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Frequency - Tension relationships in cardiac muscle

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MOTTO

"...For the life of all flesh is its blood; it is in its life..."

Leviticus 17:14

TABLE OF CONTENTS

I. SURVEY OF PERTINENT LITERATURE

A. The Effect of Serum Upon Cardiac Contraction.

Among the variables affecting the development of cardiac tension, the composition of the perfusion medium is most significant. The study of all phenomena observable in artificial media, must, in the last analysis, receive final confirmation by comparison with the in situ state. The experiments discussed in this paper deal with the determinants of cardiac tension as developed in an artificial perfusion medium. It is for this reason that the study of the development of contemporary concepts of the relationship of blood to cardiac function is of some interest.

Speculations concerning this relationship have their origins in antiquity. The biblical conception related blood to the soul (1) and forbade its consumption under threat of excommunication (2). Blood was later thought of as a carrier of natural spirits, which, produced in the liver, entered the vasculature and were dispersed throughout the body, but had no direct control over the contraction of the heart. Galen (3) conceived of cardiac control as distinct from the "succus nerveus" and postulated that it acted through "natural faculties" alone.

The elaboration of the conception of the circulation by William Harvey in 1628 (4) brought with it the necessity of distinguishing whether the blood serves as a mere "pondus" upon which the heart can contract, or whether it is essential for such contraction. Harvey, himself, was able to show that a finger wetted with saliva restored contraction of excised animal hearts. Richard Lower (1669) (5) perfused hearts with beer and effected stimulation with a needle. William Cowper (1737) (6) restored the motion of the heart by the application of "warm water or the palm of the hand." Albrecht von Haller (1755) (7) demonstrated that air blown into excised ventricles restored contraction thus eliminating the necessity of conceiving of blood as a "pondus," and commencing consideration of its constituents. Von Haller accepted the concept of the "succus nerveus" and made the widely prescient observation that,

> "the whole body, which is supposed to remain in a permanent state, is really in a perpetual flux."

Le Gallois (1812) (8) first suggested the constitution of artificial perfusing media for the study of the effects of blood on cardiac control.

It was not until Sidney Ringer (1882) discovered the effective ratio of electrolytes in artificial perfusing media that research in this field could progress with certainty.

Ringer maintained that,

> "...the effects of a small quantity of blood added to the circulating fluid cannot be all attributable to the inorganic constituents, but are in all probability due to the organic substances." (9, 10, 11)

Kronecker and his pupils (12, 13) argued that serum albumin was necessary for contractility, and that the continued contraction of the isolated, perfused, heart in Ringer's solution was due to the minute quantities of albumin remaining. The degree of recovery from hypodyanmic action was thought to be related to the concentration of serum albumin in the perfusate. Merunowicz (14), however, and later Howell and Cooke (15, 16) maintained that the ability of Ringer's solution to sustain cardiac contraction was due to its concentration of inorganic ions alone.

Efforts to extract cardioactive serum constituents have led to variable results. For example, it had been stated (17, 18) that the positive inotropic action of serum is destroyed by boiling, and slightly inactivated by mild heating. Clark (19), however, demonstrated that boiled and fresh serum are equally effective in increasing cardiac contractility. Ringer (13) had maintained that ether extraction of serum had no effect upon cardiac contractility, whereas Howell and Cooke (16) observed a lessened effect. Clark observed (19) the activity

to reside in an alcoholic extract, while Neilson and Palm (20) state that the activity resides in the non-ultra-filtrable portion of serum.

Specific serum constituents have been applied to a number of experimental systems with the object of discerning active inotropic factors. In general, factors effective were; plasma, serum, amino acids, glycogen, cholesterol, and various substances isolated from urine and blood. Ineffective were; serum proteins, purified albumin and fibrinogen, washed erythrocytes, glucose, egg albumin, and inositol. (25)

Clark (19) demonstrated that phospholipids were significant in increasing contractility, and observed phosphorus transfer into the perfusate following contraction. The perfusate was shown to develop a positive inotropic effect subsequent to contraction, when applied to another heart. He then postulated that "hypodynamic action" as usually observed with perfusion resulted from loss of lipids, and that reconstitution of the lipids resulted in restoration of permeability relationships and contractility.

Neilson and Palm (20), Salter and his associates (21, 22, 23, 24), and Green (25, 26), have studied the action of serum on the contractile properties of the papillary muscle of the cat, and have demonstrated inotropy associated with the serum albumin fraction. Emile and Bonnycastle (27) demonstrated the inotropic action of adrenal steroids and whole extracts.

B. The "Treppe" Phenomenon.

The first demonstration of the incremental relationship of cardiac frequency and tension, as well as that of the incremental tension with stimulation following a pause with constant stimulation frequency, was provided by H. P. Bowditch working with frog heart preparations in Ludwig's laboratory (1871) (28). The shape of the kymographic record, in the latter instance, suggested a "staircase," and the phenomenon was termed "Treppe". Bowditch also demonstrated the independence of the "Treppe" from the characteristics of the electrical stimulus.

("...die Gestalt, welche die Treppe annimt von der Richtung und der stärke des Inductionstromes durchaus unabhängig ist.") Significantly for the present investigation, Bowditch also attempted artificial perfusion of his preparations with an eye to studying variations in the staircase. As in many experiments in the pre-Ringer era, saline solutions in various concentrations with admixture of gum arabic were used, with no attempt to vary the electrolyte (crystalloid) concentration. It was shown that 0.5 gm. NaCl and 4 gm. gum arabic in 100 cc. of water abolished the phenomenon, as did atropine, but that muscarine diluted into dog serum only decreased the intensity of the contractions. Bowditch also attempted to study the effects of temperature variations upon the "Treppe," as well as various techniques of ligature of the atria. In these

attempts, as well as in his major discovery, Bowditch antedated most of the later observers, and, in fact correctly stated the major observables, and delineated the major significant approaches.

The discovery of the significance of the balanced electrolytic milieu by Ringer led to investigations concerning the effect of this balance and its variations upon the "Treppe" phenomenon, notably that of Hoffmann, in 1901 (29). This investigator, confirming the observation of Bowditch (28) that the staircase effect was best developed only after perfusion, and when the tension developed by the preparation had greatly diminished ("hypodynamic state"), showed also that an elevated calcium concentration in the perfusing medium not only caused cessation of spontaneous cardiac activity in systole, as had been demonstrated by Ringer (9), but that the "Treppe" effect was abolished at a less elevated concentration, in atrium and in ventricle. Woodworth (1902) (30) demonstrated that contractions at the baseline frequency subsequent to a series of shocks at an elevated frequency were greater in intensity than those preceeding the period of high frequency stimulation. This phenomenon we may term post-stimulation potentiation. As the "Treppe" effect as such was not significantly changed by the interspersed periods of high frequency stimulation, Woodworth was led to conclude that two factors were operative, one accounting for the "Treppe", and the other for the post-stimulation potentiation.

Woodworth also attempted to account for pulsus alternans on the basis of the "Treppe" effect with the conception that those contractions which followed rapidly upon a succeeding weaker contraction would have a greater developed tension since, in effect, the frequency of stimulation would have been increased, as compared with contractionw which follow after relatively long pauses of stimulation. Woodworth's investigations also showed that the time relationships of refractoriness and "Treppe" were incompatible, and that the "staircase" could not be explained on this basis. A further series of experiments by this investigator were designed to demonstrate that the phenomena of tetanus and summation could not exist in cardiac muscle. This latter observation has been shown to be incorrect. See Whitehorn (31) for demonstration of both summation and tetanus in frog and in rat ventricles.

Bornstein, in 1906, (32) also demonstrated tetanus in cardiac muscle, and showed that there existed a frequency at which the tension developed by the contracting heart muscle was optimal. Among Bornstein's observations is one which we may term rest potentiation. In the classic "Treppe" effect, the first contractions after a beat are less in tension than succeeding contractions. If, however, a rest is interposed after a period of stimulation at constant frequency, the first contractions are greater in tension than those succeeding (einleitenden Zuckungen). It was Bornstein's contention that the "Treppe"

phenomenon involved calcium primarily.

In summary then, it is clear that the major observations concerning the phenomenon itself had been made by 1906. It remained for future years to attempt to confirm and explain them.

Further investigation was concerned, not so much with the effect itself, as with variations in the external milieu which would effect changes in it, and so lead to its eventual elucidation. Clark, in 1913 (33), showed that the abolition of the "Treppe" was accomplished not only by ionic but by small-molecular organic components as well. Among these were soaps, lecithin, and alcoholic serum extracts. He then hypothesized, as we have seen, that the hypodynamic state was not so much an ionic as a permeability change. Boehm, (1914) (34) in an extensive study upon the action of electrolytes in the isolated frog heart preparation, showed that potassium was necessary in the external environment for the "Treppe" phenomenon to persist, and that lowering the potassium concentration had the effect of abolishing the "Treppe", and rendering tension independent of frequency.

