 cps (Fig. 4).

Body contractile force (BCF) responses to increasing impulse frequency (2-128 cps) increased in amplitude to maximum, then declined (Figs. 3,4, and 6). ILP increases correlated ($r=0.99$) and coincided with BCF decreases (Figs. 3,6).

Antral contractile force (ACF) responses increased to a maximum at stimulation parameters corresponding to and correlating ($r=0.98$) with ILP and BCF maxima (Fig. 6). Frequently post-stimulation ILP increases coincided with ACF increases and BCF decreases. ACF decreases

were never observed during post-stimulation ILP increases.

Decreased ILP Responses Following Atropine

ILP increases were abolished following atropine (1 mg/kg) with subsequent additions of atropine reversing the ILP response to vagal stimulation (Fig. 7). After 2 mg/kg of atropine, vagal stimulation resulted in inhibition (decreased ILP) (Fig. 8). Atropine failed to affect resting ILP in stomachs exhibiting either tonus or atony. Although vagally induced inhibition was recorded, PFI was not recorded from atropinized animals (Fig. 8).

Gastric Motility Following Barium Chloride

Barium chloride solution (administered to an atropinized animal) produced a gradual increase in resting ILP (Figs. 9,10). Vagal stimulation following each dose of barium chloride decreased ILP, with the ILP absolute value approaching that obtained following atropine (Fig. 9). At constant electrical parameters, vagally induced inhibition coincided with increased resting ILP (Fig. 11). Maximum vagally induced inhibition was obtained at frequencies less than 8 cps, with increased frequencies not only failing to augment but frequently depressing inhibition (Fig. 12).

Vagal stimulation following atropine and progressive doses of $BaCl_2$ (totaling 8.61 mg/kg administered during a 10 min period) resulted in ILP inhibition to pre-drug resting values (0.9 - 1.1 mmHg) (Fig. 12). As frequency increased, post-stimulation ILP response increased. Stimulation at 2 cps produced only inhibition with little sign of facilitation and only partial recovery upon cessation of stimulation.

Vagal stimulation following barium chloride (4 mg/kg) and atropine (1 mg/kg) resulted in an augmented ILP decrease, with BCF increases and ACF decreases being less conspicuous (Fig. 13). Barium chloride decreased pre-stimulation BCF and increased pre-stimulation ILP. Vagally induced ILP decreases recorded at each impulse frequency never exceeded that recorded at 4 cps; however, the increasing pre-stimulation ILP augmented the relative ILP decreases at 32 and 64 cps. Post-stimulation ILP increases, indicative of after-contractions, increased in amplitude as impulse frequency increased. Maximum post-stimulation ILP increases and pre-drug facilitation occurred at 16-32 cps, with 2 cps resulting in the least conspicuous response (Fig. 13, upper). Post-stimulation ILP increases (after-contractions) were often recorded when ILP changes failed to occur during stimulation.

An additional 1 mg/kg of atropine had little effect on vagally induced ILP decreases, maximum occurring at 4 cps; however, prestimulation ILP and ACF increase concomitantly (Fig. 13, middle).

Vagal stimulation following an additional 4 mg/kg $BaCl_2$ resulted in decreased ILP, increased BCF, and decreased ACF with maximums at frequencies of 16 and 32 cps (Fig. 13, lower).

Figure 3 -

Simultaneous tracings of gastric intraluminal pressure (ILP), gastric body contractile force (BCF), gastric antral contractile force (ACF) and systemic arterial blood pressure (ABP) responses to electrical stimulation of the distal end of the decentralized cervical vagi. Fifteen second trains of electrical impulses (Voltage, V, = 30 volts, impulse duration, D, = 2 msec) indicated by the heavy portion of the upper time-line, were delivered at one minute intervals at increasing frequencies (F)from 2 to 128 cycles per second (cps). In this and subsequent figures, ILP calibration bars indicate absolute pressure, with both ACF and BCF calibration bars representing grams force from zero (baseline) to indicated value.

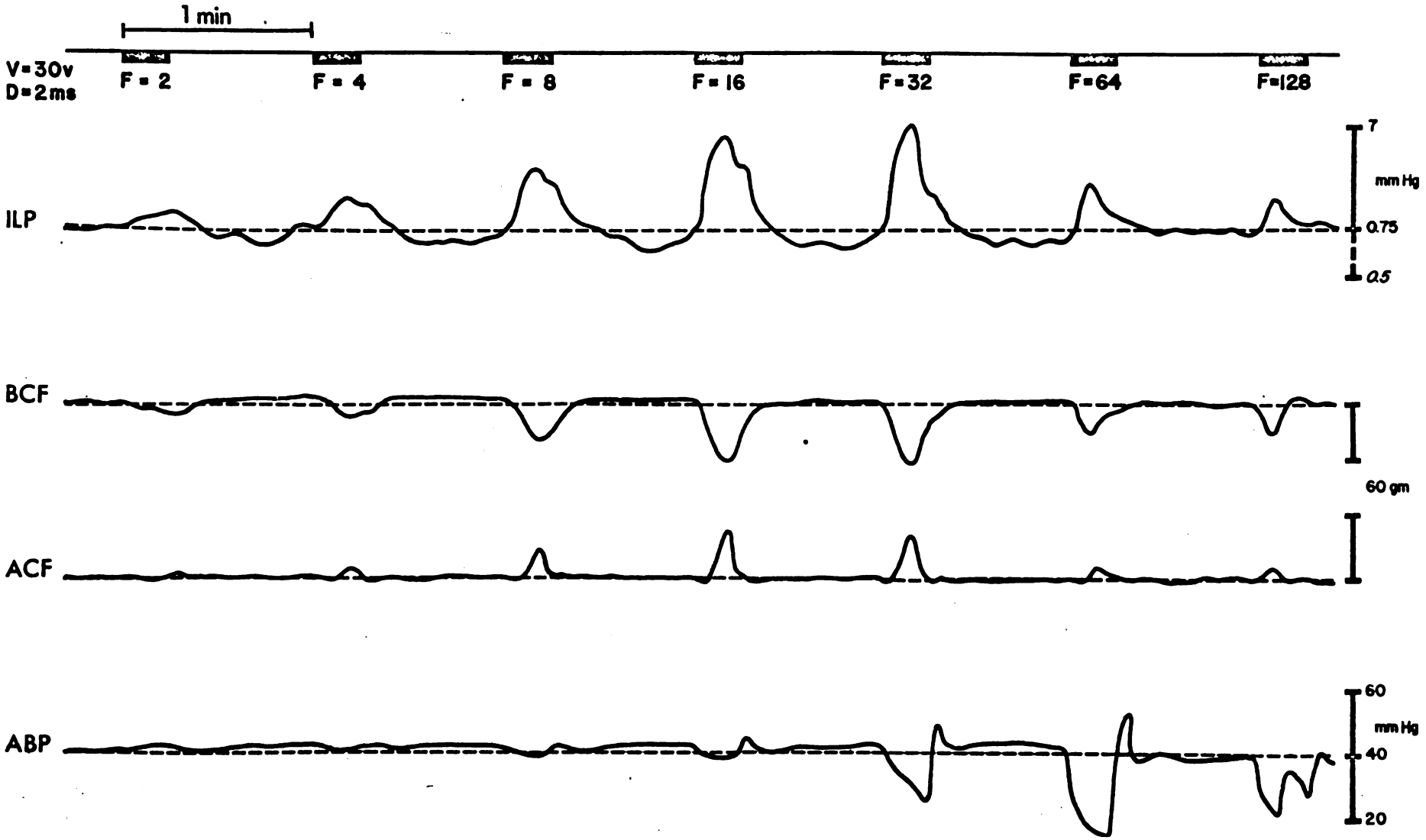

1 min
V=30v
D=2ms
F = 2
F = 4
F = 8
F = 16
F = 32
F=64
F=128
ILP
7
mm Hg
0.75
0.5
BCF
60 gm
ACF
ABP
60
mm Hg
40
20

Figure 4 -

Continuous tracings of intraluminal pressure (ILP), gastric body contractile force (BCF), and antral contractile force (ACF) responses resulting from vagal stimulation. Fifteen second trains (V = 30 volts, D = 2 msec) were delivered at varying frequencies (F = 2-64 cps).

