The Relationship of Excitability to Conduction Velocity in Canine Purkinje Tissue

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SUMMARY The relationship between interelectrode conduction time and "take-off" potential (TOP) was studied with microelectrode techniques in isolated canine false tendons. Conduction of regular or test beats initiated during phase 4 depolarization or late phase 3 repolarization speeded as TOP decreased. Similarly, beats initiated during digitalis-induced oscillatory afterpotentials demonstrated more rapid conduction at lower TOP. Because of the frequency-coupled nature of the oscillations, conduction times became rate dependent. Phenytoin antagonized digitalis oscillations and reversed speeding of conduction attributable to the oscillations. No uniform relationship between speed of conduction and maximum upstroke velocity of the action potential could be demonstrated in the above experiments or when speed of conduction was varied by changes in concentration of K+ or Ca2+. However, speed of conduction could be demonstrated to vary directly with changes in excitability as measured by intracellular current injection.

CONDUCTION velocity in excitable tissues is determined by a variety of factors, both passive and active. In mammalian cardiac tissues, action potential amplitude and the maximum rate of change of the action potential upstroke are regarded as the most important factors underlying variations in conduction velocity. This relationship has been accepted widely; published studies frequently offer only measurements of upstroke velocity as evidence for changes in conduction velocity. This practice is especially surprising in light of a number of well-known exceptions.

Exceptions to the proposed relationship were noted...
as demonstrated by Spear and Moore. The authors of each of these studies suggested that increased excitability could account for the observed speeding of conduction in response to moderate depolarization.

The effect on conduction of moderate variations of potassium concentration provides another exception. Elevation of potassium concentration from 2 to approximately 6 mM results in speeding of conduction. This occurs despite a considerable reduction of take-off potential (TOP). Dominguez and Fozard found no alteration in cable properties sufficient to explain speeding of conduction in sheep Purkinje fibers subjected to a rise in potassium concentration from 2.7 to 4.0 mM, nor was the observed reduction of membrane potential accompanied by a shift in threshold potential. They suggested that the probable cause of increased conduction velocity was the increased excitability that developed as TOP approached the threshold potential.

The concept that conduction velocity would vary as a function of excitability did not originate with those studies. Blair and Erlanger demonstrated that conduction velocity in amphibian nerve fibers varied inversely as a hyperbolic function of the minimum strength of extracellular stimuli required to recruit the fibers in question. Furthermore, the relationship with excitability was well maintained when relative excitability was appraised in terms of complete strength-duration curves. In cardiac tissues, the relative proximity of membrane potential to threshold during terminal repolarization, slow diastolic depolarization, and manipulation of the ionic environment is reflected in the excitability of the tissue as measured by intracellular current injection. In the present study, excitability and maximum upstroke velocity are evaluated as possible determinants of conduction velocity in canine ventricular specialized conducting tissue.

The previously noted exceptions to the proposed dependency of conduction on upstroke velocity occur within a physiological range of conditions. Similarly, the present study of conduction velocity is restricted to membrane potentials greater than —70 mV and to variations in ionic concentration within limits compatible with life. We studied the effects on conduction velocity of variations in TOP resulting from "normal" pacemaker activity and pacemaker activity related to digitalis-induced oscillatory afterpotentials. Variations in concentration of potassium and calcium also were studied. Variation of calcium concentration results in changes in threshold potential with little or no change in maximum diastolic potential. The results first presented constitute a reexamination of upstroke velocity as a determinant of conduction velocity. The final section is concerned primarily with excitability. The results indicate that, within the range of conditions studied, measurements of upstroke velocity are of little or no predictive value with respect to conduction velocity. Variations in conduction velocity appeared to be a function of excitability rather than of upstroke velocity or TOP.

Methods

Experiments were performed on cardiac Purkinje tissue from adult mongrel dogs of either sex. Hearts were removed from animals anesthetized with sodium pentobarbital (30-35 mg/kg, iv). The hearts were fibrillated electrically and false tendons from either ventricle were excised and placed in a reservoir of oxygenated modified Tyrode's solution. A preparation, selected for minimum branching and maximum length, was transferred to a tissue bath through which the perfusate flowed continuously. The perfusate, bubbled with a 95% O2-5% CO2 gas mixture, was maintained at 37°C and had the following millimolar composition: NaCl, 137.0; KCl, 4.0; NaH2PO4, 0.9; NaHCO3, 12.0; CaCl2, 2.5; MgSO4, 0.5; and dextrose 5.5.

