

# Conduction of the Cardiac Impulse

## III. *Characteristics of very slow conduction*

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**ABSTRACT** The excitability of short segments (5–7 mm) of bundles of canine Purkinje fibers was depressed by exposure to 15–18 mM K<sup>+</sup>, to 15–18 mM K<sup>+</sup> plus  $5 \times 10^{-6}$  M epinephrine or norepinephrine, to low K<sup>+</sup>, and to low Na<sup>+</sup>. The depressed segment was in the center chamber of a three-chamber bath; the ends of the bundle were exposed to normal Tyrode solution. Each method of depression resulted in slow and probably decremental conduction with an effective conduction velocity in the middle chamber of about 0.05 m/sec, or one-way block, or two-way block with summation of the graded responses in the depressed region. The action potential in the depressed segment (the slow response) differs from the normal action potential in its response to applied stimuli. A second active depolarization can be evoked by cathodal stimulation during much of the slow response. The response in the depressed segment is graded. The response of depressed fibers may depend on excitatory events similar to those responsible for the slow component of the cardiac action potential. It is suggested that the slow response can propagate, at least decrementally, in fibers in which the rapid, Na<sup>+</sup>-dependent upstroke is absent, and can cause reentrant excitation by so doing.

We have found that when the excitability of short segments of the ventricular conducting system of the mammalian heart is depressed certain unusual features appear. Apparent conduction velocity may fall to as low as 1% of its normal value; one-way block may develop; and a type of summation may appear (1, 2). Summation is seen in the presence of two-way block; action potentials invading the depressed segment from either end are unable to propagate throughout the depressed segment but when both ends are excited a far larger response appears at the center of the depressed segment than when either end is excited alone (2).

Slow conduction produced by such depression of excitability in short segments of Purkinje fibers often leads to reentrant excitation (3). Reentrant excitation may also appear when slow conduction is produced throughout a network of Purkinje fibers exposed to high K<sup>+</sup> and epinephrine (4). In the

present article we report other ways in which slow conduction can be brought about and we report studies of the excitability of fibers that show such slow conduction. The excitatory response of depressed cardiac fibers shows fundamental qualitative differences from that of normal cardiac muscle and appears to represent an alternative mode of conduction of excitation in the heart; we suggest that the action potential seen under such circumstances be called the "slow response."

#### METHODS

Mongrel dogs were anesthetized with sodium pentobarbital, 30 mg/kg given intravenously. The chest was opened and the heart was removed and placed in oxygenated Tyrode solution. Strands of free-running Purkinje fibers (false tendons) were removed from the right or left ventricle; each end of a false tendon usually remained attached to a bit of ventricular muscle or papillary muscle. The preparations were pinned to the wax base of a tissue bath and perfused with modified Tyrode solution containing: NaCl, 137 mM; KCl, 5.4 mM; CaCl<sub>2</sub>, 2.7 mM; MgCl<sub>2</sub>, 0.5 mM; NaHCO<sub>3</sub>, 12 mM; NaH<sub>2</sub>PO<sub>4</sub>, 1.8 mM; dextrose, 5.5 mM. The solution was gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and maintained at 35°–37°C. Perfusates for study of altered ionic environment were obtained by further modification of the Tyrode solution. Elevation of K<sup>+</sup> concentration was accomplished by adding 1 M KCl in the necessary quantity without correction for osmolarity; low K<sup>+</sup> Tyrode was made by adding less KCl to the Tyrode than was normally added, without correction for osmolarity. Solutions containing low Na<sup>+</sup> were made with choline chloride as a replacement for NaCl. The low Na<sup>+</sup> solution contained normal amounts of NaHCO<sub>3</sub> and NaH<sub>2</sub>PO<sub>4</sub> so the lowest Na<sup>+</sup> concentration was 13.8 mM. In some experiments in which choline chloride was used as a substitute for NaCl, atropine sulfate was added in a concentration of 0.015 mM. In some experiments L-epinephrine or L-norepinephrine was added to the perfusate in concentrations of  $5 \times 10^{-6}$  M. Occasionally a drop of L-epinephrine or L-norepinephrine was added directly to the fluid in the tissue bath; the usual concentration of one part of base to 1000 was used for that purpose. Epinephrine was used in the form of the chloride (Adrenalin, Parke, Davis & Co., Detroit, Mich.), norepinephrine in the bitartrate form (Levophed, Winthrop Laboratories, New York).

Nearly all experiments were performed in a three-chamber bath designed to permit two chambers to be perfused with normal Tyrode solution while a third was perfused either with normal Tyrode solution or with Tyrode solution of modified ionic composition. Apart from two enlarged areas at each end, the tissue bath had straight parallel walls and the walls had grooves into which 0.5 mm thick plastic partitions could be fitted snugly. The preparation was pinned to the wax base and the bath was divided into three chambers by the insertion of two partitions, as described below. The partitions were prepared as follows: "bottom" means the side to be applied to the wax base of the tissue bath. At the bottom of each partition a semicircular hole was cut, on a diameter of about 3 mm. A thin collar of beeswax was applied around that hole to provide mechanical support for a layer of agar that was applied as follows. To 100 cc of Tyrode solution 4 g of agar (Bacto-Agar, Difco Laboratories, Detroit, Mich.) was added; the solution was brought to the boiling point (see reference 1).

The partition was laid flat and agar was poured on it; after the agar had hardened all of it was trimmed away apart from that which filled the hole at the bottom of the partition and that under the beeswax collar. The result was a 0.5 mm thick plastic partition with a slot or hole at the bottom plugged with a layer of agar about 2 mm thick. After the preparation had been placed in the bath the partitions were inserted so that the agar-filled slot at the bottom of the partition pressed down on the false tendon. The rest of the bottom of the partition pressed into the wax base or into a groove that had been cut in the wax and filled with agar to provide a more compliant surface into which the partition could press. The middle chamber was created by inserting two such partitions; the length of the bundle in the middle chamber was either 5 or 7 mm. Separate inflow and outflow connections were available to permit each of the three chambers to be perfused separately. The quality of the seal, i.e. the quality of the separation between the three chambers, was tested by adding 3 or 4 drops of 1 M KCl directly to the fluid in the middle chamber. If rapid depolarization sufficient to abolish excitability of the fibers in the center chamber was achieved with no change in the resting potential, amplitude, upstroke velocity, or duration of the action potentials in either of the end chambers, the experiment was carried out. (The electrodes in the end chambers were 2–3 mm from the partitions so the change in duration that would be expected from local circuit electrotonic interaction would not be large.) If changes in the resting potential in either of the end chambers occurred, the partitions were removed and replaced until an adequate seal was obtained. When a good seal was obtained it often remained good for several hours.

