A comparison of alternation in myocardial action potentials and contractility

JOSEPH F. SPEAR AND E. NEIL MOORE
Department of Physiology, School of Medicine, and Comparative Cardiovascular Studies Unit, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Spear, Joseph F., and E. Neil Moore. A comparison of alternation in myocardial action potentials and contractility. Am. J. Physiol. 220(6): 1708-1716. 1971.—The transmembrane potentials and isometric force of contraction were simultaneously recorded from the isolated papillary muscles of the cat, rabbit, guinea pig, and rat right ventricles, and from frog ventricular trabeculae. The pattern of behavior of the mammalian myocardium in response to rate, rhythm, and temperature changes argues that the basis for mechanical alternation is not alternation in the action potential. Alternation in the action potential appears to occur secondarily to rate, rhythm, and temperature changes. The influence on the action potential of the underlying processes causing contraction and relaxation. In contrast to the mammalian myocardium, alternation in the mechanical activity of the frog myocardium appears to be due entirely to primary alternation in the action potentials.

Excitation contraction coupling; pulsum alternans; cardiac muscle membrane potentials; cardiac muscle contractility; sarcoplasmic reticulum; intracellular stimulation; myocardial excitability; premature activation

Alternation in the force of contraction can be induced in the isolated ventricular myocardium by increasing the rate of stimulation above a certain rate threshold value. The rate threshold for alternation can be decreased and the degree of alternation can be increased by various means including lowering the temperature (14), decreasing the calcium concentration in the external medium (2, 14), applying various drugs (2, 14) and interpolating an extra beat or pause in the regular rhythm (17). In a recent paper, Lu et al. (14) presented data from the cat and guinea pig myocardium showing that the transmembrane action potentials and contractions are always discordant when both electrical and mechanical alternation are seen, viz., the shorter duration action potential is associated with the smaller contraction. They suggested that the electrical alternation is primary and is the cause of the mechanical alternation. There are studies demonstrating that some cases of pulsum alternans which are seen clinically and experimentally in wholeheart preparations are caused by an alternating ventricular end-diastolic volume and the Frank-Starling mechanism (5, 15). It has been suggested that alternation in the isometric force of isolated papillary muscles is also due to the Frank-Starling mechanism. That is, that alternate failure of the contractile elements to completely relax against a series elastic element causes the following contraction to be smaller, since it starts at a shorter initial contractile element length (15). Other authors have attempted to refute this by showing that alternation can occur without visible changes in the end-diastolic force in the dog papillary muscle (6, 17). Also in conflict with this view is the fact that stretching an alternating papillary muscle beyond the peak active-force point of its length-force curve does not change the relationship between end-diastolic force and peak force (unpublished observation). In the case of the stretched muscle, a contraction starting from a shorter initial contractile element length, according to the argument, would be expected to give rise to a contraction of greater peak force. However, the stretched alternating muscle still shows contractions of smaller peak force arising from the larger end-diastolic force. Under normal conditions alternation has not been demonstrated in frog or turtle ventricle. However, in hypertonic solutions or in the presence of certain drugs, alternation has been induced in which the action potential and contraction are concordant (12, 14). In these cases the evidence indicated that the alternation was associated with the alternate failure of some cells to be activated.

In the present report the behavior of alternation in the action potential and contractile force of mammalian and frog myocardium was studied. The findings in the mammal indicate that alternation in the action potential occurs secondarily to alternation in the contractile force. Alternation in the isometric force appears to have its basis in alternate incomplete relaxation causing alternate decrease in the inotropic state of the muscle. In contrast to the mammalian myocardium, alternation in the contractile force in the frog ventricle depends on alternation in the action potentials.
MATERIALS AND METHODS

The experiments were carried out on papillary muscles obtained from the ventricles of 10 rabbits, 10 guinea pigs, 2 cats, and 11 rats, and strips of trabeculae carneae from 10 frogs. The mammals were either anesthetized by ether or stunned by a blow on the head. The frogs were pithed. Papillary muscles or trabecular muscles were removed from the hearts and secured in a muscle bath. Using 5-0 silk suture, one end of the muscle was secured to a force gauge by a snare and the other end tied to a steel connector and anchored to a mechanical ground.