Preparations were fixed with stainless steel pins at both ends to the wax bottom of a tissue bath. Stimulation was accomplished through chlorided silver electrodes. The anode was placed at a remote site within the tissue bath. Two cathodes, one applied to each side close to the same end of the preparation, achieved uniform wavefronts for even the earliest propagated test responses. Extracellular stimuli were rectangular pulses, 1-3 msec in duration, and were 1.5 times the threshold voltage. The exact duration used in any given experiment was the maximum, up to 3 msec, that would not overlap the upstroke of the action potential recorded from the site nearest the stimulating electrodes. Pulses were obtained from an optically isolated stimulator (Pulsar 61, Frederick Haer and Company) which was triggered by an additional digital interval generator. Unless otherwise specified, preparations were driven by trains of 10-20 members separated by 3-sec pauses.

Transmembrane activity was recorded at two sites, using glass microelectrodes filled with 2.7 M KCl (resistance 10-20 MΩ). One microelectrode was located approximately half way along the false tendon to ensure separation of the upstroke of the recorded action potential from the stimulus artifact, and the second electrode was used to record activity near the end farthest from the site of stimulation. This procedure necessarily reduced the absolute conduction time to only a very few milliseconds in most experiments. However, electronic noise and biological variability remained well below the 5-25% changes in conduction time studied (see Fig. 6). Furthermore, impalements maintained for data collection were selected to provide as nearly as possible the maximal diastolic potentials obtainable from the preparation. Therefore, the upstrokes recorded from the two sites were rapid and almost always parallel, thereby facilitating measurement of interelectrode conduction time. No measurements of data were made in those instances in which one or both impalements showed evidence of significant deterioration. Also, data collected under different sequentially applied conditions were compared only if the same two impalements were maintained throughout the sequence. Recordings from one of the sites were electronically differentiated to provide a measure of maximum action potential upstroke velocity. Prior to impalement, known voltage ramps (100-1000 V/sec) applied across a test resistance between tissue bath and ground were used to test and calibrate the entire recording and differentiating circuitry, including the microelectrode and appropriately adjusted negative capacitance circuits.
Excitability was assessed by intracellular application of current pulses of 5-msec duration. Current injection was accomplished through one of the recording microelectrodes using a technique described previously. Frequently, conduction time, maximum upstroke velocity, TOP, and threshold current requirements were all assessed for a given test beat occupying a specific point in a sequence of stimulation. To accomplish this, threshold current was first determined with current pulses of gradually increasing or decreasing magnitudes on sequential passages of the pattern. Once threshold was determined, the next test stimulus was delivered via the extracellular electrodes, thus allowing conduction time, TOP, and maximum upstroke velocity to be measured as soon as possible after the determination of threshold current.

The microelectrode records, differentiated signal, current strength, and stimulus pattern were displayed on an oscilloscope (Tektronix 5103N) and photographed with a Grass camera.

Drugs used in this study were prepared as concentrated stock solutions. Appropriate amounts were added directly to the reservoir of Tyrode’s solution to achieve the final concentrations. Acetylstrophanthidin* was prepared as a stock containing $1 \times 10^{-4}$ g/ml of a 6% aqueous solution of ethanol. Phenytion (diphenylhydantoin: Dilantin, Steri-vial; Parke-Davis) was dissolved in the diluent provided ($5 \times 10^{-3}$ g/ml). For experiments in which different concentrations of potassium or calcium were used, separate reservoirs of Tyrode’s solution were prepared with the appropriate concentrations. Since the variations were small, no attempt was made to compensate for osmotic differences.

