SP-8 cells cultures at the lowered temperature exhibited capacity to infect CRK cells as determined by the immunofluorescent technique. These findings were interpreted as an evidence supporting the view that the SPV genome, possibly a complete set of it, is persisting in these SP-8 cells.

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Vagal Blocking Action of Phentolamine and Dibenzyline Studied by Direct Perfusion of the Sinus Node* (33502)

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Phentolamine and phenoxybenzamine (Dibenzyline) are considered characteristic alpha adrenergic blocking agents, but they are also known to alter responses to serotonin, histamine and acetylcholine (1). Since the chronotropic responses of the heart to vagal and sympathetic stimuli are in opposite directions and simple to observe and record, this study was undertaken to examine the effects of selective perfusion of the sinus node with phentolamine or Dibenzyline on its reponse to cholinergic stimuli.

Methods. Twenty-seven dogs were anesthetized with sodium pentobarbital (30 mg/kg intravenously) and the tracheae were intubated for mechanical ventilation with room air. Through a right thoracotomy the pericardium was opened and the right coronary artery was dissected free in the atrioventricular sulcus. The branch supplying the sinus node (2) was isolated and a small polythylene cannula was introduced into this artery and ligated in place. Details of the technic have been reported previously (3). Ligation of the canine sinus node artery has no significant effect on the pacemaking rate because of the extensive arterial anastomoses in the region. Injections of 2-ml volume were delivered from a hand syringe into a stopcock attached to the proximal end of the cannula in the sinus node artery, perfusing selectively the sinus node and a small amount of adjacent right atrial myocardium. Duration of this injection (perfusion) was 5 to 10 sec. Because of the small volume employed, there was no significant extracardiac effect by the injectate on recirculation.

In all experiments the right atrial (central venous) pressure was monitored with a cannula placed via the jugular vein, and the central aortic pressure via a cannula placed in the femoral artery. A tachogram was constantly recorded from a small analog computer monitoring successive R waves of the

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TABLE I. Vagal Slowing Produced after Increasing Concentrations of Phentolamine or Dibenzyline Perfused Directly into the Sinus Node.[a]

Concentrations (μg/ml)	Phentolamine (14 dogs) (%)	Dibenzyline (11 dogs) (%)
100	54 ± 23	61 ± 28
500	31 ± 18	26 ± 15
1000	9 ± 6	11 ± 6

[a] The results are expressed as the mean ± one standard deviation percent decrease in heart rate. The control response to vagal stimulation was sinus arrest.

ECG (electrocardiogram). Through a slave circuit a separate single-channel ECG was recorded at 25 mm/sec throughout each experiment, to permit later examination of any changes in P wave, PR interval or QRS complexes. During the experiment, the pressures and tachogram were recorded on a multichannel polygraph at slow speeds (usually 0.25 mm/sec) and the pulses and ECG observed on a multitrace oscilloscope at sweep speeds of 25 or 50 mm/sec. The right vagus nerve was isolated in the neck and the right stellate ganglion within the chest for stimulation with an electronic square-wave stimulator at supramaximal voltage, 1 msec impulses, 30 cps, in 6-sec trains.

Test materials were prepared in Ringer's solution and were always compared with control injections of Ringer's solution alone. Substances studied were Dibenzyline (phenoxybenzamine) 1 μg to 1 mg/ml; phentolamine mesylate, 1 μg to 1 mg/ml; acetylcholine hydrochloride 0.1 to 1.0 μg/ml; methacholine (acetyl-beta-methylcholine) hydrochloride, 0.01 to 1.0 μg/ml; atropine sulfate 10 μg/ml; and *l*-norepinephrine bitartrate, 0.1 μg/ml.

The vagal blocking action of phentolamine and Dibenzyline was evaluated by testing the response of the sinus node to direct stimulation of the vagus nerve before and after selective perfusion of the node with these alpha adrenergic blocking agents. Comparative experiments were done to examine the effects of direct perfusion with acetylocholine or methacholine before and after phentolamine or Dibenzyline. The extent of negative chronotropic action was measured as the percentage fall in heart rate during stimulation or perfusion.