The end chambers were perfused with normal Tyrode solution from a common bottle; the middle chamber was perfused separately. Each end chamber had a volume of 3 cc and was perfused at a rate of about 10 cc/min; the center chamber had a volume of about 0.6 cc and was perfused at a rate of 20 cc/min. Temperature was monitored by a Yellow Springs Telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio) with sensing devices in each chamber.

Agar is not a barrier to the diffusion of water or small ions so the test of sealing was a test only for the absence of gross leak of fluid. Maintenance of differences in the ionic environment between the end chambers and the middle chamber depended on the continual flow combined with the absence of gross leaks.

In general the apparatus and methods used for stimulating and recording were as previously described (1–4). One intracellular microelectrode was placed in a cell in each end chamber and a third was placed in a cell near the middle of the center chamber. It was therefore possible to drive either end to determine the presence of slow conduction or block in each direction or, when total block occurred, to drive both ends to look for summation. In some experiments an electrode in a cell in the center chamber was used for intracellular stimulation. The apparatus and methods used for such intracellular stimulation were as previously described (5).

## RESULTS

One goal of these experiments was to determine whether slow conduction, one-way conduction, and summation would appear when the excitability of fibers in the center chamber was depressed in various ways. Another goal was

to show that these phenomena occur when the fibers in the end chamber are uniformly exposed to a normal ionic environment and when the fibers in the center are uniformly exposed to an altered ionic environment. This was considered desirable because in our previous studies with  $K^+$ -rich agar the fibers outside the agar were sometimes depressed by diffusion of  $K^+$  from the agar while diffusion of  $K^+$  also meant that depression of fibers within the agar showed both spatial and temporal variability. The difficulty of inserting electrodes into fibers imbedded in agar also makes it difficult to make frequent

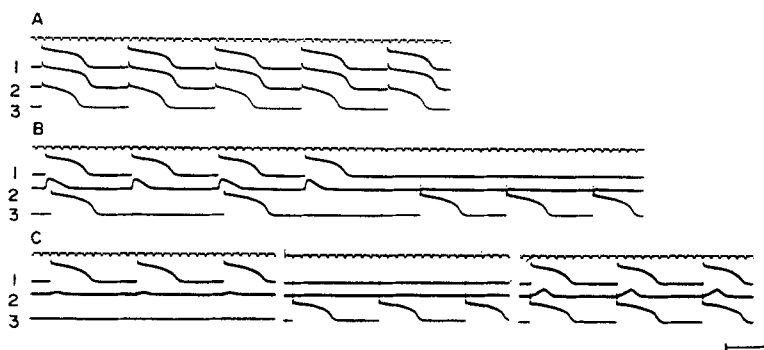


FIGURE 1. Records obtained in a three-chamber experiment with electrode 1 in one end chamber, electrode 2 in the center chamber, and electrode 3 in the other end chamber; the bundle contained no branches. In Fig. 1 A all chambers were perfused with normal Tyrode solution. In Fig. 1 B the center chamber was perfused with Tyrode solution containing 18 mM  $K^+$ . Stimulation of the end containing site 1 resulted in slow conduction with 2:1 block between the center (site 2) and the other end (site 3). Stimulation of the end containing site 3 revealed complete block in the direction from 3 to 2 to 1. In (C) complete block is seen in both directions while excitation of both ends evokes summation at site 2. The vertical calibration represents 100 mv; the horizontal calibration represents 500 msec; the time marks occur at intervals of 100 msec.

determinations of resting potential. In the present experiments typical slow responses were obtained in fibers exposed to an external  $K^+$  concentration of 16–18 mM. Such fibers have a resting potential of between 53 and 58 mv; at that level the initial  $Na^+$  inward current responsible for normal propagation is abolished in sheep Purkinje fibers (6).

The results shown in Fig. 1 were obtained from an experiment in which the perfusate to the center chamber contained 18 mM  $K^+$ . Control records shown in Fig. 1 A were obtained when all three chambers were perfused with normal Tyrode solution. Records shown in Fig. 1 B were obtained about 15 min after the  $K^+$  level in the center chamber had been raised. When one end of the preparation was stimulated, as shown at the beginning of Fig. 1 B, the center always responded with a slow, low-voltage deflection but there was 2:1 block between the center and the far end. When conduction did occur from one end

to the other it did so with a delay of about 90 msec. The tracings at the end of Fig. 1 B show that there was complete block of conduction from site 3 to site 1, so this preparation showed 2:1 conduction with delay in one direction and complete block in the other. The apparent conduction velocity from site 2 to site 3 was about 0.06 m/sec. The records in Fig. 1 C were obtained 10 min later and show the presence of two-way block and summation. This slow increase of depression with time was often noted. The first set of records shows

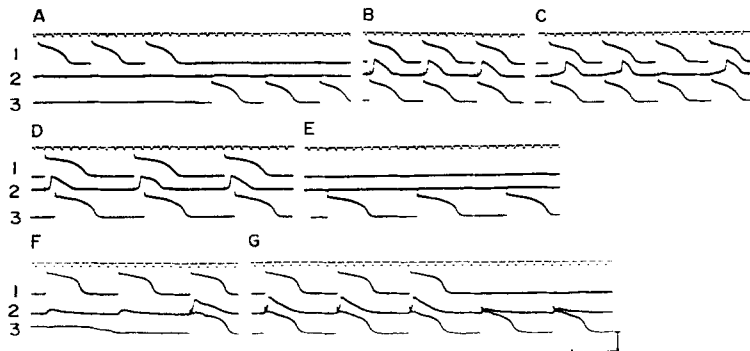


FIGURE 2. Records obtained in a three-chamber experiment with electrode 1 in one end chamber, electrode 3 in the other end chamber, and electrode 2 about 3 mm remote from the bundle in a small branch arising from that part of the bundle in the center chamber. The center chamber was perfused with Tyrode solution modified to contain 17 mM  $K^+$  and  $5 \times 10^{-6}$  M epinephrine. Complete block is shown in (A); excitation of either end does not evoke an action potential either in the branch arising in the center chamber or in the other end. When both ends are excited, (B) and (C), an action potential is seen in the branch. In (C) an action potential appears in the branch with marked delay and is blocked in one instance. The records in (D) and (E) were obtained by adding additional epinephrine to the center chamber. Conduction from 1 to the branch, and from 1 to 3, is restored in (D) but complete block from 3 to 1 persists in (E). The records in (F) and (G) were obtained from another preparation. The electrode in the center was in the bundle, not in a branch. The perfusate contained 17 mM  $K^+$  and  $5 \times 10^{-6}$  M norepinephrine. The records show two-way block and summation evoked by stimulating both ends. Calibrations: vertical, 100 mv; time marks are at 100 msec intervals.