The total volume of the muscle chamber was 3.0 ml. Physiological salt solutions at controlled temperatures were continuously passed through the muscle chamber at a rate of 10 ml/min, and discarded after overflowing out of the chamber. The composition of the Tyrode solution in millimoles per liter was: NaCl, 137.0; NaHCO₃, 12.0; dextrose, 5.5; KCl, 2.7; MgCl₂, 0.5; NaI₃PO₄, 0.9; CaCl₂, 1.0 and 1.5; the pH was 7.2. The composition of the amphibian solution in millimoles per liter was: NaCl, 101.0; NaHCO₃, 8.8; dextrose, 4.0; KCl, 2.0; MgCl₂, 0.4; Na₂HPO₄, 0.7; CaCl₂, 2.0; the pH was 7.1. The temperature of the bathing solution was monitored near the muscle by means of a thermistor probe, and maintained constant at values between 20 and 35 C. The physiological solution was oxygenated with a mixture of 95% oxygen and 5% carbon dioxide in a reservoir, and also in the muscle chamber at a location 2 cm upstream from the suspended muscle. To ensure equilibration of the isolated muscle with its environment, the experiments were begun 2 hr after the mounting of the preparation in the bath.

Electrical stimulation was usually applied to the surface of the muscle by Teflon-coated silver punctate electrodes. In one series of experiments, however, stimulation was delivered to the muscle through mass platinum electrodes located in the bath on each side of the muscle. The stimuli were biphasic and delivered by a Grass model S4E stimulator through a Grass model SIU-4B stimulus-isolation unit.

The isometric force of contraction across the whole muscle was measured by a Honeywell myocardial-force gauge and a 105-7 gauge control unit. The natural frequency of the system was 130 cycles/sec, and the oscillations were damped to one-half amplitude in 32 msec. The compliance of the system was 0.025 μ/dyne and was primarily due to the force gauge. In these experiments, the preparations were maintained under a resting preload of 200 dynes.

The transmembrane potentials of the muscles were measured by standard 3.0 mM KCl glass microelectrodes. The microelectrode was coupled to the input of a Biotronik cathode follower amplifier which had an input resistance of 10¹² ohms, and was input capacity neutralized. The resistance of the microelectrodes varied between 20 and 30 megohms. The empirical time constant of the system including the microelectrode was 0.8-1.0 msec, and the peak-to-peak noise level was usually 0.5 mv.

In some experiments the excitability of the preparation was assessed by measuring the threshold stimulus intensity to elicit a response. The test stimulus was delivered through an intracellular microelectrode and the response recorded from the same microelectrode. The circuitry for switching the microelectrode between the stimulus and record modes has been described previously (16).

All recordings were displayed on a Tektronix type 561A oscilloscope. The traces were photographed from the face of the oscilloscope on 35-mm film using a Grass model C4D kymograph camera.

RESULTS

Mammalian Myocardium

Effects of stimulation rate and bath temperature on alternation. In each of 51 papillary muscle preparations from cat, rabbit, guinea pig, and rat ventricles there was a certain rate threshold which was characteristic of the species and above which there was alternation in the isometric force of contraction. When the rate of stimulation was suddenly increased to a value greater than threshold, the peak isometric force immediately began to alternate after the rate change, and the peak isometric force of both the small and large contractions increased as the alternation progressed to a steady-state. At steady state the degree of alternation was less than initially, and the alternation remained quite constant at this level as long as the rate of stimulation was maintained. If the rate of stimulation was increased to a level just less than threshold, transient alternation was seen but this disappeared as the steady state was reached. The studies to be described in this paper were carried out during steady-state alternation. The threshold rate for alternation was defined as that rate of stimulation which just maintained a 5% degree of alternation in the peak isometric force of contraction at steady state.

The qualitative aspects of the time courses of alternating contractions were similar in cat, rabbit, guinea pig, and rat papillary muscles. An example taken from a rabbit preparation is shown in Fig. 1. In the figure are records of the isometric force of contraction during steady-state alternation in the same preparation at three rates of stimulation. In A the muscle was stimulated at 2.2/sec, in B at 2.8/sec, and in C at 2.9/sec. Notice that the contraction of smaller peak force started from a greater end-diastolic force than the larger contraction, that is, there was incomplete relaxation preceding the smaller contraction. The maximum rate of force development during the smaller contraction was always less than during the larger contraction. Also, the faster the rate of stimulation during alternation, the greater the discrepancy between larger and smaller contractions with regard to peak force, end-diastolic force, and maximum rate of force development. The appearance of alternation was uninfluenced by changes in the stimulus intensity, and, therefore, alternation probably was not due to alternate inactivity of some fibers.