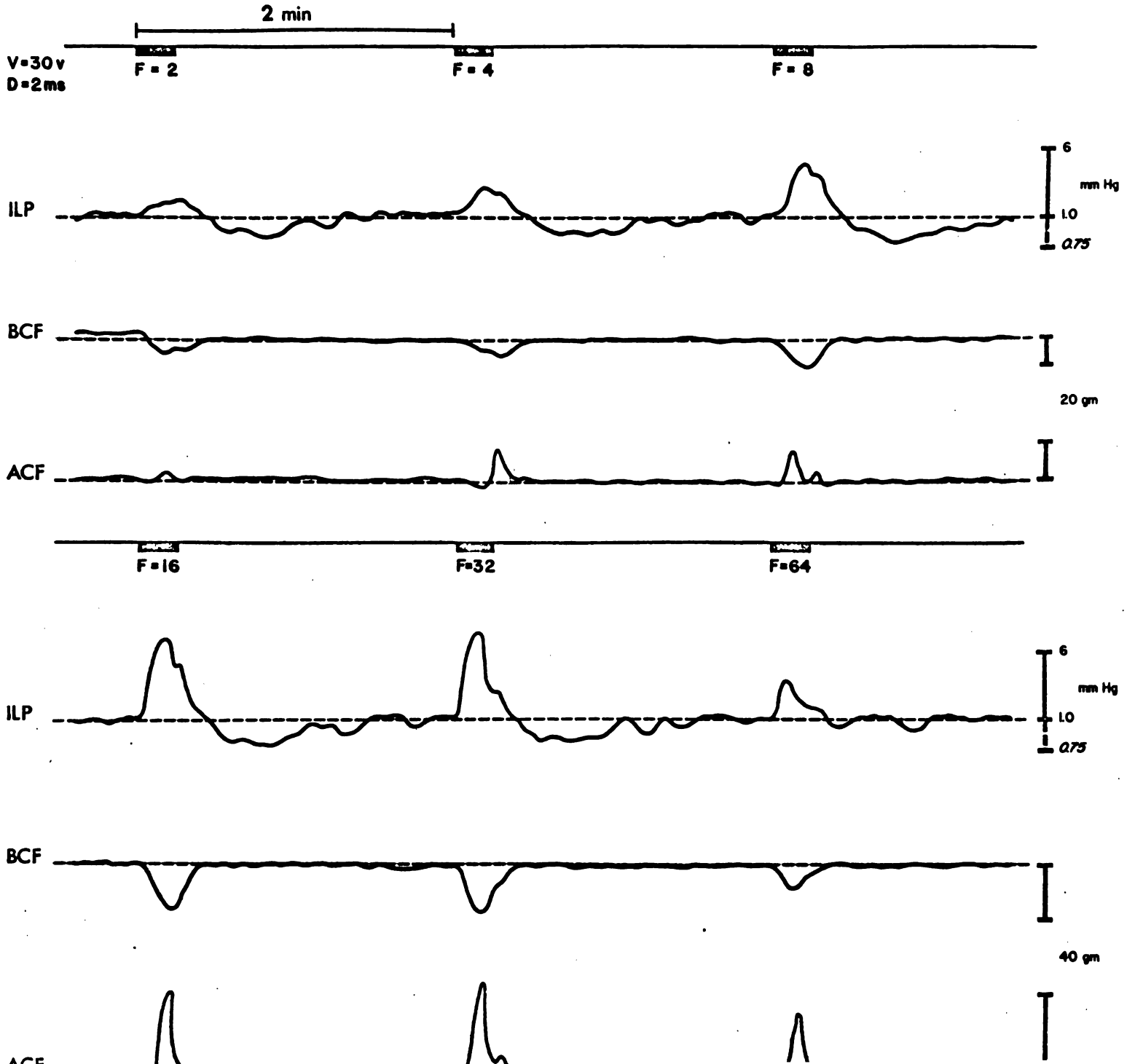

2 min
V=30v
D=2ms
F = 2
F = 4
F = 8
ILP
6
mm Hg
1.0
0.75
BCF
20 gm
ACF
F=16
F=32
F=64
ILP
6
mm Hg
1.0
0.75
BCF
40 gm
ACF

Figure 5 -

Average maximum increase in intraluminal pressure (ΔILP; mmHg) resulting from stimulation of the distal end of the decentralized vagi at frequencies (F) of 2-64 cps. Trains (15 second duration, TD) were administered to the left vagus (LV$\bar{X}$) and both vagi (BV$\bar{X}$). In this and subsequent graphs mean values ($\bar{X}$) plus standard error (SE) are graphed, with the electrical parameters of voltage (V), impulse duration (D), frequency (F), and impulse train duration (TD) indicated in the figure.

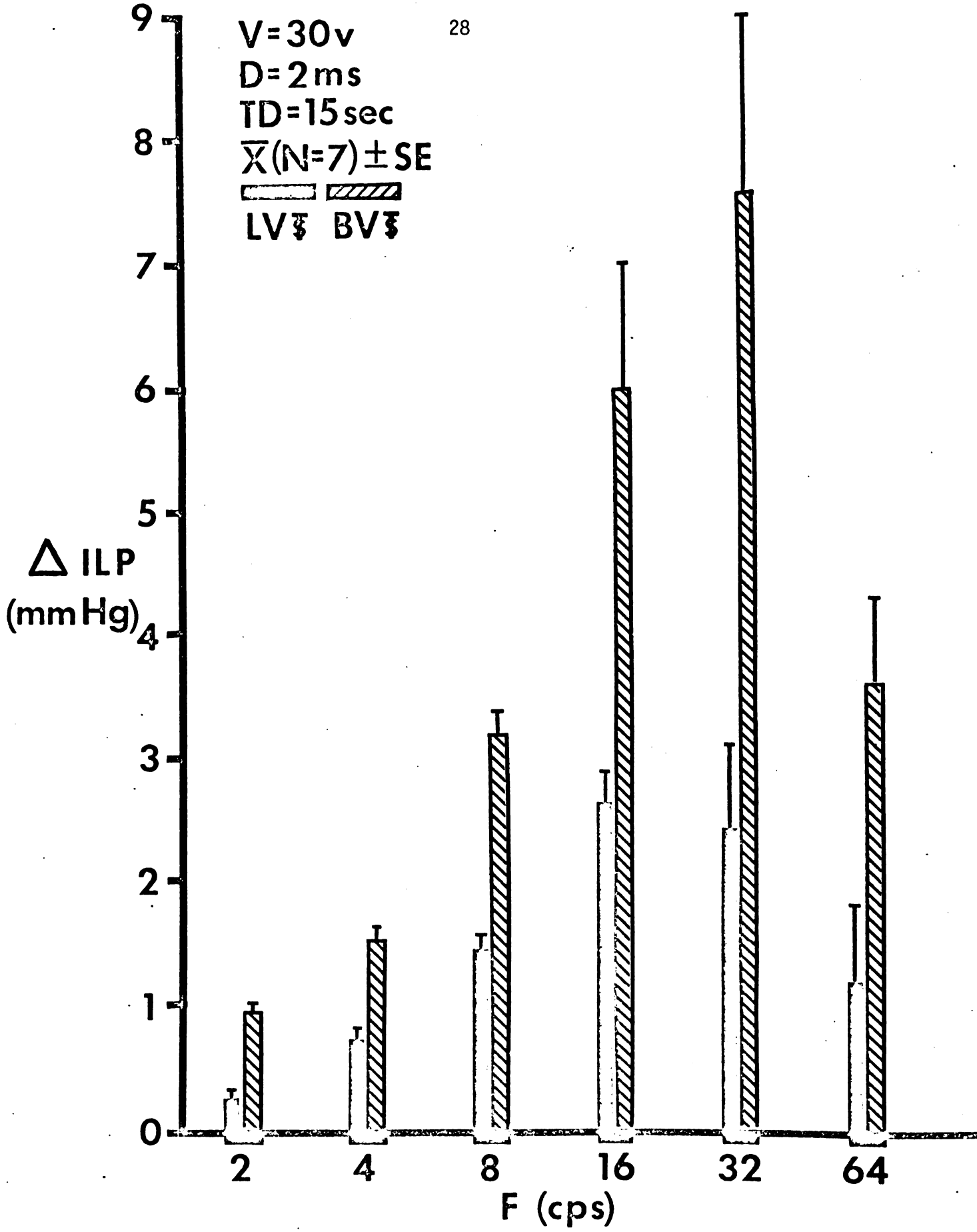

V=30v
D=2ms
TD=15sec
X̄(N=7)±SE
LV$
BV$
Δ ILP
(mmHg)
0
1
2
3
4
5
6
7
8
9
2
4
8
16
32
64
F (cps)

Figure 6 -

Average percent intraluminal pressure (ILP) increases, gastric antrum contractions (ACF) and gastric body relaxations (BCF) at varying stimulation frequency (F; cps). Correlation coefficient (r) of ILP increase and BCF decrease was 0.98 with a $p < 0.01$; while that of ILP increase and ACF increase was 0.99 with a $p < 0.01$.

