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Studies of heart rate control

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I Mechanism of Ventriculophasic Arrhythmia

II Further Characterization of the Paradoxical Effect of Vagal Stimulation on Heart Rate

III Bainbridge Reflex: Volume Infusion in the Canine Cardiac Autotransplant

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HEARTFELT GRATITUDE IS DUE MANY PERSONS with whom it was my pleasure to work during the course of these experiments.

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NOW WE SEE ONLY PUZZLING REFLECTIONS IN A MIRROR, BUT THEN WE SHALL SEE FACE TO FACE. MY KNOWLEDGE NOW IS PARTIAL, THEN IT WILL BE WHOLE, LIKE GOD'S KNOWLEDGE OF ME. IN A WORD, THERE ARE THREE THINGS THAT LAST FOREVER: FAITH, HOPE, AND LOVE; BUT THE GREATEST OF THEM ALL IS LOVE.

I Corinthians 13: 12-13 (NEB)

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STUDIES OF HEART RATE CONTROL

INTRODUCTION

In recent years clinical cardiac transplantation has become a reality. The obstacles to be overcome before clinical trial, were recognized early as being "1) preservation of the heart graft during its extracorporeal phase, 2) the surgical technique, 3) the effect of total cardiac denervation, and 4) the homograft rejection mechanism."[1] Although many of the problems remain unsolved, the development of present techniques, the assessment of denervated heart function, and the study of the immunological problems involved, have contributed to our knowledge in clinical medicine and have made the present patient trials feasible as well as provided knowledge of basic physiological mechanisms.

Transplantation of the canine heart was first reported by Carrel and Guthrie.[2,3] In their experiment the heart of a small dog was placed in the neck of a larger one by "anastomosing the cut ends of the jugular vein and the carotid artery to the aorta, the pulmonary artery, one of the vena cava and a pulmonary vein."[2] With back perfusion of the coronary circulation established, a strong contraction ensued at the rate of 88 per minute until the experiment ended at two hours. The next report of heterotopic homograft heart transplantation was that of Mann and coworkers in 1933.[4] Refinements included the use of heparin in the donor and understanding the importance of coronary air embolism; beating hearts were maintained up to 8 days post-transplant with the observation the "histologically the heart was completely infiltrated with lymphocytes,

large mononuclears and polymorphonuclears."[4] Experiments of this type began to appear more frequently in the early 1950's.[5-12] Marcus, et al, stated in 1951 that "whether (a transplanted heart) might so function as to replace its counterpart in the host is a matter of fantastic speculation for the furture."[6] They also suggested the value of a heterotopic heart graft for the study of a denervated heart,[6,7] and Luisada and Marcus first studied the effects of adrenaline, digitalis, and morphine, with comparison of the response of the graft to that of the normal recipient heart.[8]

Complete isotopic replacement of the canine heart and lungs was reported by Neptune and associates,[13] using total body hypothermia, with support of the circulation by the transplanted heart for six hours. Blanco, et al, were next to attempt isotopic replacement of the heart and both lungs;[14] with a similar technical procedure, a pump-oxygenator, and potassium arrest, they attained a maximum survival of $4\frac{1}{2}$ hours. Webb and Howard[15] performed both heart and total lung transplantation, heart and left lung alone, and autotransplantation of the heart and both lungs. With both lungs denervated they were unable to attain survival with spontaneous respiration, and thus the autograft preperations were not available for study of cardiac denervation. These same authors together with Neely,[16] reported a method for isotopic homologous cardiac transplantation involving multiple venous anastomosis, and attained survival in ten with the longest being $7\frac{1}{2}$ hours.

Two reports by Berman, Goldberg, and Akman appeared in 1958.[17,18] These authors described a method for homologous heart transplantation which consisted of a left atrial "cuff" about the pulmonary veins,

and anastomosis of the SVC, IVC, PA, and aorta. Their results with three animals were support of the circulation for up to 21 minutes with no external cardiac pacing, and two hours with external electrical stimulation, until ventricular fibrillation ended the experiment.[17] This technique was an advance over previous procedures, though the initial results were not encouraging.

A major advance in the technical feasibity of cardiac transplantation was the development by Lower and associates[19] of a rapid procedure for transplantation which involved a running atrial suture line rather than multiple venous anastomoses. With this method they were able to initially attain survival up to 21 days. From this point onwards, a technical procedure was available which would allow more complete study of the other obstacles to cardiac transplantation as well as make available a completely denervated heart preperation for long term study.

An alternative approach to the study of denervated heart had been the technique of neural ablation. Apparently the earliest attempt was that of Friedenthal in 1902.[20] He purported to show the viability of an animal (a dog that survived 8 months) with chronic cardiac denervation by means of vagotomy and stellate ganglionectomy, but neglected to study possible changes of heart rate under different circumstances. Further pioneering work was done in Cannon's laboratory,[21] with more rigorous criteria for the demonstration of denervation. This method was used by various investigators to study the effect of drugs and exercise on the heart apart from central nervous system control.[22-24] However, these experiments were sometimes contradictory and standard criteria for the completeness of denervation

were not used. In 1960, Gilbert and associates described a method for intrathoracic cardiac denervation in which the great vessels were skeletonized and middle mediastinal autonomic sturctures were excised from T3 to the diaphragm.[25,26] This technique became known as Cooper-Gilbert regional neural ablation, and has been used by other investigators, particularly Donald and Shepard.[29-31] The proof of denervation consists of no demonstrable change in heart rate following either vagal nerve or stellate ganglion stimulation, and by depletion of cardiac catacholamines.[25,26] The results of all these studies clearly showed the ability of denervated heart to support the circulation of an animal, the altered responsiveness of the denervated heart to various cardioactive drugs,[32] and the mechanism of increasing cardiac output by increasing stroke volume rather than rate.

The alternative approach of excision and reimplantation of the heart was clearly necessary, however, because of the analogy to the more extensive procedure of homograft heart transplantation. The technical difficulty of this procedure was pointed out in the first attempts by Cass and Brock.[33] The method for homograft heart transplantation developed by Lower and associates,[19] was next applied to autotransplantation by Hurley, et al,[34] and provided the first long-term survivors which could be adequately studied.[35] The success of this method was due to the atrial anastomosis and the use of an Ivalon band to bolster the fragile supravalvular aorta. With survivors of over 23 months, it was possible to study the late effects of this procedure.[36]

Simultaneously a second method for canine cardiac autotrans-

plantation was developed by Willamn and coworkers,[37] resembling the method of Berman, et al.[17] This procedure involved transecting the SVC, IVC, PA, and aorta, as well as the cuff of left atrial tissue surrounding the openings of the pulmonary veins, and then resuturing these vessels. Thirteen of the first 40 animals lived longer than two days. All developed signs of congestive heart failure, leading these authors to conclude that there was "a specific adverse effect of severing the heart from the body."[37] Animals prepared in this manner were later studied with cardioactive drugs,[38] blood volume expansion,[39] and work capacity using a right heart bypass technique under anesthesia.[40]

The experience of the Stanford group was somewhat different with autograft animals. It was found that severe congestive heart failure was not a problem; digitalis and diuretics were not routinely necessary.[34-36,41] The hemodynamic effects of denervation were characterized in these animals. It was found that the blood volume was increased, that there was a decreased urine output in response to a volume load, and that the heart rate changes gradually and to a lesser extent in response to exercise.[41] The effect of volume loading on hemodynamics in the awke unanesthetized animal has also been recently studied.[42]

The laboratory models necessary for adequate evaluation of cardiac transplantation were therefore available with the canine homograft and autograft. In addition, methods were developed for study of the rejection process and its amelioration by therapy, and for the study of unambigously denervated heart. What soon

became apparent in using the autograft animal prepared by the method of Hurley, et al,[34] was the development of a model for studying the control system of heart rate and contractility.

With excision of the right and left atria in such a manner as to leave right and left atrial cuffs about the great veins, the sino-atrial node (S-A node) is left within the animal.* In the homograft preperation of Goldberg, et al,[17] and the autograft of Willman and associates,[37] the SVC and IVC were transected and the entire right atrium removed, including the S-A node. With the S-A node intact, however, vagal and sympathetic input, carried by neural elements in the adventitia of the SVC, is able to affect the firing rate in this pacemaker according to CNS command. In engineering terminology, this is then an open-loop control system for heart rate and contractility regulation. The loop is open since feedback changes in rate and contractility are not transmitted across the suture line to the free running ventricles.

This situation differs from that in complete heart block since sympathetic input to the ventricles and vagal input to the atrioventricular node is interrupted. If heart rate control were made "closed loop" by stimulating the donor atria and ventricles off of the P-wave of the innervated atrial remnant, a pure open-loop with respect to inotropic control would exist; a condition not possible heretofore in an animal with complete heart block.

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* S-A nodal tissue is formed in the sulcus terminales at the junction of the SVC and the right atrium.

The use of these particular properties of the cardiac autograft, which developed from studies of heart transplantation, is the starting point for the experiments which constitute this thesis. This particular model is well suited for studies of heart rate control, especially of the many reflexes whose afferent or efferent arcs are manifested by heart rate.

The experiments presented here are divided into three sections. The first describes observations on the relationship between the timing of the arterial pressure pulse and the length of the atrial cycle in the autograft. This section is entitled the "Mechanism of Ventriculophasic Arrhythmia," which is a clinically seen arrythmia simulated by this model.

The second part presents a series of experiments which evolved directly from the observations in section one. These are experiments with vagal stimulation in an anesthetized normal dog which has been vagotomized and given propranolol to block sympathetic influences. This preperation serves to explain some of the findings in part one, as well as elucidate the peculiar paradoxical effect of vagal stimulation on heart rate which has been recently described. This section is entitled "Further Characterization of the Paradoxical Effect of Vagal Stimulation on Heart Rate."

The final section again concerns the autograft model. The highly controversial "Bainbridge Reflex" is studied in the awake unanesthetized animal, and evidence is presented which substantiates the original description of tachycardia associated with volume infusion.

SECTION I: MECHANISM OF VENTRICULOPHASIC ARRHYTHMIA

In studying chronic heart block in dogs, Ehrlanger and Blackman observed that "the first auricular cycle following a ventricular contraction is long but the successive auricular cycles shorten until the ventricles again contract."[43] This arrhythmia (later termed the ventriculophasic arrhythmia - VPA - since it is a variation in atrial rate associated with ventricular activity) was subsequently described in several patients with complete heart block.[44,45] A number of clinical papers have since appeared which have described the incidence of the arrhythmia,[46] the importance of efferent vagal innervation,[46-48] correlation of the arrhythmia with arterial pressure,[49-50] and calculation of an apparent latency for the reflex involved.[50-51] The majority of authors ascribe its origin to variations of vagal tone caused by the arterial pressure pulse acting on the carotid sinus, though several have suggested the local mechanical action of ventricular contraction is important.[52,53]

In recent years, the dog with an autotransplanted heart has provided an experimental model in some ways similar to complete heart block. In the standard preperation[34] a normally innervated atrial remnant (termed "recipient atrium") is apposed to completely dennervated atrial and ventricular tissue (termed "donor"). We have observed the VPA in such an animal. The purpose of this report is threefold: 1) to characterize this phenomenon in the autograft, 2) to suggest that the probable mechanism is a modulation of vagal tone together with a varying response of the atrial pacemaker to

vagal input, 3) to support this hypothesis by describing experiments of direct vagal stimulation of normal dogs "dennervated" by cervical vagotomy and propranolol (to be described in more detail in SECTION II).

MATERIALS AND METHODS

Experiments with two awake unanesthetized autotransplanted dogs were done when the animals were active and healthy 2 - 8 weeks following operation.[34] Chronic instrumentation included a totally implantable aortic pressure transducer (Microsystems) positioned in the sacrificed left subclavian artery at its origin, a bipolar recording electrode (Electro Catheter Corporation, New Jersey) sutured to the "recipient atrium" near the lateral SVC-RA junction, and a standard pacing wire sutured to the donor left atrial appendage. These instruments, their position, and the waveform obtained from them, are illustrated by Figure 1.

Experiments were done with the trained animals resting quietly in the right lateral position. The arterial pressure, the lead II electrocardiogram, and the "recipient atrium" electrical activity were simultaneously displayed on an Offner Type R Dynograph and recorded on an Ampex FR-1300 FM tape recorder for subsequent digital computer processing. Protocols consisted of recording "remnant atrium" and "donor" ventricular activity under resting conditions and with "donor" atrial pacing at intervals of 1000, 900, 800, and 700 msec. Other experiments were done in the presence of atropine sulphate (0.15 mg/kg I.V.) and with rapid I.V. infusion of isotonic saline (40-60 ml/min).

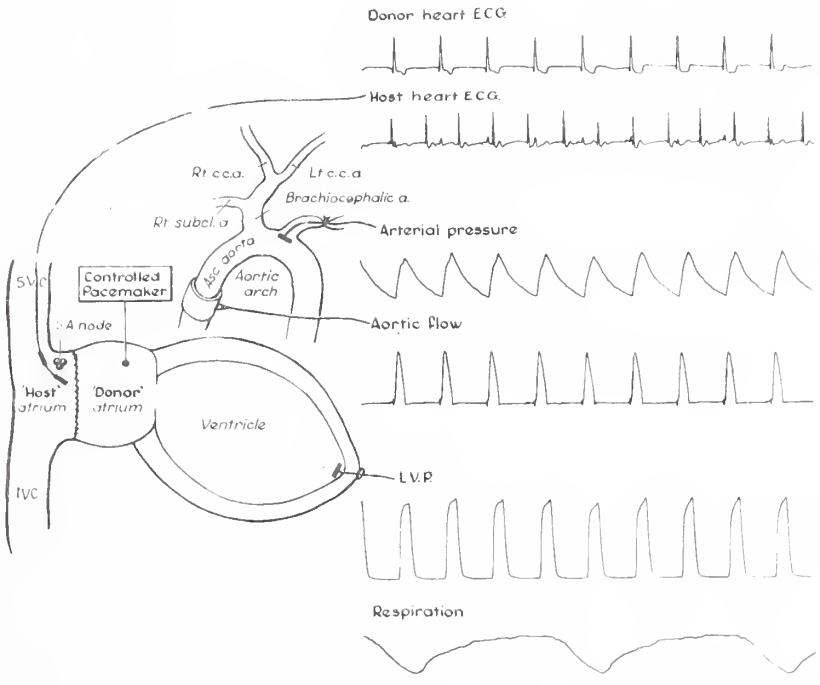


FIGURE 1: Instrumentation routinely employed in the canine cardiac autograft. Electrocardiograms from the external lead II and the "Host" heart via the bipolar lead. Chronically implanted arterial pressure transducer in the left subclavian and the left ventricle. An EMF flow meter is positioned on the ascending aorta.

A second group of experiments involved 5 normal dogs anesthetized with morphine (1mg S.C.), warmed alpha-chloralose (60 mg/kg I.V.), and urethane (600 mg/kg I.V.). Both cervical vagus nerves were transected and bipolar platinum wire electrodes placed on the cardiac end of the right vagus. Trains of one, three, and five impulses (10-14 V amplitude, 2 msec duration, 10 msec apart) were given at intervals of 1000, 900, 800, and 700 msec through a stimulus isolation unit from a Grass S4D stimulator. The stimulator was externally controlled by programmed output from the ACME IBM 360/50-1800 computing facility at the Stanford Medical Center. The stimulus delivered and activity from a bipolar electrode placed transvenously in the right atrium were recorded as described above. Propranolol (1 mg/kg I.V.) was given to block sympathetic activity.

