the histamine present in the antrum may be bound to mast cells while the major portion of corporal and fundic histamine is more closely associated with the secretory cells themselves, possibly bound by mucins(1).

Summary. The distribution of histamine and mast cells in the fundic, corporal and antral mucosa of the canine stomach was assayed. Twelve areas from each of 4 stomachs were studied. Mast cell profiles of these areas showed the cell population to be similar in all mucosal areas, although the greatest number of cells occurred deeper in the glandular mucosa of the antrum than in the glandular mucosa of the fundus and corpus. In contrast, the amount of histamine in the antral mucosa (73.3 $\pm$ 4.9 μg/g) was roughly one half of that noted in fundic and corporal mucosa (133.6 $\pm$ 1.4 μg/g), a difference which is statistically significant, ($p<.05$). One possible explanation for these observations is that the major portion of corporal and fundic histamine is not associated with mast cells.

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An Excitatory Adrenergic Alpha Receptor Mechanism of Terminal Guinea-Pig Ileum. (32017)

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The action of catecholamines on intestinal smooth muscle is generally taken to be that of inhibition of contractile activity. An exception to this expected and usual action exists in the observation of Munro(1) in which epinephrine is reported to have a definite excitatory action on terminal guinea-pig ileum. This observation was verified in a report by McDougal and West(2). Munro characterized the response to the following extent: it occurs optimally in smaller animals(3); it does not occur in fasted animals(1); it is not antagonized by atropine(1) and may even be potentiated by that agent(3); and is due to direct action on the smooth muscle that possibly involves a receptor surface(1,4).

The general inhibitory action of the catecholamines on intestinal smooth muscle has been shown to be mediated through both alpha and beta adrenergic receptor mechanisms(5). This study was undertaken to test the possibility that the excitatory action of catecholamines on terminal guinea-pig ileum involves either an alpha or beta receptor mechanism. The possibility of catecholamines acting as cholinergic, histaminic or serotonin releasing agents was tested as was the possibility that they act directly on serotonin receptors.

Methods. Ten guinea-pigs (300-400 g) were used in these studies. The animals were killed by cervical dislocation and 2 to 3 cm segments of terminal ileum were removed immediately. They were flushed with electrolyte solution and mounted on their long axis in a chamber

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in such a way that longitudinal muscle contraction was registered on a Grass FT-.03 force displacement transducer. The transducer signal was recorded on a Sanborn model 7700 polygraph. A balanced, buffered electrolyte solution of the following composition was used in the bath: 8 g NaCl, 0.2 g KCl, 0.15 g $CaCl_2$, 0.05 g Na H_2PO_4, 0.2 g $MgCl_2 \cdot 6H_2O$, 1 g $HaHCO_3$ and 1 g dextrose per liter. Both the tissue bath and electrolyte solution reservoir were bubbled with 95% O_2 and 5% CO_2 and maintained at 36-38°C. The tissue chamber was so constructed that solutions were changed by both draining and filling from the bottom. All drugs were mixed in distilled water in quantities that would allow the desired bath concentration, in terms of base, to be reached by adding 0.5 to 1 ml of solution directly to the 200 ml bath volume. All drug concentrations are expressed as g/ml of bath.

The following drugs were used as adrenergic test agents in the differential receptor blockade sequences in order to elucidate the adrenergic mechanism involved; phenylephrine hydrochloride, epinephrine hydrochloride, levarterenol bitartrate and isoproterenol hydrochloride. The action of these agents was tested on the tissue during its stimulation by acetylcholine chloride (ACh). Acetylcholine was added directly to the bath in an amount resulting in a concentration of 10^{-8} g/ml. After the ensuing contraction reached equilibrium (30-45 sec.), one of the test drugs was added to the bath at a concentration of 10^{-6} g/ml. Following a 30 second exposure the bath was drained, fresh solution replaced and the tissue allowed to reach its original base line condition. This sequence was repeated until the action of each of the adrenergic agents had been tested.