**Results**

**Conduction Velocity in Response to Phasic Changes in Transmembrane Potential**

Conduction velocity was measured in seven experiments in which slow diastolic depolarization was enhanced by superfusion of the preparations with Tyrode’s solution containing a reduced concentration of potassium (2 mM). During pauses in the regular driven rhythm, extrasystoles were initiated at various intervals during slow diastolic depolarization. An example is shown in Figure 1 (inset; bottom right). In this example, two extrasystoles were interpolated in the pause. The interrelationships between conduction time, maximum upstroke velocity, and TOP are plotted in Figure 1 for the extrasystoles and for the preceding and following regular responses. Thus the effects of four different TOP were assessed. Maximum upstroke velocity fell as TOP decreased with diastolic depolarization, but in confirmation of Arbel et al., and in direct contrast to observations of Singer et al. made at lower TOP, conduction times were shortened.

Variation of TOP in response to phase 4 depolarization is not limited to situations incorporating extrasystoles. Different TOP also can be achieved by changes in heart rate. In the presence of phase 4 depolarization, conduction velocity can become rate dependent, as shown in Figure 2. Low potassium (2 mM) was used to promote slow diastolic depolarization. The preparation was driven with trains of beats at different basic cycle lengths (BCL), four of which are illustrated in Figure 2. At the shortest

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* Generously supplied by Eli Lilly Co., Indianapolis, Indiana.
BCL, each beat was initiated at the maximum diastolic potential. At longer BCL, as diastolic depolarization progressed, the basic beats occurred at lower TOP. The resultant relationship between conduction time and TOP is graphically illustrated. Reduction of membrane potential with slow diastolic depolarization was associated with speeding of conduction. Conduction was rate dependent; slower conduction was associated with high rates and faster conduction with low rates.

The above relationships were observed with changes in TOP during diastole. A more complex situation occurs when variation of TOP is assessed at various times during repolarization of a preceding action potential. The relationships of conduction time and maximum upstroke velocity to variations in TOP achieved by this method at two different concentrations of potassium are shown in Figure 3. In Figure 3A, the potassium concentration was 2 mM and the maximum diastolic potential was 95 mV. As beats were initiated progressively earlier than full repolarization, conduction times first decreased to a minimum and then progressively lengthened. The short conduction times centering around 85 mV correspond to the supernormal period of conduction described by Spear and Moore and are probably related to the supernormal period of excitability. Although the slope of the relationship between conduction time and TOP reversed, this was not reflected in the curve of membrane responsiveness (i.e., dV/dt vs. TOP).

The data illustrated in Figure 3B were recorded at a potassium concentration of 4 mM. The relationships are essentially the same as those in Figure 3A, except for a moderate reduction in maximum diastolic potential. Again, as beats were initiated progressively earlier than full repolarization, TOP decreased and there was an initial reduction in conduction time followed by a prolongation. The magnitude of the period of supernormal conduction appears to be reduced. However, the absolute values of the minimum conduction time were essentially the same. The apparent diminution of the magnitude of supernormality was caused by the shorter conduction time of beats initiated at the reduced maximum diastolic potential characteristically achieved at the higher concentration of potassium. In experiments in which 6 mM K was studied, this effect was accentuated, and frequently repolarization proceeded no further than the TOP corresponding to “supernormal conduction.” Changes in concentration of potassium appeared not to alter the relationship between conduction time and TOP. Instead, the observations suggest that the well known speeding of conduction seen with moderate elevations of potassium concentration can be explained by failure of repolarization to proceed beyond membrane potentials corresponding to supernormal conduction.

**Digitalis: Effects of Oscillatory Afterpotentials on Conduction**

Digitalis has been shown to induce oscillatory afterpotentials (OAP) coupled to preceding action potentials. Examples of OAP, induced by acetylstrophanthidin and coupled to trains of action potentials, are illustrated in Figure 4. When trains of responses at a cycle length of 600 msec are separated by pauses of several seconds, OAP increased in amplitude with the first 8-10 members of each train, and each driven beat in the series is initiated...
at a progressively lower TOP. At higher driven rates (cycle length <400), each action potential is initiated at the maximum diastolic potential before significant loss in TOP occurs. Thus, as also illustrated in Figure 4, trains of beats at short BCL (300 msec) may demonstrate a high and almost constant TOP.