Tape records from both types of experiments were analog to digital converted using the ACME IBM 360/50-1800 computer. The digital records were then processed in one of two ways: 1) autograft experiments were displayed on a television monitor and R-onset and P-onset events tagged by hand (estimated accuracy of this method is $\pm$10 msec), 2) normal dog stimulus-stimulus and P-P intervals were automatically measured as the time between crossings of the derivative of the analog signal to some preset level (accuracy of $\pm$ 2 msec). In addition, the computer was programmed to give averaged arterial pressure waveforms, and these were plotted by a Houston Instruments Plotter.

RESULTS

Presence of VPA in the Autograft

In both the autograft animals studied the length of the "remnant" atrial cycle (P-P interval) varied according to the timing of "donor" ventricular activity as indicated by the R-wave. This is demonstrated by the records in Figure 2. Recordings from lead II of the electrocardiogram and the bipolar electrode sutured to the "remnant atrium" (the AKG) are displayed. (A) represents the animal resting quietly on his right side. The R-R interval is constant at 650 msec; the P-P intervals vary from 580-760 msec. In this record the R-waves are consistently falling near the end of the P-P interval, and it is clear that the longer P-P intervals are those in which the preceeding R-wave is closest to the initial P-wave of the interval.

Conditions which lead to a decrease in efferent vagal activity were associated with disappearance of VPA. Administering atropine sulphate (0.15 mg/kg I.V.) caused P-P intervals to decrease to a fixed value whereas ventricular activity was unchanged. Under these conditions there was no variation in "remnant" atrial cycle length within the limits of accuracy of measurements.

Part (B) of Figure 2 represents a second case where vagal activity is abolished, this time following a rapid infusion of isotonic saline to increase venous pressure (see SECTION III). In this experiment the R-R interval increased from 650 to 825 msec; the P-P intervals decreased to 420 msec with no variation in relation to the R-wave. Similar behavior could be noted if the dog was brought from the recumbent position to the standing position,

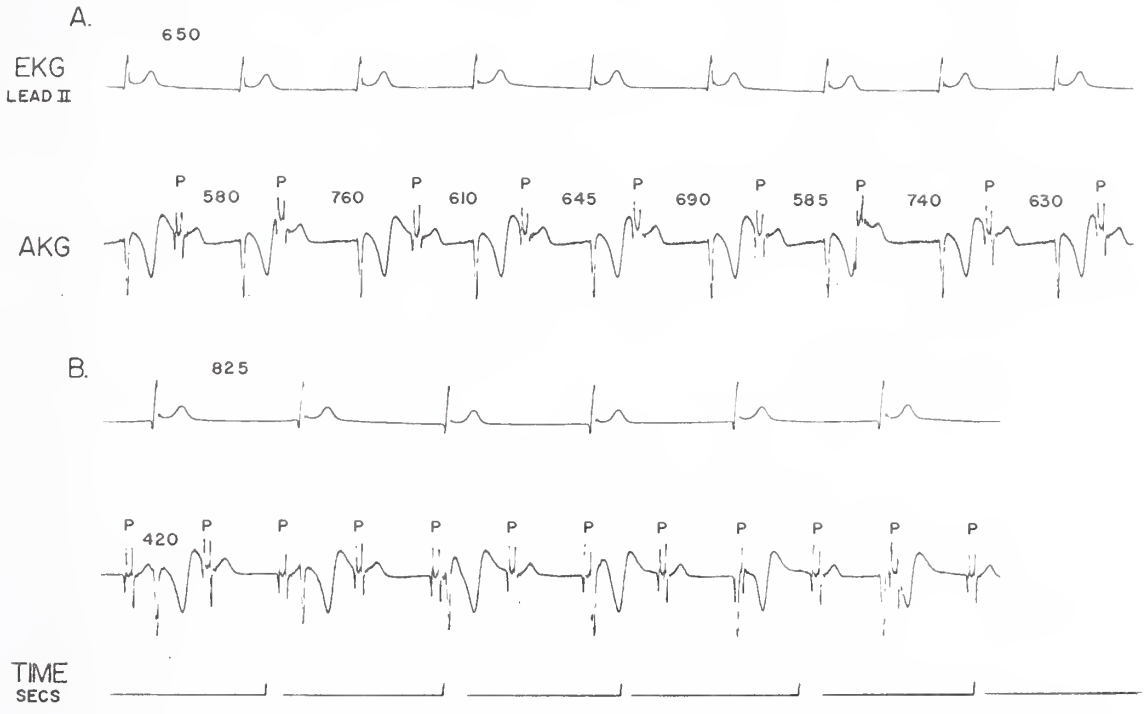


FIGURE 2: EKG recorded from a canine cardiac autotransplanted dog. The standard lead II and the atrial bipolar lead are shown (AKG). P-waves in the AKG are indicated by "P" to differentiate them from R-wave artifact. (A) Control conditions with the animal resting quietly, showing variation in the P-P interval depending on the position of the R-wave. (B) The same animal following infusion of 300 cc of isotonic saline. The R-R interval has increased to 825 msec; the P-P interval has decreased and is fixed at 420 msec.

which is associated with reciprocal sympathetic activation and vagal withdrawal. These characteristics agree with the behavior noted by previous studies of VPA in patients with complete heart block.[45-51]

Quantitative Relations in the VPA

When the "donor" heart is paced at various intervals the quantitative relationship between ventricular activity and resulting P-P intervals can be studied. Respiratory rate was not controlled in these studies and reflex respiratory changes in cycle length are seen in the autograft, increasing the variability somewhat. Respiratory fluctuations are assumed to average out in the period of time that measurements were taken. The results of recording on three seperate occasions from one autograft are plotted in Figure 3. In the upper half of the Figure the averaged arterial pressure waveforms for the four conditions have been plotted. In the lower part, the average P-P interval length is given, with intervals grouped according to the R-wave to P-wave time (R-P). Time zero represents the R-wave onset; the R-P group from 0-50 msec represents the average of all P-P intervals which begin 0-50 msec following an R-wave onset, and so on. It is first noted that the mean P-P interval increases as the R-R interval decreases, and since arterial pressure rises, this is consistent with normal autonomic innervation of the "recipient atrium". Secondly, the P-P interval length appears to be related to the time within the ventricular cycle that the initial P-wave occurs, and apparently to the arterial pressure at the beginning of the

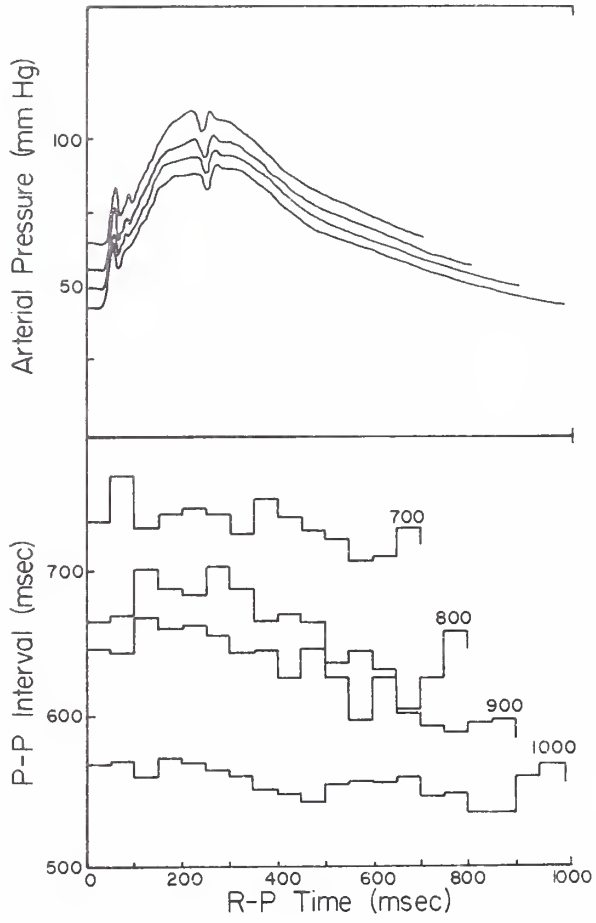


FIGURE 3: Three experiments averaged to give the arterial pressure waveform and the remnant atrial cycle length at four pacing intervals: 1000, 900, 800, and 700 msec. The atrial cycle length appears to be proportional to arterial pressure existing at the beginning of the interval, except for the last 100-150 msec of the R-R interval when atrial cycle length appears to increase.

P-P interval, as reported earlier by Bevegard, et al,[50] in patients. Thirdly, Figure 3 shows that the minimum P-P interval occurs in each instance in which the initial P-wave falls in the 150-100 msec before an R-wave; P-P intervals beginning slightly later are longer though the arterial pressure is low. This same feature was seen in the data of Bevegard, et al,[50] and is an instance in which arterial pressure is not directly related to the length of the P-P interval.

This same data is plotted in a second manner in Figure 4, such that the initial P-wave of the P-P intervals is held constant and the position of the R-wave is varied on the horizontal axis. This Figure demonstrates the same findings as in Figure 3, but accentuates the position of the minimum P-P interval in the period where the initial P-wave falls 100-150 msec before the R-wave.

Clinical studies in complete heart block have also demonstrated synchronization of atrial and ventricular activity in some cases,[54] and recently Levy, et al,[55] and Reid,[56] have shown that the basis for this phenomenon may lie in the pattern of response of the S-A node to vagal stimulation. The same type of synchronization phenomenon is seen in the autograft. Table I gives the n, mean, and standard deviation of the P-P intervals grouped by the value for R-P. The n thus represents the distribution of P-waves throughout the R-R cycle. This distribution is apparently random except for the case where the donor heart is being paced at 700 msec intervals. There is a predominance of P-waves which fall 400-500 msec after an R-wave (or 200-300 msec before an R-wave). The average value for the P-P intervals in this case is near 700 msec, so that the conditions for apparent synchronization exist.

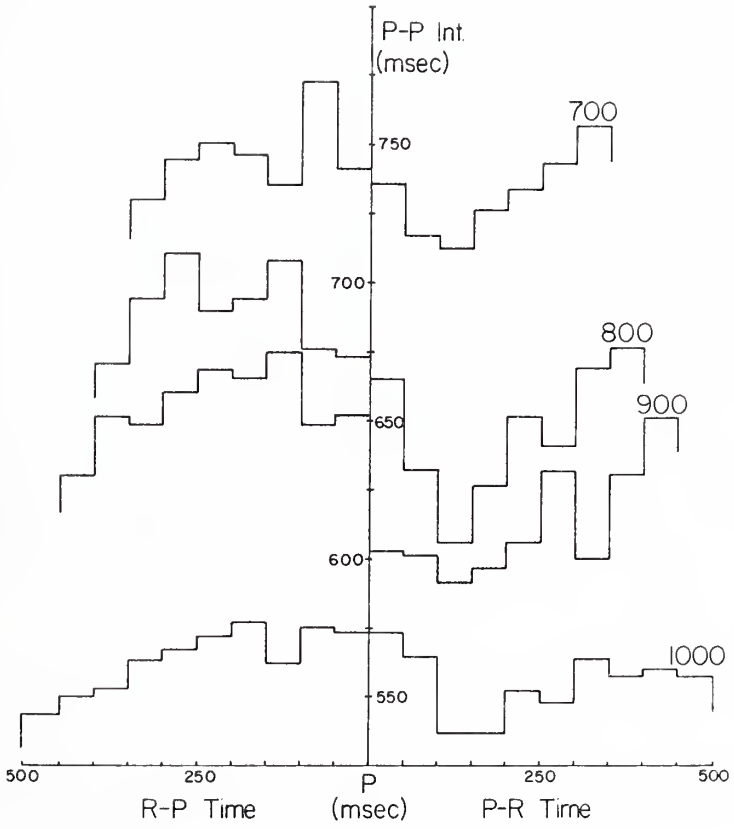


FIGURE 4: The results of three experiments averaged to give the P-P interval length for that interval which begins at the time before (P-R) or the time after (R-P) an R-wave.

R-P (msec)	R-R INTERVAL (msec) 1000 n	m	SD	900 n	m	SD	800 n	m	SD	700 n	m	SD
0-50	9	573	42	7	653	33	17	673	43	2	740	7
51-100	10	575	42	9	648	38	17	676	38	5	772	92
101-150	9	564	37	5	673	45	14	710	63	10	733	54
151-200	7	577	36	9	665	29	18	689	43	8	748	45
201-250	13	571	42	12	669	30	18	682	53	5	752	51
251-300	8	569	48	7	662	30	17	713	74	6	746	71
301-350	6	565	55	6	648	32	21	688	80	6	729	61
351-400	12	553	27	6	652	41	16	670	78	19	760	21
401-450	7	550	36	8	628	38	12	678	84	34	742	43
451-500	5	542	17	7	651	52	14	669	51	39	733	47
501-550	9	561	99	9	631	29	14	641	19	6	727	41
551-600	8	563	67	8	609	19	6	652	54	9	712	42
601-650	9	561	67	4	633	9	4	639	42	8	717	82
651-700	8	567	73	8	607	36	11	606	43	7	736	88
701-750	10	549	85	4	597	52	25	632	68			
751-800	9	553	65	4	592	60	8	665	57			
801-850	8	536	82	17	601	41						
851-900	8	536	81	8	603	22						
901-950	9	567	37									
951-1000	7	573	82									

n = number

m = mean

SD = standard deviation

TABLE I: Values for the P-P intervals from three experiments averaged, with the n and standard deviation. P-P intervals are grouped according to the R-R interval and the R-P time.

Simulation of VPA by Vagal Stimulation in the Normal Dog

Studies of direct cervical vagus stimulation in the dog, both with single stimuli[57] and constant low-frequency stimulation[55,56] have demonstrated a characteristic variation in response depending on the timing of the stimulation within the heart cycle. The pattern of response was consistent with the variation in P-P interval seen in the autograft with VPA. It seemed reasonable, therefore, to simulate the arrhythmia in normal dogs by stimulating the cut right vagus nerve following bilateral vagotomy and propranolol administration. In this simulation, vagal stimulation at 1000, 900, 800, and 700 msec is analogous to ventricular activity at these intervals (Vagal stimulation substituting for arterial baroreceptor reflexes). The number of impulses delivered per stimulation can account for ventricular activity with higher numbers of impulses simulating greater arterial pressure.

The standard stimulation protocol consisted of two minutes of stimulation at a given interval with one through five impulses per stimulation. The results of these experiments which pertain to VPA will be presented here; the results which characterize the synchronization phenomenon for different stimulus intervals and also for different numbers of impulses with the same stimulus interval will be presented in SECTION II.