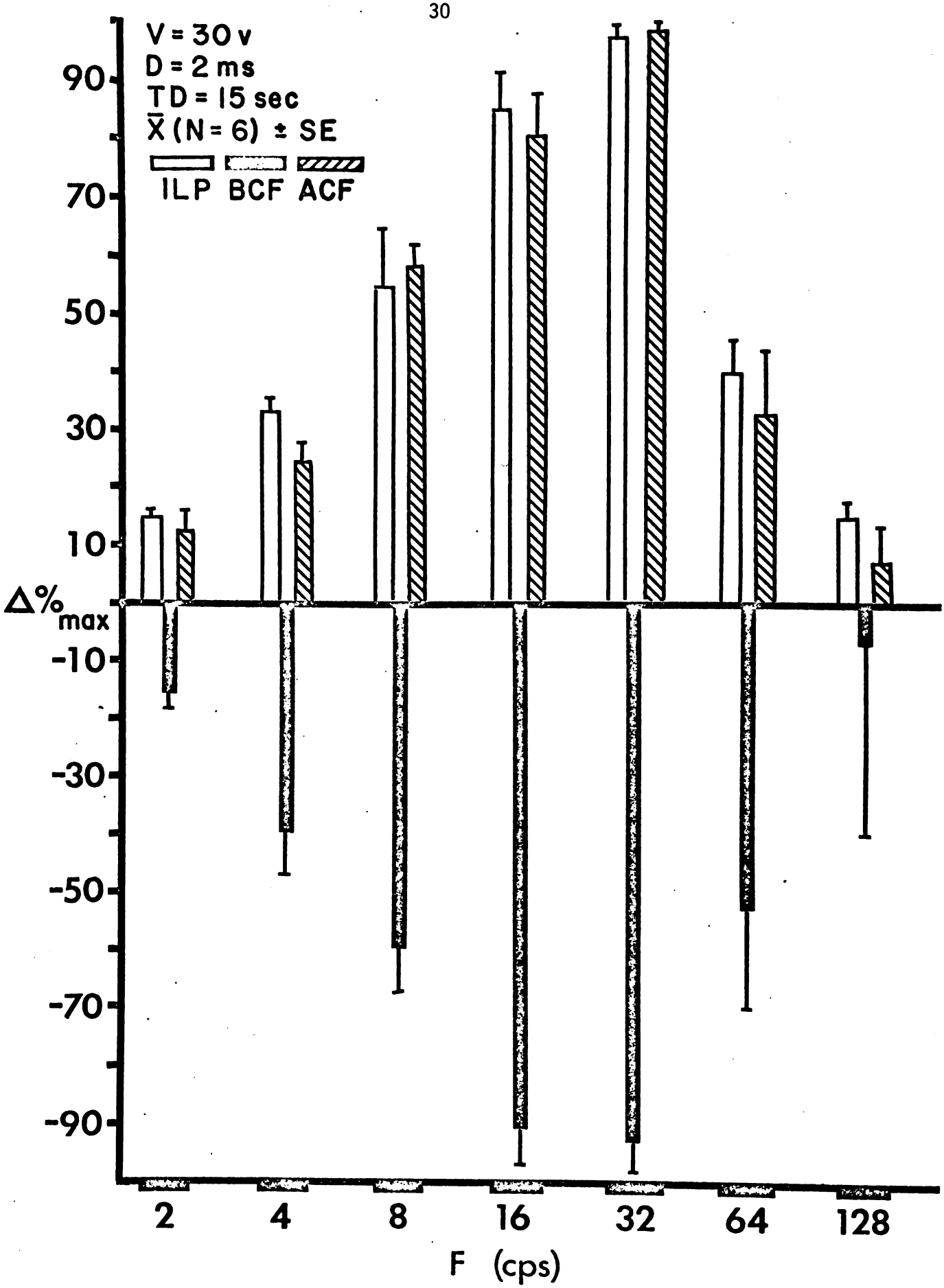

V = 30 v
D = 2 ms
TD = 15 sec
X̄ (N = 6) ± SE
ILP BCF ACF
90
70
50
30
10
Δ%max
-10
-30
-50
-70
-90
2
4
8
16
32
64
128
F (cps)

Figure 7 -

Vagally induced ILP changes (ΔILP) following atropine. Resting ILP is the absolute ILP (mmHg) previous to initial vagal stimulation. Preatropine control (C) ILP increase in response to vagal stimulation recorded 6 minutes prior to atropine administration.

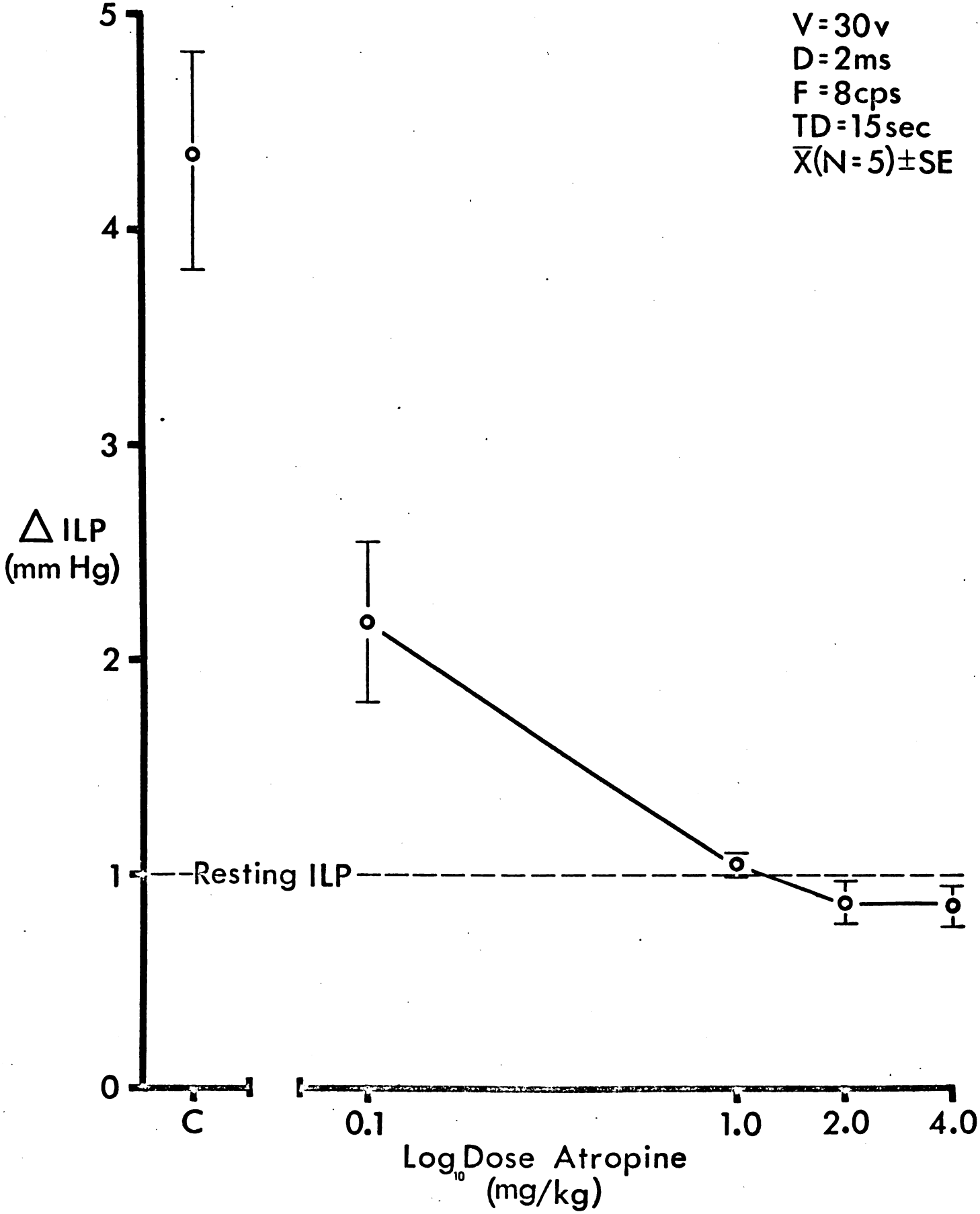

V=30v
D=2ms
F=8cps
TD=15sec
X̄(N=5)±SE
5
4
3
2
1
0
Δ ILP
(mm Hg)
Resting ILP
C
0.1
1.0
2.0
4.0
Log10 Dose Atropine
(mg/kg)

Figure 8 -

Tracings of vagal stimulation induced ILP changes before (upper tracing) and after atropine (2 mg/kg i.v.,lower tracing). Impulse trains of 15 second duration (TD) at one minute intervals. Calibration bars express absolute pressure values.

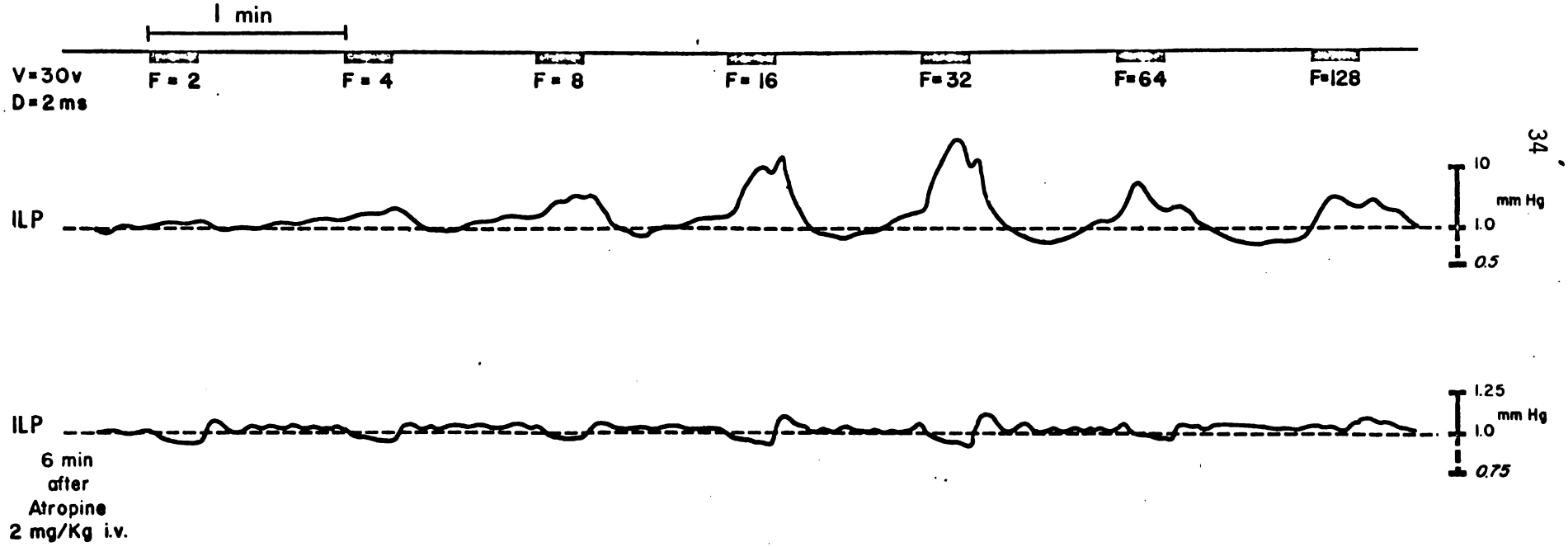

1 min
V=30v
D=2 ms
F = 2
F = 4
F = 8
F= 16
F=32
F=64
F=128
ILP
10
mm Hg
1.0
0.5
ILP
1.25
mm Hg
1.0
0.75
6 min
after
Atropine
2 mg/Kg i.v.