Results. The effect of intranodal phentolamine on the response to vagal stimulation. In 14 dogs a vagal blocking action was first apparent in 12 with concentrations of 100 μg/ml of intranodal phentolamine (Table I and Fig. 1), while in 2 dogs blockade appeared first with 500 μg/ml. An increasing effect occurred in direct relation to increasing concentration. The onset of the blocking action was prompt, being apparent directly at the termination of perfusion with phentolamine. The maximal vagal blocking effect began decreasing within 5–10 min, tested with serial vagal stimuli, but the rate of decrease was quite gradual, with partial blocking action still present at least 1 hr. In one dog followed with serial vagal stimuli for 3.5 hr, there was still detectable blockade after an initial perfusion with phentolamine, 1000 μg/ml.

The effect of intranodal Dibenzyline on the response to vagal stimulation. In 11 dogs the vagal blocking action of intranodal Dibenzyline was found similar to that of phentolamine (Table I and Fig. 2). This included minimal effective concentration, speed of onset of action, duration of action, and increasing vagal blockade with increasing test concentrations.

The effect of intranodal phentolamine on the response to intranodal acetylcholine or methacholine. In four dogs each acetylcholine and methacholine produced sinus arrest with concentrations of 0.1 or 1.0 μg/ml, and the concentration producing this effect was the one subsequently used after phentolamine. The sinus arrest produced by acetylcholine is extremely transient, lasting only during the period of perfusion, while that of methacholine is slightly longer and sinus arrest persisted for several seconds after completion of the test perfusion. Partial blocking action was apparent in two dogs after phentolamine 100 μg/ml (Fig. 3). The degree of blockade was greater with 1000 μg/ml. Increasing the test concentration of acetylcholine to 10 μg/ml permitted an overriding of the blockade. Duration of the blockade against directly administered acetylcholine was briefer than against

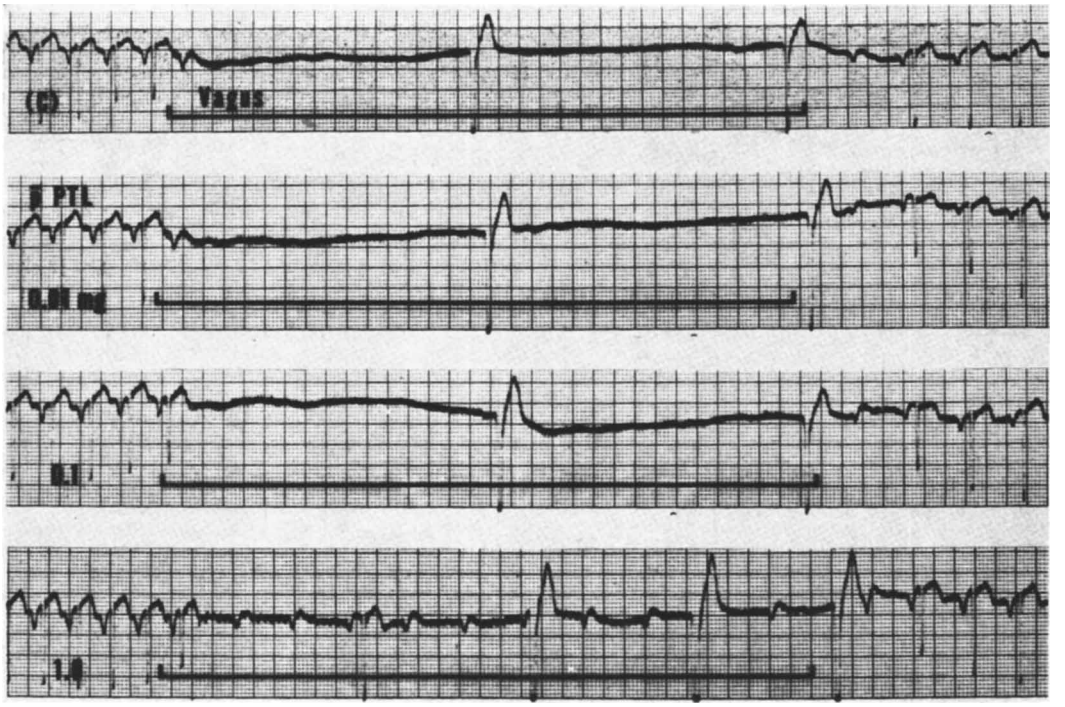

FIG. 1. Strips of lead aVR electrodiagram recorded at 25 mm/sec in a dog to illustrate the response to vagal stimulation before and after increasing concentrations of phentolamine (PTL) perfused directly into the sinus node artery.

that neurally-released by vagal stimulation, lasting only 4–12 min when serially tested with intranodal perfusions of acetylcholine.