When the stimulation interval is varied a single curve is found relating the resultant P-P interval to the P-stimulus delay. Figure 5 illustrates a vagal stimulation experiment in which four impulse trains (12 V amplitude, 2 msec duration, 10 msec apart) was delivered to the cardiac end of the cut right vagus nerve. This

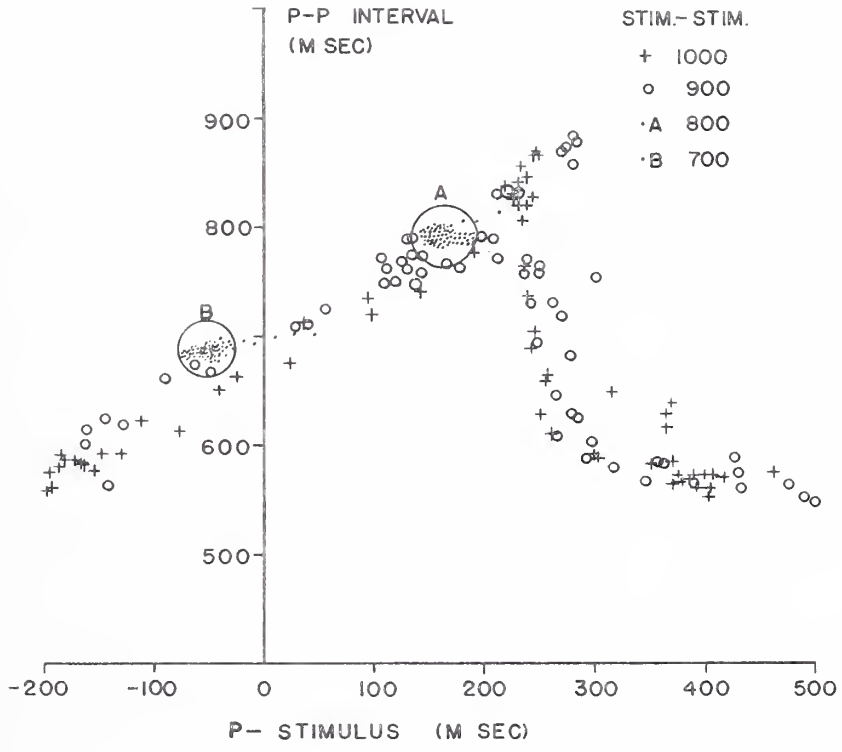


FIGURE 5: Vagal stimulation in an anesthetized normal dog after vagotomy and propranolol. Four impulses were applied to the right vagus at a stimulus-stimulus interval of 1000, 900, 800, and 700 msec. The resultant P-P interval is plotted against the P-stimulus time. Note the clustering of P-P intervals at 700 and 800 msec, when the stimulus-stimulus intervals are also 700 and 800 msec.

Figure demonstrates that as the stimulus-stimulus interval is varied from 1000 msec to 700 msec, the resulting P-P intervals fall on a single defined curve, which we will call the pacemaker response curve after Levy, et al,[55] who presented such a curve for one impulse only. At stimulus-stimulus intervals of 700 and 800 msec, the P-P intervals obtained are also 700 and 800 msec. This is the phenomenon of synchronization or entrainment, and will be described and explained more completely in SECTION II. The curves which are found at other numbers of impulses show a similar well defined form, but vary in some characteristics, especially in amplitude. When a single best-fit curve is drawn through the pacemaker response curves for one to five impulses per stimulation, a family of curves relating P-P interval to the P-stimulus delay results. This is illustrated by Figure 6, and it is clear that there is a correspondence to the family of curves presented in Figure 4, for P-P intervals in the cardiac autograft plotted against the time between R-wave and initial P-wave of the P-P interval. The difference in the timing of the minimum P-P intervals in these two Figures should be noted; 100-150 msec P-R for Figure 4, and 280-300 msec P-stimulus in the vagal stimulation experiment.

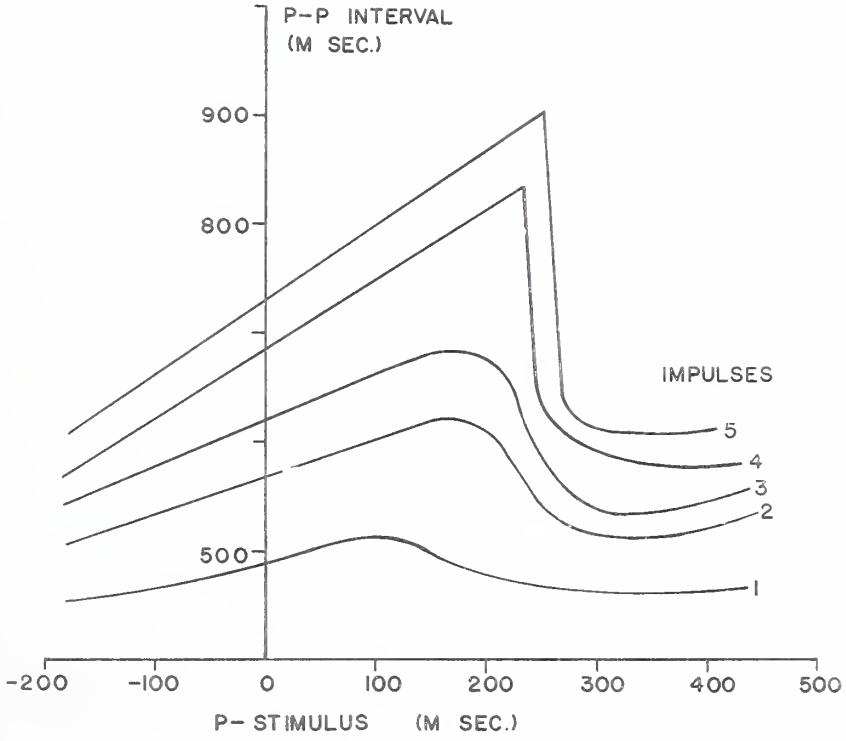


FIGURE 6: The best-fit curve for one to five impulses per stimulation drawn through the pacemaker response curve for the given number of impulses.

DISCUSSION

The cycle length of the innervated atrial remnant of the cardiac autotransplanted dog has been shown to be related to the timing of ventricular activity. The length of the P-P interval appears to be related to the arterial pressure at the beginning of the interval except for the period 150-100 msec before the following R-wave, when intervals lengthen though the pressure continues to fall. Changes which influence vagus nerve activity will predictably affect VPA. Atropine will abolish it, as will volume infusion and rising from a recumbent to a standing position. It appears, therefore, that VPA is present in the autograft, and is comparable to the arrhythmia seen in patients with complete heart block.[44-53]

Vagal stimulation in the normal dog which has been denervated by vagotomy and propranolol, appears to mimic the pattern observed in VPA. Figure 4 corresponds to the heart rate response when the vagus is stimulated at constant low frequencies and with varying number of impulses. The displacement of the curves in the horizontal axis by 130-200 msec represents the neural and mechanical conduction time between the R-wave and cervical vagus activation in the dog studied.

A further correspondence between the autograft model and previous clinical work[50] is the calculation of the apparent latency involved. The latency time of the reflex was taken to be the time between rise in arterial pressure and the end of the first P-P interval which could be lengthened by this pressure rise. This latency was found to be directly related to the length of the minimum P-P interval, with latencies between 400 and 600 msec. If

the data of Figure 3 is used to calculate latency, it is found to be directly related to the minimum P-P interval, with a slope of 45° (plotted in Figure 7). When analyzed, the factors which may contribute to the actual neural conduction latency are found to be somewhat less. The time between R-wave onset and rise of arterial pressure is 40-60 msec (from Figure 3). Direct measurements of cardioinhibitory fibers in the cervical vagus nerve of the dog by Jewett[58] show a burst of firing 80-240 msec following the rise of arterial pressure. The neural conduction time measured from the cervical vagus to the post-synaptic ganglion in the heart is estimated to be 10-20 msec.[58,59]

The long reflex time for VPA, and its variation with the heart cycle, first led us to study vagal stimulation. Reflex times, if they involve set neural pathways, are 1) usually much smaller, and 2) do not vary in the same animal as markedly. An alternative explanation was that there existed a variable response to vagal input depending on the timing. Since the study of single impulse stimulation by Brown and Eccles in 1934,[59] no quantitative study of this question existed until the recent reports already cited [55,56] which involved constant low frequency vagal stimulation. These studies, together with the present work, do demonstrate a marked variation in heart rate response to a uniform vagal input depending on its timing. When delivered early in a heart cycle, the same stimulus will increase the P-P interval to a much greater extent than when delivered only several msec later. This fact helps greatly to explain the pattern of the VPA.

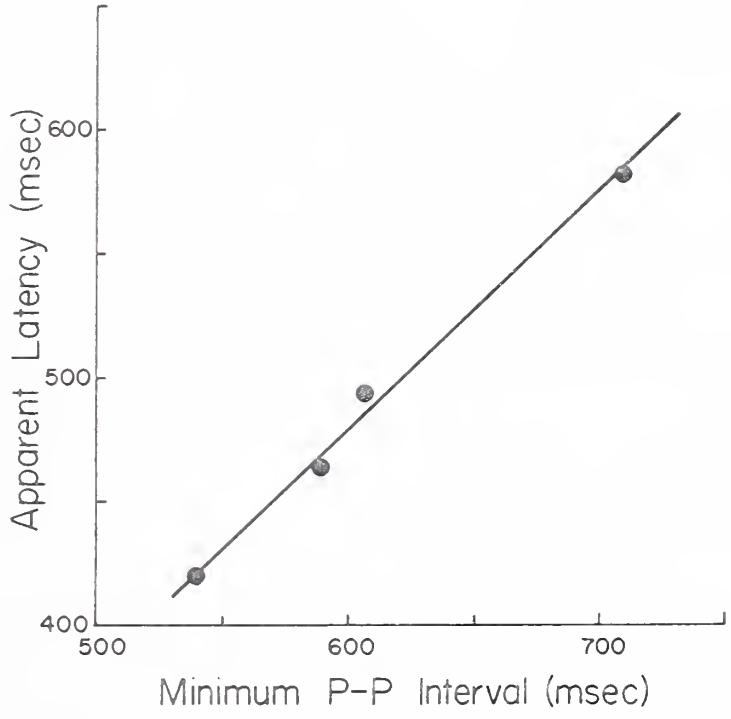


FIGURE 7: Calculated apparent latency for the VPA in the autograft after the method of Bevegard, et al.[50] Apparent latency is the time between rise in arterial pressure and the end of the first P-P interval which is lengthened above the minimum interval. This is plotted against the minimum P-P interval.

The hypothesis which is suggested by these experiments, and which would explain the pattern of VPA, can be briefly summarized. Variation in atrial rate depending on ventricular activity is primarily effected by vagal input mediated by the arterial baroreceptors. This vagal input will have a varying influence depending on the timing with which it arrives at the S-A node. Arriving in the first 250-300 msec of the interval, that same interval will be lengthened; coming later, the following interval will be lengthened, but a lesser amount. The timing relations are illustrated by the schematic diagram in Figure 8. This Figure represents what may occur in patients with complete heart block, or the canine cardiac autograft, in which the atrial and ventricular events are thought to be seperate. If we postulate a fixed sensitive period of 250-300 msec at the beginning of the atrial cycle, and an active period of vagus activity beginning 120-280 msec following the R-wave (see above), then the interactions of these two factors will result in the P-P interval observed. The minimum point in the P-P interval response curve of Figure 4, is a time when small vagal activity and the insensitive part of the atrial cycle coincide. When the sensitive period and the active period overlap, the P-P interval is lengthened. Since vagus fiber activity is greatest with the phase of rising arterial pressure,[58] the maximum lengthening results when the R-wave preceeds the P-wave in the period 100-200 msec before the P, as the active period begins to coincide best with the sensitive atrial period.

This interpretation of a sensitive early period in the atrial cycle helps to explain the apparent latencies also. If the sensitive

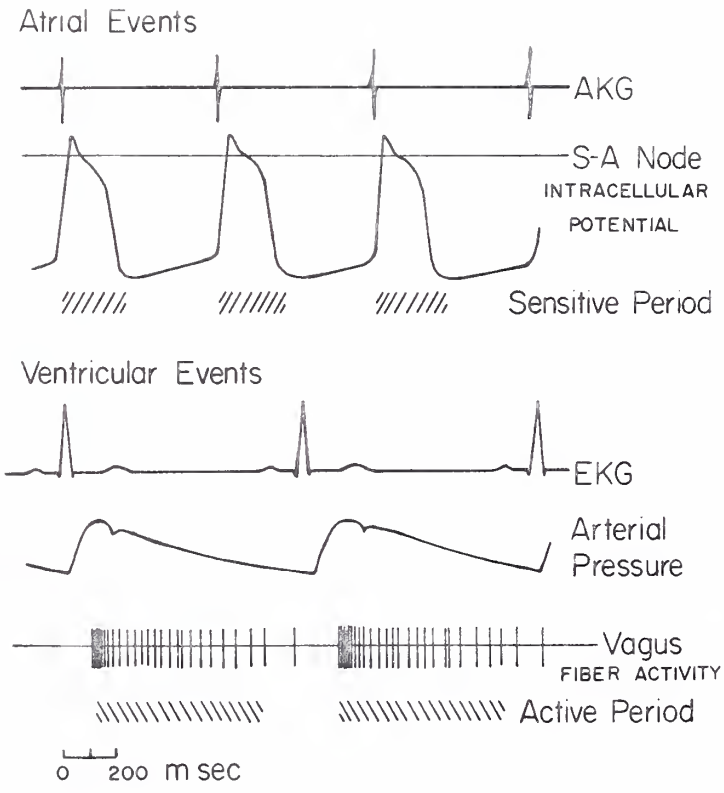


FIGURE 8: A schematic diagram of the atrial and ventricular events in patients with complete heart block and the canine cardiac autograft. A fixed sensitive period to vagal stimulation is postulated near the beginning of the atrial cycle. The active period of vagal activity derives from the arterial baroreceptors at the appropriate time (from Jewett[58]).

period is a fixed time regardless of the mean P-P interval, then the apparent latency would vary as the mean P-P interval (i.e., be proportional to the part of the atrial cycle which was "insensitive"). A vagal input arriving in the insensitive period would not affect heart rate until the next sensitive period arrived, thus making it seem as if a relatively long latency existed.

Experimental evidence suggestive of an early fixed sensitive period to vagal stimulation derives from electrophysiology. Studies of the S-A node in non-mammalian systems has shown that the effect of vagal stimulation is greatest during phase three (rapid repolarization)[60] and "least marked just prior to the upstroke of the action potential".[61] Microelectrode recordings from S-A node of the dog heart show that the period of complete repolarization of the action potential is approximately 200 msec, and that the variation in the length of the heart cycle is due to the variation in the length of the period of slow depolarization.[61] The variation in response may be due to a variation in the sensitivity of node cells to the neurotransmitter acetylcholine. Acetylcholine is well known to increase the permeability to potassium, and the potassium permeability of the membrane is changing greatly in the various parts of the heart cycle (in order to produce the action potential), so that a variation in response to acetylcholine might not be unexpected. Further experiments at the cellular level are necessary to answer this question.

The finding of synchronization at an R-R interval of 700 msec in the autograft studied, with the atrial and ventricular rates

approximately equal to each other (Table I), with a predominate P-R interval of 400-500 msec, also suggests that the sinus node response to vagal input is at work. The necessary requirement for this phenomenon, as described previously,[55,56] and in the present SECTION II, is that the pacemaker response curve have a portion with a slope which is positive. This mechanism will be more completely described below.

It appears, therefore, that the pattern of VPA is entirely explainable by the variation in response at the S-A node, given a constant vagal input by way of the baroreceptors. The relation between arterial pressure at the beginning of the P-P interval and the resulting cycle length, as observed previously,[50] is a coincidental phenomenon but not a causal mechanism.