that excitation of one end produced only a very small deflection in the center with no conduction to the other end; the second set of records shows a similar response to excitation of the other end. The third set of records shows that when both ends were excited the response in the center was larger than would be expected from mere addition of the deflections seen when either end was excited alone.

The results in Fig. 2 A-E were obtained on a preparation in which a small branch arose from that portion of the bundle that was in the center chamber. Recording site 2 was in that branch about 3 mm from the bundle proper. The center chamber was perfused with a solution containing 18 mM  $K^+$  and

$2 \times 10^{-6}$  M L-epinephrine. Fig. 2 A shows complete block between each end and the other end as well as complete block between each end and the branch. In Fig. 2 B excitation of both ends of the preparation resulted in summation with propagation out the branch. The same effect is seen in Fig. 2 C but the response in the branch appeared with great delay and, in one instance, failed to appear. Stimulation of the ends was not simultaneous in (B) or (C); the enhanced delay in (C) resulted from a very small change in the relative time of excitation of the ends, a phenomenon we have reported before (2). In Figs. 2 D and 2 E are shown the effect of increasing the epinephrine level by adding a drop of 1:1000 L-epinephrine to the center chamber. Conduction from site 1 to the branch and from site 1 to the other end reappeared temporarily, but (Fig. 2 E) excitation of the other end still failed to excite either the branch or the far end of the preparation, so one-way block was present. The records in Fig. 2 F and G were obtained from another preparation and are included to show that two-way block and summation are seen in the presence of 17 mM  $K^+$  and  $5 \times 10^{-6}$  M norepinephrine. Excitation failed to be conducted from one end to the other no matter which end was excited but when both ends were excited marked summation was seen at site 2 in the center chamber.

The effects of low  $K^+$  are shown in Fig. 3. The perfusate to the center chamber contained no  $K^+$ ; to be sure that no  $K^+$  leaked into the center chamber analysis of the effluent was made and it showed that the  $K^+$  concentration in the middle chamber was less than 1 mM. In this experiment the  $Ca^{++}$  level in the perfusate to the middle chamber was increased from 2.7 to 5.4 mM to take advantage of the fact that the depolarization during rest seen in low  $K^+$  is enhanced by elevation of  $Ca^{++}$  (7). Control records in Fig. 3 A show responses obtained when all three chambers were perfused with normal Tyrode solution. The first five action potentials at site 1 in Fig. 3 B are followed by low-voltage responses in the center; the first four responses in the center are in turn followed by a response at the far end, but there is a gradual increase in the delay between the response in the center and the response at the far end, the longest delay being seen just before conduction fails. This sort of progressive delay terminating in block resembles the so-called Wenckebach phenomenon. Excitation of the other end (last two responses at site 3) revealed the presence of complete block in that direction. The preparation thus showed a 5:4 block with the Wenckebach phenomenon in one direction and complete block of conduction in the other direction. At a later time in the experiment the preparation showed two-way block and summation (Fig. 3 C). The summation seen when both ends were excited is definite but not dramatic; we are unable to explain the curious shape of the action potential at site 2.

The records in Fig. 4 were obtained from a preparation in which the center chamber was perfused with a solution in which the  $Na^+$  concentration was

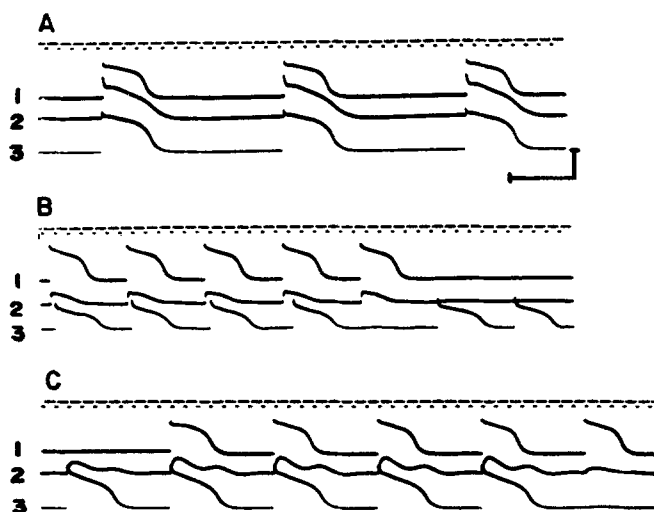


FIGURE 3. Records obtained in a three-chamber experiment with electrode 1 in one end chamber, electrode 3 in the other end chamber, and electrode 2 in the center chamber. Controls are shown in (A) with all three chambers perfused with normal Tyrode solution. In Fig. 2 B the bundle in the center chamber was exposed to low  $K^+$  (less than  $1 \text{ mM}$ ) and  $5.4 \text{ mM Ca}^{++}$ . Excitation of the end containing site 1 resulted in conduction to sites 2 and 3; excitation of the end containing site 3 revealed complete block in the direction from 3 to 2 to 1. Records obtained at another stage in the experiment are shown in (C); they show two-way block and summation. See text for discussion. Calibrations: vertical,  $100 \text{ mv}$ ; time marks at  $100 \text{ msec}$  intervals.