The different species studied under similar conditions had characteristic rate thresholds for steady-state alternation, and this rate threshold was markedly influenced by the temperature of the bathing medium. In Fig. 2 are plotted rate thresholds for alternation at temperatures between 20 and 35 C. The data were obtained from experiments on two cat, five rabbit, three guinea pig, and...
one rat papillary muscles. Each point represents one measurement. For each of the species the higher the temperature, the faster the myocardium had to be paced to induce alternation. However, at all temperatures the myocardia of the species with the shorter normal contraction durations had to be stimulated faster to induce alternation. In these experiments the rate threshold for a given species was found to be independent of the muscle diameter of the preparation.

Other investigators have noted that the calcium concentration of the bathing solution influences the rate threshold to induce alternation (2, 14). This was also verified in the present studies. All of our experiments were repeated at two different calcium concentrations (1.0 and 1.5 mM). The findings were similar with both concentrations except that at the higher calcium concentration the rate threshold to induce alternation was higher.

We found that if the rate of stimulation was increased to just greater than the threshold rate for alternation, alternation in the isometric force of contraction occurred without alternation in the simultaneously recorded transmembrane action potentials. The observation was made in four rabbit, one cat, and nine guinea pig preparations in a total of 470 cell impalements. An example is shown in Fig. 3A for the guinea pig papillary muscle. To the right of the simultaneously recorded action potential and isometric force, the smaller and larger contractions and their associated action potentials are superimposed. Notice that the action potentials are identical. This has also been observed by others (14). The consistency of the observation under these conditions among different preparations from different species argues against the effects being simply due to nonrandom sampling from a population having alternating and nonalternating cells.

Alternation in both the mechanical activity and electrical activity did occur in each of the cat, rabbit, and guinea pig preparations when the stimulation rate was increased further. The relationship between action potential contraction was always discordant for the cat and rabbit papillary muscles at all temperatures below 24.0 C. These observations were made in 300 cell impalements. In Fig. 3B discordant mechanical and electrical alternation in the rabbit papillary muscle is shown. Tracings of the action potentials corresponding to the larger and smaller contractions are superimposed at the right of the record. In all cases the action potentials with the abbreviated plateaus were associated with the contractions of greater peak force. These findings are similar to those of Lu et al.
ALTERNATION IN MYOCARDIUM

(14) for the cat and guinea pig and Greenspan et al. (6) for the dog.

If the temperature of the bathing medium was higher than 24.0°C, the guinea pig papillary muscle was unusual in that it exhibited concordant alternation between mechanical activity and action potentials. This was verified in 200 cell impalements in eight animals. In Fig. 3C a guinea pig papillary muscle was driven at 3.3 sec at 24.5°C. Tracings of the action potentials associated with the larger and smaller contractions are superimposed on the right. While the alternation in contractile force was similar to that seen for the other mammalian species and for the guinea pig at temperatures below 24.0°C, the action potential with the shorter duration and depressed amplitude was associated with the smaller contraction. Notice that the action potentials were very close together, and that the depressed action potentials started from a less completely repolarized membrane. This unusual change in the behavior of the guinea pig papillary muscle from low to higher temperatures may be the basis for the contradictory findings of Hogancamp et al. (8) and Lu et al. (14) since their experiments were carried out at different temperatures.

The rat papillary muscle preparation was interesting since typical alternation in the action potential could not be induced at even very rapid rates of stimulation. This was observed in 100 cell impalements in 10 preparations. In Fig. 3D a large degree of mechanical alternation is shown for a rat papillary muscle. The larger and smaller contractions and their accompanying action potentials have been superimposed at the right. Even during this large degree of mechanical alternation, except for slight differences in the late phase of repolarization, the action potentials were nearly identical.