SECTION II: FURTHER CHARACTERIZATION OF THE PARADOXICAL EFFECT OF VAGAL STIMULATION ON HEART RATE

In mammalian physiology courses it is commonly demonstrated and taught that increasing the frequency of vagal stimulation slows the heart rate. In mathematical models simulating the effect of inhibitory vagal stimuli on heart rate, the cardiac period is assumed to be linearly related to the instantaneous concentration of acetylcholine, and the effect of the phase of the cardiac cycle is explicitly neglected.[62] On the other hand, it has recently been found in pacemaker neurons of invertebrates and in a computer model,[63] that under certain conditions paradoxical entrainment may occur. It was demonstrated that within certain well defined zones of frequency, increasing inhibitory frequency results in an increase in pacemaker frequency; also shown was that increasing excitatory frequency may result in a decrease in pacemaker frequency. This phenomenon is defined as "paradoxical". The basic requirement as elaborated by Perkel, et al,[63] is that the pacemaker neuron must have a varying phase sensitivity to the inhibitory or excitatory input, and that the derivative of this function must be positive. Statistical methods to adequately describe this behavior, employing intervals rather than mean firing rates, was subsequently presented by these authors.[64-66]

Two recent studies in mammalian systems have demonstrated that the paradoxical behavior exists when vagus nerve stimulation interacts with the cardiac pacemaker.[55,56] Reid used cats and

rats, extirpating the stellate ganglion and stimulating both vagii with single impulses at low frequencies near the heart rate. In both these animals he showed well defined stable zones of entrainment wherein vagal stimulation frequency and heart rate were "locked", both at ratios of 1:1, and at multiples such as 1:3, 1:2, 2:3, 2:1, 3:1. These findings were those predicted by Perkel, et al.[63] However, attempts to obtain locking to bilateral accelerator nerve stimulation were rarely successful, and no stable zones could be determined as pacemaker activity continually drifted in either direction in an unpredictable manner.

Simultaneously, Levy, et al,[55] used open chested dogs with stellate ganglion decentralization, and stimulated both cervical vagii with single pulses. These workers found the same paradoxical behavior and, in addition, defined a pacemaker response curve relating the P-P interval to the P-Stimulus delay for one impulse. The shape of this response curve was sinusoidal, with a portion therefore having a positive slope, fulfilling the criteria for entrainment.[63] As they explained, in this portion of the curve a negative feedback mechanism would operate, such that regularly spaced input with an increasing interval would fall on a part of the curve which results in a greater P-P interval. And similarly, if the vagal input comes at a continually decreasing interval in this range, the P-P interval which results will be less, and this causes the heart rate to appear to lock to the inhibitory input.

The experiments to be described here are directed towards further explication of the paradoxical response to vagal stim-

ulation. First, the exact description of the pacemaker response curve for single event stimulations is presented for the first time with varying numbers of impulses per stimulation. The phase characteristics of the curves obtained predict the paradoxical phenomenon. In addition, they will be shown to have a remarkable discontinuity which provides specific regulation of heart rate without closed loop interaction.

Secondly, experiments with continuous vagal nerve stimulation are described using descrete changes in input frequency as well as continuously varying input. This is done both with one impulse per stimulation and with multiple impulses (up to 5) per stimulation. The effect of this maneuver is to vary the "mean vagal tone", altering the mean pacemaker rate, and demonstrates for the first time that locking may also occur by keeping the input frequency constant and varying the number of impulses per stimulation.

MATERIALS AND METHODS

Altogether sixteen adult mongrel dogs were used in these experiments, varying in weight from 12 to 18 kg. Anesthesia was provided by morphine sulphate (1mg S.C.), warmed alpha-chloralose (60 mg/kg I.V.), and urethane (600 mg/kg I.V.). An endotracheal tube was placed and the animal allowed to breath on his own. Both cervical vagus nerves were cut and bipolar platinum wire electrodes placed on the cardiac end of the right vagus. In most experiments, stimulation was done after administering propranolol (1mg/kg I.V.).

All experiments were controlled and processed on-line by an ACME-IBM 360/50-1800 computing facility. Stimulations were generated according to various protocols to be described below. Data was collected in each case from a transvenously placed intra-atrial electrode, which gave a clear signal of the P-wave. This record was displayed on an Offner Type R Dynograph; the signal was simultaneously differentiated and this signal also displayed on the Offner. The zero crossing of the derivative of the P-wave was then measured by the computer for the timing of this event (accuracy $\pm$ 2 msec). The stimulation was provided in each case by a Grass S4D stimulator through a stimulus isolation unit, and the timing of the first impulse from the stimulator also measured by the computer with an accuracy of $\pm$ 2 msec. In some cases these two signals were led into cardiotachometer couplers of the Offner and an analogue record of heart rate or stimulation rate obtained. All experiments were recorded on an Ampex FR-1300 FM tape recorder and subsequent high speed Offner printouts checked against the computer output for accuracy.

Various programs for administering stimuli were written depending on the protocol desired. Figure 9 is a schematic diagram of the single stimulation experiments, with one or several impulses. The bipolar intra-atrial recording electrode signal is played into the Offner channel 3; this is next differentiated and the derivative led into channel 6. The analogue signal is then carried to the IBM-1800 and analogueto digital converted and passed to the IBM 360/50. Here the signal is 1) stored on file, and 2) triggers the IBM 360/50 to begin filling a buffer

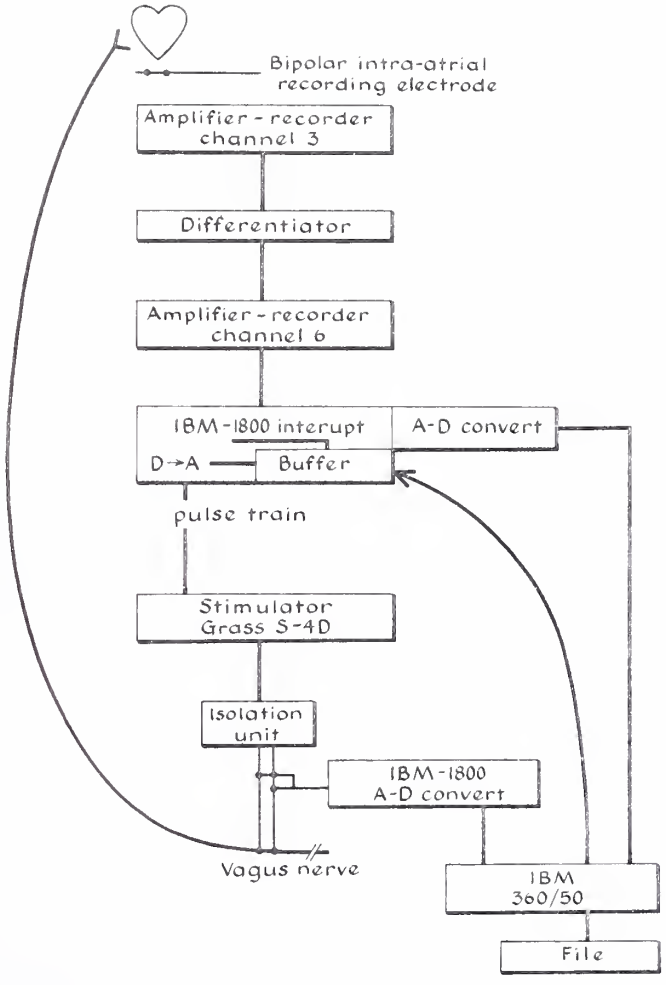


FIGURE 9: A block diagram demonstrating the experimental arrangement of vagal stimulation experiments in the normal dog. See the text for explanation.

in the IBM-1800, depending on a pre-set value for the P-stimulus delay desired, and the number of impulses desired. The buffer is simultaneously digital to analogue converted and delivered to the external control of the Grass S4D stimulator which then gives a 10-12 V, 2 msec wide, and 10 msec apart (for multiple impulses) stimulation. The stimulus is also analogue to digital converted by the IBM-1800 and filed in the IBM 360/50. In this protocol one stimulation event is given, and then 25 consecutive P-P intervals are measured before another stimulus is given.

The experimental design for constant low frequency vagal stimulation is given by Figure 10. The same basic method is used except that a standard function generator is used to trigger the IBM-1800 to deliver the impulses. With the function generator a constant stimulus-stimulus interval can be produced with any discrete value; and with the addition of a voltage controlled oscillator, could also produce continually varying stimulus-stimulus intervals about some set center period at any desired number of cycles per second. Figure 10 shows the wiring schematic for an experiment using the voltage controlled oscillator. The function generator delivers a triangular voltage input with amplitude of $\pm$ 2 volts and at 0.001 cycles per second (one cycle in 1000 seconds). With the center period set to a 500 msec interval and the variation allowed being $\pm$ 250 msec, the signal delivered to the IBM-1800 will have intervals varying from 250 to 750 msec and back again in 1000 seconds. Again the IBM-1800 triggers the stimulator, and in this case five impulses

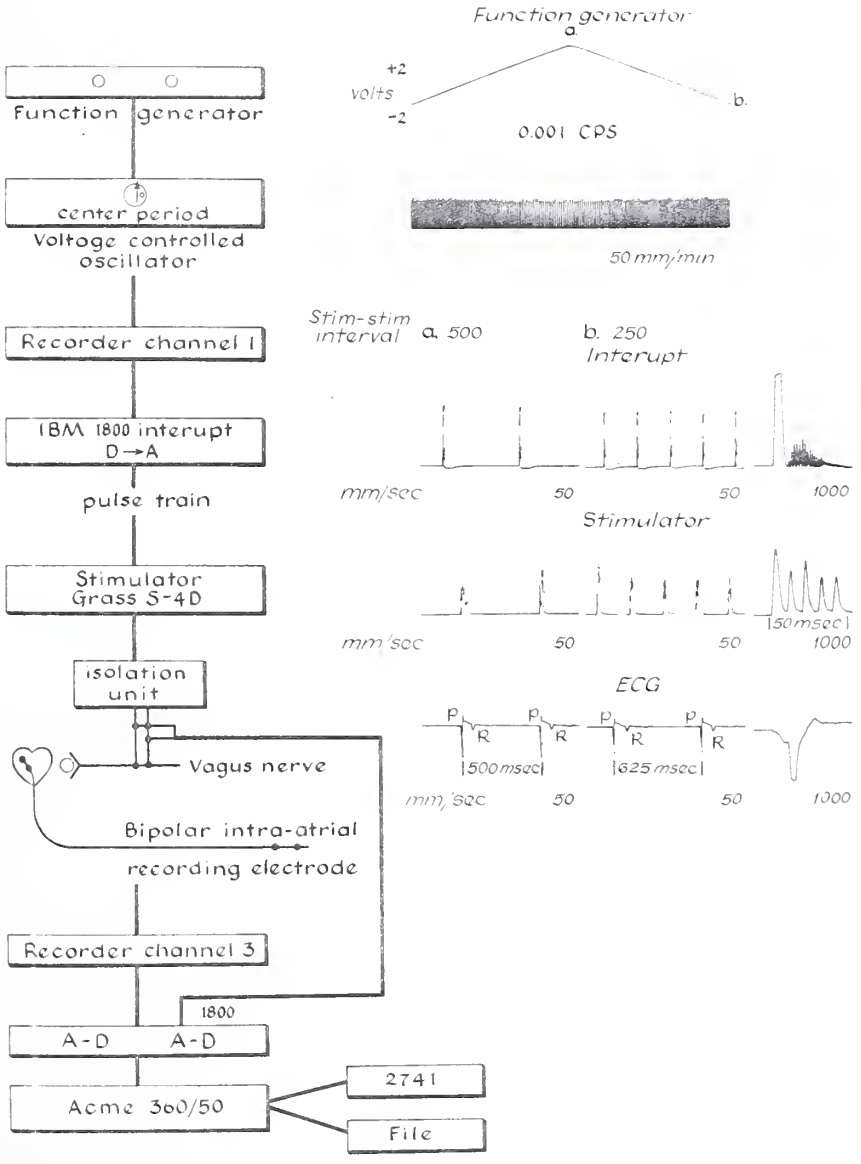


FIGURE 10: Block diagram of an alternate experimental arrangement for delivering continuously varying stimulation intervals. More complete explanation is given in the text.

are delivered. The record of the intra-atrial electrode is also displayed showing the prominent P-waves obtained.

Use of the digital computer to control and process these experiments provided a number of advantages. These may be briefly listed as 1) flexibility of stimulation protocol, 2) ability to vary the number of impulses per stimulation, 3) greater accuracy, 4) ease of processing data, and 5) subsequent ease of plotting data using automatic analogue plotters.

RESULTS

Pacemaker Response to Single Stimulation

Previous studies of the effect of single vagal stimulation on the heart rate have generally been qualitative. The only detailed experiments were those of Brown and Eccles in 1934.[59] These authors delivered single shock stimuli to the right and left vagus in cats, and showed that the right vagus is always more effective in altering heart rate than the left. In addition they quantitated the timing relations and demonstrated that a stimulus arriving in the last 170 msec of a 305 msec P-P interval, would not lengthen that interval, though the neural conduction time from the neck to the heart was 10-20 msec.

In the present work we studied vagal stimulation in eight dogs after the administration of propranolol to block sympathetic activity; two of these dogs were studied before the addition of this drug. In a typical experiment, the mean interval before vagotomy and drugs was 450-500 msec, with a prominent sinus arrhythmia. After bilateral vagotomy the interval decreased

to approximately 300 msec; with the administration of 1 mg/kg propranolol I.V. this interval promptly increased to 400-425 msec, and showed no variation.

In each case, one through five impulses were applied as a single stimulation event and the resulting P-P interval measured. The decay of the response over subsequent intervals was also studied, but will not be presented here. The records from two seperate stimulations with five impulses are shown in Figure 11. The train of five impulses placed early in the heart cycle lengthens the same interval (a); a similar stimulus delivered at 370 msec after the P-onset affects only the following interval, and lengthens it to 705 msec. Figure 12 summarizes a typical experiment for one, three, and five impulse stimuli applied at various times in the heart cycle in the presence of propranolol. The horizontal axis is the P-onset to stimulus interval the vertical axis is the length of the P-P interval beginning with the P-onset. This pacemaker response curve demonstrates a continually increasing P-P interval as the onset of the vagal stimulation is moved from a point several hundred msec before, to 268 msec within the interval. At that point, every subsequent stimulus, regardless of the number of impulses, fails to increase the length of the interval in which it falls.

The shape of the curve for one impulse is similar to that reported earlier.[59] There is a constant increase in the P-P interval as the stimulus is moved up to and through the first part of the cycle. The top part of the curve is "rounded off" to return to control values at a P-stimulus near 268 msec. The

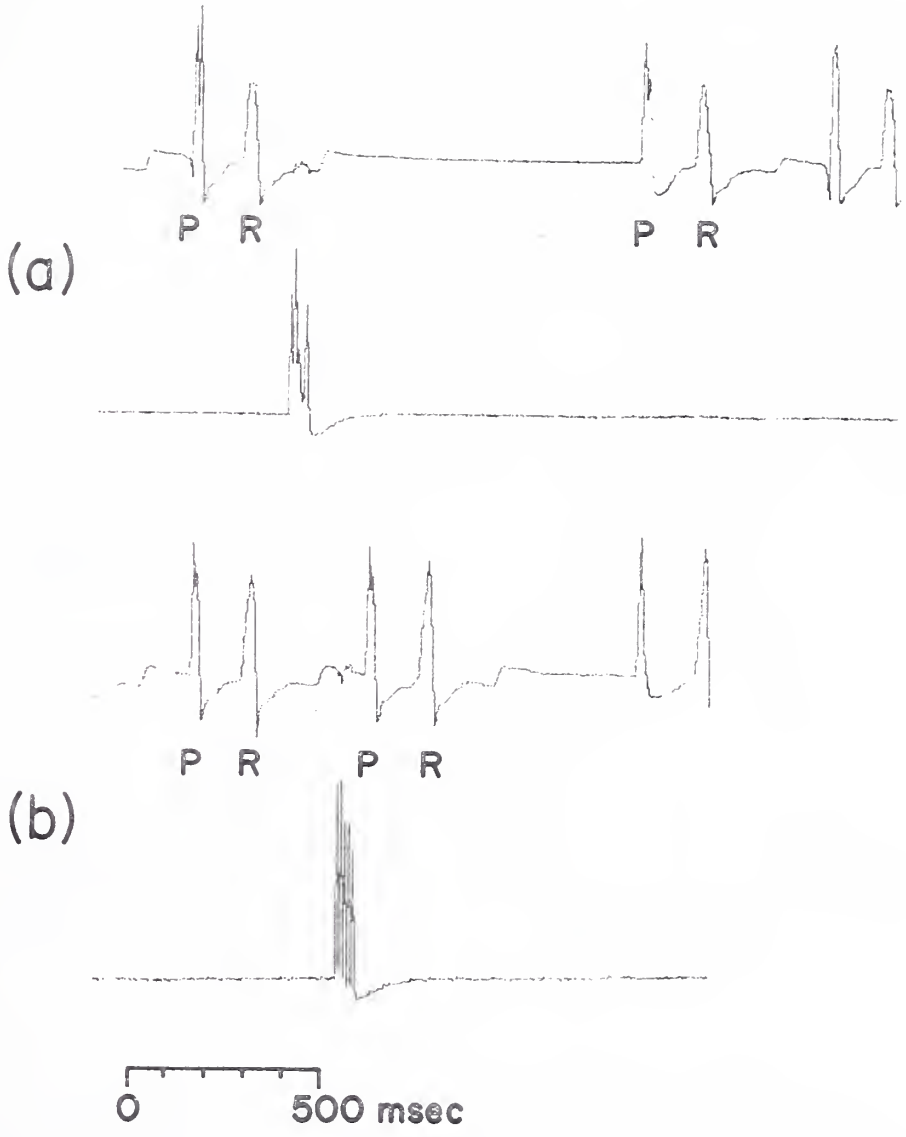


FIGURE 11: Effect of a single stimulation with five impulses to the cardiac end of the right vagus nerve of the dog. (a) Stimulus at 260 msec from the P-wave onset with lengthening of the same interval to 1190 msec (control 460 msec). (b) Stimulus at 370 msec with lengthening of the following interval to 705 msec.