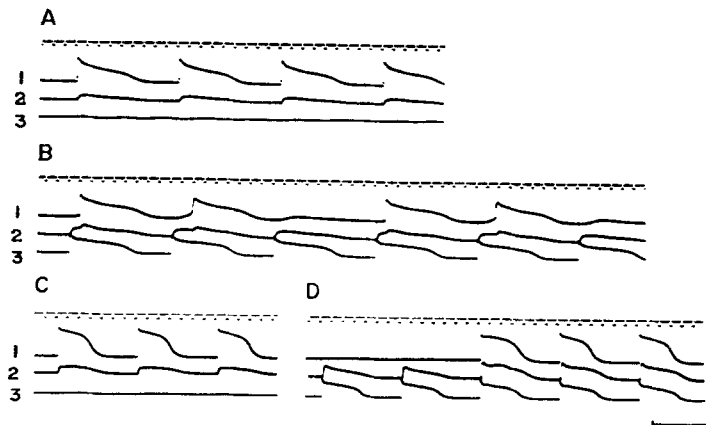


FIGURE 4. Records obtained in a three-chamber experiment with electrode 1 in one end chamber, electrode 3 in the other end chamber, and electrode 2 in the center chamber. The center chamber was perfused with a solution containing  $13.8 \text{ mM Na}^+$ . When the end containing site 1 is excited (Fig. 4 A) the response dies out in the center chamber and there is no conduction to the far end. When the end containing site 3 is excited (Fig. 4 B) the response in the center chamber is variable in amplitude and shape and conduction to the other end occurs first with delay and then with greater delay and then is blocked; that sequence then repeats itself. Figs. 4 C and 4 D show two-way block and summation; all conditions were as described above but the results were obtained on another bundle. Calibrations: vertical,  $100 \text{ mv}$ ; time marks are at  $100 \text{ msec}$  intervals.

13.8 mM, the NaCl having been replaced by choline chloride. Fig. 4 A shows that excitation of the end containing site 1 led to a very low-voltage response in the center with no conduction to the far end. Excitation of the end containing site 3 (Fig. 4 B) evoked a low-voltage response in the center. The first response showed a delayed second upstroke and excited the far end with marked delay; the second response in the center showed a more delayed second upstroke and excited the far end with even greater delay; the third response in the center was of lower amplitude, showed no second upstroke, and failed to excite the far end; that series of events was then repeated. This record thus shows two cycles of 3:2 block with the Wenckebach phenomenon. When conduction occurred with great delay the slow invasion of site 1 by excitation traveling through the center chamber produced a slow foot in the response at site 1 resembling the phase 4 depolarization seen in automatic cells. This ability of slow conduction to mimic spontaneous phase 4 depolarization has been described and discussed previously (1). The effective conduction velocity associated with this phenomenon as shown in Fig. 4 B was about 0.04 m/sec. In another preparation in which the center chamber was perfused with 13.8 mM Na<sup>+</sup> (Fig. 4 C and D), two-way block and summation were seen. All of the records shown in Fig. 4 were obtained at K<sup>+</sup> levels in the center chamber of 5.4 mM and without the addition of epinephrine. We have found that when Na<sup>+</sup> is very low and K<sup>+</sup> is normal the addition of epinephrine does not markedly facilitate conduction or summation.

We have also studied the effect of 13.8 mM Na<sup>+</sup> and 5.4 mM K<sup>+</sup> on bundles and networks of Purkinje fibers exposed over their full lengths to low Na<sup>+</sup>. Conduction over long distances fails in such preparations, as would be expected. In one preparation exposed over its entire length to 40 mM Na<sup>+</sup> and 11 mM K<sup>+</sup> we saw slow conduction throughout the length of the bundle and we frequently saw reentrant excitation of the type we have described elsewhere as reflection (3).

The experiment shown in Fig. 5 was conducted by perfusing the center chamber with 13.8 mM Na<sup>+</sup> and 11 mM K<sup>+</sup>. The records in Fig. 5 A show that excitation only of the end containing site 3 results in the appearance of a low-voltage, notched response in the center which succeeds in exciting the far end of the fiber with a fairly marked delay, while excitation only of the end containing site 1 evokes a much smaller response in the center and no conduction to site 3. This preparation thus showed one-way conduction with delay. At a later stage in this experiment the preparation showed two-way block with marked summation (Fig. 5 B). At the end of this experiment the center chamber was perfused with Tyrode solution containing a normal concentration of NaCl and 11 mM K<sup>+</sup>; full excitability in the center chamber and rapid two-way conduction through the center chamber occurred very soon, showing that, as would be expected, no severe depression occurs from the



effect of 11 mM K<sup>+</sup> when the Na<sup>+</sup> level is normal. The response at site 2 in the center chamber in Fig. 5 A shows one feature that requires further attention. When site 2 is excited by propagation from site 3 it responds with a slow, rounded upstroke that is eventually interrupted by a small but more rapid second upstroke. That second upstroke *follows* the upstroke at site 1; a similar effect is seen at site 2 in Fig. 4 B. We have reported this phenomenon

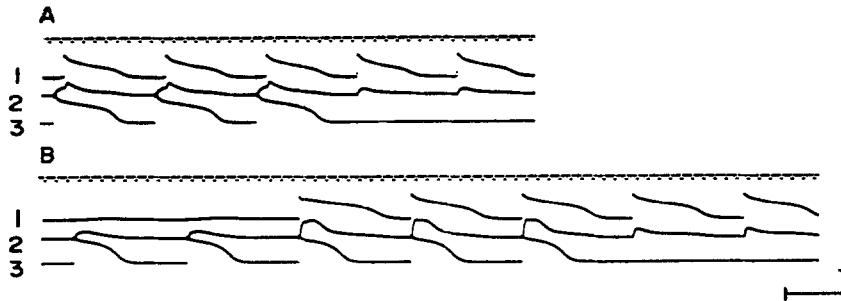


FIGURE 5. Records obtained in a three-chamber experiment with electrode 1 in one end chamber, electrode 3 in the other end chamber, and electrode 2 in the center chamber. The center chamber was perfused with Tyrode solution modified to contain 13.8 mM Na<sup>+</sup> and 11 mM K<sup>+</sup>. The records in (A) show that conduction from 2 to 1 occurs with delay while excitation from 1 to 3 is blocked in the center chamber. The response at 2 is discussed in the text. At another stage, shown in (B), two-way block and summation were seen. Calibrations: vertical, 100 mv; time marks are at 100 msec intervals.