Data from an experiment in which TOP was modified by OAP are shown in Figure 5. The BCL was 600 msec and each train contained 10 members. The data illustrated are from a single train. Figure 5A shows that the first beat of the train, occurring after a 3-second pause, was initiated at a TOP of −79 mV, corresponding to the resting potential. The second beat was the first to occur at an interval of 600 msec and was initiated at a higher TOP than the first. The next 10 beats demonstrated a sequentially decreasing TOP. Concurrently, the interelectrode conduction times indicated a speeding of conduction. Figure 5B illustrates a progressive loss of TOP, and also a progressive decrease of maximum upstroke velocity as the OAP developed. Reduction of TOP, whether caused by digitalis-induced OAP or associated with late phase 3 repolarization or phase 4 depolarization, is accompanied by acceleration of conduction at a time when the maximum upstroke velocity is depressed.

One of the main advantages of using OAP to alter TOP is that a large array of TOP with resultant conduction times can be recorded within a very brief time. Statistical analysis of values from several sequential trains allows an estimate of the variability of the observed relationship between conduction times and TOP. An example of such an analysis of data collected from seven sequentially recorded trains is shown in Figure 6. The data presented in this figure are from the same experiment as that illustrated in Figure 9. Both the horizontal and vertical bars represent standard errors of the mean for values associated with each member of the train. The regression line was fitted by the least squares method. The regression coefficient \( r \) had a value of 0.506 and the corresponding \( P \) value was less than 0.001. Thus the data suggest a direct linear relationship between conduction time and TOP. The occurrence and direction of this relationship was confirmed in 25 experiments in which TOP was varied by superimposition of test beats on OAP developing within trains of driven beats. In many experiments, the same relationship also was demonstrated by superimposition of a test beat at different positions on a large OAP following each of several sequential trains similar to that illustrated on the right in Figure 4. In all cases, data were collected only from preparations exhibiting maximum diastolic potentials greater than −75 mV during exposure to acetylstrophanthinid (AS).

![Figure 4](image1.png)

**Figure 4** Variation in TOP in canine Purkinje tissue by acetylstrophanthidin-induced OAP (1 × 10^{-7} g/ml). The top trace is an intracellular recording and the bottom trace illustrates the pattern of stimulation. The BCL is indicated below each train of 10 driven responses. OAP coupled to preceding driven activity are apparent at both BCL. At long BCL, developing OAP caused a progressive loss in TOP. At short BCL, each beat occurred at a high and almost constant TOP.

![Figure 5](image2.png)

**Figure 5** Progressive changes in TOP, conduction time, and maximum upstroke velocity \((dV/dt)\) caused by progressive increments in amplitude of acetylstrophanthidin (AS)-induced OAP. The numbers on the abscissas indicate the numerical position of individual driven beats within trains. Panel A shows that, after the first pair of driven responses, the progressive loss in TOP was associated with a reduction in interelectrode conduction time. Panel B shows that, despite speeding of conduction, loss of TOP is associated with depression of maximum upstroke velocity. BCL = basic cycle length.
In illustrations presented thus far, conduction velocity was an inverse function of upstroke velocity and of TOP. However, within the high range of membrane potentials studied (−75 to −105 mV), a similar relation between conduction velocity and TOP also could be demonstrated when the maximum dv/dt remained constant. An example of this is shown in Figure 7A. The data were collected from a preparation treated with AS and driven with trains of stimuli at a BCL = 600 msec. Thus, the first beat was initiated at the resting potential, the second beat at the maximum diastolic potential, and the remaining beats exhibit a progressive loss of TOP due to developing OAP.

Conduction times show an initial lengthening corresponding to the change of TOP from resting levels to maximum diastolic potential. Subsequently, conduction times shortened as the OAP increased in amplitude and thereby reduced the TOP. Maximum upstroke velocity, shown at the top of the figure, remained essentially constant throughout.

Because of the relationship between conduction times and TOP, conduction time becomes rate dependent when slow diastolic depolarization is enhanced (see Fig. 2). Changes in TOP due to OAP are also rate dependent. At moderate BCL, OAP asymptotically increase in amplitude with approximately the first 10 beats. Subsequently, if intoxication is moderate, the TOP remains constant at a value determined by the BCL, and the amplitude and time course of the OAP. However, at short BCL, each beat is initiated at close to the maximum diastolic potential (Fig. 7B; also see Fig. 4, right). Figure 7B shows that, following the first beat which is initiated at the resting potential, TOP show little variation, and the conduction times remain almost constant. Furthermore, conduction times are longer than observed at the end of the train at BCL = 600 msec (Fig. 7A).