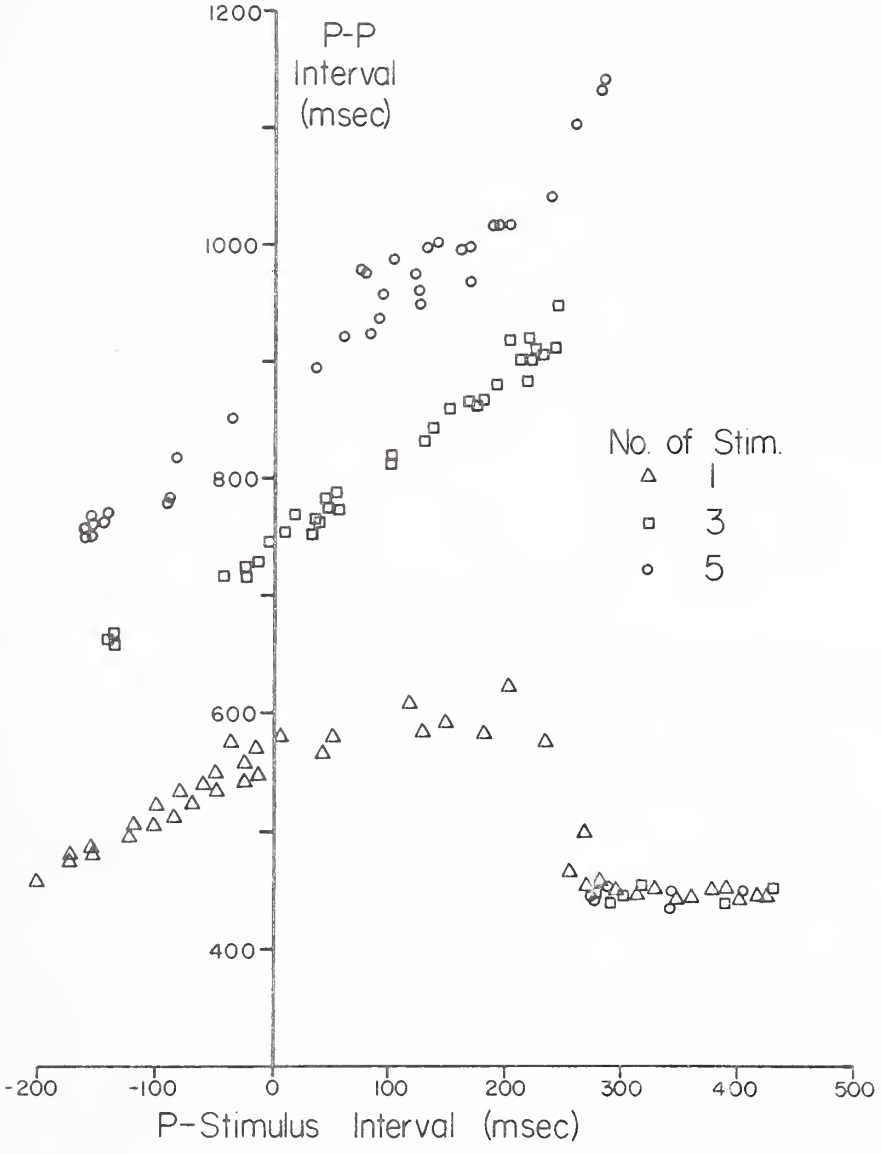


FIGURE 12: Effect of one, three, and five impulses applied as a single stimulation to the cardiac end of the right cervical vagus. The resulting length of the P-P interval is plotted in relation to placement of the stimulus.

shape of the curves for three to five impulses were different, however, in that the linear increase in interval length approached a sharp turnover point where a stimulus beginning but 2 msec later would not affect that interval. The slopes increased slightly with an increase in the number of impulses, approaching one with five impulses. The form of the pacemaker response curve for the same animal without propranolol was similar with control P-P intervals of 360 msec, an increased slope, and a rapid turnover from maximal lengthening to no lengthening at a P-stimulus of 170 msec.

Constant Low Frequency Vagal Stimulation

Continous vagal stimulation increased the P-P interval and increasing the number of impulses per stimulation increased the response. Stimulation with one impulse delivered at intervals between 1200 and 500 msec reproduced the results described by Levy and coworkers,[55] with a sinusoidal pacemaker response curve and the phenomenon of synchronization. When more than one impulse per stimulation was used, the pacemaker response curve was increased in amplitude and with a slightly different form. The rising part of the curve was linear, becoming sharply discontinuous at a point 250-300 msec within the P-P interval. This is shown in Figure 5 for an experiment with four impulses per stimulation, with the stimulus-stimulus intervals varying from 1000 to 700 msec. Clustering of the P-stimulus intervals is noted at 160 msec for a P-P interval and stimulus-stimulus interval of 800 msec; and at -50 msec for a P-P and stimulus-

stimulus intervals of 700 msec. The synchronization came in each case on the portion of the curve which had a positive slope. Impulses delivered at 1000 and 900 msec in this case, wander randomly throughout the heart cycle. If a single best-fit line is drawn through the pacemaker response curves obtained by this method at different numbers of impulses per stimulation, a family of curves is obtained, and this is plotted in Figure 6.

When the stimulation was carried out for multiple impulses at a fixed stimulus-stimulus interval, clustering of the P-stimulus intervals was again found. This observation supports the concept that synchroniztion can also occur without varying the input frequency, but rather by modulating the mean rate of the output (the pacemaker). The results of stimulation at a constant interval of 1000 msec, and with the number of impulses per stimulation varied from one to five impulses, are presented in Figure 13. The coordinates are the same as those of Figure 5; the clustering of P-stimulus intervals is again noted. A seperate and distinct pacemaker response curve is produced by each stimulation with a given number of impulses. This is especially pronounced with one and three impulses where stimulations fall randomly throughout the heart cycle, and thus more clearly delineate the response curves. When two impulses were given, the P-stimulus times were clustered with either of two values, 200 or 360 msec; four and five impulses resulted in single clusters at approximately 240 and 140 msec respectively. It is apparent that the points are falling on the appropriate curves delineated by Figure 6, as the pacemaker response curves for the given number

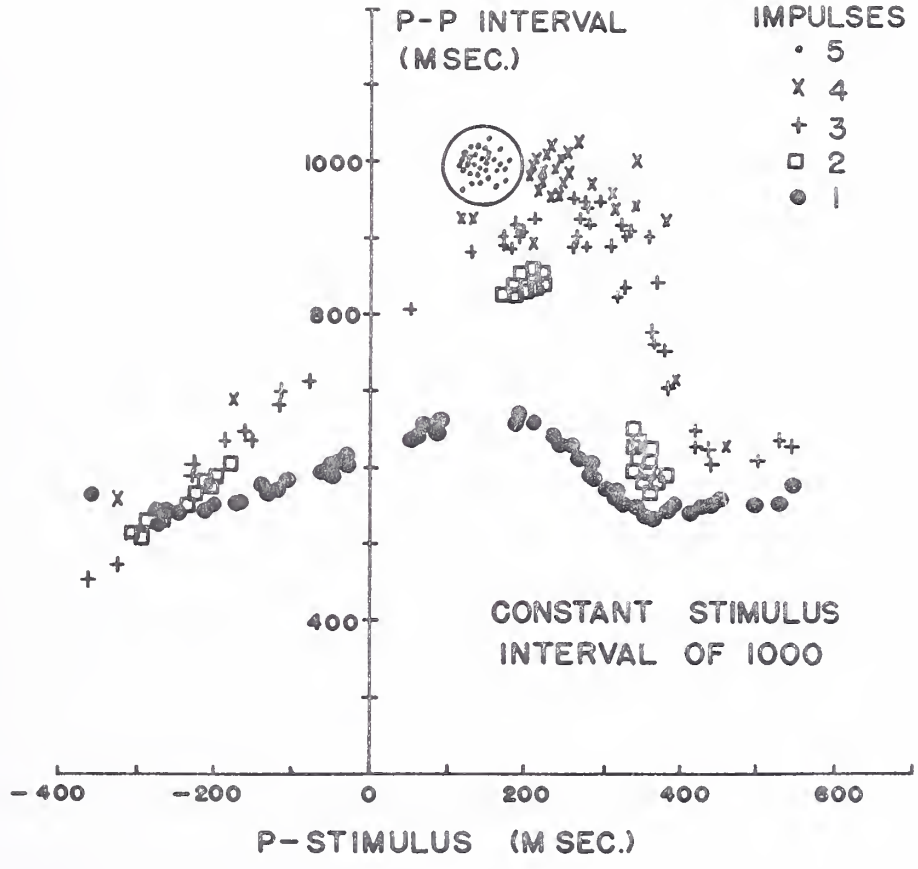


FIGURE 13: The P-P interval which results when one through five impulses per stimulation is delivered to the right cervical vagus at a constant stimulus-stimulus interval of 1000 msec.

of impulses.

Similar patterns of behavior are noted at each stimulation interval. Methods of describing the phenomenon of locking of two interacting oscillators have been worked out for similar neuronal models,[65,66] and involve the use of frequency histograms to describe the relation of the output oscillator response to the phase of the input. Such histograms are given in Figure 14; the per cent distribution of the P-stimulus intervals for a representative experiment is plotted. The number of stimulation events for each seperate histogram ranged from 60 to 86. The per cent of stimulations at each P-stimulus value is given on the vertical axis; P-stimulus values on the horizontal axis are in groups of 20 msec from 0 to 700 msec. Different dogs varied slightly in the values obtained, but the same patterns were clear. Each "set" of histograms where either the number of impulses were constant and the stimulation interval varied, or the stimulation interval constant and the number of impulses incremented, showed one of three types of patterns in the conditions studied. Pattern one is an apparent random distribution of P-stimulus intervals throughout the heart cycle, such as at three impulses and 900 msec stimulation interval. A second pattern showed one predominate P-stimulus interval such as the group at 600-640 msec with four impulses at 700 msec stimulation interval. The third pattern seen in the ranges studied is that there are two predominate modes of P-stimulus intervals, as in four impulses at 1000 msec. The interpretation of these findings will be discussed below.

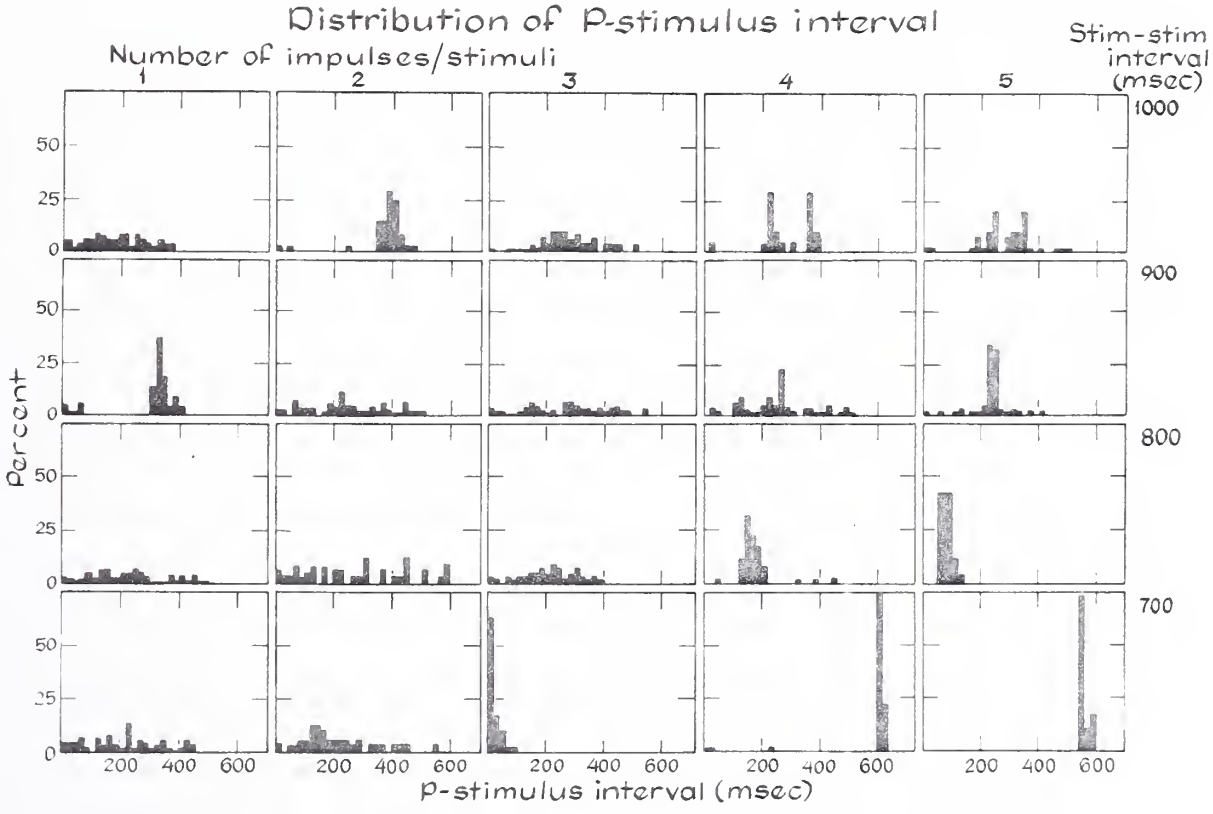


FIGURE 14: Frequency histograms of the distribution of P-stimulus intervals obtained when the number of impulses per stimulation and the stimulus-stimulus interval is varied.

