resemble properties of enterovirus type receptors. Therefore, an analogy between receptor material and enhancer for $m+$ virus must be considered. Studies of cell receptors for $m+$ and m mutants and comparison of receptor and enhancer activity are in progress. Preliminary studies indicated that receptor concentration for $m+$ virus was similar in AV3 and monkey cells. Therefore, the low efficiency of $m+$ infection in monkey cells did not appear to be related to a deficiency of receptor material. In contrast to enhancer, receptor material was not inactivated by phenol, chymotrypsin, or trypsin, but was destroyed by ether treatment. These results suggest that enhanced and receptor activity may be different.

Summary. A transferable enhancer has been demonstrated in cultured human cells which increases the efficiency of infection of the $m+$ mutant of echovirus 6 in monkey cells. Enhancer was present in a variety of human cells but not in cells of other species. In general, presence of enhancer coincided with the greater susceptibility of human cells for $m+$ infection. Enhancer in AV3 cells was detected in viable and dead cells, cellular debris and subcellular fractions. Most of the enhancer activity sedimented with the microsomal fraction. Enhancer was thermolabile and destroyed by phenol, proteolytic enzymes, acid and alkali. Debris (AV_d) obtained by homogenization of AV3 cells enhanced the $m+$ titer (pfu and TCD_{50}) in monkey cells by 5- to 100-fold. Plaques appeared 2 days earlier in the presence of AV_d. There was a linear relationship between enhancer activity and concentration of cells ($10^{4.5}$ to $10^{6.5}$ cells/ml) used for preparation of AV_d. Prior *in vitro* contact of virus and AV_d was not required for enhancement. Full enhancement was evident when monolayers were treated before, during, or after addition of virus. The data suggest that enhancer binds rapidly and irreversibly to the cell surface and acts beyond the virus adsorption step.

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Ventricular Augmentor Fibers in the Cervical Vagosympathetic Trunk.* (31979)

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Conventional concepts of vagal control of the heart are limited to influences upon atrial excitability, conductivity and contractility and upon heart rate as determined by the sinoatrial node. In a survey of pertinent literature, DeGeest *et al* found that investigators were about equally divided between those who concluded that vagus had little or no influence on contractility of the ventricles and those who deduced that it elicits a negative inotropic effect(1). In their own experi-

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ments employing an isovolumetric preparation of the dog heart, DeGeest *et al* have shown conclusively that efferent vagal stimulation exerts a potent negative inotropic influence upon the left ventricular myocardium (1,2).

The presence of cardioaccelerator fibers in the cervical vagus has been well documented (3-5) and the modest elevation in arterial blood pressure associated with vagal stimulation in the atropinized preparation is generally associated with this influence. However, preliminary observations of changes in ventricular myocardial contractile force in such a preparation suggested a direct sympathetic-like augmentation. The importance of recent demonstrations of vagal inhibitory innervation of the ventricles would be further amplified by demonstration of positive inotropic fibers in these nerves.

Materials and methods. Two groups of experiments upon dogs anesthetized with Sernylan‡ (2 mg/kg), α-chloralose (60-80 mg/kg), and atropine (0.5 mg/kg) have been carried out, one employing application of multiple strain gauge arches (modified Walton gauges) to the ventricular surfaces (6) and the other using pressure recording from each of the 4 cardiac chambers(7). Central arterial blood pressure and lead II of the ECG were also generally recorded. Electrical stimulation of the distal ends of severed vagi was accomplished by means of bipolar electrodes and an AEL Model 104 square wave generator, while all stimulation parameters were continuously monitored by means of a cathode ray oscilloscope.