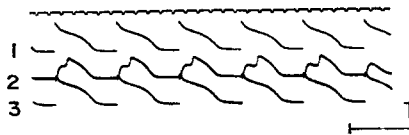


FIGURE 6. The upper (1) and lower (3) traces were obtained from each end of a bundle exposed to normal Tyrode solution in a three-chamber bath. The middle (2) trace was obtained from a fiber in the center chamber that was exposed to 17 mM K<sup>+</sup>. The preparation showed two-way block. When both ends were stimulated, as shown here, summation evoked a response in the center that had two upstrokes. See text for discussion. Calibrations: vertical, 100 mv; horizontal, 500 msec.

previously and have suggested that the appearance of excitation beyond the depressed segment might cause a retrograde electrotonic depolarization that would evoke the second upstroke in the depressed area (1).

The appearance of a second upstroke during the course of a slow response is also seen in Fig. 6 in records from a preparation in which the level of K<sup>+</sup> in the center chamber was 17 mM. Two-way block was present and all records shown were obtained during drive of both ends. The response at site 2 has two upstrokes in every tracing but the last one, in which only one upstroke is seen. The delay between the first upstroke and the second upstroke increases

progressively throughout the tracing until the last tracing when the second upstroke drops out. This suggests that the first upstroke was evoked by activity entering one end of the depressed segment and that the second upstroke was evoked by activity originating at the other end and reaching the depressed segment with progressive delay. The presence of the second upstroke suggests that the system may be shifted into a region of negative conductance by a rather small additional depolarization introduced in the course of the slow response.

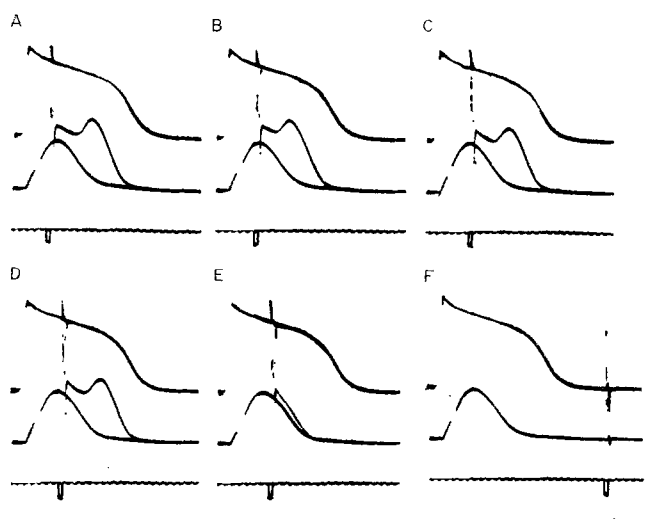


FIGURE 7. The upper traces were obtained from that part of a bundle exposed to normal Tyrode solution in a two-chamber bath. The lower traces were obtained from that part of the bundle exposed to 17 mM  $K^+$  in the other chamber. The basic drive was applied to the part of the fiber in the normal solution. A test stimulus 10 msec long was applied via an intracellular electrode about 1 mm away from the recording site in the end of the bundle exposed to high  $K^+$ . A regenerative depolarization and a second upstroke are seen in (A)–(D), but not in (E) where the stimulus was applied only 10 msec later than in (D), nor in (F) where the stimulus was applied in diastole. The stimulus strength was the same in all records. The vertical calibration represents 100 mv for the upper trace and 50 mv for the lower trace; the horizontal calibration is 100 msec.

The results shown in Fig. 7 show that an externally applied depolarizing stimulus can evoke a second regenerative depolarization during the course of the slow response. In the experiment shown in Fig. 7 the bath was used as a two chamber bath by inserting only one partition. Part of a bundle of Purkinje fibers was exposed to normal Tyrode solution in one chamber while part was exposed to 19 mM  $K^+$  in the other chamber. The preparation was driven through stimulating electrodes applied to the part of the bundle exposed to normal Tyrode solution. Two electrodes were placed in the part of

the bundle exposed to high  $K^+$ ; one was used to record while the other was used to apply intracellular stimuli. The recording and stimulating electrodes were less than 0.5 mm apart. Several features of interest appear in Fig. 7, in which an intracellular depolarizing square wave 10 msec long was applied at different intervals; the strength of the stimulus was  $5 \times 10^{-8}$  amp. A second regenerative depolarization was evoked when the second stimulus was applied slightly before, during, and slightly after the peak of the response (Fig. 7 A–D), but a further delay of 10 msec (Fig. 7 E) caused the stimulus to be ineffective. The second upstroke appears with a marked delay after the application of the stimulus. We have found the same phenomena with stimuli of 5 msec and with stimuli of 20–30 msec duration; in all cases a second upstroke can be

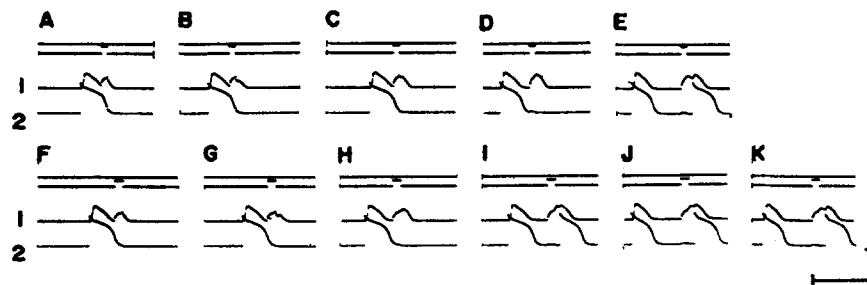


FIGURE 8. The lower traces (2) show records from that part of a bundle of Purkinje fibers exposed to normal Tyrode solution in one chamber of a two-chamber bath, the basic drive was applied to that part of the bundle. The upper traces (1) show records from that part of the bundle exposed to 16 mM  $K^+$  in the other chamber. Premature stimuli were applied intracellularly at a site in the bundle exposed to high  $K^+$  less than 1 mm from the recording site. In (A)–(E) the premature stimulus was 50 msec long and  $9 \times 10^{-8}$  amp in strength. In (F)–(K) the premature stimulus was 80 msec long and  $7 \times 10^{-8}$  amp in strength. See text for discussion. Calibrations: vertical, 100 mg; horizontal, 500 msec.

evoked when the stimulus is applied in the middle of the response but not when it is applied during the period of rapid repolarization. This behavior accords completely with that seen when summation and a second upstroke result from the addition of two action potentials; indeed, the responses in Fig. 7 are very similar to those in Fig. 6. It should be noted that when the stimulus was applied in diastole (Fig. 7 F) no active response was elicited.