Abolition of OAP by Phenytoin: Effects on Conduction

Clinically, digitalis arrhythmias are frequently suppressed with phenytoin (diphenylhydantoin, DPH). Rosen et al.15 have reported that they were able to decrease the amplitude but did not abolish ouabain-induced OAP with DPH. We have studied the effects of DPH on canine false tendons treated with AS in a total of 17 experiments. Figure 8 shows records from one of five experiments in which both electrical and contractile activities were recorded. During the control period (Fig. 8, top), pauses in
driven activity exposed only moderate slow diastolic depolarization. Following the first beat of the train, the TOP of subsequent beats remained essentially constant at close to the maximum diastolic potential. The second panel was recorded after the preparation was exposed to $1 \times 10^{-7}$ g/ml of AS for approximately 1 hour. Prominent OAP followed the driven trains. In addition, the OAP increased in amplitude during the first 8-10 beats of the train and thereby caused a progressive reduction in TOP at the BCL illustrated. The contractile record demonstrates a strong positive inotropic effect. Shortly after these traces were recorded, DPH ($1 \times 10^{-6}$ g/ml) was added to the perfusate. After 20 minutes of exposure to the two agents (third panel), the OAP and the associated progressive change in TOP were reduced in amplitude. These effects were accompanied by a reversal of the positive inotropic effect of AS. The bottom panel, recorded 9 minutes later, demonstrates further reversal of the positive inotropic effect and complete abolition of OAP. The TOP once again remained constant at near the maximum diastolic potential after the first beat of the train. Similar changes were observed in all five experiments.

In six other experiments, the effects of DPH were assessed only after the development of spontaneous beats attributable to OAP. Spontaneous activity was greatly reduced by DPH in two preparations and was reversibly abolished in the remaining four. In six additional preparations exhibiting AS-induced OAP, an appropriate concentration of the commercial solvent was tested and found to be without effect.

The effects of DPH on OAP amplitude and the associated changes in TOP suggested that DPH might cause related changes in conduction times in AS-treated preparations. We investigated this in seven experiments, one of which is illustrated in Figure 9. Figure 9A shows the TOP for each beat within trains of nine at a BCL = 600 msec. During the control period, the first beat was initiated at the resting potential achieved after a 3-second pause in driven activity. All of the remaining members of the train started from a constant maximum diastolic potential. After exposure of the preparation to AS, the TOP for the first two beats were similar to those of the corresponding control beats. However, the remaining members of the train demonstrated a conspicuous beat-by-beat reduction of TOP because of the developing OAP. Upon exposure of the preparation to DPH for 45 minutes, the amplitude of the OAP observable at the end of the train of driven responses was greatly reduced, as was the slope of the curve relating TOP to beat number.

The above changes in TOP resulted in corresponding alterations in conduction times of successive members of the train of beats. This was confirmed in all seven experiments and is illustrated for the present example in Figure 9B. The first beat in the control period was initiated from a lower TOP than the subsequent responses and was conducted relatively quickly. Conduction times were constant for the remaining beats. In the presence of AS, a progressive acceleration of conduction accompanied the developing OAP. In this experiment, the later beats of the train were conducted with conduction times briefer than those of the control series. Speeding of conduction relative to control was observed in several preparations but was not a consistent effect of AS. Responses superimposed on OAP, however, always were conducted more quickly than beats initiated at higher potentials either preceding or following OAP. The remaining curve in Figure 9B shows conduction times of beats recorded after the amplitudes of OAP were greatly reduced by DPH. The curve indicates an overall shift toward longer conduction times. This shift was not present in all experiments. However, DPH greatly reduced the slope of the curve attributable to underlying OAP. This effect was observed in all seven experiments. Thus the effects of DPH on conduction in preparations exposed to digitalis may be compounded by effects on TOP mediated via actions on OAP.