Results. Fig 1 illustrates alterations in myocardial force of contraction as recorded from strain gauge arches stitched to the epicardium of both right and left ventricles. The upper channel represents contractile force from a segment of the anterior surface of the right ventricle midway between apex and base, while the middle and lower force recordings were derived from the base (channel 2) and apex (channel 3) of the anterior surface of the left ventricle. Channel 4

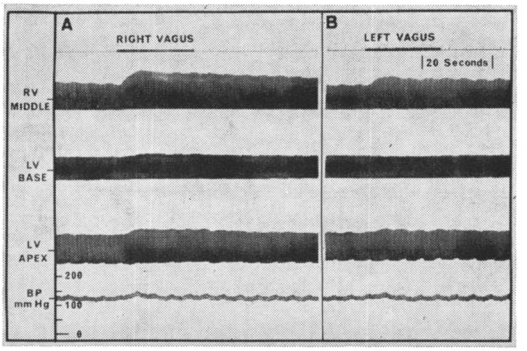

FIG. 1. Myocardial force of contraction on right ventricle (channel 1), left ventricular base (channel 2) and apex (channel 3) during electrical stimulation (5 volts, 5 msec, 30 cps) of the right (panel A) and left (panel B) cervical vagosympathetic trunks. Blood pressure was recorded from the carotid artery.

is a recording of central arterial blood pressure. In panel A the distal end of the sectioned right cervical vagus was stimulated at the signal. Distinctly increased contractility was clearly apparent in all 3 segments of the heart, and was sufficient to elevate arterial pressure from approximately 130/110 to a peak of 155/130 mm Hg. The response was detectable within 2 seconds after onset of stimulation and was sustained approximately 2 minutes before returning to control levels. Cardiac acceleration from 210 to 255/min occurred. The general characteristics of these responses correspond closely (except in magnitude) to those induced during electrical excitation of the stellate ganglion. Panel B reveals responses to identical stimulation of the left cervical vagosympathetic trunk. The prompt rise in contractile force of the right ventricle was similar in onset to that observed during stimulation of the right vagus, although of lesser amplitude. The positive inotropic change in left ventricular apex was considerably smaller than in panel A, and no response was induced in the left ventricular base. There was no change in heart rate, and the elevation in arterial blood pressure was less pronounced.

Direct pressure recordings from the 4 cardiac chambers (Fig. 2) confirm the conclusion that fibers within the cervical vagosympathetic trunk have a direct augmentor action on both right and left ventricles. Both pulsatile and mean pressures are shown from

‡ Sernylan Phencyclidine HCl employed in these experiments was generously furnished by Parke, Davis and Co.

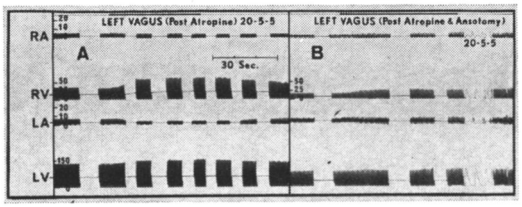

FIG. 2. Intracardiac pulsatile and mean pressure recordings during electrical stimulation of left cervical vagosympathetic trunk before (A) and after (B) bilateral section of the ansa subclavia.

each of the chambers, with prompt and distinct increase in intraventricular pressures in both chambers during electrical stimulation of the left vagus in the atropinized animal. The change was relatively greater in the low pressure chamber, but the absolute change was approximately 20-25 mm Hg in each. Mean pressure became elevated in both ventricles, relatively more in the right than in the left, and fell slightly in both atria.

Since it is conceivable that augmentation in ventricular pressure during vagal stimulation may be related to pathways in the vagus which interact (or interconnect) with fibers in the stellate ganglion by way of the ansa subclavia, bilateral ansotomy was performed immediately following the production of panel A (Fig. 2). Upon cutting the ansae, pressures in both ventricles fell dramatically and heart rate decreased from 180 to 135 beats/min (Fig. 2B). This response reveals a remarkably high sympathetic tone acting by way of the thoracic sympathetic outflows directly upon nodal and contractile tissues of the heart. Electrical stimulation of the left vagus employing parameters identical to those in panel A elicited positive augmentor responses in both right and left ventricles although the magnitude of change was considerably less. This undoubtedly reflects the important interaction of the extrinsic innervation upon existing myocardial tone. Nevertheless, a positive response indicates the patency of nervous pathways directly from the cervical vagosympathetic trunk to the ventricular musculature.