Effects of premature activations during alternation. Experiments on three guinea pig and three rabbit papillary muscles during steady-state alternation showed that carefully timed premature activations could either increase or decrease the degree of alternation. The findings were consistent that when the premature activation followed the larger contraction of the alternation, then the degree of alternation increased and only gradually returned to control value after several contractions. If the premature activation followed the smaller contraction of the alternation, then the degree of alternation was reduced or eliminated for several contractions. This effect is shown in Fig. 4A for a rabbit papillary muscle. At the first arrow indicated by 1 the preparation was prematurely activated following the larger contraction. At the second arrow indicated by 2 the preparation was prematurely activated following the smaller contraction with a stimulus of exactly the same timing as in 1. Notice that the premature activation following the larger contraction increased the degree of alternation while the premature activation following the smaller contraction decreased it.
Simultaneous records of isometric contractions and action potentials of premature activations during alternation show that the ability of the membrane to generate a full action potential is depressed to a greater degree following the larger contraction than following the smaller contraction. This was demonstrated during 70 cell impalements in one rabbit papillary and four guinea pig muscles. The results from a guinea pig papillary muscle are shown in Fig. 5. Control alternation is shown in A. The preparation was stimulated just fast enough to produce mechanical alternation without alternation occurring in the action potentials. At the first arrow, 1, in B, the muscle was prematurely activated following the larger contraction by abbreviating the cycle length by 74 msec. At the second arrow, 2, in B, the muscle was excited following the smaller contraction by a stimulus of exactly the same timing as in 1. In C are superimposed tracings of the premature action potentials of 1 and 2. Notice that the action potential duration following the larger contraction was depressed to a greater degree than following the smaller contraction. This indicates that the excitable membrane was less completely recovered and earlier repolarization occurred following the action potential associated with the larger contraction.

**Relative refractory period during alternation.** A premature action potential during alternation was associated with a greater increase in the refractoriness of the membrane to stimulation after the larger contraction than following the smaller. This was verified during 20 cell impalements in one rabbit and four guinea pig preparations, using premature stimuli delivered across the whole muscle through mass electrodes, and by stimulation through an intracellular microelectrode. The data of Fig. 6 show a guinea pig papillary muscle exhibiting mechanical but not electrical alternation as in Fig. 5A. The muscle was being driven by mass electrodes. The intensity of the stimulation was just greater than threshold. In Fig. 6A the arrow indicates a premature stimulus interposed just before the impaled cell completely depolarized in association with the larger contraction. The premature stimulus did not excite the muscle. In B, a premature stimulus of exactly the same timing and intensity as used previously was interposed after the action potential associated with the smaller contraction and a response was evoked (at the arrow in B). This indicates that the preparation was more refractory to mass stimulation following the action potential associated with the larger contraction, even though there was no apparent difference between the contours of the action potentials associated with the larger and smaller contractions. Even during discordant alternation when the action potentials with depressed plateaus are associated with the larger contractions, the preparation is still more refractory following the action potentials associated with the larger contractions.

Similar experiments on refractoriness were performed during 25 cell impalements in a rabbit and guinea pig preparation using stimuli delivered through the recording microelectrode. The results from a rabbit preparation are shown in Fig. 7. Two superimposed records of intracellular potentials recorded from the base of the anterior papillary muscle are shown. The basic driving stimuli were delivered through punctate electrodes applied to the right septal myocardium. The stronger contraction was associated with...
that the action potentials are well separated and that the action potentials with the depressed plateau phase are associated with the larger contractions. This is discordant alternation. In B the rate of stimulation was increased to 2.3/sec. Now the action potentials are closer together. The action potential associated with the larger contraction still has the depressed plateau phase but the action potential associated with the smaller contraction has a slightly depressed amplitude. In C at a stimulation rate of 2.5/sec the action potential associated with the smaller contraction is of shorter duration and lower amplitude than that associated with the larger contraction. This is concordant alternation. The phenomenon was consistently repeated during 15 cell impalements in this experiment.