The rather confusing data relating to the staircase effect, as well as its picturesqueness, led to a multiplicity of explanatory hypotheses, none of them completely adequate to explain all aspects, but some lending themselves to the understanding of isolated facets, and others showing great ingenuity and developed with consummate experimental skill. Among these

may be mentioned the work of Skramlik (1920) (35) who maintained that succeeding stimulation increased cardiac conductivity and thus facilitated contraction, and that of Adrian (1920) (36) who maintained that,

> "...the increased contractile power demonstrated by Bowditch and others is one expression of the supernormal phase of recovery"

and who also showed that the so-called "Treppe" phenomenon in skeletal muscle was a recording artifact and was related to fatigue at high frequencies of contraction, slowing the contraction, and thus causing overshoot by the highly inertial recording systems then used. It is obvious, however, that Adrian's hypothesis concerning the staircase cannot be used as an explanation at extremely low frequencies, as these are out of range of action of supernormal periods of excitability as generally understood. The hypothesis of Wastle (1922) (37) that the "Treppe" is explained by recruitment of previously asynchronous fibers into the contraction group appeared attractive enough until the appearance of the second very significant paper of Hoffmann (1926) (38) on the subject of the "Treppe" and the relationships between the electrogram and mechanogram in the contraction of cardiac muscle. Hoffmann was here concerned with the relationships of frequency and tension about the point of the optimal frequency, or, more appropriately, about the point of the optimal interval between

stimuli (Optimum des Reizintervalle). The relationship between the amplitude and duration of the action potential and the amplitude and duration of the contraction are not constant and depend upon the frequency of stimulation, or specifically upon whether the frequency is above or below the optimum. Below the optimum frequency increments in frequency will produce increments in contraction tension, and also in duration of contraction. The action potential will be increased in amplitude and duration, but the time to the action potential maximum (Gipfelzeit) is not increased. Above the optimal frequency, increments of frequency result in decrements in contraction tension and duration and also in decrements of action potential amplitude and duration. Depending upon the position of the frequency in relation to the optimum both increments and decrements of action potential can be produced by frequency increments. It would be difficult, therefore, to imagine that frequency increments are responsible for contraction synchronization and therefore increased tension, as this should result uniformly in action potential increment with frequency increment unless a second factor operates to account for the reversal of behavior above the frequency optimum. In the records later to be presented, it will be shown that some synchronization undoubtedly does occur in the early contractions of the frog heart preparation following a long pause, but this appears to have a minimal effect on the "Treppe" phenomenon as

such, and may be related almost entirely to anoxia or rapid frequency shifts, and is a transient rather than a steady state phenomenon.

It was during this period (the early 1920's) that Neiderhoff's researches (1925) (39) demonstrated again the action of elevated calcium concentrations in the abolition of the "Treppe" effect. Demoor (40) and Kruta (1937) (41) further elaborated upon the mechanism of the "Treppe" with confirmation of the original observations and restatement of the two-factor theoretical approach. Kruta (41) based his work upon the phenomena of post stimulation and rest potentiation, (contractions d'entreé) giving full priority, however, to Bornstein (32).

With the possible exception of the work of Clark (42) and associates on the metabolism of the frog's heart, and their investigations into the effects of temperature upon frequency-tension relationships, the subject of the "Treppe" lay dormant until the attempt by Spadolini (1949) (43) to account for the "Treppe" on the basis of acetylcholine production. Spadolini demonstrated that graded responses of the toad ventricle occurred following variation in stimulation frequency, and demonstrated further that acetylcholine was produced during the contractions elicited. Transfer of perfusion fluid to a bioassay heart produced inhibition of atria and excitation of ventricles. Atropine in concentrations of 10^{-4} M. changed inhibitory to excitatory responses. That

the effects in the test heart were produced by acetylcholine seems probable, but that the simple release of acetycholine can account for all the observable phenomena with which the "Treppe" is associated seems doubtful, and indeed, these ancillary phenomena were not discussed by the author.

A truly significant contribution to the literature on the "Treppe" effect was made through the collaboration of Szent-Gyorgyi and Hajdu at the Institute for Muscle Research at Wood's Hole, Massachusetts. (44, 45, 46, 47) Using a preparation designed for recording the isometric tension developed by perfused whole frog heart preparations, the "Treppe" phenomenon, and its relationship to temperature, potassium concentration, and bovine serum constituents, was studied. The value of the steady state isometric tension developed at different frequencies of stimulation was used as an index, and it was possible to demonstrate that lowering of the potassium concentration abolished the "Treppe", and that lowering of the temperature produced a "reversed Treppe," i. e., the frequency increment being followed by a tension decrement even at frequencies below the optimum frequency. It was confirmed that the "Treppe" effect develops only after a rather long period of perfusion, and the addition of various sera rendered tension independent of frequency over a rather wide range. An empirical search undertaken with various bovine serum constituents, after it had been shown that a factor active

in abolishing the staircase existed in the lipid fraction, revealed that desoxycorticosterone and progesterone share the activity of serum in this regard. However, neither progesterone nor desoxycorticosterone were shown to be the active components of bovine serum inasmuch as the heart had a greater pharmacological affinity, as demonstrated by washing experiments, for the active components, than it did for the two steroids.

It was thought by Szent-Gyorgyi and Hajdu that substances abolishing the staircase belong in three groupe: (1) Substances with a very high affinity for the heart which cannot be washed out at all within the time limits of the experiment. (2) Substances with a high affinity which can be washed out slowly, and which act in very low concentrations. (3) Substances with a low affinity, which can be washed out in a few moments and require high concentrations for their activity. The active agents of serum, it was thought belong to the first two groups, desoxycorticosterone and progesterone to the last one. In regard to isolation, then, it would seem as if substances with high pharmacological affinity should be present in extremely low concentrations, for if they existed in high concentrations in the serum, contracture of the heart would most probably result.

Extremely significant from the medical standpoint were the results relating to the action of digitalis bodies (digitan) which were found to have an action similar to that of progesterone

and desoxycorticosterone, and in concentrations adequate to cover the surface of the frog heart to the extent only of 1-5%, indicating the possibility of specific affinity to specific loci on the cell surface.

Epinephrine and acteylcholine were found to have opposite effects on the staircase, epinephrine abolishing it, while acetylcholine favored it, but only at relatively elevated temperatures and in the presence of potassium in the perfusate. ATP was found to have an effect similar to that of epinephrine. It was also shown that, when under the influence of agents with effects similar to that of acetylcholine, the heart is extremely sensitive to CO_2 and a profound drop in tension results with decreases in CO_2 tension that would not have resulted had the acetylcholine like agents not been present.

The theoretical approach of Szent-Gyorgyi and Hajdu to the action of the active substance ("AS") of serum and of digitalis was that a "favorable state" for contraction existed following each contraction, and that the deterioration of this state was a rather rapid phenomenon, analagous to the deterioration of the "supernormal recovery period" of Adrian (36).

> "Contraction being an all or none reaction of actomyosin units, maximal tension means that the whole mass of actomyosin has contracted, while a low tension means that only part of it has done so." (44)

At constant temperature the most significant variable affecting the ATP-actomyosin complex is the concentration of the external ionic milieu. It is thus probable that the staircase "actually reflects the intracellular ionic concentration, and the 'favorable condition' is an ionic balance which favors contraction while its deterioration is the establishment of a balance which favors the relaxed state, that is, the dissociation of actomyosin." The favorable state is thought to be the loss of potassium from the internal environment. Digitalis, by preventing the reentry of lost potassium into the cell prevents further loss by the establishment of an unfavorable concentration gradient, and thus stabilizes the internal environment at relatively higher levels of potassium, with the effect of counteracting the development of a potentiated favorable state.

Quantitative evidence of the potassium hypothesis as related to the staircase effect was provided by Hajdu (47). Loss of 3 mEq. of potassium out of a total concentration of 106.8 mEq./l. sodium plus potassium results in an increase in tension from 0-100%. The released potassium is followed by 127 micro-Eq./l. of fiber water which reenters the cell during rest. Loss of internal sodium was found to have the same effect as loss of potassium on tension, but loss of fiber water alone had no effect. Digitalis and related substances were found to block reentry of potassium into the cell.