Phenoxybenzamine hydrochloride (Dibenzyline®) was used as an alpha and propranolol (Inderal®) as a beta adrenergic receptor site blocking agent. Alpha receptor blockade resulted from a 30 minute exposure to 2.5×10^{-9} phenoxybenzamine while propranolol (4×10^{-6}) for 30 minutes produced an effective, though not complete, beta block. The antagonists were not present in the bath when the action of the adrenergic drugs was tested.

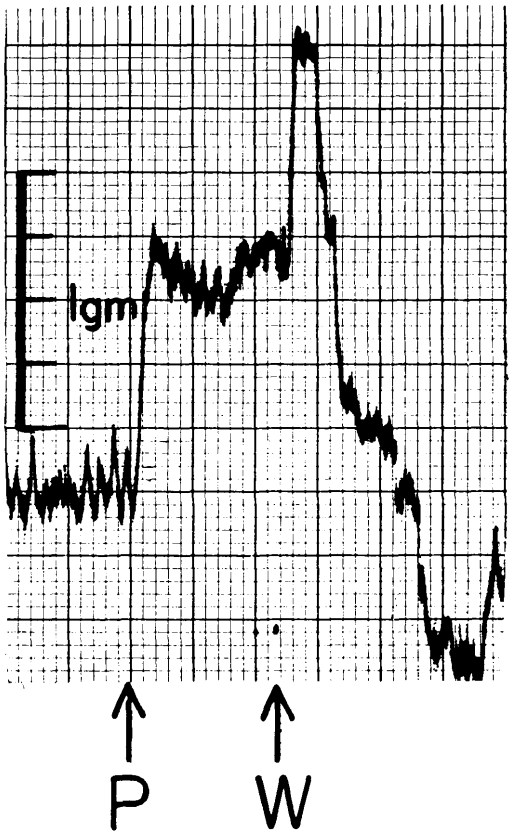

FIG. 1. Effect of phenylephrine (P) on terminal guinea-pig ileum without previous acetylcholine stimulation. W—wash artifact during drainage of chamber. Calibration—1 g/division.

Other drugs used were; histamine dihydrochloride, diphenhydramine hydrochloride (Benadryl®), serotonin creatinin sulfate, reserpine hydrochloride (Serpasil®), atropine sulfate, BOL-148 (2 Br LSD, Sandoz) and morphine sulfate.

Results. Addition of phenylephrine (1 μg/ml) to the bath, with the ileal segment at equilibrium and with no ACh stimulation, caused a definite contractile response. This response developed rapidly and reached a steady tension that was held until the system was drained (Fig. 1). Procedures were carried out in 4 animals to test the possibility of phenylephrine causing a release of acetylcholine, histamine or 5-hydroxytryptamine (5HT) or that it acts by combining directly with serotonin receptors of intestinal smooth