The effect of intranodal Dibenzyline on the response to intranodal acetylcholine or methacholine. Experiments similar to those with intranodal phentolamine were performed in six dogs with Dibenzyline, and similar results were obtained (Fig. 4). Blockade by intranodal Dibenzyline of the negative chronotropic effect of either acetylcholine or methacholine was again less profound than the vagal blocking action, both as to maximal effect and duration of action. In all the experiments a final comparative response was made with intranodal perfusion of atropine, 10 μg/ml, which completely blocks both vagal and acetylcholine effects (4). In no dog was the blocking action of either phentolamine or Dibenzyline as effective as that of atropine.

The effect of intranodal atropine on the chronotropic response to adrenergic stimuli. In three dogs the sinus acceleration produced

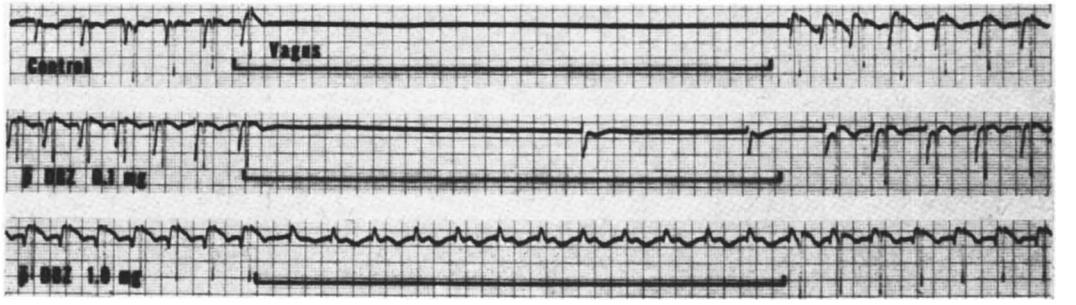

FIG. 2. There is only minimal vagal blocking action by intranodal Dibenzyline (DBZ) with a low concentration (0.1 mg/ml) but a marked blocking effect occurs with 1.0 mg/ml.

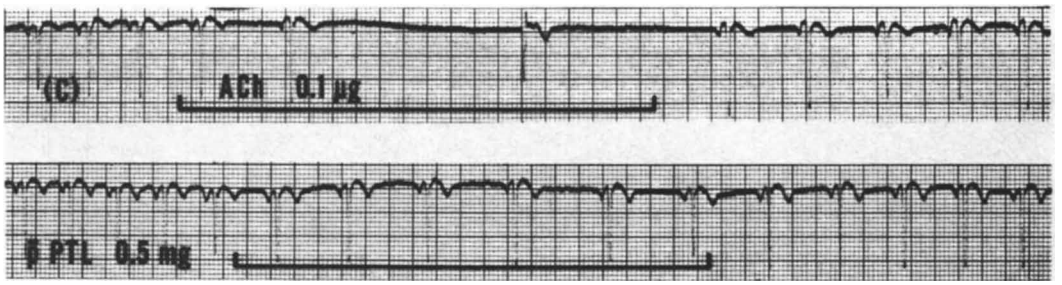

FIG. 3. Sinus arrest produced by direct perfusion of the sinus node with acetylcholine (ACh) is partially blocked after intranodal phentolamine.

by either stimulation of the right stellate ganglion or intranodal perfusion with norepinephrine, 0.1 μg/ml, was not significantly changed by prior perfusion with intranodal atropine, 10 μg/ml.

Discussion. Inhibition of vagal responses with an adrenergic blocking agent seems a paradox. It serves as a reminder that most pharmacologic blocking agents are only relatively specific (1) rather than being absolute or precise antagonists. In previous experiments with direct perfusion of the sinus node, for example, we have found that typical beta receptor blocking agents also have an associated vagal blocking action (5, 6), although as in the present experiments this effect is apparent only at relatively high concentrations. In the canine sinus node it is thus apparent that blocking agents for both alpha and beta adrenergic receptors have a concomitant vagal blocking effect (and against acetycholine as well), but that this anticholinergic effect becomes apparent only with concentrations considerably greater than those required for their more familiar adrenergic blocking action.