Figure 9 -

Effects of barium chloride ($BaCl_2$) on ILP changes resulting from vagal electrical stimulation (30 volts, 2 msec, 2 cps). Progressive i.v. doses of $BaCl_2$ totaled 8.61 mg/kg. Impulse trains (15 sec) were delivered at 1 minute intervals. Calibration bars indicate absolute ILP pressures recorded at an oscillograph sensitivity of 0.05 mv/cm. Upper time line represents time of indicated tracings with each tracing break representing 6 minutes.

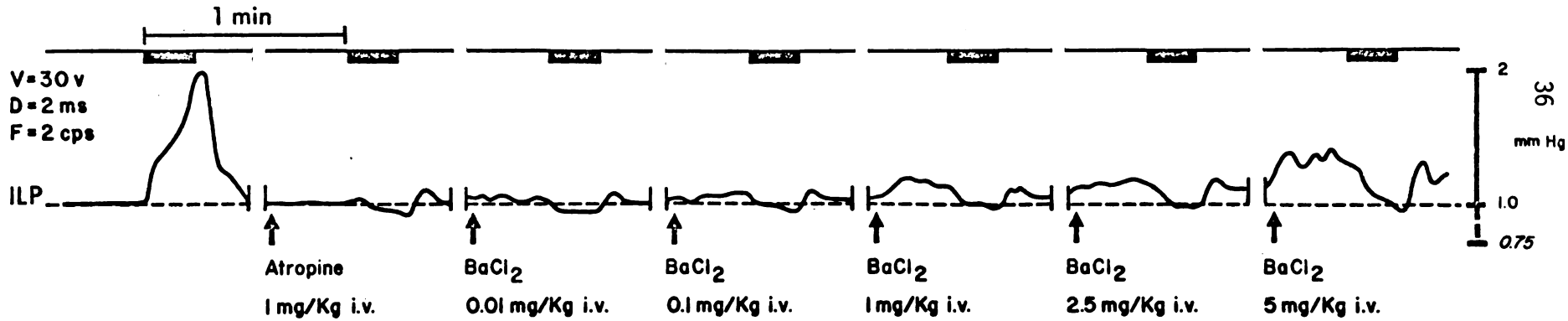

1 min
V=30 v
D=2 ms
F=2 cps
ILP
2
1.0
0.75
mm Hg
Atropine
1 mg/Kg i.v.
BaCl2
0.01 mg/Kg i.v.
BaCl2
0.1 mg/Kg i.v.
BaCl2
1 mg/Kg i.v.
BaCl2
2.5 mg/Kg i.v.
BaCl2
5 mg/Kg i.v.

Figure 10 -

Changes in baseline (prestimulation) intraluminal pressure (ILP_b) resulting from i.v. infusion of $BaCl_2$. Barium chloride doses (mg/kg) expressed on a log scale. Mean values ($\overline{X}$) $\pm$ standard error (SE).

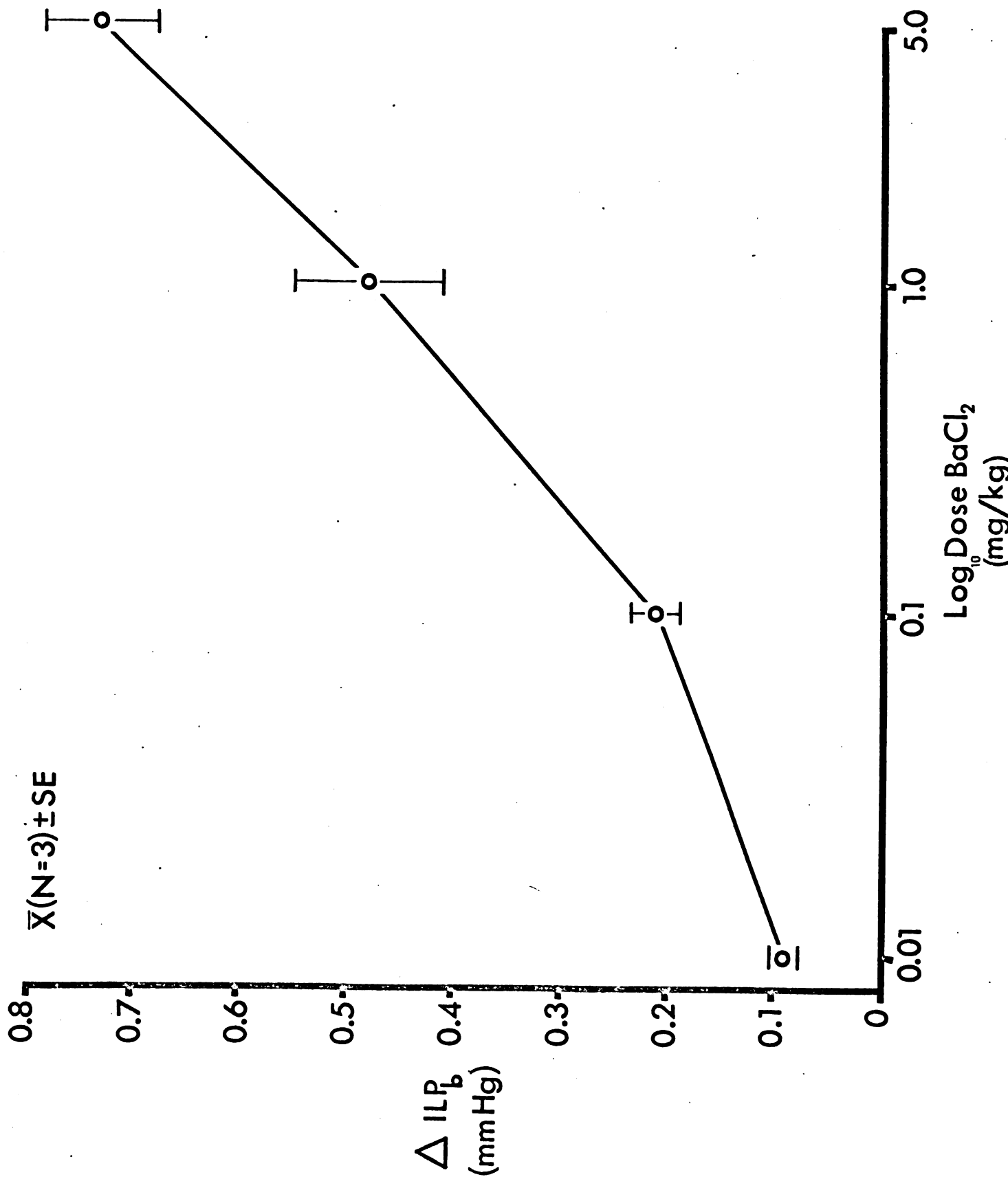

$\bar{X}$(N=3)±SE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Δ ILP$_b$ (mm Hg)
0.01
0.1
1.0
5.0
Log$_{10}$ Dose $BaCl_2$ (mg/kg)

Figure 11 -

Increased vagally induced inhibition (I) (decreased ILP) plotted as a function of increased pre-stimulatory baseline intraluminal pressure (ILP_b) following barium chloride in the atropinized rabbit.