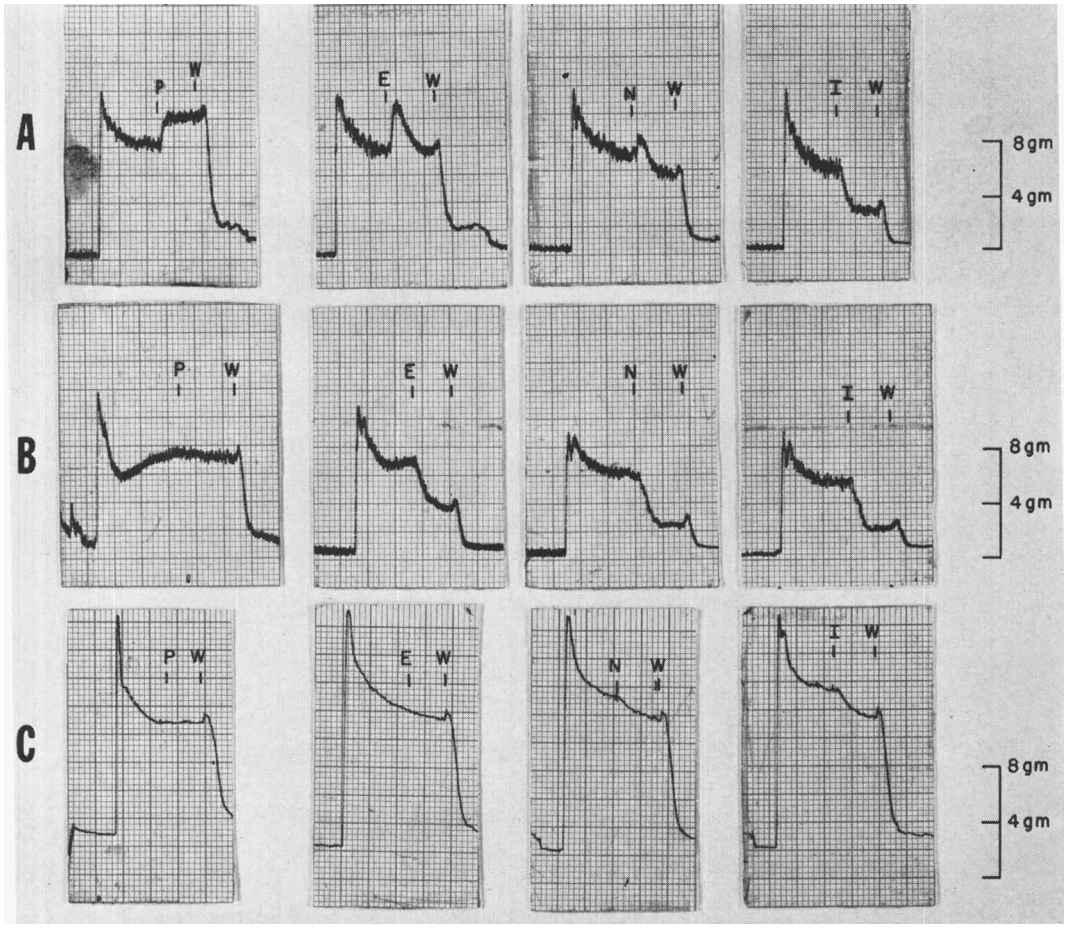

FIG. 2. Effects of adrenergic agents on terminal guinea-pig ileum. Initial contractions due to stimultion with 10^{-8} g/ml acetylcholine. P—10^{-6} g/ml phenylephrine, E—10^{-6} g/ml epinephrine, N—10^{-8} g/ml norepinephrine, I—10^{-6} g/ml isoproterenol. W—wash artifact during drainage of chamber. A—control series, B—following 30 min. exposure to 2.5 x 10^{-9} g/ml phenoxybenzamine, C—following 30 min exposure to 4 x 10^{-6} g/ml propranolol.

muscle. Incubation of the tissue with atropine (10^{-7}) for 5 minutes virtually abolished the contractile response to acetylcholine (10^{-7}) while the response to a test dose of phenylephrine (10^{-6}) remained relatively unchanged. Incubation for a like period with diphenhydramine (10^{-7}) abolished subsequent histamine contraction and possibly potentiated the phenylephrine response. Five minute exposure to BOL-148 abolished the 5-HT contraction and attenuated that resulting from phenylephrine. In 2 experiments, 30 minute exposure to 10^{-7} reserpine abolished the 5-HT response but did not effect the phenylephrine action. The addition of 10^{-7} morphine to the bath caused a greatly reduced 5-HT response and augmented the phenylephrine response.