Constant Low Frequency Vagal Stimulation With Continually Varying Stimulus-Stimulus Interval

A more complete description of the synchronization phenomenon, and especially of the ranges within which it occurs, was obtained by experiments in which the stimulation interval was continuously varied from 1050 to 450 msec, and back again, in a period of ten minutes. The results of such an experiment are illustrated by Figure 15. Here the cardiotachometer record from the Offner for atrial rate and stimulation rate is given for vagal stimulation with two and three impulses, and with stimulation rate varying from 58 to 132 per minute. Both tracings clearly delineate the range in which the paradoxical increase in stimulation rate (the "stable zone", entrainment, and synchronization[55,56,63]) causes a 1:1 increase in heart rate. For two impulses, this extends from 84 to 92 beats per minute; at three impulses, the stable zone is between 70 and 90 beats per minute.

In the ranges of stimulation rate studied outside of this stable zone, increasing frequency of stimulation results in a decrease in the mean rate of the heart as expected. However, a second period of entrainment is noted at three impulses in the range of stimulation from 124 to 132 stimulations per minute. This area shows a second stable zone with locking at a ratio of two stimulations per one heart cycle. This kind of second order locking is more clearly seen with five impulses per stimulation as shown in Figure 16. In this experiment, locking at 1:1 ratio occurred from the bottom rate of 58 to 92 beats per minute. The

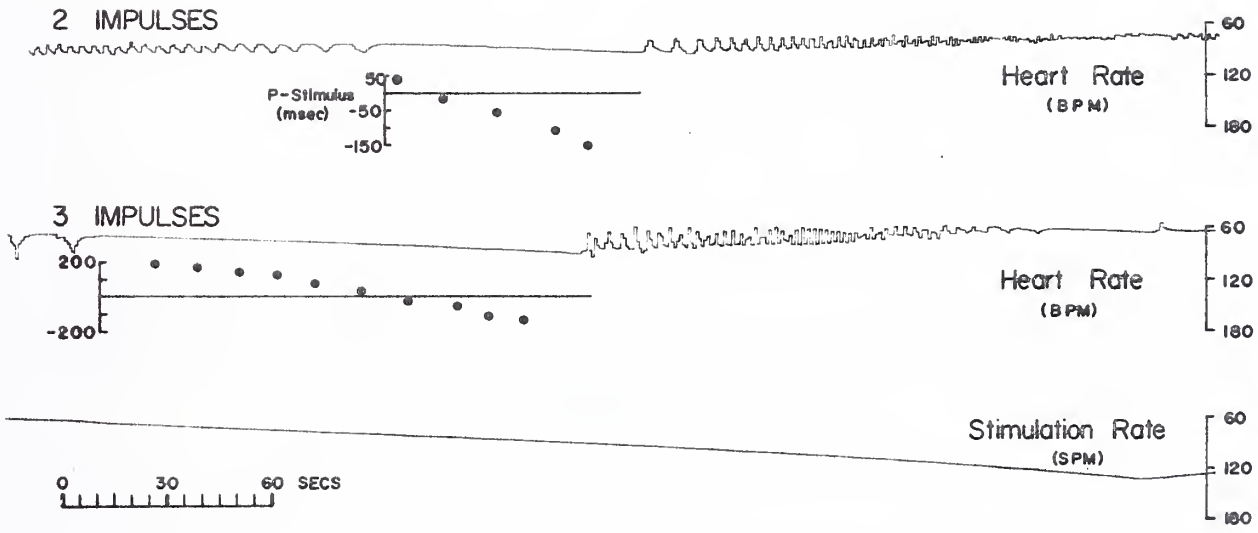


FIGURE 15: The cardiotachometer record from the Offner dynograph of the heart rate and the stimulation rate in an experiment with vagal stimulation using two and three impulses per stimulation. The stimulation rate is continuously varied. The P-stimulus existing during the periods of synchronization have been indicated in each case.

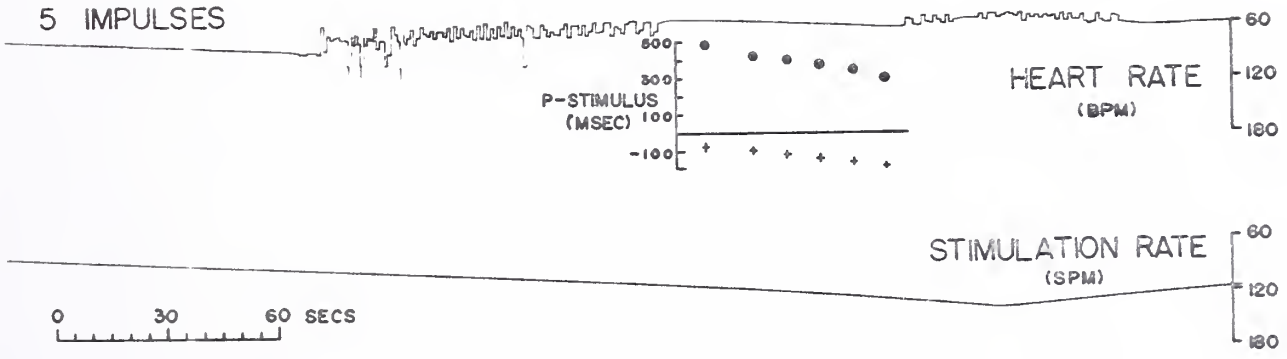


FIGURE 16: Similar to Figure 15, except that five impulses per stimulation is used and entrainment with two stimulations per one heart cycle is demonstrated.

stability zone for two stimuli per one heart cycle extends from a stimulation rate of 88 to 126 (heart rate exactly half over this range).

When the individual records for each experiment were analyzed for the P-stimulus interval which pertained over each stability zone, the interval was found to continuously vary. The data for two and three impulses is given directly under the stable zone in Figure 15; with five impulses per stimulation in Figure 16. When the stimulation rate increases, the P-stimulus interval goes from a positive value (P preceding stimulus) to a negative value (P following stimulus). The opposite trend holds when the stimulation rate decreases. An experimental record which graphicly demonstrates this phase-shift within the period of paradoxical response is given in Figure 17. In this experiment five impulses per stimulation was given and the stimulus-stimulus interval continously varied. Entrainment exists in the record with the P-P interval following the stimulus-stimulus interval. As the stimulus-stimulus interval decreases from (a) to (d), the P-P interval decreases also, and the P-stimulus goes from an initial positive 250 msec to a negative 150 msec.

The upper limit of heart rate in the 1:1 stability zone appears fairly constant near 90-92 beats per minute. It is noted that the P-stimulus interval is also fairly constant at this point with a value near -150 msec. The values for the P-stimulus intervals with 2:1 locking also follows similar trends in regards to the direction of change as shown by the inset graph of P-stimulus in Figure 16.

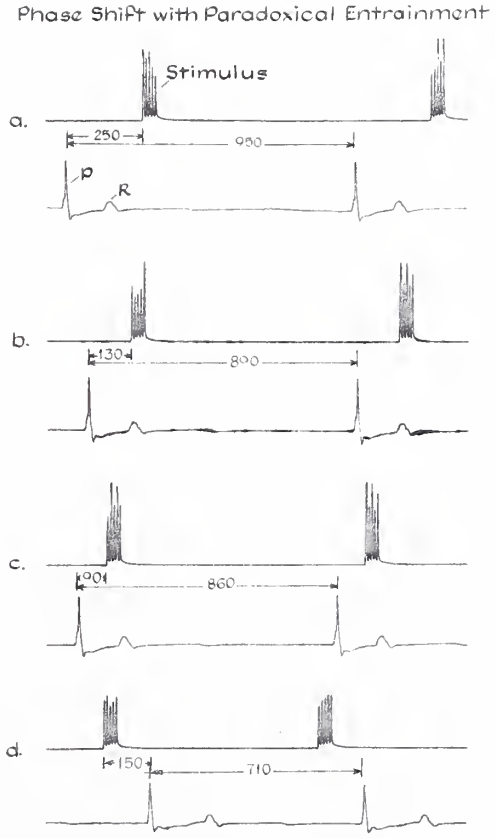


FIGURE 17: An experimental record demonstrating the P-stimulus phase shift during a period of entrainment with decreasing stimulus-stimulus interval.

DISCUSSION

Pacemaker Response to Single Stimulation

These results demonstrate the dependence of atrial cycle length on the timing as well as the strength of vagal stimulation. The description of pacemaker response curves for multiple impulse stimulations extends the quantitative data provided by Brown and Eccles.[59] The finding of an insensitive period 150-200 msec at the end of the P-P interval is confirmed, and found to hold when the amount of neurotransmitter released is increased by giving a train of impulses. The shape of the response curves, especially the linear positive slope, fulfills the criteria set forth in the model of Perkel, et al,[63] and predicts that a paradoxical heart rate response will occur in the vagal-cardiac system.

The shape of the delay-interval curve also implies that the time of arrival of a vagal volley could be important for controlling heart rate on a beat to beat basis in the intact dog. Increased vagal activity at the point of maximum response lengthens the same interval; coming just slightly later, the same activity would not only not lengthen that interval, but would lengthen the following interval a lesser amount. This behavior is a form of negative feedback with respect to time existing at the heart. When vagal activity is present, those factors which accelerate the conduction of the beat (short atrioventricular conduction time, rapid ventricular ejection and accelerated pulse wave transmission) might result in the arrival of the vagal volley in the sensitive period with greater slowing. When those factors

are depressed, cardio-acceleration would result.

Calculations of the time between P-wave onset and cervical vagal activity from arterial baroreceptor response vary from 200-420 msec, taking into consideration the normal ranges for P-R interval and Jewett's data for single cardioinhibitory fiber activity in the cervical vagus of the dog.[58] The shortest time is within the highly inhibitory zone and the longest is outside.

The question remains as to whether the last period of 150-200 msec in the atrial cycle, when stimulation has no effect on that cycle, represents a latency as suggested by Brown and Eccles, or whether it is a refractoriness to the neurotransmitter acetylcholine as discussed in SECTION I. The actual neural conduction time between cervical vagus and the heart is 10-20 msec,[58,59] and virtually all of the latency would have to exist between presynaptic nerve endings and the pacemaker cells. Regardless, if this lack of response is due to latency or refractoriness or some other mechanism, the sharp turnover point provides a way to control heart rate at the heart itself.

Pacemaker Response to Constant Low Frequency Stimulation

The recent studies of the paradoxical effect of vagal stimulation on heart rate have been confirmed. Within well defined ranges of vagal stimulation rate, increasing the frequency of stimulation is accompanied by an increase in the heart rate. A sinusoidal pacemaker response curve for one impulse was confirmed. With the use of multiple impulses per stimulation a series of pacemaker response curves was defined. In addition, the previous

results were extended by demonstrating that a second type of interaction occurs when the number of impulses per stimulation (the "strength of stimulation") was increased for the same rates of stimulation. In this situation, the mean rate of the output oscillator (the pacemaker) is lowered, while the input rate is maintained.

The second type of entrainment is explained by considering the pacemaker response curves for various numbers of impulses given in Figure 6. With increasing number of impulses, the amplitude of the curves is increased as is the maximum slope. Thus, the range over which entrainment will occur is increased. The shape of the response curves is predicted from the shape of the response to single event stimulations (Figure 12). The sharp discontinuity with four and five impulses is seen in both. The rising part of the curves with continuous stimulation in the last part of the P-P interval is best explained as the result of the preceding stimulation coming in closer to the beginning of the measured interval. With single event stimulation, and thus no preceding stimulation, this portion of the pacemaker response curve is flat. Likewise, the fact that with continuous stimulation this portion of the curve is higher for five impulses than for one, reflects the increased mean concentration of acetylcholine (the increased mean vagal tone).

The result of the second type of entrainment on the P-stimulus interval is demonstrated by the histograms of Figure 14. The first pattern where P-stimulus intervals are random throughout the atrial cycle, describes conditions where either the number

of impulses (strength) of stimulation, or the stimulation rate, is not sufficient to allow impulses to fall on the positive part of the sinusoidal pacemaker response curve. The stimulation interval must equal a P-P interval which will result with the given number of impulses per stimulation arriving at a part of the response curve where the slope is positive. Under these conditions, small changes in either input or output will result in exactly compensatory changes by means of negative feedback as previously described.[55,63]

The single predominate grouping of P-stimulus intervals in most cases describes the situation where one vagal stimulus falls in one heart cycle. The stimulation is of sufficient "strength" to increase the P-P interval to the stimulation interval, and be on the positive part of the pacemaker response curve. The histograms of Figure 14 illustrates what happens to such a stable situation when the stimulation rate, or the number of impulses, is changed. In the first case, a stable 1:1 ratio is demonstrated by five impulses at 900 msec stimulation interval; decreasing the stimulation interval to 800 msec must then decrease the P-stimulus at which locking will occur since the P-P interval resulting from the stimulation must equal the new stimulation rate, and this is a point to the left on the pacemaker response curve. The same thing happens when the stimulation interval is changed to 700 msec. With a constant stimulus-stimulus interval a second mechanism operates. If a locking situation exists, as in three impulses at 700 msec, when the number of impulses is changed to four, the pacemaker curve becomes the one for four impulses, which

is of greater amplitude and slightly increased slope. The new locking point will then simply be the intersection of a horizontal line at the locking interval with the new response curve, and the P-stimulus will necessarily be less. In this case it goes slightly negative (stimulus precedes P) which shows upon the histogram as being in the last part of the P-P interval; when changed to five impulses, it moves to the left more.

The third pattern of histogram seen, where there are two predominate groups of P-stimulus intervals accounts for complex ratios of entrainment. For example, two groups of P-stimulus intervals are seen in Figure 13 in the case of two impulses. There are groupings near 200 and 350 msec. When a stimulation falls in the interval at 200 msec, that interval becomes 840 msec long. The next stimulation will then fall (1000-640) equals 360 msec into the next interval, accounting for the second group. The second stimulation lengthens its interval to only 600 msec, so that the following interval (described by the negative P-stimulua points to the left of the zero point) will be 1000-[(600-360)+560] equals 200, and the conditions are set for a repeat cycling. This is then a 2:3 locking situation, with two stimulation cycles equal to three heart cycles.

These same principles are well demonstrated in the P-stimulus values shown in Figures 15, 16, and 17. With increasing stimulation rate in the stable zone, the P-stimulus interval must continuously change from a positive to a negative value. This is the necessary adjustment to allow locking to occur. With multiple impulses, the upper limit of the stable zone appears fixed at

90-92 beats per minute. With increasing numbers of impulses per stimulation the bottom rate of the stable zone decreases. This is explained by the pacemaker response curves. The minimum interval of each curve is nearly equivalent; the maximum interval, however, varies greatly depending on the number of impulses. Thus the range of the stable zone increases with increasing numbers of impulses, but the upper rate (minimum interval) is relatively fixed.

As demonstrated here and previously,[55,56] the locking phenomenon may be seen at many multiples of the input:output ratios. For example 2:1, 1:1, 2:3, 1:2, etc., may be seen. All of the patterns can be explained by the pacemaker response curve for the particular strength of stimulus used. It should be emphasized that changing the rate of stimulation does not change the response curve, whereas changing the number of impulses will.

The locking phenomenon herein described is almost certainly observed in clinical situations of complete heart block,[67] in the canine cardiac autotransplant,[68] in the human cardiac transplanted patient,[69] in so-called isorhythmic A-V dissociation,[70-72] and in the locking of the atrial rate to the ventricular rate in patients with pacemakers.[73] Segars, et al,[67] presented a patient with complete heart block who demonstrated 2:1 atrial to ventricular entrainment, with only small fluctuations in P-R and R-P intervals. With exercise, an acceleration of atrial rate occurred and entrainment disappeared. Many previous attempts have been made to explain the mechanism of isorhythmic A-V dissociation, and it

is clear that a number of different mechanisms may operate[72] including retrograde activation of the atria from a nodal pacemaker.[74] In some records presented in the literature,[71] it is possible that entrainment due to vagal input from arterial baroreceptors is active, though this possibility has not heretofore been considered.