Hajdu concluded that the effect of the released potassium

was exerted through a membrane potential, as was shown by the linear correlation established between the internal ionic content and the membrane potential required to prevent contraction. The resting potential was thought to exert a static action on actomyosin which favors dissociation, while the loss of potassium upsets this potential and leads to conditions favoring association and contraction.

During recent years the staircase effect has attracted the attention of a number of workers who have studied both the gross observables and the effects of variations in the ionic and organic milieu with modern instrumentation and improved techniques of chemical analysis. Wezler and Hangst (48) in studies upon the isolated frog heart demonstrated the effect of decreased carbohydrate and sodium concentrations in the production of the hypodynamic state, and of calcium in the production of contracture and in the elimination of hypodynamic activity. High phosphate concentrations were found to increase stroke volume and extensibility, and to decrease residual volume in this preparation. Whitehorn (31) showed that summation and incomplete tetanus could be produced in frog heart preparations by elevated temperatures, while digitoxin potentiated this summation and caused it to appear at lower temperatures. Epinephrine in concentrations of 10^{-6} M. prevented summation due to digitoxin or elevated temperatures. Rat ventricle was shown to be capable of summation and incomplete tetanus at 38° C. without drugs. Halbach (49)

studied the effect of elevated pressure upon binding of dyes in the frog heart, while Moulin and Wilbrandt (50) established a quantitative relationship between the decrease in potassium and the elevation in calcium concentrations necessary for the abolition of the staircase effect. These authors also advance the hypothesis that the "Treppe" effect is related to the concentration of the internal ionic environment.

Rosin and Farah (51, 52) working with isolated rabbit auricles, studied the post stimulation and rest potentiation phenomena, as well as the staircase. In a comprehensive discussion of the factors relating to the "Treppe", it is developed that neither the characteristics of the action potential, nor the increasing synchrony of contraction, nor the nature of the stimulation whether "natural" or artificial, was responsible for the effect.

In an apt criticism of the Hajdu-Szent Gyorgyi hypothesis, Rosin and Farah point out that since diastolic periods of up to four minutes do not eliminate either post stimulation or rest potentiation, these cannot depend on the relatively depleted state of the cell as far as potassium is concerned, since the potassium should have been well rediffused in this time period. The authors also state their feeling that the rest potentiation is not due to metabolic recovery of the muscle cells, since, in their experiments the first post-rest contractions are not greater than the succeeding contractions. This is in conflict, so far as experimental

evidence goes, with previous authors (32, 39, 41, 53) and with the results to be described in the present paper, where it will be shown that the first rest contraction is greater in tension than succeeding contractions under certain conditions (Figure III, 3-D).

A substance PS (potentiating substance) relatively stable, is thought to be produced intracellularly during each contraction, and it is this material which potentiates succeeding contractions. A further hypothesis of slow diffusion of PS to its intracellular locus of action would explain the rest potentiation, and a slow extracellular destruction of PS would explain the failure of longer rest periods to produce potentiation. Although admittedly this hypothesis is useful in unifying the conceptions relating to the staircase effect, post stimulation and post rest potentiations, it does not explain the decrement in tension with frequency above the frequency optima. The postulation of "potentiating substances" serves merely to describe and not explain the phenomena.

Katzung and Farah (54) demonstrated that reduction in temperature could produce a reversed staircase which contrasts with the work of Hajdu and Szent-Gyorgyi (44) who could not obtain reversed staircase above 0° C. without the use of drugs. Post-stimulation potentiation was found to be present at all temperatures above 14° C.

Cattell and Gold (55) described the "Treppe" effect in

the papillary muscle of the cat. Following an abrupt change in rate, the force of contraction was found only gradually to assume the characteristics of the new frequency, rising when the rate was increased, and falling when decreased. A single extra stimulus applied close to one in the regular series resulted in a marked increase in the succeeding response. The influence of the extra stimulus persisted for several minutes but was quickly dissipated.

Garb and Penna (56) presented a quantitative analysis of the Cattell and Gold phenomena, and extended them to the study of interspersed beats. In most instances, an extra beat, interspersed during a series of regularly spaced contractions, produced an augmentation of the force of the next beat and a successively decreasing augmentation of succeeding beats. As the interval between the last regular beat and the extra beat decreased, the augmentation of the succeeding contraction increased. If the muscle was allowed to rest after the extra beat, the augmentation effect reached a peak in about ten seconds and persisted for about five minutes. In effect, then these investigators studied post-stimulation potentiation with single beats.

Trautwine and Dudel (57) demonstrated that the duration, but not the amplitude, of the action potential changes with increased frequency. They also demonstrated the great instability of the optimal frequency from experiment to experiment, and may

also have demonstrated post-stimulation potentiation.

Neidergerke (58) in a recent paper in which refined instrumentation was used to study the staircase and its variation with changes in potassium and calcium concentration concluded, after a stimulating discussion, that it is the concentration of calcium in a superficially located portion of the cell which influences the "Treppe".

Most recently, Hajdu and Leonard (59) have assayed plasma with frog heart preparations for strophanthidin-like activity, and have shown a slight rise in activity in patients with essential hypertension. More significant is the work of Titus, Weiss and Hajdu (60) who, utilizing the isometric perfused frog heart preparation as bioassay have succeeded in isolating from beef adrenal medulla a material which has decided AS (44) action. This material, monopalmitoyl lysolecithin, is produced as an inactive precursor, and becomes active after exposure to pH 2 for several hours. It is also found in liver and in plasma in decreased concentrations and not at all in adrenal cortex or skeletal muscle. It has the properties of:

(1) increasing the tension in the perfused frog heart preparation;

(2) abolishing the staircase effect in the perfused frog heart;

(3) increasing the tension of pigeon ventricular muscle and carotid artery strip;

(4) high affinity to the heart.

The active isomer is the beta form, the alpha form being produced as an isolation artifact.

We can summarize, at this point, the most plausible conclusions available to us from a perusal of the literature concerning the mechanism of the "Treppe" effect. These are, in the main, negative in character. We have seen that neither stimulus intensity (28), refractory period (30), increased conductivity (35), supernormality, (36), recruitment and synchronization (37), loss of potassium (47), or gain in calcium (58) in an intracellular locus, can satisfactorily explain all facets of the effect.

Consideration of the metabolism of the frog heart will enable us to evaluate the effects of anoxia.

C. The Metabolism of the Frog's Heart.

The frog's heart has proved itself extremely useful as an experimental physiological system, both for its ready availability and economy, and because its anatomy is almost entirely suited for perfusion studies. An understanding of the total metabolism and some aspects of the intermediary metabolism of the frog heart system is essential to further discussion of the mechanism of contraction in this species. Metabolic data for this discussion were taken from "The Metabolism of the Frog's Heart" by A. J. Clark, M.G. Eggleton, R. Gaddie, and C. P. Stewart; Oliver and Boyd, 1938.

The average weight of the frog's heart is 0.14-0.16 grams. As the heart is not supplied with a coronary circulation, but attains its oxygen and nutritive supply through diffusion alone, it is essential that the thickness of its various components and particularly the thickness of the ventricular trabeculae be small. That this is so is seen from the fact that the depth of diffusion to reach all parts of the trabeculae is nowhere greater than 20 micra. The thickness of portions of the frog's heart and great vessels is dependent upon the stage of distention as follows: (first figure empty, second distended)

thickness of sinus venosus		0.025 cm.	----
atrium	thickness	0.040	0.011
	surface area (cm^2/gm.)	48	176
ventricle	thickness	0.300	0.200
	pericardial surface	12	22
trabeculae	thickness	0.006	0.004
	endocardial surface	330-680	500-1000

In addition to its smaller surface area, the pericardial surface has a lower coefficient of permeability than the endocardium, as demonstrated by various experimental techniques.

The ventricles comprise 70% of the heart weight. The heart weight x 10^3 body weight, or the heart ratio is 1.76-2.65, and is greater in males than in females.

Chemical analysis of cardiac components reveal inorganic constituents as follows:

(1) Water	
atrium	90.5%
ventricle	84.0
whole heart	86.0
(2) Sodium	0.047
(3) Potassium	
atrium	0.38
ventricle	0.23
whole heart	0.21-0.33
(4) Calcium	0.07
atrium	0.07
ventricle	0.025
(5) Phosphate factors	
orthophosphate	0.011
creatine phosphate	0.007
pyrophosphate	0.011
hexose phosphates	-----
organic phosphates	
Ba soluble	0.043
Ba insoluble	0.011
total acid soluble	0.085

The dimensions of the cells of the frog heart have also been measured and are as follows:

Sinus	0.073 x 0.0054 mm.
Atrium	0.193 x 0.0057
A-V ring	0.116 x 0.0091
Ventricle	0.131 x 0.0092
Bulbus	0.130 x 0.0060

The cells are cone-shaped, and the folume of the average frog heart cell is 2.9×10^{-6} cm.^{3}, and its surface area is 19×10^{-4} cm.^{2}. One gram of heart tissue contains 345×10^{6} cells, with a total calculated surface area per gram of 6.6×10^{5} cm.^{2}.