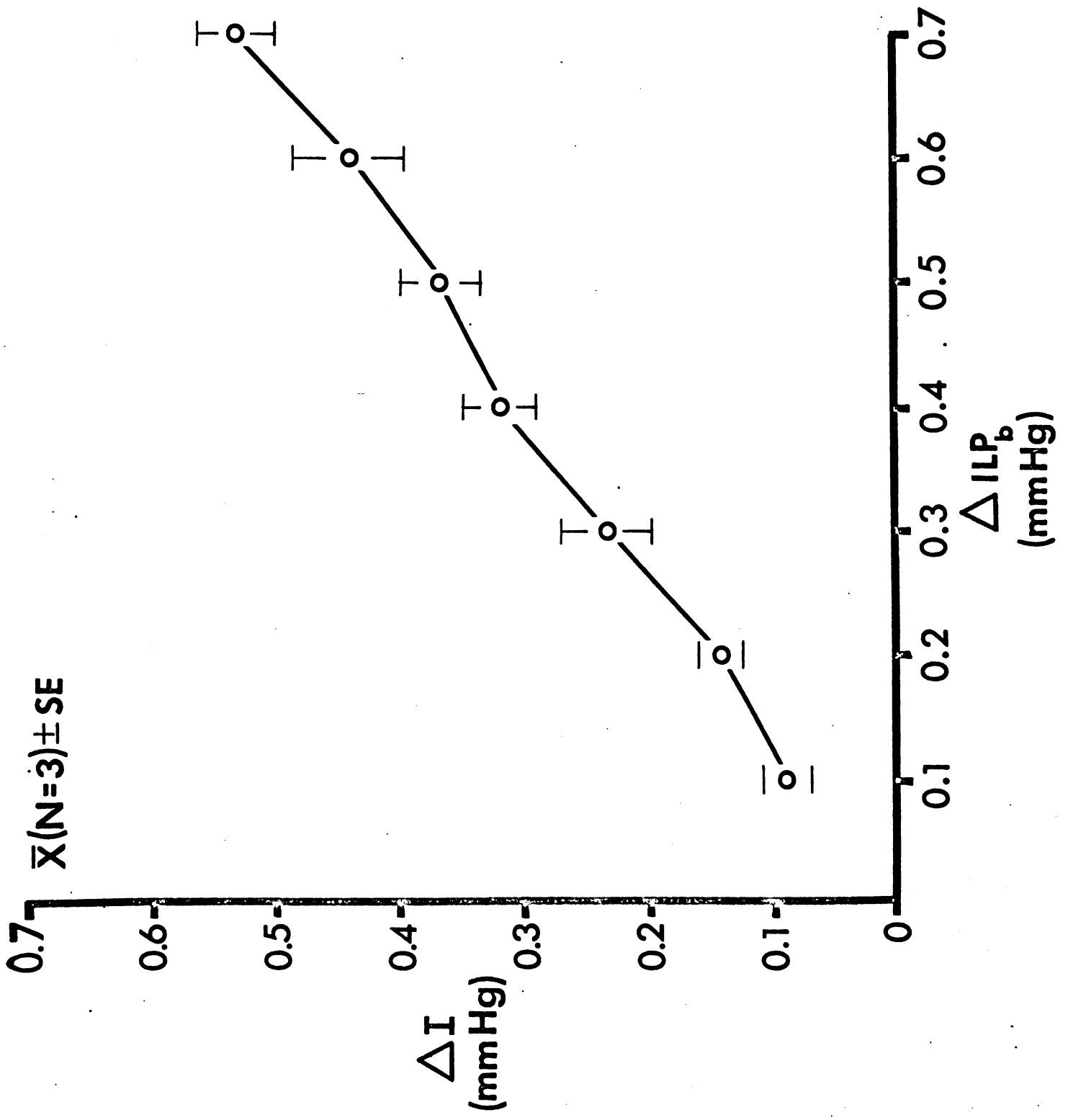

$\bar{X}$(N=3)±SE
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ΔI (mmHg)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
ΔILP$_b$ (mmHg)

Figure 12 -

Effects of changing stimulation frequency (F = 2-32 cps) on intraluminal pressure (ILP) in the atropinized animal following progressive doses of barium chloride ($BaCl_2$ totaling 8.61 mg.kg). Impulse (30 volts, 2 msec, varied frequency) trains (15 sec) were delivered at one minute intervals. Atropine and barium chloride were administered over 12 min. period after recording spontaneous gastric motility. Tracing break represent 6 minutes.

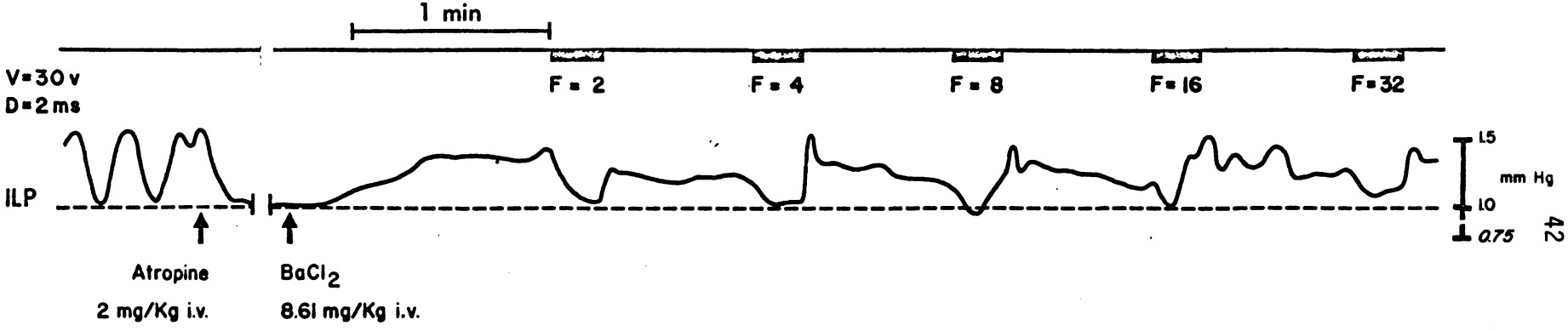

1 min
V=30 v
D=2 ms
F = 2
F = 4
F = 8
F = 16
F = 32
ILP
1.5
mm Hg
1.0
0.75
Atropine
2 mg/Kg i.v.
BaCl2
8.61 mg/Kg i.v.

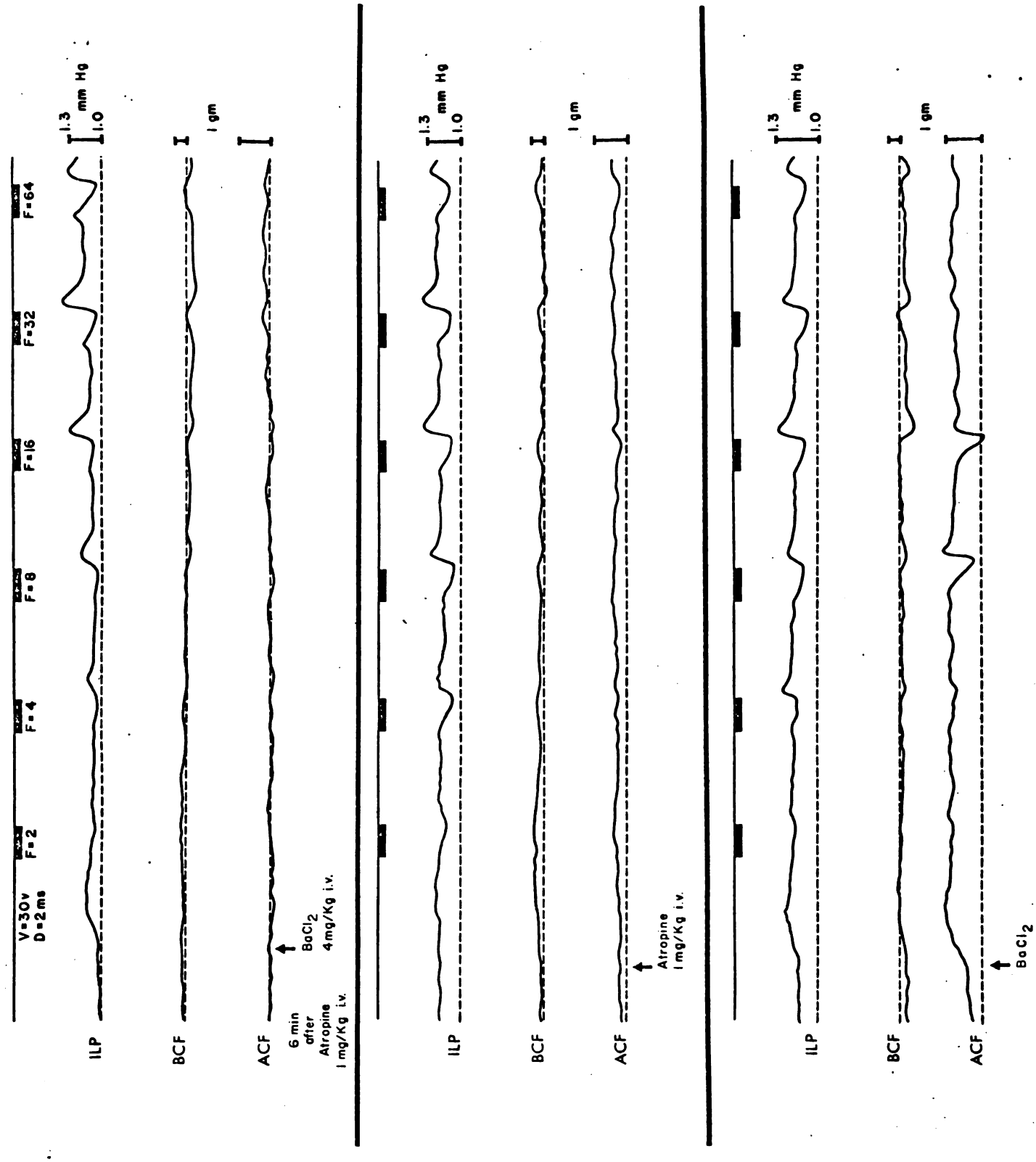
Figure 13 -

Continuous ILP, BCF, and ACF tracings showing the effects of changing stimulus frequency, following atropine and $BaCl_2$. 15 second trains of electrical impulses (30 volts, 2 msec) were delivered at 1 minute intervals.