It may also be an important mechanism for heart rate control in the intact cardiac system. The information of an arterial pressure rise reaches the cervical vagus in 80-240 msec.[58] This is information in discrete bursts of vagal activity which will in some cases fall on the rising part of the pacemaker response curves. The results presented here demonstrate that the range over which stability occurs is increased as the strength of stimulus increases, perhaps allowing for smooth changes in heart rate to occur where discrete jumps may have resulted with a flat pacemaker response curve. The exact role for this extremely interesting general biological mechanism remains to be determined in the vagal cardiac system.

SECTION III: Bainbridge Reflex: Volume Infusion in the Canine Cardiac Autotransplant

"The influence of venous filling upon rate of the heart" was the title of Bainbridge's paper[75] in 1915, which started the continuing controversy as to whether an increase in central venous pressure will reflexly cause an increase in heart rate. In dogs anesthetized with morphine, chloroform, and ether, he showed that rapid intravenous infusion of 200-400 cc of isothermic, isotonic saline would result in a tachycardia of 20-80 beats per minute above controls (70-110). A slow injection, without a rise in venous pressure, would not cause tachycardia. Dogs with adrenalectomy continued to show the same response. However, with vagotomy and stellate ganglionectomy there was no infusion tachycardia. With atropine alone, the control heart rate increased to 170; infusion was associated with an apparent small increase of rate by 10-20 beats per minute. With vagotomy alone, there was the increased control rate, but no detectable tachycardia. Bainbridge then concluded that 1) the adequate stimulus was increased central venous pressure, 2) afferents for the reflex traveled in the vagus, 3) the efferent arc was mediated by primarily a release of vagus tone, but also a slight sympathetic stimulation. In his experiments arterial pressure did not consistently rise with rapid infusion, and sometimes fell or stayed the same; heart rate uniformly increased.

Five years later Sassa and Miyasaki[76] confirmed this work in dogs and cats, and found that rabbits and frogs, which had practic-

ally no vagus tone, did not exhibit the infusion tachycardia. Arterial pressure was said not to vary in these experiments, and atropine and vagotomy abolished the tachycardia with a rise in the mean control heart rate.

These results were confirmed by Anrep and Segall[77] in 1926, in anesthetized dogs. They studied "Marey's Law" as well, which defines the inverse relation between blood pressure and heart rate. At this time the role of the arterial baroreceptors was not understood. They confirmed the infusion tachycardia, its abolishment with vagotomy, the partial response with atropine alone and with stellate ganglionectomy alone. He stressed the point that blood pressure did not change with the infusion, and thus did not interfere by way of "Marey's Law".

This seemed to be the end of agreement among physiologists concerning the reflex. Papers began to appear which questioned the existence of the reflex. DeGraff and Sands[78] used dogs anesthetized with morphine and chloretone, and found heart rate increase with infusion in only 50 % of trials. Vagotomy did not completely abolish the tachycardia, which still occurred in 1/3 of the experiments. However, they cannulated the right and left carotid arteries simultaneously to measure mean and pulsatile pressure, thus excluding the baroreceptors in an unpredictable way from the circulation.

Warthen in 1935,[79] gave massive infusions (up to $2\frac{1}{2}$ liters) and found tachycardia consistently in anesthetized dogs. He did not do vagotomy or study the effects of atropine. Ballin and Katz[80] studied both chloralose anesthetized dogs and those without anesthesia. No definite cardiac acceleration was noted.

Further contradictory results were presented by Aviado, et al,[81] in complex cross circulation experiments in dogs. They found both infusion tachycardia and bradycardia and could not determine which factors governed these responses. Blood pressure changes were also inconstant.

A new concept for understanding the infusion tachycardia was advanced by English workers in the mid 1950's. Coleridge and Linden,[82] and later Jones,[83] found that the initial heart rate determined the direction of heart rate changes. When the control rate was less than 110, the rate would increase; when greater than 110, the rate would slow. This was interpreted as an attempt by the circulation to maximize cardiac output and thus restore venous pressure to its initial level. For example, a high initial heart rate would imply inadequate or compromised filling were an infusion given, so that some mechanism for decreasing rate would allow cardiac output to increase, thus reducing venous pressure. Unfortunately they do not suggest what the actual mechanisms may be.

These same results were verified in unanesthetized animals by Hirsch and associates.[84] They found that the initial heart rate determined the direction of change. By means of vagotomy and pharmacologic sympathectomy (dibenamine) they concluded that the tachycardia depended on sympathetic activity and that the bradycardia was due to local mechanisms.

At this time an alternative, non-reflex mechanism for infusion tachycardia was demonstrated in isolated heart preperations. Blinks[85] used isolated dog heart and rabbit atrium and found that a rise of atrial pressure or stretch of the atria would cause tachycardia.

Local anesthetic would not abolish the rise in heart rate, suggesting this was an intrinsic property of the myocardium. These findings were confirmed for frog's auricle[86] and mammalian heart as well.[87,88] It is interesting that these changes had not been seen earlier in Starling's isolated heart-lung preperation, with wide variations in filling pressure.[89]

Donald and Shepard[90] reported the only study of heart rate changes in chronically denervated dogs. They showed some slight increase in heart rate with infusion. Initial rates ranged from 70-94 beats per minute; with saline or dextran infusion, the rate increased by 4-31 beats per minute. These increases were not striking, and occurred from $2\frac{1}{2}$ to 45 minutes following infusion, when venous pressure had returned to normal, and other factors such as change in the level of anesthesia may have been occurring.

Finally, a recent study by Kinnison, et al,[91] has cleared some of the controversy over the existence of the reflex.[92] These workers used a technique of small balloon catheters in the anesthetized but intact dog to selectively modify pressures within various parts of the circulation. When arterial blood pressure was held constant a quantitative relationship between right atrial pressure and heart rate was observed. The relationship was positive regardless of the initial heart rate though the slope decreased as the initial control rate increased, suggesting a maximum attainable heart rate of 240. This apparently represented a pure right sided reflex, and was abolished by vagotomy, fulfilling Bainbridge's criteria. In other experiments, with right atrial pressure held constant, an inverse relation was seen between arterial pressure and heart rate.

This appeared to be the pure left sided reflex mediated by baroreceptors and responsible for "Marey's Law". In situations where both venous and arterial pressures changed, "simultaneous right and left sided heart rate influence can be predicted by taking the algebraic summation of their independent influence."[91]

It becomes clear from examining the controversy over the existence of the infusion tachycardia that there are multiple factors of importance. The level and type of anesthesia is important, with some kinds of anesthetics having deletorious effects on cardiac reflexes as well as cardiac performance.[93] The type of animal used and the mean level of vagus tone must be considered.[76] The type of fluid, the temperature, and the possible change in hematocrit will influence the experiment.[94] The influence of local, direct stretch on the irritability of the myocardium must be considered.[85-87] And finally, any analysis of reflexes which change heart rate must take into account all other reflexes which influence rate. In the case of the right atrial pressure reflex, it must be adequately demonstrated that arterial pressure is held constant.

Studies in the canine cardiac autotransplant were undertaken with these factors in mind. With a completely denervated and free running pump, and the innervated atrial remnant intact, it may be possible to answer the following questions. Does volume infusion cause tachycardia of 1) the denervated heart by way of local stretch, 2) the innervated atrial remnant in any consistent way; and does atropine or beta-blockade modify the response of either.

MATERIALS AND METHODS

Experiments were done using four canine cardiac autotransplants prepared according to the procedure described previously.[34] The instrumentation in these animals included chronically implanted aortic EMF flow probe (Biotronex), pressure transducers in the left subclavian artery and the left ventricle (Microsystems), left atrial and right ventricular pacing wires, and a bipolar recording electrode sutured to the remnant atrial wall. Since right atrial pressure was not measured directly in these experiments, data is also presented from volume infusion in non-rejecting canine homografts[95] which were instrumented with pulmonary artery EMF flow probe, pacing wires, left subclavian catheter, and right and left atrial catheters for pressure measurements using the Statham strain gauges.

The animals were awake, unanesthetized, and resting quietly in the right lateral position. Infusions were given through a #19 gauge Butterfly needle into the main foreleg vein. Both isotonic saline and 6% dextran (M.W. 40,000) were used, administered at a rate of 20-80 cc per minute. The volume of infusion varied between 300 cc of dextran to 800 cc of saline. Infusions in homografts were administered in the same manner, but 300 cc of 6% dextran was consistently used. Recordings from all instruments and the EKG were simultaneously displayed on an Offner type R Dynograph and recorded on an Ampex FR-1300 tape recorder for subsequent digital computer processing.

The taped recordings of the EKG and the remnant atrium were analogue to digital converted using the ACME IBM 360/50-1800

computer facility. The digital records were then displayed on a television monitor and processed according to the technique described in SECTION I. The mean and standard deviation of the P-P interval and the R-R interval were computed for a ten second period each 50 cc of infusion. Arterial pressure, aortic flow, left ventricular end diastolic pressure, right and left atrial pressure, were all obtained from the analogue print-out of the original Offner recording.

RESULTS

Four canine cardiac autotransplanted animals were used for eleven volume infusion experiments. In all of these animals, volume infusion was accompanied by 1) no change in donor heart rate, or 2) a small change in donor rate which could be of either direction, and 3) a consistent, immediate increase in the remnant atrial rate. This is demonstrated by an experiment shown in Figure 18; 300 cc of saline was administered, and it is seen that the R-R interval of 730 msec remains fixed, whereas the atrial rate, P-P interval, decreases from 725 to 400 msec. The results from the eleven experiments are displayed in Figure 19. The atrial rate from the remnant atrium is indicated by the circles, and is seen to increase in each case, regardless of the initial heart rate. It is also noticed that the slopes of the heart rate change decrease as the pre-infusion rate is increased, consistent with the findings of Kinnison, et al.[91] The donor heart rate (triangles) may stay the same, increase slightly, or decrease slightly. Table II lists the values for the mean and standard deviation of pre and

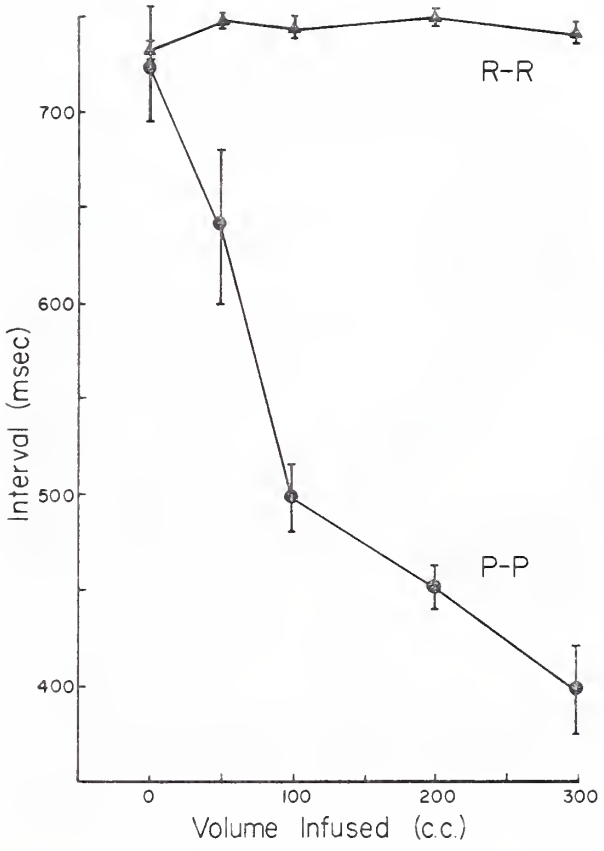


FIGURE 18: The response of the remnant atrium (P-P interval) and of the donor heart (R-R interval) to a rapid intravenous infusion of 300 cc of isotonic saline.

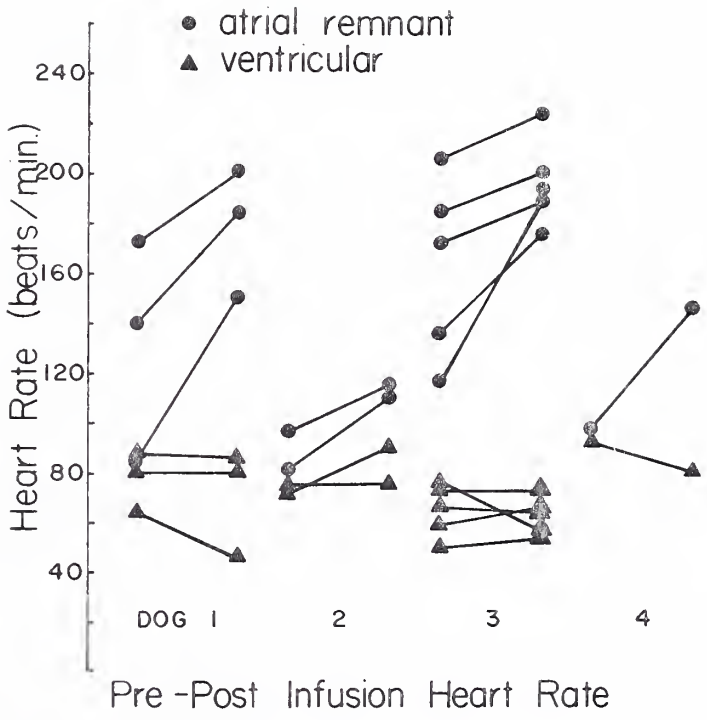


FIGURE 19: Eleven volume infusion experiments in four canine cardiac autotransplanted dogs. Remnant atrium rate (circles) uniformly increased; ventricular rate (triangles) changes slightly or not at all.

TABLE I: Effect of Volume Infusion on Heart Rate in the Autograft

DOG		INFUSION cc	INFUSION type**	INTERVALS* R-R Pre	INTERVALS* R-R Post	INTERVALS* P-P Pre	INTERVALS* P-P Post
Hamlet	2-17	500	S	935 ± 29	1036 ± 42	430 ± 21	338 ± 10
"	3-12	500	S	685 ± 10	700 ± 10	700 ± 8	309 ± 8
"	3-27	300	S	735 ± 5	750 ± 4	725 ± 22	400 ± 21
Everything	4-13	500	S	650 ± 8	615 ± 11	750 ± 9	558 ± 20
"	4-25	800	S	855 ± 14	682 ± 12	626 ± 15	530 ± 6
Omnibus	6-9	500	S	1015 ± 11	930 ± 12	356 ± 23	318 ± 19
"	6-16	800	S	830 ± 15	825 ± 10	290 ± 9	283 ± 9
"	6-20	700	S	914 ± 11	940 ± 11	325 ± 20	300 ± 9
"	8-11	500	S	1232 ± 12	1080 ± 5	512 ± 19	315 ± 11
"	8-29	500	S	800 ± 18	1040 ± 30	445 ± 17	348 ± 7
WWA	9-18	300	D	650 ± 10	720 ± 46	620 ± 41	410 ± 12

* Mean ± standard deviation for a ten second period

** Type: S = isotonic saline, D = 6% dextran

post-infusion intervals, together with the type and amount of fluid used in each case.