**Alternation in Frog Myocardium**

The frog ventricle was found to behave very differently from the mammalian ventricle when attempts were made to demonstrate the contribution of closely evoked action potentials during alternation in the guinea pig preparation, the stimulation rate of a papillary muscle initially alternating discordantly was gradually increased. This is shown in Fig. 8. In A the preparation was stimulated at 1.5/sec. Notice
to induce alternation. Experiments on 10 frog preparations showed that they had to be stimulated very rapidly to induce contractile behavior vaguely resembling alternation in the mammalian ventricle. The results of increasing the rate of stimulation on the transmembrane potential and isometric force of a frog trabeculum are shown in the three records of Fig. 9A. The transmembrane potentials in the three records of Fig. 9A are from the same cell impalement. Alternation in the transmembrane potentials appeared as seen in the third record at a stimulation rate of 3.3/sec. Alternation in the contractile force also occurred at this rate, and mechanical alternation was never seen in the absence of electrical alternation. Faster stimulation produced block. These phenomena were seen during 60 cell impalements. Alternation in the end-diastolic force was the prominent feature of mechanical alternation in the frog ventricle. The direction of alternation was such that the small action potential was always associated with the contraction arising from the higher end-diastolic force. The small action potential was characterized by reduced amplitude and rate of depolarization and decreased duration. The alternation in peak force was always very small, and in about 90% of the cases, the contraction of reduced peak force was associated with the smaller action potential as in Fig. 9A. However, occasionally the smaller peak force contraction was associated with the larger action potential as in Fig. 9B and there was also occasionally no alternation in peak force during alternation in action potential and end-diastolic force as shown in Fig. 9C. All of these phenomena were demonstrated in normal amphibian solution and they were independent of the intensity of the stimulation. Since changing the intensity of stimulation did not modify the responses, it is felt that they did not have their basis in the alternate failure of some cells to be activated, but that they resulted from alternation in the individual cells of the preparation.

**Discussion**

The present studies demonstrate that there is a loose coupling between the form of the action potential and the force of contraction (Fig. 3). First, with increasing rates of stimulation, alternation in the force of contraction appears before alternation in the form of the action potential. Second, in the guinea pig myocardium, alternation in the force of contraction and action potentials can be concordant as well as discordant. Third, in the rat myocardium even large degrees of alternation in the force of contraction are not accompanied by alternation in the action potentials. Therefore, we do not feel that, during rapid stimulation, the alternation of the action potential is the cause of alternation in the force of contraction in the isolated mammalian myocardium.

Our investigations do support the idea, however, that there are two factors secondary to alternation in the contractile force which influence the action potential and cause it to alternate. First, in most cardiac muscle the plateaus of the action potentials last until the peak of the contraction and, therefore, occur during the time when large changes in the intracellular concentration of free calcium are accompanying contraction (9). This allows the possibility that the increase in intracellular free calcium may have an influence on the time course of the action potential. There is considerable evidence that intracellular calcium does influence the electrical properties of excitable membranes (3, 7, 13, 18). Studies which show that an increase in contractile force is accompanied by an increase in efflux of cellular potassium (4, 21) suggest that mechanisms at the basis of contraction may interact with ionic mechanisms involved with the action potential. Figures 5, 6, and 7 demonstrate that the excitability of the membrane of an alternating cardiac muscle cell is different during the larger and smaller contractions. Following the action potential associated with the larger contraction, a premature stimulus requires more current to excite the muscle (Figs. 6 and 7), and when the muscle is excited the premature action potential following the larger contraction has a shorter duration than it does following the smaller contraction (Fig. 5). In all of the myocardia except the rat discordant alternation in the action potential appears if the degree of mechanical alternation is severe enough (Fig. 3D). It is felt that this alternation of the action potential is due to the influence on the excitable membrane of the action potential.
of some factor accompanying alternating contraction. The rat myocardial action potential does not show typical alternation because the action potential is so brief it is nearly over before the peak of contraction occurs. However, the slight alternation seen during the late phase of repolarization in the rat action potentials does occur near the peak force and therefore may be due to this mechanism (Fig. 3D).

The data of Figs. 3C and 8 demonstrate the second influence that can cause alternation in the time course of the action potentials. Noble (19) suggested that the potassium permeability of the membrane remains high following an action potential immediately after repolarization of the membrane, and that a premature action potential evoked during this time would have a reduced duration. Successive action potentials evoked close together would alternate according to this mechanism. The data of Fig. 8 show the gradual change from discordant to concordant alternation in the same cell as the action potentials are brought closer together. This demonstrates how the discordant alternation was gradually shifted to concordant alternation caused primarily by the alternate incomplete recovery of closely evoked action potentials.