Upper group of tracings - ILP, BCF, ACF responses following atropine (1mg/kg) and $BaCl_2$ (4 mg/kg).

Middle group of tracings - ILP, BCF, ACF responses following an additional dose of atropine (1 mg/kg).

Lower group of tracings - ILP, BCF, ACF responses following additional dose of $BaCl_2$ (4 mg/kg).


V=30v
D=2ms
F=2
F=4
F=8
F=16
F=32
F=64
1.3
1.0
mm Hg
ILP
BCF
1 gm
ACF
6 min after Atropine 1 mg/Kg i.v.
BaCl2 4mg/Kg i.v.
Atropine 1mg/Kg i.v.

DISCUSSION

Vagally induced facilitation of rabbit gastric motility is manifested by antral contractions occurring simultaneously with passive distention of the gastric body (Figs. 3,4,6). PFI appears to be related to the magnitude of the preceding facilitation (Figs. 3,4). Vagally induced gastric inhibition following atropine suggests a noncholinergic mechanism (Figs. 7,8). Augmentation by barium chloride suggests that inhibition is related to the contractile state (tonus) of the gastric muscle (Figs. 9,11).

Vagally Induced Gastric Facilitation

The characteristic pattern of vagally induced gastric motility facilitation (increases in ILP with increasing stimulation impulse frequency to maximum, followed by decreases in ILP) which has been observed in cats (44), guinea pigs (12) and rabbits (Figs. 3,4), has been interpreted as the response to stimulation of two different classes of vagal fibers: low-threshold excitatory fibers and high-threshold inhibitory fibers (46). This hypothesis was based on the data of other investigators who obtained a more sustained postfacilitation inhibition (PFI, decrease in ILP following cessation of stimulation to a level below prestimulatory ILP) at frequencies producing decreasing facilitation (42-46). Although PFI augmentation was obtained in the present study (Fig. 8), the more typical response was a gradual decrease in PFI which corresponded to the decreasing facilitation (Figs. 3,4). Vagally induced gastric facilitation decreased when the left vagus was stimulated (LVS) and both vagi stimulated (BVS) (Fig. 5), suggesting that the neural mechanism regulating gastric function, influences each contractile unit and stimulation of both vagi recruits more units. When the responses, exhibiting the character-

istic pattern, were expressed as a percent of maximum for any given series of frequencies, there were no statistical differences between LV$ and BV$; suggesting that the regulating mechanism is consistent for each contractile unit and the quantitative difference observed was indicative of the number of units participating.

The characteristic pattern of gastric facilitation observed in these studies suggests that as frequency is increased above some critical level, impulse conduction through the neurocontractile complex (defined here as a complex consisting of pre- and post-ganglionic fibers and the muscle cell or cells innervated) might be inhibited. The relation of this decreased conduction to the natural refractory period of the neurocontractile complex (60) as suggested by Langley (40) and Veach (59) requires elucidation.

Postfacilitation Inhibition (PFI)

PFI, which appears to be related to vagally induced gastric motility facilitation, differs characteristically from the results obtained by Martinson and Muren (42-46). Martinson and Muren (42-46) reported an augmented atropine resistant postfacilitation inhibition (PFI) at impulse frequencies greater than that which produced maximum facilitation. However, in this study PFI failed to increase in magnitude at high impulse frequencies, and was absent in the atropinized rabbit. If PFI results from high-threshold fiber excitation, one would expect postfacilitation inhibition at high impulse frequencies equal to or greater in magnitude and duration than that obtained following maximum facilitation. Failure to observe consistently this expected pattern, in this study, suggests either the absence of specific high-threshold inhibitory fibers in the rabbit or decreased efficiency of the neurocontractile

mechanism. However, the former explanation is unlikely because the decrease in ILP recorded during vagal stimulation of the atropinized animals indicated the activation of some mechanism capable of relaxing gastric smooth muscle. The appearance of vagally induced gastric inhibition at 2 cps fails to support a high-threshold frequency concept.

Three hypotheses for postfacilitation inhibition in nonatropinized animals are:

1. Hyperpolarization of the neurocontractile complex following facilitation.
2. accummulation of biological substances capable of relaxing smooth muscle.
3. Activation of specific fibers, thereby releasing an inhibitory transmitter.

Eccles (22), Hughes (33) and Lloyd (41), discussing post-tetanic potentiation, reported that repetitive stimulation caused hyperpolarization of presynaptic fibers. Postganglionic firing resulting from repetitive preganglionic impulses (61) might result in subsequent hyperpolarization of the postganglionic neurocontractile complex, thereby decreasing tonic postganglionic firing by increasing the relative depolarization required to maintain basal contractile tone.

The local accummulation of physiologically active agents has been suggested by Grundfest (28), explaining postactivation potentials, and Scott et al. (56), explaining local regulation of vasodilation. During gastric facilitation, the increased metabolic demands may result in a similar accummulation of metabolites which upon cessation of stimulation produces relaxation either by direct action on the gastric smooth muscle or indirectly via nerve fibers. It is well

documented that certain substances (carbon dioxide, potassium ions, hydrogen ions, adenosine, adenine nucleotides, and Kreb's metabolites) are capable of relaxing smooth muscle (17,29,30). One or more of these agents may be involved in the PFI phenomenon; for example, efflux of potassium from the guinea-pig taenia coli has been reported by Bulbring (8) to be high when contractile tension is high and low when contractile tension is low. Eccles (22) suggests that after-hyperpolarization, which is abolished when considerable intracellular potassium is replaced by sodium or intercellular potassium becomes depleted, is produced entirely by the net outward movement of K^+ ions. Martinson (44) reported that excitation of high-threshold vagal inhibitory fibers resulted in an increased gastric flow suggesting a decrease in cat gastric vascular resistance occurring concomitantly with increased gastric HCl and pepsinogen secretion. The decrease in gastric vascular resistance suggests the relaxation of vascular smooth muscle, however this relaxation may be a functional response to increased metabolic demands or passive vasodilation (16) resulting from diminished gastric tone.

The third explanation of PFI, specific inhibitory fibers, gains its greatest support from the work reported by Martinson and Muren (42-46). Although specific vagal inhibitory fibers to the stomach have not been histologically or pharmacologically identified, vagal stimulation of the isolated atropinized guinea-pig stomach results in a more efficient relaxation than that produced by perivascular nerve stimulation (12). Campbell (12) suggested that the post-ganglionic fibers in the vagal inhibitory pathway were similar to the intramural inhibitory fibers reported by Burnstock *et al.* (10). The specific

chemical transmitter in this inhibitory pathway has so far elluded investigators of the vagal inhibitory fibers; however, Bulbring and Gershon (9) have shown that 5-hydroxytryptamine is capable of characteristically duplicating vagally induced gastric inhibition in the isolated scopolaminized guinea pig stomach. From their study they concluded that 5-HT participates preganglionically in this inhibitory pathway. The postganglionic mechanism still requires ellucidation.

Effects of Atropine on Vagally Induced Gastric Motility

Although gastric facilitation is mediated through vagal preganglionic cholinergic fibers (38), increasing doses of atropine gradually converted vagally induced gastric facilitation to inhibition (Figs. 7,8).

The important tone-response relationship of gastric motility (40,49) forces consideration of the effects vagotomy might have on basal tone. Past investigators fail to report consistent results of the effect of vagotomy on postoperative gastric tone. However, references to the importance of gastric tone on motility appear frequently (40,45,49). Harper et al. (31) and Martinson (44) report increased tone (as a result of bilateral vagotomy in anesthetized cats) which they interpret as evidence for a tonic central inhibition. Thomas and Komarov (58) report hypoactivity and hyposecretion in the unanesthetized dog, following vagotomy, which they interpreted as loss of tonic excitation. Although Alvarex et al. (2) report little change in rabbit gastric tone following unilateral vagotomy, bilateral vagotomy resulted in dilatation, diminution of tone, slow and weak peristalsis, and delayed emptying. In the present study, it was assumed that gastric atony (decreased resting and spontaneous contrac-

tile activity) existed in those animals in which atropine administration failed to yield vagally induced gastric inhibition. Vagally induced gastric inhibition could be produced in those animals exhibiting gastric atony by increasing resting tone with barium chloride.

The failure of atropine administration to affect the level of basal tone in vagally decentralized stomach preparation subjected to low transmural pressure, confirms reports by Martinson (44) who suggested that the failure of atropine to lower basal tone indicated that basal tone was not maintained by continuous nerve discharge. Martinson (45) produced an increase in cat gastric volume (relaxation) only if relaxation was initiated from a state of high tone (low volume). This finding was similar to that of Langley (40) who obtained relaxation in rabbits with high gastric tone and facilitation when gastric tone was low.

Existence of atropine resistant cholinergic fibers (34) may play an important role in gastric function. Atropine resistant after-contractions recorded in the present study, confirm those reported by Langley (40) and indicated the possible existence of atropine resistant cholinergic fibers, non-cholinergic gastric facilitory fibers, or a transient overshoot of a recovery response within the hyperpolarized neurocontractile complex.