The most essential factor in the metabolism of this tissue mass is the provision of an adequate oxygen supply based upon thoroughness of irrigation, adequate oxygen pressure in perfusion fluids, and minimal diffusion depth. Frog's blood, when fully saturated with oxygen contains 20 volumes per cent oxygen, while air-saturated Ringer's solution at room temperature and atmospheric pressure will contain 7 mm.^{3} of oxygen (STP). Experimental work by Clark et al. (61) has shown that the frog heart under conditions of moderate activity utilizes 1 cc. oxygen/gram/hour or 16 mm.^{3} oxygen/gram/minute. Therefore, a frog's heart weighing 0.16 gram requires 3 mm.^{3} oxygen/minute which is contained in 0.4 cc. of air-saturated Ringer's solution. Since frog heart tissues cannot extract all the oxygen available in the perfusion fluid, at least 1-2 cc. of air-saturated Ringer's solution is required each minute to maintain adequate oxygenation.

A minimum oxygen pressure must also be provided in perfusion fluids regardless of the rapidity of perfusion. This has been shown for the sinus venosus to be 5 mm. Hg or 0.0066 atmosphere. At this pressure the sinus venosus has a barely detectable uptake of oxygen. The lowest limit of oxygen pressure at which a strip of frog's atrium is functional is at 40 mm. Hg. Tests with iodoacetate-poisoned muscle show that the sinus venosus requires 20-30 mm. Hg oxygen pressure.

Insofar as diffusion is concerned, Warburg's law has been found applicable. That is

$$d = (8c \times D/A)^{\frac{1}{2}}$$

where

d = thickness in cm.
c = required oxygen pressure in atm.
A = oxygen utilization in cc./gm./min.
D = diffusion coefficient of oxygen in water which is 1.3×10^{-5} at 15° C.

For sinus and atrium the thicknesses are in accord with this formula except at lower levels of oxygen pressure. Total body weight and total heart weight have not been found to affect the per gram utilization of oxygen in the cardiac tissue of the frog.

Frequency of stimulation, however, does affect oxygen utilization. With low frequencies there is a linear correlation between frequency and oxygen uptake of the cardiac tissue, but the curve flattens at higher frequencies, much more with Ringer's

solution than with blood. Clark and White (61) have presented evidence of a quantitative nature as follows:

Frequency/minute	0	.15	20	40
Oxygen usage of heart (cc./gm/hr.)	0.33	0.84	0.97	1.02

As seen, the resting metabolism is between 20-25% of metabolism at a frequency of 15/minute.

Other factors affecting oxygen uptake include distention of the ventricles, where it can be shown that at constant frequency the oxygen uptake for the distended ventricle is greater than that for the undistended ventricle.

Temperature, too, exerts its effect on oxygen utilization, both directly and by increasing the natural frequency of contraction 2-3x for each 10° C. rise.

The hypodynamic state, being primarily as defined a decrease in tension associated with perfusion or variation of external electrolyte concentrations, is also associated with a decrease in oxygen consumption. Serum, alcoholic extract of serum, lecithin, sodium oletae, charcoal, colloidal metals, hexose diphosphate, and ATP, all restore oxygen consumption to normal. The significance for experiments involving the "Treppe" effect is obvious.

It is interesting to review, at this point, some other factors, both pharmacologic and physiologic in nature which affect the metabolism and thus the tension of the frog heart.

These can be considered to act in one, two, or all of three ways: (1) by inhibition of metabolism, (2) by inhibition of the contractile process, and (3) by inhibition of the recovery process.

Cyanides abolish oxygen uptake and cause inhibition of contraction, while at higher levels glycolysis is inhibited as well. Iodoacetate abolishes the Meyer-Embden pathway and forces dependency on other pathways. pH changes also affect energy metabolism. Not all agents can be considered as affecting metabolism alone. Calcium, narcotics, and acetylcholine act too quickly, while in the case of potassium the absolute refractory period could be seen to be increased with decreased concentrations, thus pointing to an effect on the recovery period.

A comprehensive discussion of cardiac intermediary metabolism is beyond the scope of this paper, but it is well to point out that the mechanism of coupling between the mechanical and chemical components of contraction is as yet unclarified. Most recently, the leading hypothesis of such coupling, namely, the breakdown of ATP preceeding contraction, has been questioned (62, 63).

II. Experimental Design and Technique.

At the commencement of the present series of experiments, it was thought to be of interest to study the frequency-tension relationships of the frog heart when perfused (the classic "Treppe") with a view of eliciting the more recently clarified phenomena (rest potentiation, post-stimulation potentiation) which had been described mainly in mammals, and to study the effects of digitalis and human serum on the frog heart preparation, which latter effect, though under study at the time in a different laboratory, has not to date been published.

The hearts of 25 average-sized R. pipiens, both male and female, were used for these studies. (Obtained from Earl Jarvis, Alburg, Vermont.) Summer frogs were placed in a refrigerator at 10° C. for 2 weeks, while winter frogs (Nov.-Feb.) were used immediately upon arrival, at room temperature. Frogs which showed obvious signs of disease, as tremors, apparent unresponsivness at room temperature, "red leg," or heavy nematodal parasitization, were discarded, and hearts excessively erythematous or inadequately pigmented were also discarded. Thus, a total of approximately fifty frogs were sacrificed for these experiments. (An interesting aspect of experimentation with frogs is the high mortality noted in the newly arrived colony within the first week after

arrival. It was our practice to place the animals in glass jars in groups of six, and, since entire groups would often succumb en masse, while their confreres in a separate glass case went unscathed, it would appear probable that the mortality was related to infection.)

Isometric tensions of the frog ventricle were recorded with varying frequencies of stimulation by means of a modified Hajdu-Szent-Gyorgyi cannula, which facilitates recording of isometric tensions of whole heart mounts without the necessity for clamps or ventricular strips. The cannula consists of the Straub frog heart perfusion cannula fitted with a small rubber stopper at the cannula end through which is passed a fine platinum wire tipped with a glass bead. The frog was pithed, placed immediately under a binocular dissecting microscope, the pericardium was removed with fine forceps and scissors, and the dissection with scissors and forceps was continued to demonstrate the great vessels and the sinus venosus to the upper border of the liver. The aorta was lifted with fine forceps and a transverse incision was placed in its substance about one-half way across and one-half centimeter from the distal end of the bulbus cordis. The cannula, with the glass bead pressed tightly against its end by traction of the platinum wire, and filled with Boyle-Conway solution (vide infra), was then passed into the aorta, through the bulbus, the semilunar valves (at which point a soft

give could be felt) and then into the ventricle. The great vessels and sinus venosus were then incised, freeing the entire heart. With fine silk, the upper border of the aorta was tied to the cannula. Another turn was then taken about the aorta with fine silk including the atrioventricular groove and isolating the atria from the ventricles. At this point, it was usually observed that the ventricles, which had increased the frequency of their contractions during the procedure, ceased beating if no mechanical stimulation were applied. The cannula was immediately refilled with Boyle-Conway solution, and the ventricle gently stimulated mechanically, resulting in expulsion of retained blood, which, diluted in the cannula, was withdrawn, and replaced with fresh solution. This procedure was repeated until there was no tinge of red in the fluid expelled from the ventricle, the perfusion fluid then being replaced.

The cannula with heart attached was then placed in a beaker filled with Boyle-Conway solution, which in turn was placed in a larger beaker, through which water was kept circulating at any desired temperature. (Most of the time experimentation was done at room temperature.)

The platinum rod, projecting from the cannula, the bead of which was now resting upon the ventricular apex interiorly, was placed gently in the center of a rubber cushion suspended from the rod of a Statham Strain Gauge (5608 Gl-1.5-30 ± 1.5 oz. 9 v. max.) arranged in such fashion that with ventricular

contraction pressure would be exerted, through the rod and the cushion, upon the sensitive recording element. Electrodes, connected to a Grass Model C Physiologic Stimulator set for a 25 v., 5 millisecond square wave stimulus, were placed, one within the perfusion cannula and the other within the outer chamber, the negative electrode being inside the perfusion cannula. The strain gauge was connected to a Grass Direct Writing Oscillograph through a Grass Bridge Amplifier Model A Series III.