Differential blockade experiments. Fig. 2 and 3 illustrate the results of differential blockade on the action of phenylephrine, epinephrine, norepinephrine and isoproterenol on ACh stimulated terminal guinea-pig ileum. Fig. 4 represents the action of ACh on this segment of ileum unhindered by secondary drug effects. Both Fig. 2A and 3A represent the actions of the above adrenergic agents prior to any receptor blockade. Phenylephrine produces a prompt contraction that rapidly reaches maximal tone which is held until the bath solution is changed. Both epinephrine and noreinephrine cause an initial increase in

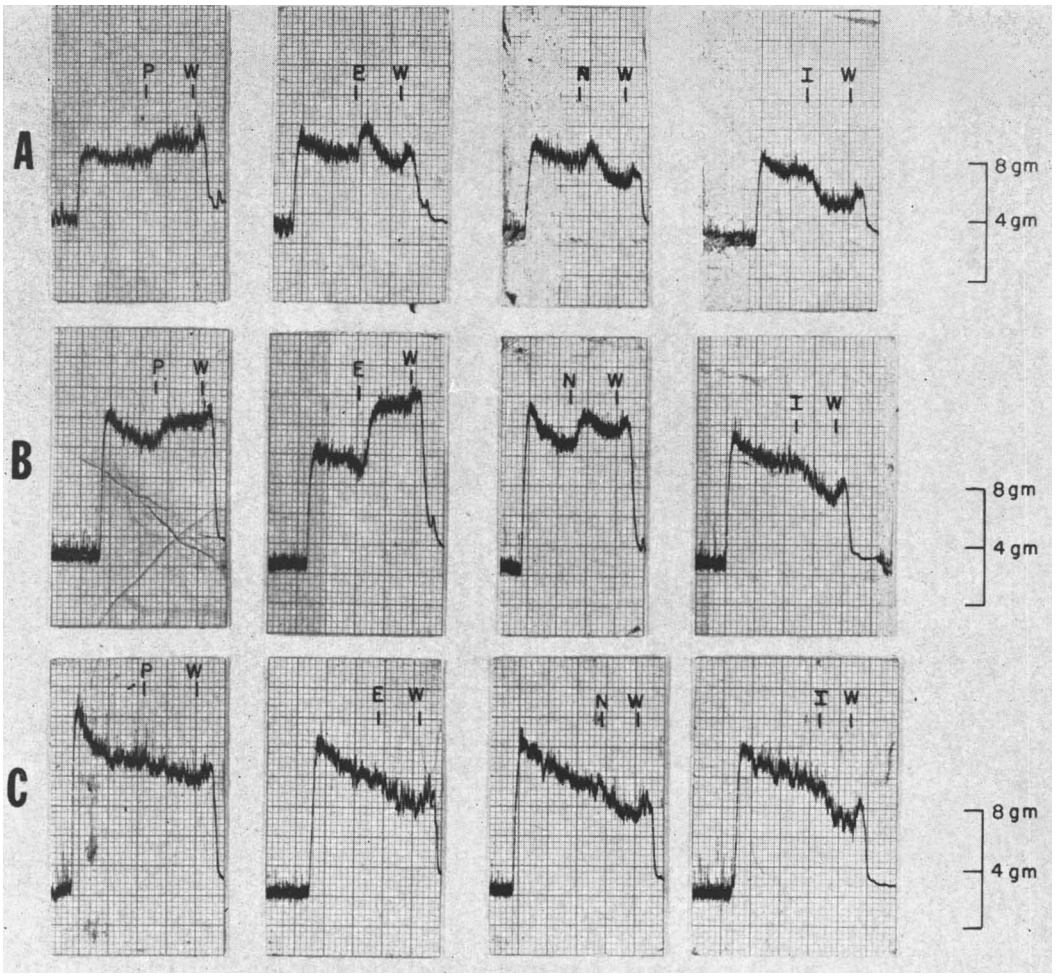

FIG. 3. Effects of adrenergic agents on terminal guinea-pig ileum. Initial contractions due to stimulation with 10^{-8} g/ml acetylcholine. P—10^{-6} g/ml phenylephrine, E—10^{-6} g/ml epinephrine, N—10^{-6} g/ml norepinephrine, I—10^{-6} g/ml isoproterenol. W—wash artifact during drainage of chamber. A—control series, B—following 30 min exposure to 4 x 10^{-6} g/ml propranolol, C—following 30 min exposure to 2.5 x 10^{-9} g/ml phenoxybenzamine.

contractile tension followed by a secondary loss of tone. In all instances, epinephrine produced a greater contraction than did norepinephrine while norepinephrine appeared to have a more effective inhibitory phase to its action than did epinephrine. Isoproterenol universally resulted in marked inhibition.