In 12 animals, the most pronounced changes induced by stimulation of the vagosympathetic trunk were elevation in right intraventricular pressure and increased heart rate. The former averaged approximately 45% increase above control systolic pressure (from control of 22 mm Hg to 31 mm Hg during stimulation) and the latter showed acceleration from a mean control value of 170/minute to 196 during stimulation of the right vagus and to 176 during stimulation of the left. There simultaneously occurred a 10% elevation in left ventricular systolic pressure (from control of 91 to 100 mm Hg). However, in 3 of the 12 dogs, changes were more profound in the left than in the right ventricle, and in 2, cardiac acceleration was more prominent during excitation of the left vagus. This may be interpreted to indicate a greater supply of sympathetic augmentor fibers by way of the right vagosympathetic trunk to the right ventricle. However, in many animals moderately positive inotropic responses were elicited in both cardiac chambers and during excitation of either vagosympathetic trunk.

Fig. 3 illustrates responses in an experiment in which few if any cardioinhibitory fibers appeared to be present in the left vagosympathetic trunk, thus permitting direct excitation of the sympathetic-like fibers even in the absence of atropine. In panel A, electrical stimulation of the right vagus induced cardiac arrest with a few escape beats late in the period of stimulation. One minute later, identical stimulation of the left vagus (panel B) not only failed to induce bradycardia, but elicited marked acceleration in rate (from 156 to 216/minute) as well as significant elevations

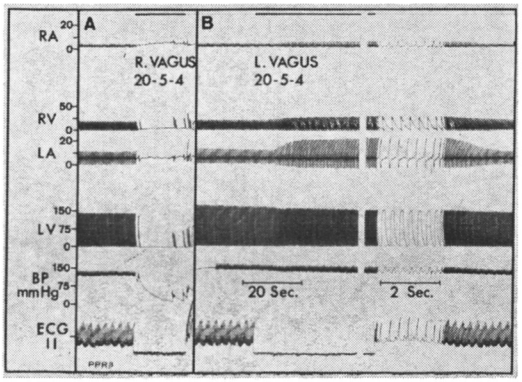

FIG. 3. Pressures within the 4 cardiac chambers together with systemic arterial pressure and ECG during stimulation of right (A) and left (B) vagosympathetic trunks in the absence of atropine.

in atrial pressure. Reference to the fast traces reveals marked augmentation in amplitude of the a-waves in both RA and LA, a portion of which was due to contraction against closed A-V valves. The ECG tracing indicates maintenance of a sinus rhythm. RV systolic pressure progressively increased from a control level of 20 to a maximum of 30 mm Hg. LV systolic pressure declined slightly and was accompanied by an appropriate decrease in systemic arterial pressure. All pressures and heart rate returned to normal values within approximately one minute after cessation of stimulation.

Discussion. From these experiments there can be little question that the cervical vagosympathetic trunk contains fibers with adrenergic properties and which directly supply the ventricular musculature as well as the SA node and the atria. In the presence of the well-known inhibitory fibers, their action has not previously been demonstrated during routine electrical stimulation of the vagus nerves, because of overwhelming inhibitory action on the heart. Functionally, however, such antagonistic action of mixed fibers descending within the vagosympathetic trunk could play an extremely important role in reflex regulation of cardiac action.

The occurrence of vasoconstriction in the pulmonary vascular bed could account, at least in part, for the greater elevation in right ventricular pressure. In some instances elevated right ventricular pressure was accompanied by reduced pressure in the left heart. Thus, the present studies do not rule out the possibility of such important distribution of sympathetic vasoconstrictor fibers from the cervical vagosympathetic trunk to the pulmonary blood vessels, but this is believed not to account for the major alterations in ventricular contractility. Preliminary experiments with a bilateral isovolumetric ventricular preparation have revealed similar augmentation in intraventricular pressures during vagal stimulation (atropinized preparation) and in such experiments, possible participation of pulmonary resistance changes was completely eliminated.

There is also reason to interpret positive inotropic responses to vagal stimulation in the atropinized preparation in terms of cholinergic release of myocardial catecholamines which in turn induce augmentation in contractility. Hollenberg *et al*(8) observed a characteristic biphasic response of the ventricle during intracoronary infusion of acetylcholine, and Levy *et al*(9) noted similar rebound of left ventricular pressure after vagal stimulation. In some of the present experiments post-stimulation rebound or overshoot in ventricular pressure was observed prior to the administration of atropine, but these changes were invariably small. If a cholinergic link in release of myocardial catecholamine were responsible for the prompt augmentor response reported here, one might expect it to be exaggerated by eserine. It was not. It was suppressed or abolished by the β-blocking agent, propranolol, and was markedly attenuated in animals whose superior cervical ganglia had been removed two weeks earlier.