Thus, with electrical pulse stimulation variably controlled through the Stimulator, isometric direct recording was possible. A time line was inscribed on several records when necessary by means of a Haydon Time Marker (Haydon Manufacturing Corporation, Torrington, Conn.). The frog heart cannula itself was obtained from the A. S. Aloe Corporation, St. Louis, Missouri.

The basic perfusion fluid was adapted from that of Boyle and Conway (64) and was intended as a close electrolytic stimulation of frog plasma. It has the following composition:

Na	103.8 mEq./1/	HCO_3	25.0 mEq./1.
K	2.5	PO_4	3.0
Ca	0.9	SO_4	1.8
Mg	1.2	gluconate	1.9
		Cl	74.5

Glucose in the original solution was eliminated as per Szent-Gyorgyi (44), as the glucose tended to make preparations irritable. The solution was made up as follows:

Four stock solutions were prepared, containing (1) 21.2% $NaCl$; (2) 5.25% $NaHCO_3$, 0.89% Na_2HPO_4; (3) 1.70% KH_2PO_4, 3.70% KCl, 7.39% $MgSO_4$, 2.30% Na_2SO_4 (anhydrous); (4) 10% calcium gluconate. For 500 ml. of solution, 10 ml. of (1) was introduced into a 500 ml. flask, then 20 ml. of (2) added. The solution was diluted to near the full volume, then 2 ml. each of (3) and (4) were added and the mixture made up to the mark with water. The solution had a turbid white appearance due to the insolubility of the calcium ion. This was remedied by bubbling through the solution a mixture of 97% oxygen and 3% carbon dioxide. This gas mixture was also continuously bubbled through the perfusion cannula and the outer chamber as well throughout the duration of the experiments.

The preparation, placed in the perfusion chamber with gas bubbling was allowed to contract at the rate of 1-2/minute for one-half hour to promote oxygenation and adequate perfusion. It was found that if the preparation was allowed to contract in a non-isometric fashion during the time required for perfusion, and the fluid frequently (4-5x) changed during this period, that the "Treppe" effect was most marked.

It is clear that the second ligature is in effect a Stannius II ligature, and ventricular beats should occur only through either an "escape" mechanism or an inadequate ligature.

The escape mechanism was obviated by reducing idioventricular irritability by the removal of glucose from the solution, and conducted supraventricular stimulation was removed when necessary by thermocoagulation of the atria with a heated soldering iron. Small pieces of heavy paper were first wrapped around the preparation to prevent excessive burning of the ventricules, the preparation was revolved, and the soldering iron held steady. This last procedure was the most critical of all, failure adequately to suppress spontaneous cardiac rhythmicity invalidating results, particularly at the lower frequencies, where spontaneous rhythms were most likely to emerge.

A fair number of preparations, however, did not show ventricular responses to supraventricular stimuli, and the experments proved satisfactory without atrial thermocoagulation.

The procedure followed in most experiments was to allow perfusion, then to determine the frequency optimum and also to demonstrate the sharpness of the "Treppe". If the latter seemed satisfactory, the conclusion was drawn that a satisfactory stage of perfusion had been reached. If this was not the case, further perfusion, up to two hours, was performed. If the "Treppe" effect was not clearly marked, after the prolonged perfusion, it was generally found to be due either to retained coagulated frog blood (which could be seen after dissection of the heart) or to imbalance in the recording apparatus. The "Treppe" was seen to

develop with greater intensity if the bridge amplifier was then properly rebalanced.

III. Critique of Experimental Design.

Although for short-term perfusion experiments the experimental apparatus and design appeared quite adequate (in spite of several critical manual adjustments, which, however, required only time in which to acquire precision) several fundamental flaws become obvious when the procedure is used for longer term observations and when quantification of results is desired.

(1) With the apparatus as described, and with manual adjustment of the tension of the unstimulated ventricle, fair comparison between two or more hearts is impossible, each heart being required to be its own control. In addition, quantitative estimation of actual tensions developed from recorded strain gauge voltages either in different hearts or in the same heart is inaccurate. In the same heart, however, differences in voltage, and thus in tension, can be derived, but this was not found to be of great utility in expressing the experimental results.

(2) Although those muscle fibers which contribute to the tension as expressed in the movement of the recording oscillograph do not greatly change their lengths upon stimulation, and thus the contractions can be thought of as nearly isometric, it must not be supposed that the contractions are isovolumetric, as indeed, muscle fascicles not involved in the recording do move laterally, producing a reflux of perfusion fluid, and

thus oxygen and metabolite exchange. Further, continued observation during long periods of experimentation indicated that the ventricle generally underwent a globular transformation during perfusion, no longer appearing "heart-shaped", but rather soggy and round. That this is a function of perfusion is confirmed when serum is added and the ventricle is seen to resume its former shape rapidly. Here again the subtle transformation in fiber length, with the resultant change in resting tension interferes with the isometric characteristics of the record.

(3) Continuous bubbling of the oxygen-carbon dioxide gas mixture produced recording artifacts at high amplifications, which render measurements of curve peak values difficult. The vibration error was not obvious, however, at the lower amplifications which were used for most of the recordings.

(4) A basic defect in the cannula used in these experiments was the necessity for continuous cardiac contraction to effect perfusion fluid exchange. The more rapid the contraction, the more frequently was the fluid exchanged, and the currents set up in the larger, upper, portion of the cannula assured fresh perfusate. However, as the contractions increased in rapidity, pari passu, oxygen availability increased. In the ranges of contraction frequencies utilized in this experimental series (1/10 - 3/second) the requirement for air-saturated Ringer's solution was 1-2 cc./minute as we have calculated. Observations

on the volume of the frog's heart, as well as consideration of the increased oxygen pressure in the perfusate supplied by the high-oxygen mixture will lead to the conclusion that the preparation is fairly well oxygenated at rapid rates. At low rates, however, or at rest, the lack of fluid exchange overbalances the reduction in oxygen requirements and the preparation is hypoxic. Therefore, no conclusions concerning the effect of variable oxygenation on the early portions of the classic "Treppe" effect can be drawn, as these can readily be ascribed to hypoxia. Other data, on more fully oxygenated ventricular strips, indicate, however, that anoxic effects are minimal in the early stages of the "Treppe". (61) The data reported in this paper are from records taken after an adequate period of rapid stimulation was used subsequent to the originally slow perfusion rate.

(5) The bridge amplifier showed a disturbing tendency to stray from balance during the course of the experiment. Attempts were made to control this effect by prewarming all equipment for one hour before use.

(6) It could be assumed that the transducing system developed voltages within the limits of linearity for the accuracy desired in this experimental series. However, during the contraction which approached maximal tension the platinum rod coupling the ventricle to the strain gauge was seen to bend slightly. This,

too, adds to the difficulty in quantitative interpretation of results, but does not detract from the qualitative comparison of the recordings.

IV. Experimental Results.

A. Spontaneous Rhythms. (Figure I)

Recording was generally not attempted during the period of early perfusion. However, immediately upon completion of one-half hour of perfusion with Boyle-Conway solution saturated with 97% O_2-3% CO_2 isometric recording without artificial stimulation was attempted. During this period various spontaneous contractions were noted, of some interest. Most of these were arrhythmic and displayed little periodicity. (1-B) Others showed remarkable regularity and displayed a rising staircase. Of greatest interest was the alternating rhythm demonstrated throughout almost all the experiments. (1-A) This consisted of a small, sharp contraction, followed by a contraction which developed greater tension, but succeeded by a relatively long diastolic interval. There are at least two ways of classifying this rhythm under staircase phenomena. The first would be to assume that due to the closeness of the second beat in time it is of necessity larger. Another explanation would be that the increased tension developed by the second beat is a manifestation not only of the short inter-stimulation interval but also of post-stimulation potentiation which may be a separate phenomenon.

When the frequency of stimulation was lowered to circa one stimulus each 5-6 seconds, the preparation often responded in non-phasic fashion, such responses often continuing when stimulation was removed.

B. The "Treppe" Effect.

If isometric recording was begun immediately after the cannulation and before perfusion was completed, no "Treppe" effect could be seen. The "Treppe" effect gradually developed throughout the period of perfusion, provided the preparation had been washed as described.

If pauses as long as 15-20 minutes were allowed to occur in the stimulation of the frog heart preparation, the oscillographic record showed, first, what appeared to be abortive contractions manifested only by slight elevations in the base line, followed by arrhythmic and often coupled contractions, each pair showing the development of increased tension, and finally by the development of contractions of incremental tension, in phase with the stimulation frequency. (Figure III, 3-B) Thus, although the voltage used was supraliminal, the response of the total frog heart system was not initially in phase with the stimulus frequency. It thus seems plausible that not only is the contractile system subject to frequency effects, but the stimulus transmitting or generating system itself and its coupling to the contractile mechanism is facilitated by previous stimulation.