The refractory period of the slow response also differs from that of the normal action potential as is shown in Fig. 8. The basic drive was applied to the part of the bundle in normal Tyrode solution; the premature stimulus was applied intracellularly to a fiber in the portion of the bundle depressed by exposure to 16 mM  $K^+$ . The records in Fig. 8 therefore show not only whether a response was evoked in the depressed fiber by a premature stimulus but also whether it could propagate into the normal part of the bundle. In Fig.

8 A–E the stimulus was 50 msec long and  $9 \times 10^{-8}$  amp in strength. The response in (A) appears to be purely passive; a response evoked as late as that seen in (D), well after full repolarization of both the depressed and normal parts of the bundle, failed to propagate. Only the very late response in (E) propagated into the normal part of the bundle; it shows a small second upstroke that follows the upstroke of the normal part of the bundle. A similar range of effects is seen in (F)–(K) in which the intracellular stimulus was 80 msec long and  $7 \times 10^{-8}$  amp in strength.

That the response varies with the stimulus is shown in Fig. 9. The conditions were as in Fig. 8, but the interval at which the stimulus was applied remained fixed and was well into diastole. The duration of the stimulus was 70 msec and the strength varied from  $4 \times 10^{-8}$  amp in (A) to  $8 \times 10^{-8}$  amp

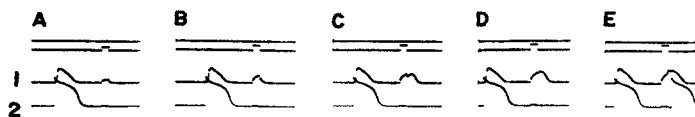


FIGURE 9. All conditions were the same as those in Fig. 8 except that a premature stimulus 70 msec long was applied at a fixed interval in diastole and its strength was varied from  $4 \times 10^{-8}$  amp in (A) to  $9 \times 10^{-8}$  amp in (E). See text for discussion. Calibrations: vertical, 100 mv; horizontal, 500 msec.

in (E). The responses in (A) and (B) appear to be purely passive; an active component is present in (C) and (D) but not until (E) does propagation back into the normal part of the bundle occur.

The records in Fig. 10 were obtained from a bundle of canine Purkinje fibers in a single-chamber bath. The recording electrodes were 15 mm apart. The records in Fig. 10 A show the response in normal Tyrode solution. The bundle was then exposed to 18 mM  $K^+$ ; after 20 min the bundle was completely inexcitable, whereupon  $5 \times 10^{-6}$  M epinephrine was added and the records in Fig. 10 B were obtained at three stimulus strengths. The stimuli were applied at one end of the bundle in (A) and at the other end in (B). In (B), at the weakest strength (response 1) a small local response, or perhaps merely a passive depolarization, was seen near the stimulus site (upper trace) but no deflection was seen 15 mm away from the stimulus site (lower trace). At a higher stimulus strength (response 2) a definitely regenerative response was seen near the stimulus site but only a very low-voltage deflection appeared 15 mm away. At a still higher stimulus strength (response 3) the response had all of the characteristics of the "propagated" response seen in depressed fibers. The record 15 mm away showed that the response undoubtedly had propagated and was capable of further propagation. It is equally clear that the response 15 mm away had a slightly lower amplitude, a shorter duration, and a definitely slower upstroke than it had had near the stimulus site.

The records shown in Fig. 11 were obtained in an experiment using a two-chamber bath. One electrode (upper trace) was in the part of the bundle exposed to normal Tyrode solution; two electrodes (middle and lower traces) were in the part of the bundle exposed to 18 mM K<sup>+</sup>. The middle trace was recorded about 1 mm from the partition; the lower trace was recorded about

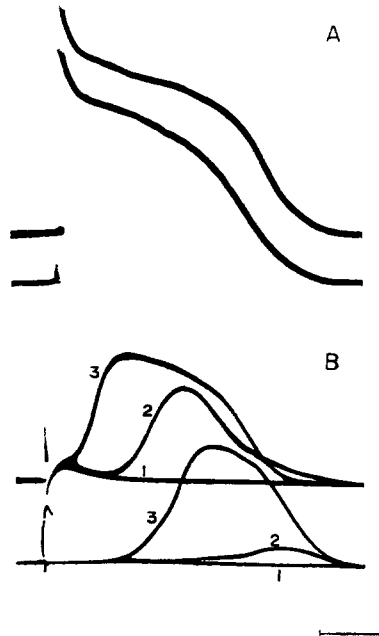


FIGURE 10. Records from a bundle of Purkinje fibers exposed to normal Tyrode in (A) and to 18 mM K<sup>+</sup> and  $5 \times 10^{-6}$  M epinephrine in (B). The bundle was stimulated at one end in (A), at the other end in (B). Normal action potentials are seen in (A), slow responses in (B). The recording sites for the upper and lower traces in (A) and (B) were 15 mm apart. The three responses in (B) were obtained with three different stimulus strengths and show gradation of response both near the stimulus site and remote from it. The stimulus in (B) was applied through bipolar external electrodes and was 15 msec long. See text for discussion. Calibrations: vertical, 50 mv; horizontal, 100 msec.

8 mm from the partition. When the normal end of the bundle was driven (Fig. 11 A) a normal action potential in it was followed by a typical slow response at site 2 (middle trace); the response at site 3 (bottom trace) had, in comparison with the response at site 2, a slightly lower amplitude and a definitely lower upstroke velocity. When the depressed end of the bundle was stimulated (Fig. 11 B) the response at site 3 had a slightly greater upstroke velocity than the response at site 3 in (A), while the response at site 2 had a far lower upstroke velocity than the comparable response in (A). Conduction did occur into the normal end, as is seen by the appearance of an action

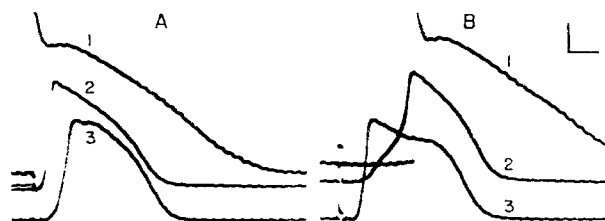


FIGURE 11. The upper traces (1) show records from part of a bundle of Purkinje fibers exposed to normal Tyrode in one part of a two-chamber bath. The middle (2) and lower (3) traces show records obtained from the part of the bundle exposed to 18 mM  $K^+$  in the other chamber. In (A) the stimulus is applied to the normal end of the bundle; in (B) the stimulus is applied to the depressed end of the bundle. See text for discussion. Calibrations: vertical, 20 mv; horizontal, 50 msec.

potential at site 1. The impulse thus conducted with decrement, losing amplitude and upstroke velocity as it traversed the depressed segment. The response at site 2 had a far greater upstroke velocity when evoked by propagation into site 2 from site 1 than when evoked by propagation into site 2 from site 3; this is further evidence for the stimulus dependence of the slow response since the stimulus for site 2 in (B) was a slow response depressed by traveling a considerable distance in partially depolarized fibers, whereas in (A) the impulse arose in normal fibers and underwent very little decrement before evoking the response at site 2.