In light of findings that rabbits possess the ability to hydrolyze belladona alkaloids thereby making this species less susceptible to atropine blockade than some other, the validity of the present results along with other data collected from rabbits, may be questionable. However, not only is there species variation of atropinesterase, but enzymatic activity within species varies (3). Ambache et al. (3) report that only 45% of the animals tested exhibited

atropinesterase activity and in those animals having the most potent activity, 1mg/kg of atropine was effective in abolishing the light reflex for at least 45 minutes. In this study, atropine dosage at least equaled 1 mg/kg, and all subsequent data was collected in less than 45 minutes. Therefore the use of these data appear to be justifiable in light of the findings of Ambach et al. (3).

Effects of Barium Chloride on Vagally Induced Gastric Inhibition

Vagally induced gastric inhibition in the atropinized animal is more easily obtained with presence of an elevated gastric tone (Figs. 9,12). Relaxation amplitude did not increase appreciably with increased frequency (Fig. 12), suggesting that under these conditions maximum activation of vagally induced inhibitory fibers was obtained at 2 cps. and that the inhibition was not a frequency dependent response. The frequency response curves, at submaximum intensities, reported by Jansson and Martinson (37) may be criticized on the point that stimulation of an entire nerve trunk at submaximum intensities fails to assure activation of every nerve fiber, therefore, in their preparations increasing frequency may be facilitating recruitment by increasing intraneural current density. However, if supramaximum intensities are used, increasing frequency would elicit only frequency dependent responses. The failure of high impulse frequency to augment vagally induced gastric inhibition supports the suggestion that the neurocontractile mechanism may be hindered (40,59) by these supraphysiological frequencies (greater than 10 cps) (23,24, 25). The apparent effects of barium chloride on gastric motility, increased tonus and facilitation of spontaneous motility, may be due to

the ability of barium ions to substitute for sodium ions, thereby producing prolonged action potentials in B and C fibers (26), and stimulating the release of acetylcholine from cholinergic nerves (21). Barium chloride at doses greater than 4 mg/kg often produced erratic spontaneous gastric motility. Acknowledging that muscle cells are most active at low membrane potentials (39,57) and that barium ions potentiate depolarization of certain nerve fibers (26), barium chloride might be affecting the muscle cell by lowering membrane potential.

Gastric inhibition occurred not only in the gastric body, but also in the gastric antrum (Fig. 13). At increased tone levels, decreased ILP occurred simultaneously with relaxation in the gastric antrum and contraction in the gastric body (Fig. 13). Blair et al. (7) reported small contractions superimposed on a background of decreased gastric tone of etherized cats. This superimposed pattern was also observed in this study, although infrequently.

Neuroregulation of Vagally Induced Gastric Inhibition

Little evidence has been reported ellucidating the neuroregulatory mechanism for vagally induced gastric inhibition, or supporting those mechanisms hypothesized by past investigators. However, vagally induced gastric inhibition, recorded in the present study, occurred well within the physiological frequency range of less than 10 cps reported by Folkow (23) and Garry and Gillespie (25). Campbell (12), working with in vitro guinea pig stomachs, recorded maximum gastric relaxation at about 30 cps. The fact that 65% of maximum was reached at 3 cps strengthens the concept that autonomic control of gastrointestinal effector cells is maintained by low frequency impulses (23,24,25,39). In the present study impulse

frequencies greater than 32 cps depressed facilitation and impulses less than 10 cps produced inhibition in the atropinized rabbit.

Vagally induced gastric inhibition was postulated to be adrenergically mediated because cholinergic blocking drugs abolished vagally induced gastric facilitation but had little effect on vagally induced gastric inhibiton (32,52); however, Bulbring and Gershon (9), confirming Martinson (43) and Campbell (12) abolished splanchnically induced relaxation with propranolol (10^{-5}g/ml) and phenoxybenzamine (10^{-7}g/ml), having little effect on vagally induced relaxation.

Adrenergic inhibition of the cat's gastrointestinal tract has been reported to be postganglionic (39), but Martinson (44) suggests vagally induced gastric inhibition in the cat to be controlled by preganglionic fibers regulating the intrinsic nerve plexus and differing from adrenergically mediated inhibition (42,43,44). In the present study atropine failed to abolish vagally induced gastric inhibition suggesting a noncholinergic muscarinic site and possibly a ganglionic action; however, atropine given in doses 4-8 times greater than those abolishing facilitation failed to abolish inhibition suggesting that a noncholinergic ganglionic transmitter is involved. The indication of a noncholinergic ganglionic transmitter has been reported by Bulbring and Gershon (9). Using an <u>in vitro</u> preparation of the guinea pig stomach, they were able to obtain evidence indicating the possible complimentary role of acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) in the vagal inhibitory pathway. Addition of 5-HT (10^{-5}g/ml) to the bath medium produced a sustained gastric relaxation indicating that 5-HT is capable of activating an inhibitory mechanism. Chemical analysis of the bath medium revealed the release of 5-HT

during gastric vagal inhibition. Tetrodotoxin abolished the release suggesting that 5-HT originates from a neural tissue. When vagal induced gastric inhibition was abolished following cholinergic blockade combined with desensitization of 5-HT receptors, Bulbring and Gershon hypothesized that ACh and 5-HT participated synergistically as preganglionic transmitters with separate postganglionic receptors.

1. Vagal influence on gastric motility was studied in anesthetized rabbits by means of cervical vagal stimulation with graded impulse frequencies (2-128 cps) at supramaximum intensities.

2. Gastric antral and body contractile activities, monitored by extraluminal contractile force transducers, were correlated with gastric intraluminal pressure changes.

3. Noncholinergic gastric inhibition elicited by vagal stimulation was studied following atropine administration, i.v., and tonus related effects of vagal stimulation were studied following varied doses of barium chloride.

4. On the basis of the data obtained in the present investigation the following conclusions seem justified:
   a. Gastric facilitation (ILP increases, BCF decreases, and ACF increases, recorded simultaneously) had a consistent characteristic pattern, increasing in amplitude with increasing impulse frequencies, and had maxima at 16-32 cps. Depression of gastric facilitation at impulse frequencies greater than those producing maximum facilitation, suggests a decrease in nerve impulse conduction.

   b. Gastric postfacilitation inhibition increased in amplitude and duration at increasing impulse frequencies with a

pattern coinciding to vagally induced gastric facilitation, suggesting a relationship to the previous facilitory response. This postfacilitation inhibition, which failed to occur in the atropinized rabbit, may be the result of hyperpolarization of the neuromuscular complex, or a neuromuscular inhibiting substance released during and related to the metabolic process.

c. Atropine converted the vagally induced gastric facilitation to inhibition, with impulse frequency of 2 cps often producing near maximum inhibition, suggesting a noncholinergic intensity-dependent, not frequency-dependent pathway.

d. Barium chloride administration increased prestimulatory gastric tone and augmented vagally induced gastric inhibition with the absolute inhibition obtained never exceeding postatropine level. The inhibition which was recorded in ILP and ACF tracings appeared to decrease at frequencies greater than 10 cps, suggesting that conduction through the neuromuscular inhibitory pathway is also hindered and that the facilitory and inhibitory pathways may have a common component.

REFERENCES

1. Agostoni, E.J., H.E. Chinnock, D.M. Deburgh, and J.F. Murray.
   Functional and histological studies of the vagus nerve and its branches to the heart, lungs, and abdominal viscera in the cat.
   J. Physiol., London, 135: 182-205, 1957.

2. Alvarez, W.C., K. Hosoi, A. Overgard, and H. Ascanio.
   The effects of degenerative section of the vagi and the splanchnics on the digestive tracts.
   Amer. J. Physiol., 90: 631-655, 1929.

3. Ambache, N., L. Kavanaugh, and D.W. Shapiro.
   A rapid procedure for selection of atropinesterase-free rabbits.
   J. Physiol., London, 171: 1P-2P, 1964.

4. Bass, P. and M.R. Callantine.
   Simultaneous recording of electrical and mechanical activity of the uterus in the unanesthetized animal.
   Nature, London, 203: 1365, 1964.

5. Bayliss, W.M. and E.H. Starling.
   The movements and innervation of the small intestine.
   J. Physiol., London, 24: 99-143, 1899.

6. Bercovitz, A. and F.T. Rogers.
   The influence of the vagi on gastric tonus and motility in the turtle.
   Amer. J. Physiol., 55: 323-338, 1921.

7. Blair, E.L., A.A. Harper, C. Kidd, and T. Scratcherd.
   Post-activation potentiation of gastric and intestinal contractions in response to stimulation of the vagus nerves.
   J. Physiol., London, 148: 437-449, 1959.

8. Bulbring, E.
   Smooth muscle of the alimentary tract.
   Modern Trends in Gastroenterology., Hoeber Medical Division, Harper and Row, New York: 1-11, 1958.

9. Bulbring, E. and M.D. Gershon.
   5-hydroxytryptamine participation in the vagal inhibitory innervation of the stomach.
   J. Physiol., London, 192: 823-846, 1967.

10. Burnstock, G., G. Campbell, and M.J. Rand.
    The inhibitory innervation of the taenia of the guinea-pig caecum.
    J. Physiol., London, 182: 504-526, 1966.

11. Callantine, M.R., O.P. O'Brien, B.L. Windson, and R.J. Brown.
Inhibition of uterine contractions in vivo in the unanesthetized rabbit.
Nature, London, 213: 507, 1967.