The Relationship between Conduction Time and Excitability as Measured by Intracellular Stimulation

At membrane potentials greater than $-70$ mV, one cannot reliably predict changes in conduction times from changes in maximum upstroke velocity. Within the range of membrane potentials tested, concurrent depression of conduction and maximum upstroke velocity was observed.

![Figure 8](http://circres.ahajournals.org/Downloadedfrom)

**Figure 8** Effects of phenytoin (diphenylhydantoin, DPH) on electrical and contractile activity of a canine false tendon superfused with a toxic concentration of AS ($1 \times 10^{-7}$ g/ml). In each panel, the top trace is the electrical record, the middle trace shows the corresponding contractions (resting tension 200 mg), and the bottom trace indicates the pattern of stimulation. Abolition of OAP by phenytoin was accompanied by reversal of most of the positive inotropic effect of acetylstrophanthidin.
Changes in conduction time attributable to effects of phenytoin (DPH) on OAP. Open circles: control; filled circles: acetylstrophanthidin (1 × 10^{-7} g/ml); filled squares: acetylstrophanthidin plus DPH (1 × 10^{-6} g/ml). "Beat number" indicates the numerical position of a driven response within a train of 9.

Only for beats initiated before complete repolarization of a preceding action potential and then only for beats earlier than the supernormal period of conduction.

Other possible determinants of conduction time were considered in five experiments in which potassium and calcium concentrations were varied. The results of one experiment, representative of the series, are shown in Figure 10. For each change in ionic concentration, data from three responses are included. Each of the three responses was initiated at an early, intermediate, or late position during the diastolic interval accompanying a 3-second pause in regular stimulation. Thus, variation of TOP of measured responses was maximized, especially at low concentrations of potassium. Changes in the concentrations of both ions were accompanied by variation in maximum upstroke velocity. However, conduction times demonstrated no uniform correlation with those variations (Fig. 10A).

In other experiments described in this study, we showed a direct relationship between conduction times and TOP (e.g., Figs. 2 and 6). Figure 10B shows an attempted correlation between changes in conduction times and changes in TOP for variations in potassium and calcium concentrations. A linear relationship well describes the relationship between conduction time and TOP for changes resulting from alterations of potassium concentration, but when calcium concentrations were altered, equally large changes in conduction times occurred with little or no change in TOP. Obviously, another variable in addition to TOP must be important in determining speed of conduction.

Because calcium is known to affect threshold voltage, we considered excitation requirements to be a possible determinant of conduction velocity. In the series of experiments illustrated by Figure 10C, we determined the relationship between intracellularly injected current required to initiate propagated responses (threshold current) and TOP. Threshold current clearly demonstrated a linear correlation with changes in TOP induced by alterations of potassium concentrations within the range known to cause little variation of threshold voltage. In contrast, changes in calcium concentration resulted in large changes in threshold current with little variation in TOP. Threshold current would be expected to be directly proportional to the difference between threshold potential and TOP, when changes in membrane resistance are not excessive. When calcium concentration is varied, the membrane potential is little affected but threshold voltage varies inversely with the calcium concentration. Thus, our observed changes in threshold current in response to variations in calcium concentration are also predictable on the basis of the difference between threshold voltage and TOP. The similarity between variations of both conduction times and threshold current with TOP is striking (Fig. 10, B and C, respectively). The close similarity between these graphs suggests that conduction times might better be expressed as a direct function of threshold current. Figure 10D shows this to be true for alterations of both potassium and calcium concentrations. These results, similar in all five experiments, suggest that excitation requirements are a major determinant of conduction time.

Discussion

The present results demonstrate that the maximum upstroke velocity of action potentials cannot be used as a reliable index of conduction velocity. Speeding of conduction was observed in the present study under circum-
Excitability and Conduction

Changes in potassium and calcium concentrations affect conduction time, maximum upstroke velocity ($dV/dt$), TOP, and threshold current. Threshold current was measured as the minimum current necessary to initiate a propagated response when the current was delivered intracellularly via a microelectrode. A detailed explanation of the series of plots is given in the text. All lines are hand drawn.