When given systemically, phentolamine and Dibenzyline take several hours to become maximally effective and then their effect persists for hours or days (1). The more prompt onset of action and briefer duration in the present experiments most likely reflect the consequences of selective brief but direct perfusion of target cells, although there may be other explanations. The greater effectiveness against vagal stimuli than against directly perfused acetylcholine most likely represents a difference in intranodal concentration or volume of released as compared to administered acetylcholine, since the effectiveness of most pharmacologic antagonists can readily be overcome with sufficiently high concentrations of agonists. This point is supported by the demonstration that effective blockade by phentolamine or Dibenzyline

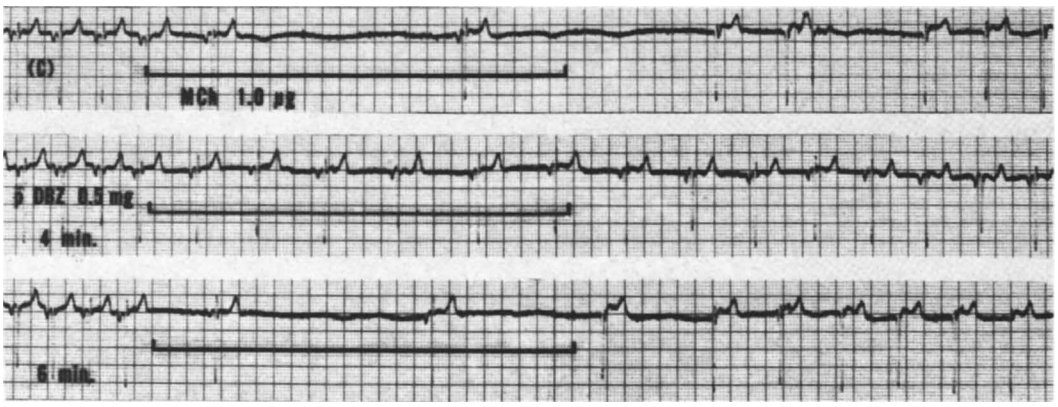

FIG. 4. Sinus arrest produced by direct perfusion of the sinus node with methacholine (MCh) is partially blocked by intranodal Dibenzyline for 4 min, but the blocking action is gone by 6 min.

against a given concentration of acetylcholine could be overcome with a higher concentration.

Although it is conventionally thought that there is no significant adrenergic alpha receptor activity in the heart (1), we have recently presented evidence (7) which suggests that the same interplay of opposing adrenergic receptors exists in the canine sinus node as is generally considered to exist in peripheral vascular smooth muscle, except at that in the sinus node it is the beta receptors which are predominant and more easily demonstrated. Just as beta receptor (vasodilating) activity is weaker, more subtle, and more difficult to demonstrate consistently in peripheral arterial smooth muscle, we consider alpha receptor activity in the sinus node to be weaker, more subtle and more difficult to demonstrate or study. Among other lines of evidence to support such an interpretation, it was shown that the negative chronotropic action of intranodal methoxamine could be reversed with either Dibenzyline or phentolamine (7).

Relative to the present experiments, the elimination of a postulated alpha adrenergic depressant activity in the sinus node by use of intranodal phentolamine or Dibenzyline may in part be responsible for their effects on vagal responses, since the control heart rate was higher during the initial vagal challenges after phentolamine or Dibenzyline. However, both the vagal blockade and that of responses to acetylcholine or methacholine were present even when heart rate had returned to control levels. Furthermore, concentrations of phentolamine and Dibenzyline required to produce vagal blockade were 10 to 100 times those required to reverse the negative chronotropic effect of alpha adrenergic stimulation with methoxamine (7). We believe previous interpretations by Leimdorfer (8, 9) and by others (10–12) are correct in attributing the vagal blocking action to an antagonism against acetylcholine, although relatively high concentrations of phentolamine or Dibenzyline are required for this effect.