12. Campbell, G.
The inhibitory nerve fibers in the vagal supply to the guinea-pig stomach.
J. Physiol., London, 185: 600-612, 1966.

13. Cannon, W.B. and C.W. Lieb.
The receptive relaxation of the stomach.
Amer. J. Physiol. 27: XIII, 1911.

14. Carlson, A.J., T.E. Boyd, and J.F. Pearcy.
Studies on the visceral sensory nervous system.
Amer. J. Physiol., 61: 14-41, 1922.

15. Celander, O.
Are there any centrally controlled sympathetic inhibitory fibers to the musculature of the intestines?
Acta Physiol. Scand., 47: 299-309, 1959.

16. Chou, C.C. and J.M. Dabney.
Interrelation of ileal wall compliance and vascular resistance.
Amer. J. of Dig. Dis., 12: 1198-1208, 1967.

17. Daugherty, R.M., J.B. Scott, J.M. Dabney, and F.J. Haddy.
Local effects of O_2 and CO_2 on limb, renal, and coronary vascular resistance.
Amer. J. Physiol. 213: 1102-1110, 1967.

18. Day, M.D.
Effect of sympathomimetic amines on the blocking action of quanethidine, bretylium and xylocholine.
Br. J. Pharmac. Chemother. 18: 421-439, 1962.

19. Dominic, J.A.
Contractile Activity of the Rabbit Uterus Monitored In Vivo with Extraluminal Force Transducers.
Thesis for the Degree of M.S., Michigan State University, 1967.

20. Dominic, J.A. and D.A. Reinke.
Extraluminal force transducer for in vivo measurement of rabbit uterine contractile activity.
Fertility and Sterility, 19: 945-953, 1968.

21. Douglas, W.W. and J.M. Ritchie.
On excitation of non-medullated afferent fibers in the vagus and aortic nerves by pharmacological agents.
J. Physiol., London, 138: 31-43, 1957.

22. Eccles, J.C.
The Physiology of Nerve Cells.
London: Oxford University Press: 76-83, 206-211, 1957.

23. Folkow, B.
Impulse frequency in sympathetic vasomotor fibers correlated to the release and elimination of the transmitter.
Acta Physiol. Scand., 25: 49-76, 1952.

24. Folkow, B.
Nervous control of the blood vessels.
Physiol. Rev., 35: 629-663, 1955.

25. Garry, R.C. and Gillespie, J.S.
The responses of the musculature of the colon of the rabbit to stimulation, in vitro of parasympathetic and of the sympathetic outflows.
J. Physiol., London, 128: 557-576, 1955.

26. Goodman, L.S. and A. Gilman.
The Pharmacological Basis of Therapeutics. 3rd ed., New York; The Macmillan Company, 804, 1965.

27. Greeff, K., H. Kasperat, and W. Osswald.
Paradoxe Wirkungen der elektrischen Vagusreizun am isolierten Magen- und Herzvorhofpraparat des Meerschweinchens sowie deren Beeinflussung durch Ganglienblocker, Sympathicolyticia, Reserpin and Cocain.
Naunyn-Schmiedegerg's Arch. exp. Path. Pharmak. 243: 528-545, 1962. [cited by Martinson, 1965 (44)]

28. Grundfest, H.
Excitation triggers in post-junctional cells.
Physiological Triggers and Discontinuous Rate Processes.
Ed. - Bullock, T.H., Baltimore Waverly Press, 1957.

29. Haddy, F.J., C.C. Chou, J.B. Scott, and J.M. Dabney.
Intestinal vascular responses to naturally occurring vasoactive substances.
Gastroenterology, 52: 444-451, 1967.

30. Haddy, F.J., H.W. Overbeck, and R.M. Daugherty.
Peripheral vascular resistance.
Annual Rev. of Med., 19: 167-194, 1968.

31. Harper, A.A., C. Kidd, and T. Scratcherd.
Vago-vagal reflex effects of gastric and pancreatic secretion and gastrointestinal motility.
J. Physiol., London, 148: 417-443, 1959.

32. Harrison, J.S. and B.A. McSwiney.
The chemical transmitter of motor impulses to the stomach.
J. Physiol., London, 87: 79-86, 1936.

33. Hughes, J.R.
Post-tetanic potentiation.
Physiol. Rev., 38: 91-113, 1958.

34. Hukovic, S., M.J. Rand and S. Vanov.
Observations on an innervated preparation of rat urinary bladder.
Br. J. Pharmac. Chemoth., 24: 178-188, 1965.

35. Jacoby, H.I.
An Extraluminal Gastrointestinal Smooth Muscle Contractile Force Transducer for Intact Unanesthetized Dogs. Doctoral Dissertation. University of Michigan, Ann Arbor, Michigan, 1963.

36. Jacoby, H.I., F. Bass, and D.R. Bennett.
In vivo extraluminal contractile force transducer for gastrointestinal muscle.
J. Appl. Physiology., 18: 658-665, 1963.

37. Jansson, G. and J. Martinson.
Some quantitative considerations on vagally induced relaxation of the gastric smooth muscle in the cat.
Acta Physiol. Scand., 63: 351-357, 1965.

38. Kay, A.W. and A.N. Smith.
The effect of the ganglion-blocking methonium salts on gastric secretion and motility.
Gastroenterology, 18: 503-517, 1951.

39. Kewenter, J.
The vagal control of jejunal and ileal motility and blood flow.
Acta Physiol. Scand. Suppl., 251: 1-68, 1965.

40. Langley, J.N.
On inhibitory fibers in the vagus for the end of the oesophagus and the stomach.
J. Physiol., London, 23: 407-414, 1898.

41. Lloyd, D.P.
Post-tetanic potentiation of response in monosynaptic reflex pathways of the spinal cord.
J. Amer. Physiol., 33: 147-170, 1949.

42. Martinson, J.
The effect of graded stimulation of efferent vagal nerve fibers on gastric motility.
Acta Physiol. Scand., 62: 256-262, 1965.

43. Martinson, J.
Vagal relaxation of the stomach. Experimental re-investigation of the concept of the transmission mechanism.
Acta Physiol. Scand., 64: 453-462, 1965.

44. Martinson, J.
Studies on the efferent vagal control of the stomach.
Acta Physiol. Scand., 65, Suppl. 255: 1-14, 1965.

45. Martinson, J. and A. Muren.
Studies on vagal excitation and inhibition of gastric motility.
Acta Physiol. Scand. Suppl., 175: 103-104, 1960.

46. Martinson, J. and A. Muren.
Excitatory and inhibitory effects of vagus stimulation on gastric motility in the cat.
Acta Physiol. Scand., 57: 309-316, 1963.

47. May, W.F.
The innervation of the sphincters and musculature of the stomach.
J. Physiol., London, 31: 260-261, 1904, (Cited by McSwiney, 1931).

48. McSwiney, B.A.
Innervation of the stomach.
Physiol. Rev., 11: 478-514, 1931.

49. McSwiney, B.A. and W.B. Spurrell.
The gastric fibers of the vagus nerve.
J. Physiol., London, 77: 447-458, 1933.

50. McSwiney, B.A. and W.J. Wadge.
Effects of variations in intensity and frequency on the contractions of the stomach obtained by stimulation of the vagus nerve.
J. Physiol., London, 65: 350-356, 1928.

51. Openchowski.
Centralbl f. d. med Wissensch, 31: 546, 1883.
Zentralbl f. physiol. 3, 1, 1889. (Cited by Langley 1898, and Veach, 1925).

52. Paton, W.D. and J.R. Vane.
An analysis of the response of the isolated stomach to electrical stimulation and to drugs.
J. Physiol., London, 165: 10-46, 1963.

53. Reinke, D.A.
Patterns and Parameters of Dog Gastrointestinal Contractile Activity Monitored by Extraluminal Force Transducers.
Doctoral Dissertation. University of Michigan, Ann Arbor, Michigan, 1964.

54. Reinke, D.A., A.H. Rosenbaum, and D.R. Bennett.
Patterns of dog gastrointestinal contractile activity monitored in vivo with extraluminal force transducers.
Amer. J. Dig. Dis., 12: 113-141, 1967.

55. Rosenbaum, A.H., D.A. Reinke, and D.R. Bennett.
In-vivo force, frequency, and velocity of dog gastrointestinal contractile activity.
Amer. J. Dig. Dis., 12: 142-153, 1967.

56. Scott, J.B., R.M. Daugherty, J.M. Dabney, and F. J. Haddy.
Role of chemical factors in regulation of flow through kidney, hindlimb, and heart.
Amer. J. Physiol., 208: 813-824, 1965.

57. Texter, E.C., Jr.
Motility in the gastrointestinal tract.
J. Amer. Med. Assoc.,184: 640-646, 1965.

58. Thomas, J.E. and S.A. Komarov.
Physiological aspects of vagotomy.
Gastroenterology, 11: 413-418, 1948.

59. Veach, H.O.
Studies of the innervation of smooth muscle.
Amer. J. Physiol., 71: 229-264, 1925.

60. Wedensky, W.N.
Dans quella partie de l'appareil neuromusculaire se produit l' inhibition.
Compt. Red. Acad. Sci. CXIII, 805-807, 1891. (Cited by Veach, 1925).

61. Wilson, V.J.
Post-tetanic potentiation of polysynaptic reflexes of the spinal cord.
J. Gen. Physiol., 39: 197-206, 1955.