In all of the above examples, excitability changed in a direction appropriate to account for the observed changes in conduction. Increased excitability associated with depolarization in response to elevation of potassium concentrations was tested directly in the present study and has been called upon previously to explain this well known example of speeding of conduction. Phase 4 depolarization also has been shown to be accompanied by increased excitability. Weidmann measured excitability during phase 4 with intracellular injection of current and found that progressively less current was needed to initiate a response as depolarization progressed. Block of conduction related to phase 4 depolarization and suggested as a mechanism of arrhythmia would necessarily be restricted, as Singer et al. suggested, to situations involving substantial depression of excitability and depolarization to levels well below −70 mV. Phase 4 depolarization associated with normal pacemaker activity, because of increased excitability and more rapid conduction, would tend to ensure invasion of all specialized conducting tissue by a propagating wavefront. Digitalis-induced pacemaker activity provides conditions similar to that of phase 4 depolarization. In a previous study, we demonstrated increased excitability associated with the peak and ascending limb of oscillatory afterpotentials induced by acetylstrophanthidin in canine Purkinje tissue. As noted in the results of the present study, increased conduction velocity associated with oscillatory afterpotentials was on several occasions of sufficient magnitude to reduce conduction times below control. Speeding of conduction in response to exposure to digitalis is not without precedent. Moe and Mendez observed speeding of intraventricular conduction in situ canine hearts exposed to moderate doses of cardiac glycosides. Speeding of conduction was accompanied by increased excitability. Swain and Weidner confirmed these observations in canine heart-lung preparations treated with ouabain. It is, of course, not known whether oscillatory afterpotentials were a part of the mechanism underlying these earlier examples. It should be emphasized that speeding of conduction in
specialized conducting tissues was observed at an early stage of digitalis intoxication. As confirmed in our previous study, later more advanced stages of intoxication are characterized by block of conduction. This level of intoxication was associated with very large oscillatory afterpotentials and greatly depressed excitability as measured by intracellular current injection.

The effects of varying the concentration of calcium were of special interest to us since conduction could be related to excitability in the absence of changes in TOP. The observation that conduction is related similarly to threshold current requirements, whether the latter is varied by changing TOP with different potassium concentrations or threshold potential with different calcium concentrations, lends considerable support to the contention that excitability is an important variable in the determination of conduction velocity.

In Purkinje tissue depolarized during diastole to potentials between approximately −70 and −55 mV, loss of TOP and depression of maximum upstroke velocity is accompanied by slowing of conduction. Singer et al. assigned the accompanying depression of excitability a permissive rather than determinant role, but excitability might also be a major determinant of conduction velocity in this range of potentials. However, it would be difficult to evaluate the relative importance of excitability and maximum upstroke velocity because most agents or procedures would be expected to change both in parallel in preparations depolarized to this level.

It may be instructive to compare our observations to the behavior predicted by mathematical models of excitable tissues. Hunter et al. have recently considered the relative influence of various determinants of conduction velocity in several analytical models. Of the determinants considered, the term most comparable to excitability was safety factor. Their expressions for safety factor quantified the extent to which the ability of the tissue to be excited and conduct exceeded the minimum conditions necessary for these events. The analysis predicted that conduction velocity would vary as a function of the square root of the safety factor. Furthermore, the definition used in their cubic model predicts that safety factor will increase greatly as membrane potential approaches threshold in the range of membrane potentials normally associated with phase 4 depolarization. Although the mathematical models considered by Hunter et al. were not derived specifically for canine Purkinje tissue, the behavior predicted by these models is analogous to our experimental findings.

The phenomenon of supernormal conduction must be considered in the context of the present study. Supernormal conduction has often been considered as a separate entity distinctly different from "normal" conduction. In light of the present study, supernormal conduction is easily incorporated within a single relationship applying equally well to beats later in diastole. In preparations exhibiting phase 4 depolarization, one may consider the point in time at which maximum diastolic potential is reached as a reference. The membrane potential either earlier or later than this point is less and is closer to threshold. Excitability is greater during both terminal repolarization and diastolic depolarization, and conduction is more rapid than for beats initiated at the maximum diastolic potential. A single continuous relationship to excitability appears to govern conduction velocity through terminal repolarization and diastole. Conduction during terminal repolarization gives the greatest impression of being "supernormal" in those preparations in which slow diastolic depolarization is suppressed by previous rapid activity. Diastolic potentials remain high and conduction slow.