Since action potential alternation does not appear to be the cause of mechanical alternation in the mammalian myocardium, the basis for mechanical alternation must reside in the contractile mechanism itself. Our data indicate that alternate incomplete relaxation is the basis for the mechanical alternation in the mammalian myocardium. The data of Fig. 2 show that the papillary muscles of the species with the faster normal heart rates must be stimulated faster to induce alternation. Species with faster normal heart rates have papillary muscles that exhibit faster and briefer contractions under similar in vitro conditions. Therefore it would be expected that species with faster normal heart rates would have to be stimulated faster before incomplete relaxation occurred. Procedures which tend to prolong contractions, such as low temperature and metabolic depressant drugs (14), tend to lower the rate threshold to induce alternation. Procedures which tend to shorten the duration of contractions, such as application of norepinephrine or epinephrine (10, 22), increase the rate threshold for alternation (2, 14).

The behavior of the alternating mammalian myocardium following premature contractions (Fig. 4A and B) suggests that premature activations disrupt some self-sustaining recycling process which is associated with incomplete relaxation. Following the larger contraction, a premature stimulus of appropriate timing reactivates the muscle in a less completely relaxed state than following the smaller contraction and increases the degree of alternation for several cycles; whereas, following the smaller contraction, it decreases the degree of alternation for several cycles. Following very early premature activations the muscle is allowed to relax more fully and the degree of alternation is always transiently increased suggesting the alternation process is being reset (Fig. 4B).

The data on the mammalian myocardium suggest that alternation in the peak force of contraction is due to alternate incomplete relaxation which occurs at rapid rates of stimulation. In situations where isolated mammalian myocardium is being paced at rapid rates the muscle is able to be reexcited before it has completely relaxed from the previous contraction. Reexciting the muscle before it has completely relaxed prevents the contractile elements of the muscle from being fully reactivated, and thus less force is developed and relaxation proceeds to a greater degree before the next activation. With reexcitation the contractile elements can now become more fully activated and relaxation is, therefore, prolonged. The next excitation repeats the cycle.

It is generally thought that excitation and contraction coupling in the frog myocardium is different than in the mammalian myocardium and that the duration of the action potential in the frog closely determines the contraction-relaxation cycle (1, 11, 20, 23). Our studies demonstrated that the frog myocardium does not show the alternation that is observed in the mammalian myocardium even during large degrees of alternate incomplete relaxation. We believe that these differences are due to differences in the mechanism of relaxation of the frog myocardium as compared to the mammal. Contractile behavior of an alternating type does not occur in the frog myocardium until the rate of stimulation is so rapid that the action potentials infringe upon each other. When this occurs the action potentials alternate. This is manifested by alternation in duration, amplitude, and depolarization velocity of the action potentials. These observations suggest that mechanical alternation in the frog myocardium is secondary to alternation in the action potentials due to alternate incomplete recovery of the depolarization mechanisms of the membrane. The variability of the peak force which accompanies large degrees of alternation in the end-diastolic force in the frog cannot be explained (Fig. 9). The form of the contractions is not well defined in the frog at the rapid rates of stimulation necessary to produce alternation since the small contractions ride on a greatly elevated base line (partial contracture). For this reason the possibility that the variability of the small degree of alternation in peak force may be artifactual cannot be ruled out.

In summary, since a regular and stable increase and decrease in the force of contraction can be induced in the mammalian myocardium under the same in vitro conditions and stimulation rate, alternation offers the unique opportunity to study the myocardium at two different inotropic states simultaneously. The pattern of the behavior of the mammalian myocardium in response to rate, rhythm, and temperature changes argues that the basis for alternation is in the incomplete relaxation of the contractile machinery itself and not in the action potential. Alternation in the action potential is secondary to the influence of some factor associated with contraction, or the influence of closely evoked action potentials, on the electrical properties of the membrane. In contrast, alternation in the mechanical activity of the frog myocardium appears to be entirely due to primary alternation in the action potential.

The authors thank Dr. Saul Winegrad, Dr. Lloyd Barr, and Dr. C. Paul Bianchi for their helpful comments and Mr. Ralph Iannuzzi for his technical assistance.

This work was supported, in part, by grants from the American Heart Association, 65-G-31 and 68-737, and a United States Public Health Service Predoctoral Traineeship.

The study was taken, in part, from a dissertation submitted by J. F.
Spear to the Graduate School of Arts and Sciences, University of Pennsylvania, 1969, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. J. F. Spear is presently a Postdoctoral Fellow of the Southeastern Pennsylvania Heart Association.

REFERENCES