Fig. 2B represents the action of these same agents following initial incubation with phenoxybenzamine. Phenylephrine no longer has a stimulating effect and epinephrine and norepinephrine retain only their inhibitory actions, which are augmented. The action of isoproterenol remained unchanged. Subsequent incubation with propranolol, Fig. 2C, greatly attenuates the inhibitory action of epinephrine, norepinephrine and isoproterenol.

The series shown in Fig. 3 represents the action of these same sympathomimetic agents when beta receptor blockade was accomplished first. Fig. 3B shows the responses occurring following incubation with propranolol. Phenylephrine produces a contractile response similar to its control. However, the response to epinephrine and norepinephrine is one of sustained contraction that is of greater magnitude than that occurring during the control series. Isoproterenol only retained a slightly in-

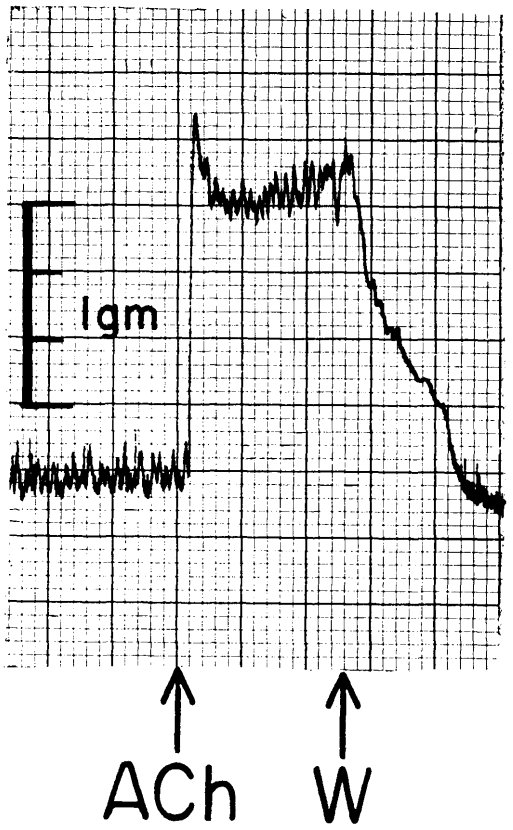

FIG. 4. Effect of acetylcholine (ACh) on terminal guinea-pig ileum without secondary drug effects. W—wash artifact. Calibration—1 g/division.

hibitory effect. The record of 3 C follows exposure to phenoxybenzamine. It was found that the effects of the propranolol tended to wear off during the 30-minute time lapse of the phenoxybenzamine treatment. To prevent this occurrence, 2×10^{-6} propranolol was added to the bath with phenoxybenzamine. Under these conditions all excitatory actions are abolished and only slight inhibitory actions remained. This particular segment was allowed to recover for 30 minutes following completion of the series. This time lapse allowed appreciable recovery from the beta block with no recovery of alpha mechanisms. The result was one of effective recovery of all inhibitory actions with no recovery of excitatory actions.

Discussion. The facts that: 1) atropine blocked acetylcholine contraction without preventing phenylephrine induced contraction; 2) diphenhydramine blocked histamine stimulated contraction while possibly facilitating that resulting from phenylephrine; and 3) 2 Br LSD and reserpine prevented serotonin stimulated contractions without greatly affecting that produced with phenylephrine negates the possibility that phenylephrine contraction results through an interaction involving these agents. The fact that morphine abolished 5-HT stimulated contraction while augmenting that caused by phenylephrine indicates that phenylephrine is not acting directly on 5-HT receptors.