The effect on "supernormal" conduction of elevating the potassium concentration provides a counterpart to the last example. Elevation of potassium concentration has been shown to eliminate the period of "supernormal" conduction in isolated canine Purkinje tissues. The present results indicate that this effect is accomplished not by antagonism of supernormal conduction but by elimination of subsequent slowing of conduction. This effect can be seen in our results (Fig. 3) and in the illustrations published by Spear and Moore. In both studies, phase 3 repolarization, in the presence of moderately elevated potassium concentrations, proceeded to levels associated with full recovery of excitability. Further repolarization to higher membrane potentials and lower excitability was antagonized by the effects of the higher levels of potassium on maximum diastolic potential. Thus, diastolic and "normal" conduction velocity approached and frequently equalled that previously labeled "supernormal." Strongly supporting this interpretation is the observation of Spear and Moore that supernormal excitability also is eliminated by elevation of potassium, and this occurs by enhancement of diastolic excitability rather than by suppression of excitability during the period of supernormality.

In the course of our study of the effects of DPH on Purkinje tissue treated with AS, we confirmed previous observations that DPH reduced the amplitude of OAP. In addition, we frequently observed that DPH abolished OAP and suppressed digitalis-induced automaticity. These effects were accompanied by a marked reversal of the positive inotropic effect of digitalis. Direct negative inotropic effects of DPH in the absence of digitalis pre-treatment have been reported in studies of isolated rabbit atrial muscle and of left ventricular function in the dog. In addition, although some reports suggest that DPH may dissociate arrhythmic and inotropic effects of digitalis, others indicate depression of left ventricular function by DPH in patients receiving digitalis. Comparison of the present observations with those of previous studies is impeded by differences in tissue studied, in drug concentration, and criteria used in assessing inotropic and antiarrhythmic effects. However, our observations are consonant with the close association of oscillatory phenomena and inotropic actions of digitalis we previously reported.

Studies of the effects of DPH on conduction have also provided a diversity of results. Bigger et al. observed speeding of conduction in isolated preparations in which conduction was depressed prior to exposure to DPH. However, studies of the in situ canine heart demonstrated little or no change in intraventricular conduction either
with26 or without27 pretreatment with digitalis. In addition, Katzung and Jensen26 reported that the effects of DPH were dependent on the concentration of potassium to which the tissue was exposed. They observed slowing of conduction in the presence of high potassium concentrations (5.6 and 7.6 mM) and no change in conduction at low concentrations (2.6 mM). In light of the present study, one may speculate that slowing of conduction in high potassium might be mediated by an action of DPH to reverse the depolarizing effect of high potassium and thus increase the difference between TOP and threshold potential. In the absence of other agents, DPH reportedly increases excitability by shifting the threshold voltage of canine Purkinje fibers to more negative values.27 In our experiments, any negative shift in threshold potential must have been more than counterbalanced by reduction of the amplitude of OAP as evidenced by the cessation of excitability. The present study suggests that the exceptions were more likely the rule. Although upstroke velocity and conduction velocity may both decrease in relatively refractory tissues or in tissues in which membrane potentials of less than −70 mV are reached, over a broad range of transmembrane potentials (−70 to −110 mV), conduction velocity was not observed to vary as a function of upstroke velocity. Speed of conduction appeared to vary more directly with changes in excitability as measured by intracellular current injection. Thus, upstroke velocity cannot be used as a reliable index of conduction velocity in assessing new pharmacological agents for possible cardiac effects. Similarly, interpretation of previous studies in which only upstroke velocity was measured must be reserved until direct measurements of conduction velocity are made.

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Erratum

In the paper by Ralph D. Tanz et al., "Negative Chronotropic and Antiarrhythmic Properties of Atropine and Other Tropine Analogues on Isolated Cat Heart Preparations," Circ. Res. 42: 467-473 (April) 1978, Figures 1 and 3 were inadvertently reversed. The figure at the bottom of the page is Figure 1 and should have been placed at the top of the page. The figure at the top of page 469 is Figure 3 and should have been placed at the bottom of the page.
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