After shorter pauses, the first contraction is not manifest by a variation in baseline height but begins immediately in phase with the stimulus frequency. Out-of-phase phenomena occur only with increases in frequency above the optimum frequency. (Figure III, 3-A)

If the pause is made much smaller (within the order of 1-2 seconds) the first beat following the pause is greater in tension than the succeeding beats, which themselves show the incremental staircase. (Figure III, 3-D) This is a manifesta- of rest potentiation, as described by Rosin and Farah.

When the frequency level is changed from a lower frequency (one stimulation every 3-4 seconds) to a higher frequency (one stimulus per second) and the higher frequency is either the optimum frequency or below it, the contractions occurring at the higher frequency range manifest an incremental staircase. This demonstrates that the staircase is not in itself dependent on a period of complete lack of stimulation for its appearance. (Figure IV, 4-D)

When the frequency of stimulation is lowered, and the upper frequency is either the optimum frequency or below this value, the contractions occurring at the lower rate of stimulation are decremental until a stable value is reached. This value will depend on the difference in frequency, a change to a relatively low frequency of stimulation requiring several minutes for stabilization of contraction intensity. (Figure IV, 4-D)

If, after stimulation at the optimum frequency, stimulation was changed to an extremely low frequency (1/10/second) an estimate of the decrease in the "favorable state" (44) could be obtained and shown graphically. This was analagous to the decremental relationship of tension with frequency at frequencies below the optimal. (Figure IV, 4-C)

C. Optimal Frequency.

For every experiment, there exists a frequency at which the isometric tensions developed are maximal. Below this frequency the equilibrium-developed tensions are directly related to the frequency, while above this frequency developed equilibrium tension is inversely related (Figure IV, 4-B)

When the frequency of stimulation is raised above the optimum frequency, there results, in addition to early nonphasic response and decrease in steady-state developed tension, an incomplete <u>relaxation</u> manifested by a rising base line. With increasing frequency over the optimal frequency, as the developed equilibrium tension falls, so the baseline rises, showing decreased relaxation, until, as the frequency is further raised, the preparation is no longer responsive. These phenomena resemble summation and tetanus, but examination of the curves reveal that the incomplete relaxation does not appear to be homologous to tetanus in skeletal muscle. (Figure IV, 4-A, 4-B, Figure II, 2-B).

The optimal frequency varies for each experiment. As the temperature was generally 20-27° C., it would be expected that the optimum would be at high levels. . Most often it remained at one stimulus/second, but occasionally fell to one stimulus/2 seconds.

D. Effect of Digitoxin.

Five frogs were prepared and perfused in the stated fashion, their frequency optima were obtained, and then varying doses of

digitoxin were added to the perfusion medium in 0.25, 0.5, 1.0, 1.5, 2.0 microgram/milliliter concentrations, each application of higher concentration separated from the others by five washings with Boyle-Conway solution. In the higher dosage groups (1.0, 1.5, 2.0 micrograms/milliliter) digitoxin had the effect of flattening the "Treppe" at frequencies below the optimum, and thus rendering contraction tension relatively independent of frequency. Above the optimum frequency, the height of developed tension was not markedly affected, but the tendency toward incomplete relaxation and elevation of the base line was reduced for frequencies near the optimum frequency (Figure V)

E. Effect of Human Serum

Nine frogs were prepared and perfused with Boyle-Conway solution in the stated fashion, the frequency optima were obtained and the staircase phenomena were demonstrated. When the effect was considered to have been well demonstrated human serum diluted with distilled water to isotonicity with frog plasma was placed in the perfusion cannula, and the preparation stimulated. Sera used were obtained from three separate healthy donors, and were tested on three separate preparations on the day obtained. At frequencies below the optimum frequency it was observed that the replacement of Boyle-Conway solution by diluted serum rendered tension independent of frequency over a considerable range. At frequencies above the optimal frequency, tension was still decremental with increased frequency. There was manifest lowering of the base line of contraction, which

was normally expected to be elevated in these frequency ranges. Washout was effective, both with serum and digitalis in restoring the pre-mixture conditions, with the progressive return of the baseline to normal in the higher frequency ranges, and the redevelopment of the "Treppe" in the lower frequency ranges. In addition, a powerful inotropic effect was noted upon the addition of serum, digitalis, and levophed (norepinephrine), which was not related to the "Treppe", and which required a change in amplification to keep on the record. (Figure VI, Figure VII)

V. Discussion.

The Bowditch phenomenon or "Treppe" can most conveniently be considered as one manifestation of a complex relationship existing between frequency of contraction and developed tension of myocardial fibers. That it is not the only accompaniment of frequency change is obvious upon the examination of the work of previous investigators, as well as from the work detailed in this paper. A theory of frequency-tension relationships must include, among others, the following developed facts:

(1) Developed tension of myocardial fibers is relatively stable over wide ranges of frequency in the presence of serum, and changes occur only after perfusion for fairly long periods (44).

(2) An optimum frequency exists, above which and below which developed tension in response to a supraliminal stimulus

decreases. At frequencies above optimum relaxation becomes progressively less complete, and a limit of response is reached when the heart may be said to enter into contracture (32); Figure IV).

(3) Below the optimum frequency, increments in frequency result in increments indeveloped tension and in duration of contraction. The action potential is also increased in amplitude and duration, although its time-to-maximum is not changed. Above the optimal frequency, increments of frequency result in decrements of developed tension and duration of contraction, as well as in decrements of the action potential amplitude and duration (38).

(4) Following stimulation at frequencies above a steady-state frequency, succeeding contractions at the steady-state frequency are potentiated (post-stimulation potentiation). (52, 53) Interpolated stimuli have a similar effect (55, 56).

(5) Following a long pause, contractions at constant frequency in response to uniform supramaximal stimulation show a progressive rise in developed tension to a steady state. As the period of rest is shortened, the first contraction following the pause is relatively greater than the first contraction following the longer pause. As the period of rest is shortened still further, however, the first contraction following the pause is greater in developed tension than succeeding contractions (rest potentiation).

(6) Reduction in potassium concentration of the perfusate, elevation of calcium concentration, addition of lipoid material and soaps, and monopalmitoyl lysolecithin, the addition of digitalis bodies, desoxycorticosterone-like steroids, and epinephrine, as well as the addition of serum, all render tension independent of frequency over rather wide ranges (44, 58, 59, Figure VI).

(7) Acetylcholine promotes degeneration of the "favorable contraction state" and sensitizes heart muscle to the effects of hypercapnia (44).

(8) The above observations relate specifically to the relationship between frequency and developed cardiac tension. These effects must be distinguished from the so-called "hypodynamic state" which develops independently of frequency, and can also be reversed with the addition of serum.

The investigations described in this paper do not lend themselves readily to consideration of the mechanism of the "Treppe" effect. It is of interest, however, to examine the possible significance of the observed phenomena for the understanding of some mechanisms of the control of the cardiac force.

Wiggers (65) has reviewed in detail the historical aspects of the development of the conception of cardiac function and control. Frank (66) in 1895 recorded isometric and isotonic contractions of frog hearts, and demonstrated that developed

tension was related to resting length similar to the effect in skeletal muscle. Patterson, Piper and Starling (67), by means of investigations using the heart-lung preparation enunciated a principle of cardiac control as follows:

> "We...find no connection between the diastolic tension and the succeeding contraction, though as a rule these two quantities will be altered together. But we do find a direct proportion between the diastolic volume of the heart (i.e. the length of its muscle fibers) and the energy set free in the following systole.
>
> "The Law of the Heart is, therefore, the same as in skeletal muscle, namely that the mechanical energy set free on passage from the resting to the contracted state depends on the area of 'chemically active surfaces' i. e. on the length of the muscle fibers."

Wiggers and Katz (68) repeated Starling's experiments with improved techniques, and confirmed his results with regard to the concept that increase in stroke volume was attended with increase in diastolic volume.

Rushmer (69) has stated some rules which had been held fundamental to the explanation of the control of stroke volume:

> "(a) The cardiac output is controlled by the venous return. (b) If the heart rate is constant the stroke volume is determined by the venous return. (c) Stroke

volume of the ventricles depends directly upon the diastolic filling. (d) The tension of the myocardial fibers depends directly upon their length. (e) Diastolic filling and diastolic volume of the ventricles is determined by effective filling pressure. (f) The mechanical energy set free on passage from the rested to the contracted state depends on the length of the myocardial fibers. (g) The tension developed during contraction depends on the initial length of the myocardial fibers."