Examination of the control records in Fig. 2 and 3 indicates that all of the adrenergic test drugs used elicited responses commensurate with their known actions on alpha and beta receptors. Phenylephrine, acting as a stimulant, produced a characteristic pure alpha monophasic response. That is, the increase in contractile tension was immediate with peak tension being reached rapidly and then maintained until the agent was washed out of the bath. In those tissues where alpha and beta receptors produce antagonistic responses, epinephrine and to a lesser extent norepinephrine produce biphasic responses. Since the interaction of the catecholamines with alpha receptor is more rapid than it is with beta receptor(6,7), the results of the present study are compatible with the idea of the epinephrine and norepinephrine responses being biphasic alpha/beta activities. In this particular tissue, both epinephrine and norepinephrine produce an initial pressor response *via* alpha excitation followed by beta activation leading to inhibition and loss of contractile tension. Isoproterenol acts in its predictable manner as a beta stimulant and only causes a marked decrease in tension.

The results of the differential receptor blocking procedures support the above ideas exactly. In the sequence where alpha blockade was accomplished initially, phenylephrine loses its excitatory activity and its addition to the tissue bath system is without effect. During initial alpha blockade both epinephrine and norepinephrine responses become monophasic. Due to the loss of alpha mechanism these agents no longer produce excitation. The remaining beta inhibition is potentiated as a direct result. The inhibitory

acton of isoproterenol is unaltered by alpha blockade. Subsequent beta blockade (Fig. 2C) results in an almost complete attenuation of the inhibitory responses in addition to the complete loss of the phenylephrine contractile action.

In the sequence beginning with beta blockade (Fig. 3B), the phenylephrine contractile action is unchanged from control. Once again the epinephrine and norepinephrine responses become monophasic ones. In this case the loss of beta activity effectively potentiates the initial excitation. The ensuing contractions resemble pure alpha responses in that they occur rapidly and peak contraction is held as a plateau. The beta blockade can be seen to have been highly effective by the marked attenuation of isoproterenol inhibition. Following phenoxybenzamine exposure (Fig. 3C) all alpha excitatory responses are lost in addition to the previously blocked beta inhibition.

Summary. Experiments were performed on segments of terminal guinea-pig ileum utilizing differential alpha and beta adrenergic receptor site blocking techniques. Phenylephrine caused a contraction of this segment of guinea-pig intestine which resembled a pure alpha receptor site response. Both epinephrine and noreinephrine produced biphasic responses which follow a pattern of alpha excitation followed by beta inhibition while isoproterenol elicited a pure beta inhibitory action. The use of phenoxybenzamine as an alpha blocking agent abolished all excitatory responses while augmenting beta actions. Propranolol, through beta adrenergic receptor site blockade, potentiated alpha excitatory actions. The results are discussed in terms of the guinea-pig terminal ileum possessing an unique alpha adrenergic receptor mechanism which is excitatory in nature.

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Detection of Five Components Having Antibody Activity in Rat Antisera.*† (32018)

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The immunoglobulins of the rat have been described by several investigators. Precipitating antibody against bovine serum albumin (BSA) was found to reside mainly in a component designated by Arnason *et al*(1) as γA; however, these authors found the anti-BSA hemagglutinating antibody to be present largely in the γG component. Using specifically purified rat antibodies against the 2,4-dinitrophenyl (DNP) determinant, Nussenzweig and Binaghi(2) demonstrated that precipitins are present in the γ_2 component. Immunoelectrophoretic analysis of the purified antibody with one of their rabbit anti-rat antisera showed two parallel lines in the γ-globulin region. Both of these components are apparently different from the component designated as γA by Arnason *et al*(1). Nussenzweig and Binaghi(2) also detected 19 S antibody in this anti-DNP antibody preparation.

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