Brown (13) has recently reported the blockade of sympathetic pathways to the heart by use of atropine. However, in the canine sinus node concentrations of atropine which completely block the response to vagal stimulation (7) had no apparent effect on the positive chronotropic response to stimulation of the stellate ganglion or direct perfusion of the sinus node with norepinephrine. Furthermore, inhibition of acetylcholine synthesis with hemicholinium to the point of completely abolishing vagal response likewise has no significant effect on the cardioaccelerator response to stimulation of the stellate ganglion (4).

Summary. In the canine sinus node the negative chronotropic effect of vagal stimulation or of directly administered acetylcholine or methacholine can be blocked with directly administered phentolamine or Dibenzyline. Both these classical alpha adrenergic blocking agents are approximately equally effective, but relatively high concentrations are required for the vagal blocking action. The onset of vagal blockade is prompt but is seldom complete, and can be overcome with sufficiently high concentrations of acetylcholine. Considering the concentrations of phentolamine and Dibenzyline required during direct perfusion of the sinus node to produce effective vagal blockade, it seems unlikely that this experimental pharmacologic effect is of much significance during the use of these substances for their more familiar alpha adrenergic blocking action.

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Treatment of Leukemic (L-1210) Mice with Double-Stranded Polyribonucleotides (33503)

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Many animal leukemias are induced by viruses, and human leukemia may also be a viral etiology; therefore, inhibition of viral multiplication has been attempted in order to treat leukemia. In a recent paper Wheelock and Larke (1) reported on the efficacy of interferon in prolonging the life span of mice with established Friend virus leukemia. The authors also demonstrated that injection of Statolon (an interferon-inducing substance) prolonged the life of mice with established leukemia. Wheelock (2) had previously shown that some nontumorogenic viruses (which can induce interferon) inoculated into mice prior to the Friend leukemia virus could prolong the life of the mice.

Among the most potent inducers of interferon are the double-stranded polyribonucleotides described by Hilleman and co-workers (3). These polyribonucleotide complexes can induce high levels of circulating interferon and thus protect animals from lethal viral infections.

The present communication describes experiments in which the life span of leukemic (L-1210) mice is prolonged by administration of polyribocytidylic acid: polyriboinosinic acid (1:1) copolymers.

Materials and Methods. (i) *Mice.* Female BDF_1 mice, 18–20 g were obtained from Jackson Memorial Laboratories.

(ii) *L-1210 leukemia.* Leukemic mice were obtained from Dr. I. Wodinsky, Arthur D. Little, Inc., and maintained in an ascitic form, by weekly transfer of 10^6 cells intraperitoneally (i.p.) in BDF_1 mice.

(iii) *Dougle-stranded polyribonucleotides.* Polyribonucleotides were products of Miles Laboratories, Inc. Dougle-stranded complexes of polyinosinic acid and polycytidylic acid (1:1) [poly I:poly C] were prepared by dissolving equal weights of each polynucleotide in 0.15 *M* NaCl at a concentration of 10 mg/ml. The solutions were heated to 80° mixed thoroughly and kept at 80° for 15 min. The solution was then allowed to cool slowly to room temperature over a period of 3 hr. An alternate solvent for the polynucleotides was 0.5 *M* NaCl–0.02 *M* citrate–0.01 *M* Tris, pH 7.3–0.001 *M* EDTA. Two batches of poly I:poly C were prepared similarly; however, slightly varying results were obtained.

(iv) *Inoculation of BDF_1 mice.* Groups of 10 mice were inoculated with 10^6 L-1210 ascites cells i.p. The polynucleotide complex was given i.p. unless otherwise mentioned. The standard deviation and percentage increase in life span were calculated by published procedures (4).

Results. Multiple injections of poly I: poly C prolonged the life span of leukemic mice. Control mice died 7.5–8 days after inoculation, with a standard deviation ranging from 0.65 to 0.81 in different experiments. The mice treated with 5 mg/kg poly I: poly C lived 25–40% longer than control mice. Doses of poly I:poly C above 10 mg/kg were toxic resulting in early deaths of the mice. The results are given in Table Ia.

Mice treated with a single dose of poly I:poly C at 5 or 20 mg/kg 1 day prior to inoculation with L-1210 cells showed an insignificant increase in survival time. Thus, the effect does not seem to be simply an increase in nonspecific host-resistance.