With the relatively large amounts of fluid used in these studies, the hemodynamic variables changed surprisingly little. The only consistent change which can be seen in these autografts and non-rejecting homografts is a rise of atrial pressures and of ventricular end-diastolic pressures. The arterial blood pressure, left ventricular pressure, and aortic and pulmonary artery flow, do not significantly change with increasing end-diastolic and atrial pressures. Ventricular function curves are therefore essentially flat for this denervated heart preperation. These results are demonstrated in Figure 20, where it is shown that arterial pressure and flow in two non-rejecting homografts (4 days post-operative and the R-wave voltage greater than 1 mv) and one autograft are constant during an infusion, whereas atrial pressures rise. This apparently fulfills the criteria for a controlled and constant arterial pressure stressed by Kinnison.

Pharmacologic Alteration of the Response

In an attempt to assess the importance of sympathetic and parasympathetic innervation for the infusion tachycardia, atropine (2mg I.V.) and propranolol (1 mg/kg I.V.) were administered to two autografts. In two dogs, propranolol was given prior to volume infusion; in one dog atropine alone was given; and in one dog atropine and propranolol together were used. In one additional experiment, propranolol was given immediately following the infusion of 300 cc of 6% dextran.

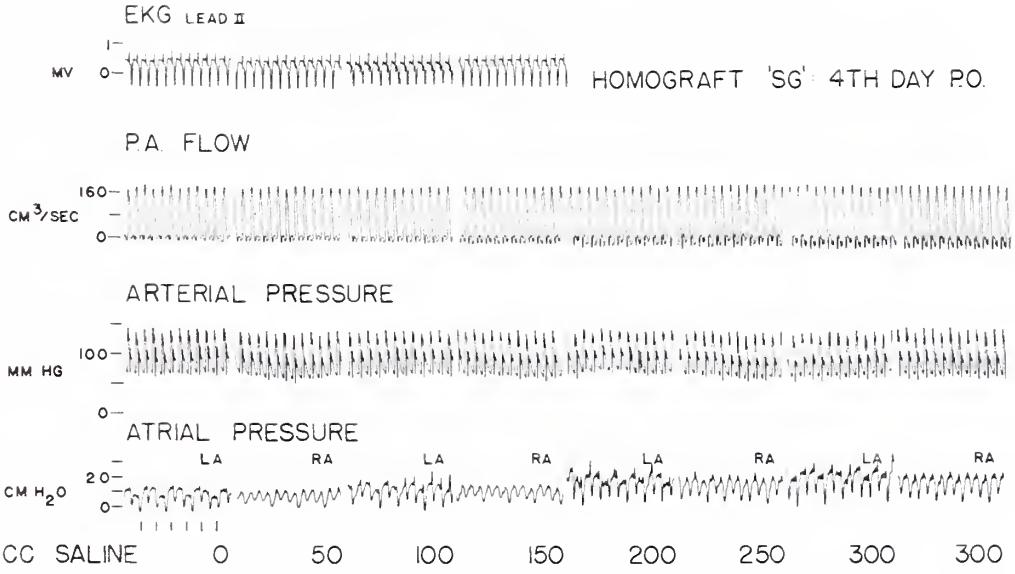
FIGURE 20: The Offner Dynograph print-out records of three complete volume infusion experiments.

(A) Homograft, 4th day post-operative, 300 cc of saline given. The lead II EKG, PA flow, arterial pressure, and right and left atrial pressures are displayed for a short segment each 50 cc of infusion. Time markers indicating one second intervals are placed under the LA pressure trace of the control condition.

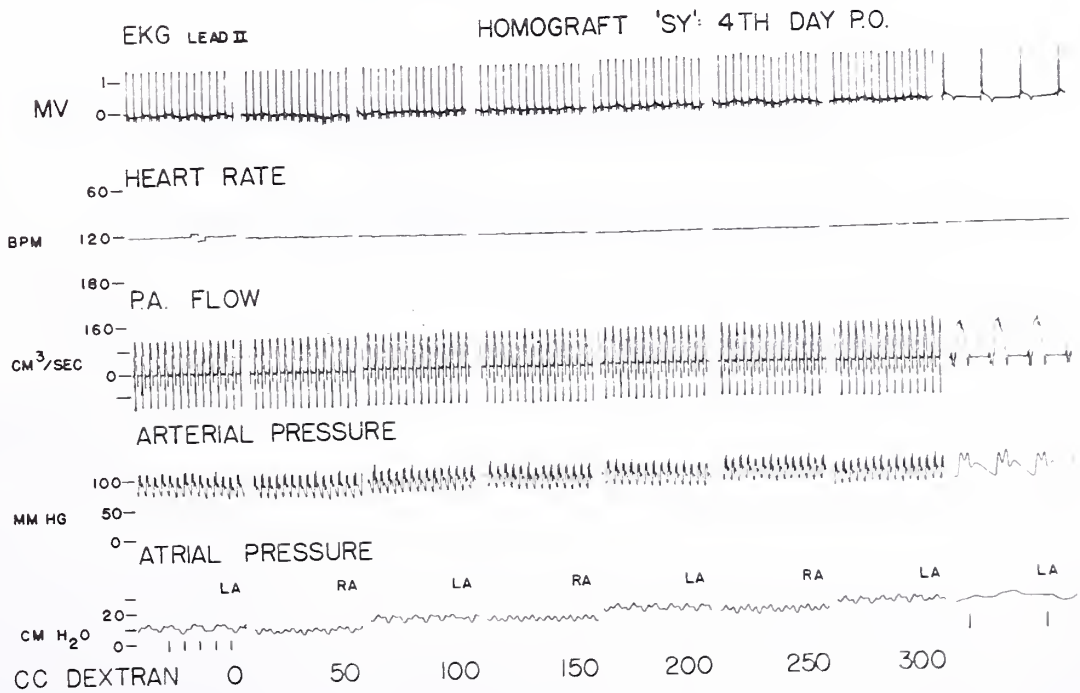
(B) Homograft, 4th day post-operative, 300 cc dextran given. Same as in (A), except that the heart rate is also displayed.

(C) Autograft, 9th day post-operative, 300 cc dextran given. The heart rate, PA flow, LV pressure, and the arterial pressure is displayed. Time marker indicates 5 seconds.

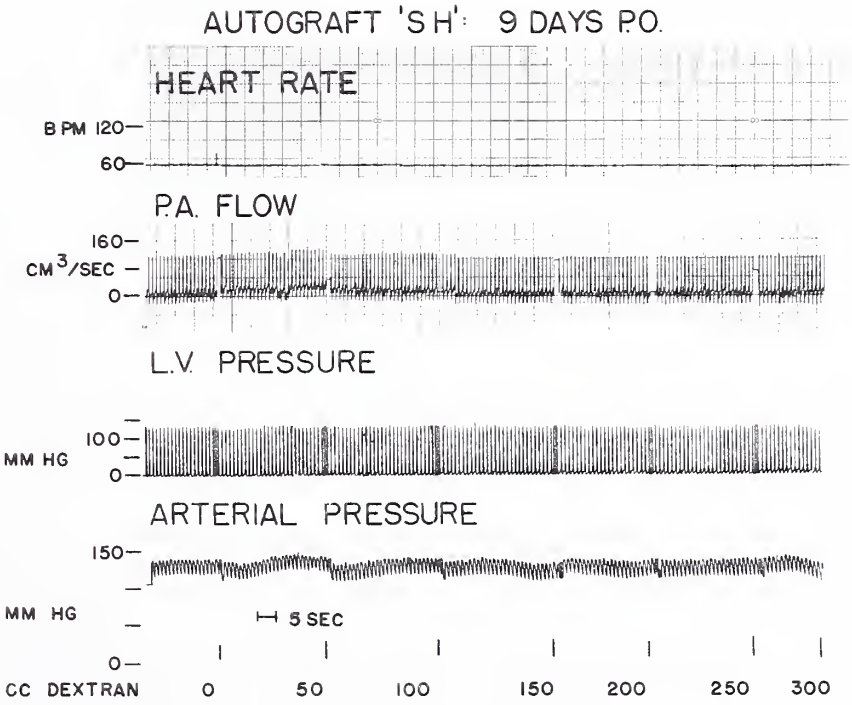
(A)


EKG LEAD II
MV
1
0
HOMOGRAFT 'SG' 4TH DAY P.O.
P.A. FLOW
160
CM³/SEC
0
ARTERIAL PRESSURE
100
MM HG
0
ATRIAL PRESSURE
LA
RA
LA
RA
LA
RA
LA
RA
20
CM H₂O
0
CC SALINE
0
50
100
150
200
250
300
300

(B)


EKG LEAD II
HOMOGRAFT 'SY' 4TH DAY P.O.
MV
1
0
60
HEART RATE
BPM
120
180
P.A. FLOW
160
CM³/SEC
0
ARTERIAL PRESSURE
100
MM HG
50
0
ATRIAL PRESSURE
LA
RA
LA
RA
LA
RA
LA
LA
20
CM H₂O
0
CC DEXTRAN
0
50
100
150
200
250
300

(C)


AUTOGRAFT 'S H': 9 DAYS P.O.
HEART RATE
B PM 120
60
P.A. FLOW
160
CM 3/SEC
0
L.V. PRESSURE
MM HG
100
0
ARTERIAL PRESSURE
150
MM HG
0
5 SEC
CC DEXTRAN
0
50
100
150
200
250
300

With beta-blockade the infusion tachycardia was found to be greatly reduced. Figure 21 shows the atrial remnant heart rate of a single autograft before and after infusion both with and without drugs. It is seen that propranolol (Inderal) reduced the control rate from 128 to 110 beats per minute; the donor heart rate was similarly affected, changing from 82 to 74. With infusion of 500 cc of 6% dextran, the atrial rate gradually increased from 110 $\pm$ 3.2 to 122 $\pm$ 2.8 beats per minute. The donor rate in the same period changed from 74 $\pm$ 2 to 84 $\pm$ 0.5 beats per minute. In a second dog, propranolol changed the remnant atrial rate from 126 $\pm$ 10 to 109 $\pm$ 6; with the infusion of 300 cc dextran this rate increased slightly to 115 $\pm$ 7. When propranolol is given to an autograft following a normal volume infusion experiment, the accelerated atrial remnant rate will return to near control values within two minutes.

Atropine was given I.V. to the same autograft prior to infusion. Figure 21 shows the control pre-infusion rate increased from 132 $\pm$ 8 to 174 $\pm$ 2; following 500 cc of saline, the atrial rate was 176 $\pm$ 2 beats per minute. The ventricular rate was unaffected by atropine and increased slightly from 58 to 61 with the infusion.

When both atropine and propranolol were given simultaneously the control atrial rate went from 164 $\pm$ 3 to 148 $\pm$ 3. At the same time the ventricular rate decreased from 67 to 63 with the drugs. Then 500 cc of 6% dextran was given accompanied by essentially no change in atrial rate (152 $\pm$ 2) and a further small decrease of ventricular rate to 60 $\pm$ 2 beats per minute.

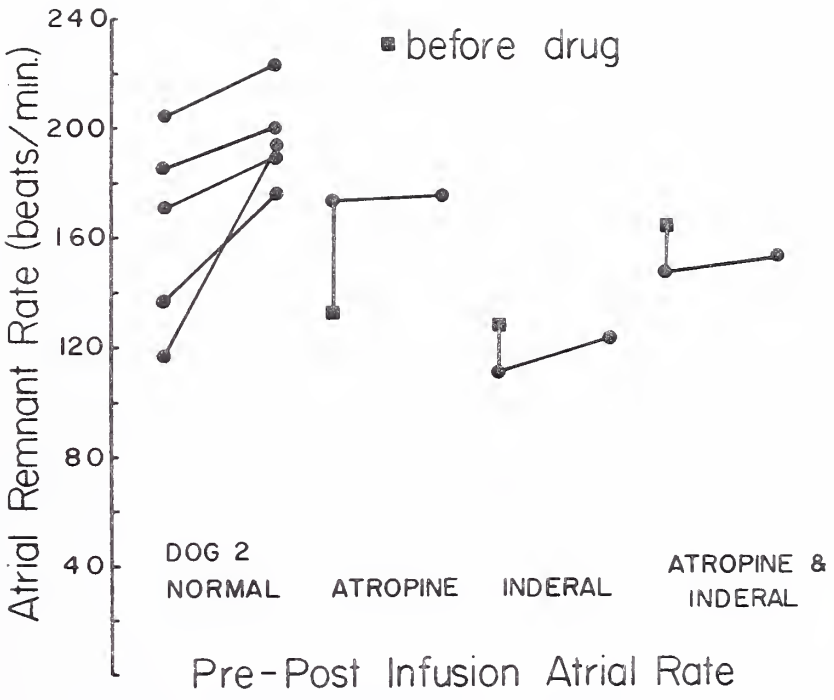


FIGURE 21: The pre and post-infusion atrial rates of a single autograft both with and without drugs: atropine (2 mg I.V.) and propranolol (inderal) (1 mg/kg I.V.). The control rate before the drug is given by the squares.

DISCUSSION

Volume infusion in the canine cardiac autotransplant is associated with an increase in remnant atrial rate and inconstant or no change in the donor ventricular rate. The measurement of pressures and flows during infusion demonstrate that cardiac output and left sided pressures change very little if at all, whereas right sided venous pressures increase.

The lack of response of the totally denervated heart to an increase in end-diastolic pressure is contrary to previous assumptions of Starling's law of the heart.[96,97] These experiments may demonstrate that homeometric autoragulation[98] does not exist in the heart deprived of catacholamines or of some other factor supplied by autonomic innervation. A second interpretation may be that these animals are chronically on the "failure" end of the ventricular function curve due to increased resting blood volumes, and subsequent addition of volume will thus not lead to an increase in contractility, work, and cardiac output. It is interesting that ventricular function curves of stroke work against right atrial pressure in dogs denervated by chronic neural ablation are of the normal form.[99]

The demonstration of atrial remnant tachycardia with volume infusion appears to substantiate the concept of a Bainbridge reflex. The rapid, immediate increase in rate which is greatly diminished or abolished by atropine and propranolol support the interpretation that this is a reflex phenomenon mediated by vagus withdrawal and sympathetic activity. Not enough experiments were done to determine which factor is of relatively greater importance; both appear

to function.

The contribution of stretch of the myocardium and local changes in irritability is inferred by these experiments. Though we describe the donor heart rate by R-R intervals and speak of "ventricular" rate, more than half of these experiments were done when the donor heart was in atrial rhythm, the dominate pacemaker being in the denervated atrial tissue. In the remainder of the experiments the donor heart was in nodal rhythm. If local stretch is of importance, and could account for the infusion tachycardia as some suggest,[92] then both rates should change simultaneously since both "pacemakers" are exposed to the same degree of stretching pressure. This, however, is not the case and donor heart rate may either stay the same, increase, or decrease slightly, leading to the conclusion that in the awake dog this mechanism for control of heart rate is relatively unimportant.

No turnover point is seen in these experiments for the change in atrial rate, in agreement with Kinnison, et al.[91] This agreement is thought due to the fact that arterial pressure is constant during infusion. The maximum heart rate attainable (near 230 in these animals) is apparently the limiting factor for the tachycardia; when the control heart rate is low there is a relatively greater increase in rate than when the control rate is high. These results suggest that in the normal dog experiments with no control over arterial pressure, an infusion to a dog with a high control heart rate will influence left-sided control mechanisms through an increase in arterial pressure; when the control rate is low, the right-sided reflex would dominate, causing an infusion tachycardia.

That this occurs, and the mechanism for it, must be studied by further experiment.

Finally, these experiments also support the concept that the atrial stretch receptors lie in the back wall of the atria. Some workers[100,101] have shown evidence that pressure (stretch) sensitive receptors lie in the junction of the pulmonary veins and the left atria. Again, the fortuitous use of the atrial cuff would allow these reseptors their innervation while depriving the remainder of the heart its own.

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