Summary. The existence of ventricular augmentor fibers in the cervical vagosympathetic trunk of the dog has been established by electrical stimulation after atropine. These fibers are distributed directly to both right and left ventricles, although preliminary evidence indicates disproportionate supply to the right ventricle. Augmentation in atrial systole during vagal stimulation after atropine also indicates significant innervation of atrial musculature, but these influences may often be subordinate to alterations in mean atrial pressure. Cardioaccelerator responses may or may not accompany the augmentor changes in intraventricular pressure, and are usually more characteristic of right vagal stimulation.

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ATP Hydrolysis by Isolated Embryonic Heart Nuclei Using a Histochemical Method.* (31980)

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Embryonic myocardial cell nuclei of the chick are able to hydrolyze adenosine triphosphate (ATP) enzymatically; this hydrolysis is characteristic of an ATP phosphohydrolase, EC 3.6.1.3 (ATPase)(1). A number of similar physical and chemical properties of the enzyme can be demonstrated both biochemically using purified isolated nuclei and histochemically using unfixed (or fixed) cryostat sections. A modified method of Wachstein's and Meisel's(2) was used in the latter procedure.

The present investigation was undertaken to demonstrate that the histochemical test for presumptive ATPase activity can be applied to isolated nuclear preparations from myocardial tissue. It is also given as further proof that earlier interpretations using cryostat sections could not be due to diffusion artifacts resulting from phosphate liberation by other cell components.

Methods. Myocardial cell nuclei of the chick were isolated from 12-day embryos and purified as described previously(1). The final concentrated suspension of nuclei was in 0.5 M sucrose. Aliquots were spread on clean glass slides with the edge of a cover slip and allowed to air dry for 5 minutes. Slides with adhered nuclei were placed on a petri dish in a Technicon water bath at 37.5°C and flooded with the desired incubating solution at the same temperature. The enzyme reaction medium contained: 5 mM Mg^{++} (Cl^-, NO_3^- or SO_4^{3-} salt), 5 mM $tris_4$-ATP, 20 mM tris-HCl or tris-maleate-NaOH buffer at the desired pH, 0-130 mM Na^+ (Cl^- or NO_3^- salt) and 5×10^{-4} M Pb $(NO_3)_2$ as the phosphate trapping agent. Calcium ions were substituted for Mg^{++}, and nucleoside and other phosphates for ATP in equal concentration. Media were kept isotonic with sucrose when below 0.31 M in non-electrolyte equivalents. Mersalyl (Salyrgan), ouabain and 2,4-dinitrophenol (2,4-DNP) were used at concentrations between 10^{-7} and 5×10^{-4} M.

Preparations were preincubated for 5 minutes in reaction media without substrate and lead ions. Upon the addition of the latter components, reactions were allowed to proceed for 30 minutes with several medium changes. They were stopped by rinsing with ice cold medium without substrate or lead ions, bathed 3 minutes in cold 0.1% $(NH_4)_2S$, rinsed and mounted in dilute gelatin medium without further staining.

Results. The concentration of Pb^{++} is comparatively low to that often employed. Approximately 3×10^{-4} M gives near maximal staining intensity using hatched chick myocardial sections(1). With isolated nuclei from 12-day embryo heart, about 5×10^{-4} M Pb^{++} is required. Higher concentrations are considered unwarranted as they would only cause increased enzyme inhibition and poorer nuclear preservation. In addition higher concentrations of Pb^{++} can cause non-enzymatic hydrolysis of ATP(3). The latter was tested under present conditions and 5×10^{-4} M Pb^{++} produces no measurable hydrolysis of ATP, or is this affected by Na^+ or Mg^{++}.

Differential analyses of ATP hydrolysis by other cell fractions (myofibrillar, actomyosin, microsomal and mitochondrial) under a vari-

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