#### DISCUSSION

*The Slow Response* A low conduction velocity, a tendency to block, the presence of graded response, and the presence of summation during part of the response are properties of depressed fibers that are very different from those seen in normal cardiac tissue. These phenomena appear when the normal upstroke is abolished by depolarization or by removal of external  $Na^+$ , i.e. by changes that abolish the  $Na^+$ -dependent events that are assumed to play a major role in the fast phase of depolarization in the action potential of normal cardiac cells.

That cardiac fibers can show regenerative depolarization when the rapid  $Na^+$ -dependent upstroke has been suppressed may be considered as established. Paes de Carvalho et al. have examined in detail the idea that the cardiac action potential might be composed of a fast component and a slow component, and a careful review of earlier studies pointing to the same conclusion is found in their article (8). Paes de Carvalho et al. particularly emphasized the possibility that the action potential of A-V nodal fibers might represent the slow component seen in isolation from the fast component. A slow response is presumably seen in normal cells of the atrioventricular node (8); in cells of the ventricular conducting system exposed to high  $K^+$  (1), to high  $K^+$  plus

epinephrine (9, 10), to low  $\text{Na}^+$ , to low  $\text{Na}^+$  plus epinephrine, or to tetrodotoxin (9); in atrial cells treated with high  $\text{K}^+$  or high  $\text{K}^+$  and epinephrine (11); in atrial and ventricular myocardial cells subjected to high  $\text{K}^+$  (12) or to high  $\text{K}^+$  and low  $\text{Na}^+$  (13); and in Purkinje fibers exposed to solutions containing no  $\text{Na}^+$  and no  $\text{Ca}^{++}$  to which 10 mM  $\text{Sr}^{++}$  was added (14, 15).

*The Ionic Basis of the Slow Response* It is not certain that the slow responses seen in fibers depressed in different ways are identical in every respect, although they may all depend upon ion flux through the same channels and have characteristics determined by the same permeability changes (see reference 15). The various slow responses certainly have much in common and differ greatly from the response seen in normal cells of the myocardium or ventricular conducting system. The fact that in many of our records, and in many other studies, some degree of local  $\text{Na}^+$  activation may persist does not alter the fact that when the rapid upstroke is abolished the characteristics of the response are chiefly determined by the subsequent flow of current through channels whose time- and voltage-dependent conductance changes differ markedly from those responsible for the normal rapid upstroke.

In general the slow response has been found to be tetrodotoxin insensitive and to be suppressed by 2–4 mM  $\text{Mn}^{++}$ , while the amplitude and upstroke velocity of the slow response are sensitive to external  $\text{Ca}^{++}$  concentration. Carmeliet and Vereecke (9), Pappano (11), and Mascher (12) have therefore suggested that the response of cells subjected to high  $\text{K}^+$ , low  $\text{Na}^+$ , or tetrodotoxin may depend upon a  $\text{Ca}^{++}$  current. Mascher has suggested that the electrical response of dog and cat papillary muscle depolarized by high  $\text{K}^+$  “may be the equivalent of the slow component of inward current found in voltage clamp experiments” (12), while Rougier et al. have spoken of the possibility that the “slow channel might well constitute a primary system more or less hidden under normal conditions” (16). These suggestions naturally rely on a variety of studies on the role of  $\text{Ca}^{++}$  currents in the action potential of normal cardiac cells (16–19). At the present time voltage-clamp studies and studies of the slow response have established the presence of permeability changes that show the characteristics needed to explain many of the properties of the plateau and of the slow response, but they have not shown whether the membrane current is carried by  $\text{Ca}^{++}$ , by  $\text{Na}^+$ , or by both.

*Graded Response* Most studies of the slow response have shown that its amplitude and upstroke velocity are stimulus dependent (9, 11–13). Graded responses are well known in many excitable tissues whose “normal” response is an all-or-nothing propagated action potential. Such responses are seen in normal cells of the myocardium or in normal cardiac Purkinje fibers during the refractory period (7) and in the giant axon of the squid during the refractory period or after various experimental insults (20); they are also seen

in electroplaques (21). The fact that the upstroke velocity and amplitude of the slow response are graded and stimulus dependent is not of fundamental importance in terms of membrane biophysics; its importance derives from the relationship of graded responses to summation and to decremental and incremental conduction, as discussed below.

*The Slow Response and the Normal Action Potential* When the fast component or rapid upstroke is present it evokes and is followed by the slow component or plateau, the two together making up the action potential of normal cardiac muscle and of normal cells of the ventricular conducting system. It appears that there are three possibilities for electrical response in the heart: (a) the fast upstroke followed by fast repolarization, (b) the slow response, and (c) the normal action potential in which the fast and slow components both appear. It is important, however, to note that the normal action potential cannot be regarded as the fast upstroke followed by the slow response, for, if it were, the plateau would show all of the properties of the slow response such as summation and the absence of a total refractory period. This suggests that the sequence of time- and voltage-dependent permeability changes responsible for the fast upstroke continue to exert an influence throughout the entire course of the action potential, even though the ionic currents responsible for the slow response surely play an important role in determining the characteristics of the plateau.