Sarnoff (70) and his associates have developed the Starling concept further to demonstrate that there is not a constant relationship between stroke work and diastolic filling but that this varies and one may produce a family of "Starling-Frank curves" by, for example, coronary occlusion or addition of epinephrine. It was thus made clear that the Starling hypothesis was the result of experimentation under restricted conditions, and that the data were not controlled for variables which were, in effect, determining cardiac output and stroke volume by adjustment of what Rushmer has called the "contractility" of the heart. Experiments with clased-chest, intact, unanesthetized animals (72) revealed basic incompatibilities with the hypothesis of the control of stroke volume by length alone, and led to

consideration of the alternate views of Sarnoff (71).

Rushmer has presented some of the factors which affect cardiac "contractility" (69).

Myocardial tension is affected, in addition to fiber length, by "the condition of the myocardium" as affected in turn by coronary oxygen supply, epinephrine, acetylcholine, autonomic stimulation, and a host of unknown factors and metabolites. Tension is also affected by viscous and elastic resistance to contraction.

In addition, the Law of Laplace, the effect of which is that greater contractile tension is required to sustain a given ventricular pressure as the radius of the myocardial chambers is increased, is of significance. The advantages of eliminating viscosity and increasing resting length which were gained by increasing diastolic volume are balanced against the greater tension necessary for filling, and for contraction.

Rushmer summarizes his discussion with the following statement:

> "As a working hypothesis, it is proposed that certain factors which tend to produce an increase in heart rate may also act simultaneously to augment stroke volume, so that effective filling pressure is maintained at relatively low levels, unless the cardiac output fails to meet the circulatory demands."

It is at this point, i. e. in the failing heart that Rushmer sees the applicability of the Starling hypothesis and other data derived from the "failing" heart-lung preparation.

Thus, during cardiac contraction under normal conditions, it is the "condition of the myocardium" which leads to tension variation, this condition determined not only now by length, but also by oxygen supply and humoral influences. As we have seen throughout this paper, frequency also affects the "condition of the myocardium" in a profound way and tension in the perfused heart is a function of frequency.

It is known that an almost universal effect of heart failure is increase in heart rate (73). If the chief determinant of cardiac tension were resting fiber length and, therefore, diastolic volume, this reflex tachycardia would be disadvantageous to the circulation in that diastolic filling time would be decreased (74). If, however, the "Treppe" effect were in evidence in heart failure, it would appear as if the reflex increase in frequency in and of itself were a compensatory mechanism, and, by increasing cardiac force increased cardiac output despite decreasing diastolic inflow time.

The observations reported in this paper to the effect that at frequencies above the optimum incomplete relaxation of the whole heart occurs, and the addition of digitalis in "therapeutic" doses or of serum results in more complete relaxation of

the myocardium, is useful in our consideration of this effect. The reflex tachycardia (which we have viewed as a compensatory affect) is useful only if the rate were to remain at or below the frequency optimum. If it rose above this optimum, further rises would result both in decreased developed tension and incomplete relaxation. The presence of digitalis facilitates stroke volume by allowing an increased use of the length-tension relationships and cancelling the frequency effects.

Assuming, then, that an etiological factor in heart failure is a deficit of those humoral factors essential in maintaining the independence of frequency and tension, low cardiac rates would be extremely disadvantageous. The reflex tachycardia is compensatory in accordance with the now active "Treppe" effect and is so not only for the circulation (i.e. the minute volume) but for the heart itself. If the compensation is overshot, and that would imply an increase of rate over the optimum frequency, not only is the diastolic filling compromised by a shorter filling time, but ventricular relaxation would be incomplete as well. The presence of digitalis, however, eliminates incomplete ventricular relaxation at high cardiac rates, and also has the effect of eliminating the "Treppe" effect at lower rates. The fact that digitalis restores

cardiac rate to its pre-failure levels by intracardiac block would be disadvantageous to the cardiac output, were not its use accompanied by the simultaneous abolition of the "Treppe".

It is thus possible that a significant portion of the effect of digitalis may be manifested through the "Treppe".

SUMMARY

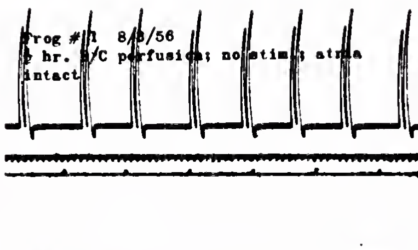
The relationship between cardiac frequency and developed tension was studied with the isometric whole perfused frog heart preparation as a model system, prepared after the technique of Szent-Gyorgyi and Hajdu (44). The effects of non-stimulation, variable frequency stimulation, length of pauses, superstimulation, and of digitoxin, human serum, and norepinephrine were determined.

Data of previous investigators were either confirmed or denied in the species used, and it was shown that at frequencies above the optimum incomplete cardiac relaxation occurs, eliminated by serum, norepinephrine, and digitoxin.

The major observables were systematically described, the literature of the "Treppe" effect since 1871 was reviewed, the reflex tachycardia of heart failure was discussed as a cardiac, as well as a circulatory, compensatory adjustment.

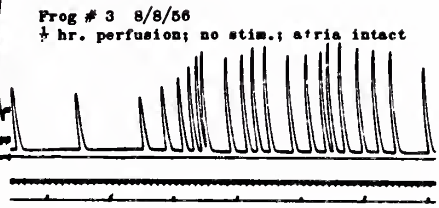
APPENDIX

1-A



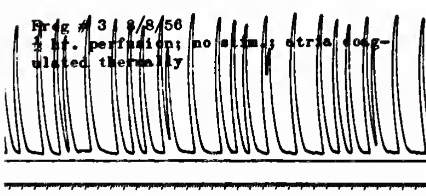
Bigeminally coupled alternating rhythm without summation, spontaneous.
Time line = 1 and 10 seconds.

1-B



Spontaneous irregular contractions with occasional summation and treppe.
Time line = 1 and 10 seconds.

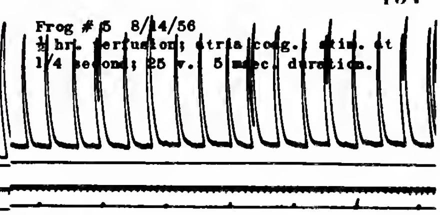
1-C



Spontaneous irregular contractions at steady state, with summation.
Time line = 1 and 10 seconds.

1-D

437



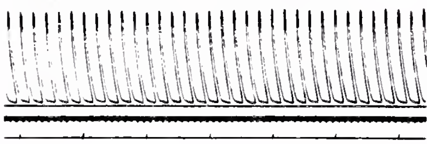
Spontaneous bigeminal coupling, with alternation and summation, superimposed upon steady state electrical stimulation.
Time line = 1 and 10 seconds.

Figure I

Spontaneous Rhythmicity

2-A

Frog # 6 8/14/56
½ hr. perfusion; atria coagulated
stim. at 1/sec.; 25 v.; 5 msec. duration

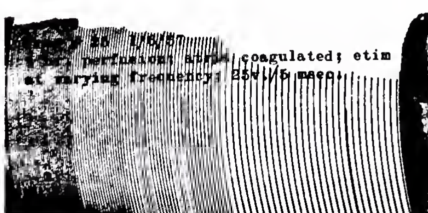


Steady state contraction under electrical stimulation.

2-B

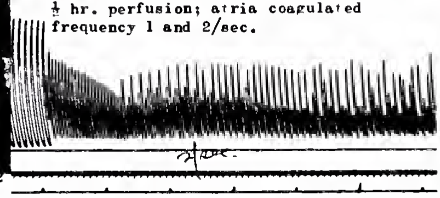
Frog # 6 8/14/56
½ hr. perfusion; atria coagulated
stim. at 1.5 and 2/sec.; 25 v; 5 msec

Change in amplitude with frequency increment; oscillograph at high speed.



Treppe phenomenon below optimal frequency. Oscillograph at slow speed.

2-D
Frog # 7 8/15/'6
½ hr. perfusion; atria coagulated
frequency 1 and 2/sec.



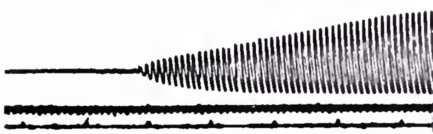
Decrement in developed tension, decrease in relaxation, and uncoupling with alternation at frequencies above optimum. Time line = 1 and 10 sec.

Figure II

Steady State, Recording Effects, and Uncoupling

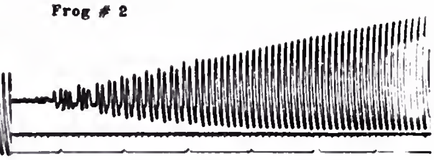
093

3-A
Frog #2 8/3/56
½ hr. perfusion; atria coagulated; stim. at 1/sec; 25 volts; 5 msec. duration



Classic treppe effect after moderate pause; note increased developed tension and degree of relaxation.