*Summation* We have used the term summation to describe a phenomenon seen in a depressed segment in which two-way block is present (2). On a descriptive level, summation is present when excitation at both ends of such a segment produces a depolarizing response at the center of the segment that is greater in amplitude than that expected from the mere addition of the depolarizations seen when either end is excited alone. This nonlinear behavior may result from several factors, some of which have nothing to do with the slow response. It is due in part to the fact that when both ends are excited so that impulses invade the depressed segment from both ends the cable properties are changed, as we have pointed out before (2). Summation might also be predicted from the property of anomalous rectification seen in normal as well as in high  $K^+$  Tyrode solution (22). That summation is nonlinear does not, therefore, prove the presence of active or regenerative depolarization. The functional importance of summation is that it may lead to active depolarization since the level of depolarization attained by summation may be sufficient to reach the threshold of a branch that arises at the center of a depressed segment and evoke an action potential that propagates out that branch. In addition, the level of depolarization attained by summation may be sufficient to evoke a second active depolarization in the course of a slow



response. Such a second active depolarization could also cause propagation out a branch and, even if no branch were present, it could alter the refractoriness of the depressed segment, thereby altering its response to the next action potential conducted into it.

*Conduction of the Slow Response* We have found in previous studies (1, 2), as well as in the present one, that when a short segment of a bundle of Purkinje fibers is depressed conduction through that segment becomes slow, often shows block, and may occur with decrement. In the present study we have found that these phenomena occur under a variety of conditions that lead to depression or abolition of the rapid, Na<sup>+</sup>-dependent upstroke and have suggested that when that upstroke is abolished conduction persists because of the persistence of the slow response.

The slow response has a slow upstroke velocity and the inward ionic currents upon which it depends are undoubtedly far smaller than those associated with the rapid, Na<sup>+</sup>-dependent upstroke. In addition the slow response is stimulus dependent. These characteristics are sufficient to cause slow conduction; they are also sufficient to cause decremental conduction (23, 24).

Since we have actually seen decremental conduction in our preparations it is tempting to regard all of the properties of conduction through a depressed segment as properties of decremental conduction of the slow response. To do so is misleading, however, since it overlooks the importance of the syncytial nature of Purkinje fibers. Those properties make it possible, for example, for decrementing slow responses in a pair of converging fibers to give rise to summation at the point of convergence of those fibers, thereby causing conduction to become incremental over a short distance. Decrement, or apparent decrement, could also be greatly exaggerated if inhibition (2) occurs at a point of convergence of two fibers. In addition, the safety factor of conduction of the slow response is so low that conduction could be altered by very minor anatomical features that would have no effect on the propagation of the normal action potential. We believe, therefore, that, while the presence of the slow response is necessary for the appearance of the phenomena we have described, a full and detailed explanation of them must take into account the syncytial properties of the tissue. For that reason neither uniform slow conduction nor simple decremental conduction in an unbranched fiber can safely be taken as a model of conduction through a depressed segment of cardiac Purkinje fibers.

*Significance for the Whole Heart* The difference between the rapid all-or-nothing conduction characteristic of the normal action potential and the slow and faltering conduction characteristic of the slow response may not be fundamental from the point of view of membrane biophysics but it is fundamental from the point of view of the spread of excitation in a network parts of which

are depressed. The following recapitulation of the properties of the normal action potential and of the slow response emphasizes those aspects of the slow response that seem particularly likely to play a role in the genesis of abnormal activation of the ventricle or in the genesis of cardiac arrhythmia.

The fast response has an amplitude of about 120 mv; the slow response varies in amplitude but may propagate when its amplitude is 60 mv or less. The fast response is abolished when depolarization reduces the resting potential to about 60 mv; the slow response can arise in more depolarized fibers. The fast response has a conduction velocity from 0.5 to 4 m/sec according to the tissue in which it appears; the slow response has an apparent conduction velocity of from 0.01 to 0.1 m/sec. The rate of depolarization of the upstroke of the fast response may approach 1000 v/sec; the rate of depolarization of the upstroke of the slow response may be less than 10 v/sec. The response of normal fibers is independent of the strength of the stimulus provided the stimulus reaches or exceeds threshold; in depressed fibers a longer or stronger stimulus evokes a response with a faster upstroke, a greater amplitude, and a longer duration than are seen in responses evoked by weaker or shorter stimuli. The application of a second depolarizing stimulus during the normal action potential produces little effect; the slow response shows a further depolarizing response to a second stimulus applied during its course. Recovery of excitability accompanies repolarization in the fast response; refractoriness far outlasts repolarization in the slow response. The fast response follows high rates of drive on a 1:1 basis; conduction of the slow response tends to fail at high rates of stimulation. The fast response has a high safety factor of conduction and overrides minor electrical and anatomical impediments to its spread; the slow response has a low safety factor of conduction and is correspondingly prone to block at branch points or other impediments to forward conduction.

Reentrant excitation may well play a role in causing ventricular extrasystoles, ventricular tachycardia and ventricular fibrillation. The presence of areas of depolarization in an otherwise normal syncytial network produces conditions favorable to reentrant excitation. An impulse conducted rapidly through the normal parts of such a network may linger in the depressed parts long enough to reemerge after the end of the refractory period of the normal portion and reexcite it (1-3) or may evoke circus movement of excitation and repetitive reexcitation of the depressed and the normal portions (4).

Reentrant excitation in the ventricle may thus depend upon the appearance of discrete areas in which depolarization causes the normal action potential to be replaced by the slow response. Reentrant arrhythmia is especially common in association with the localized ischemia caused by myocardial infarction. Such ischemia causes depolarization and, quite probably, local release of catecholamines (25, 26), thereby creating conditions particularly favorable to the appearance of the slow response.

## ADDENDUM

Since the above article was written we have found that the alpha-adrenergic agent methoxamine inhibits the slow response at concentrations that do not affect the normal action potential (Cranfield, P. F., B. F. Hoffman, and A. L. Wit. 1971. Block of conduction in partially depolarized cardiac Purkinje fibres induced by an alpha-adrenergic agent *Nature (New Biol.) (London)* **234**:159), and Dr. Wit has found that when portions of the ventricular conducting system of very old dogs are studied in the tissue bath normal action potentials are found in most of the preparation but typical slow responses are found in isolated areas even when the tissue is exposed to a normal ionic environment. The 15-yr old dogs from which these preparations were taken showed chronic reentrant arrhythmia.

In addition, Lieberman has shown, in tissue culture preparations of cardiac muscle, that when the strand of tissue narrows down to a few fibers the response has the characteristics of a slow response, showing slow conduction, decrement, and a tendency to block (Lieberman, M., J. E. Purdy, A. E. Roggeveen, and E. A. Johnson. 1971. Structure-function implications of a synthetic strand of cardiac muscle. *J. Gen. Physiol.* **58**:711. [Abstr.]).

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