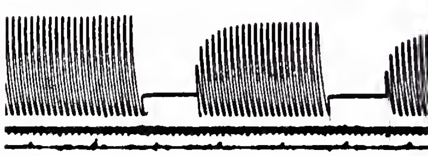
3-B
Frog # 2



Time line = 1 and 10 seconds.
Very fine lines = 1/5 second.

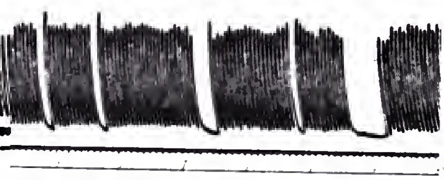
Classic treppe effect after 20 minute pause; note phases of beginning of contraction as discussed in text.

3-C
Frog # 2



Effect of short pauses on treppe effect. Note rapid rise to steady state.

3-D
Frog # 2

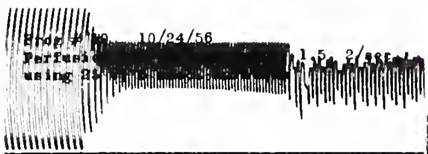


As pause shortens rest potentiation is shown. As pause lengthens, first contraction following decreases in amplitude.

Figure III

Effect of Pause in Stimulation upon the Treppe Phenomenon

4-A

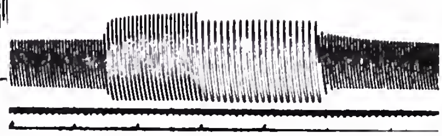


Time line = 1 and 10 seconds.

As frequency is shifted from 1 to 1.5/second, contractions are decremental in developed tension and in relaxation, and are uncoupled and alternating in relaxation until steady state is reached. As frequency is further elevated, complete uncoupling occurs.

4-B

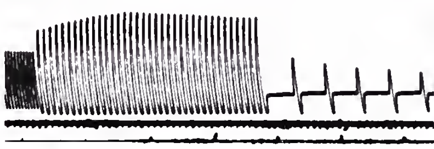
Frog # 10



1.5/second is demonstrated as the optimal frequency in this record.

4-C

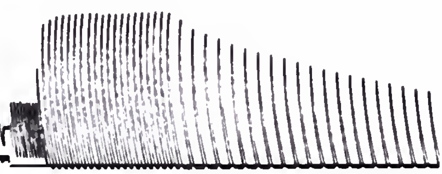
Frog # 10 10/24/56



Stimuli of 1/10 second frequency demonstrate waning of "favorable" contraction state.

4-D

Frog # 1^



1/second is optimum. As frequency is lowered from 2/sec., tension is incremental, as it is lowered from 1/second, tension is decremental

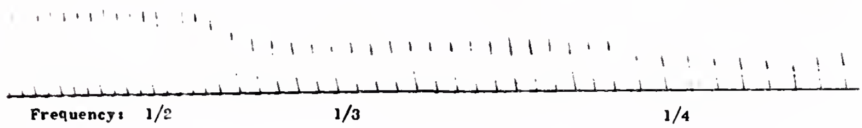
Figure IV

Optimal Frequency Phenomena

Figure V
Effect of Digitoxin on Treppe

5-A
Frog # 8 8/17/56
perfusion for 1 hour; atria coagulated;
stim. at varying frequency with 25v.
for 5 msec.

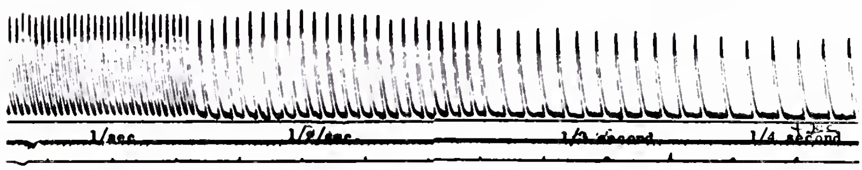
5-B



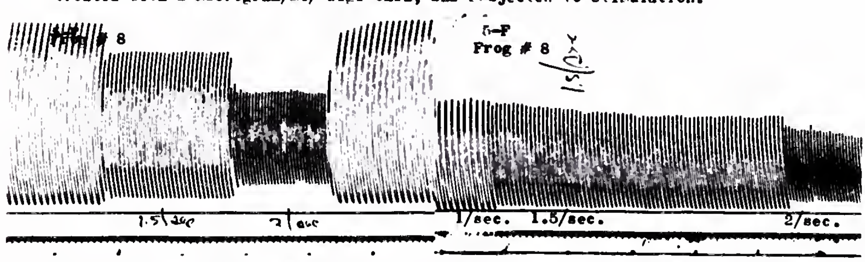
Treppe effect below frequency optimum in perfused, isolated, frog hearts subjected to stimulation at decremental frequency. Observe both steady state and transient decrements in contraction amplitudes (developed tension).

5-C
Frog # 8

5-D
Frog # 8



Treppe effect below frequency optimum in isolated, perfused, frog hearts treated with 2 microgram/ml/ digitoxin, and subjected to stimulation.



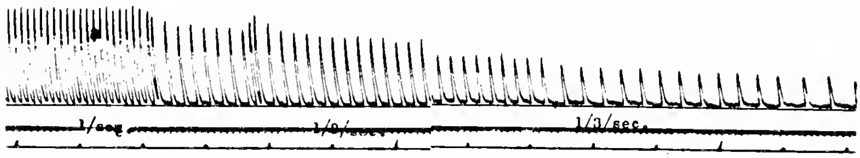
Treppe effect above optimum frequency

Treppe effect above optimum frequency with 2 micrograms/milliliter digitoxin.

Figure VI
Effect of Serum on Treppe

6-A
Frog # 17 10/21/56
¼ hour perfusion; atria coagulated;
no added serum.

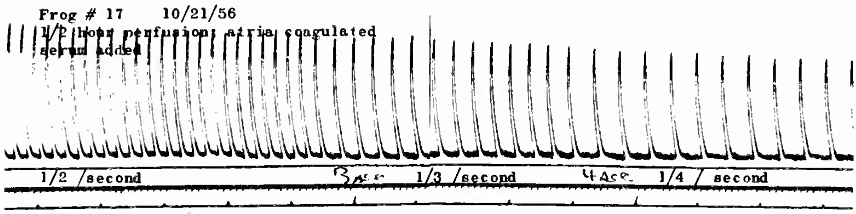
6-B
Frog # 17 10/21/56



Decremental tension with decremental frequency in perfused frog heart. Note emergence of spontaneity, in spite of atrial coagulation.

6-C
Frog # 17 10/21/56
1/2 hour perfusion; atria coagulated
serum added

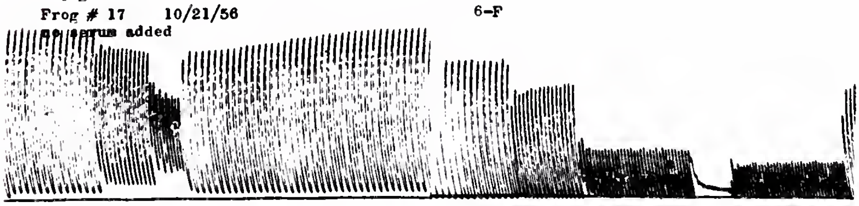
6-D



Flattening of treppe with decremental frequency stimulation after addition of serum diluted to isotonicity with frog plasma, to perfusate.

6-E
Frog # 17 10/21/56
no serum added

6-F



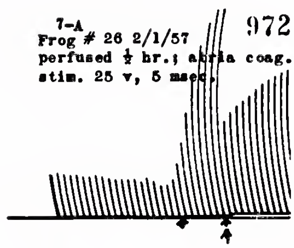
Effect of stimulation above frequency optimum without added serum

Addition of serum to perfusate, and stimulation above frequency optimum

Figure VII
Effect of Levarterenol

7-A
Frog # 26 2/1/57
perfused ½ hr.; atria coag.
stim. 25 v, 5 msec.

972



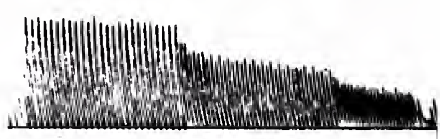
Single arrow marks point of addition of 2 microgram/ml. levarterenol. Double arrow marks point of halving amplification.

7-B
Frog # 26 2/1/57

7-C

Frequency above optimum, at height of levarterenol potentiation of tension.

7-D
Frog # 26
2/1/25



Continued development of levarterenol effect

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