Two Components of the Cardiac Action Potential

I. Voltage-time course and the effect of acetylcholine on atrial and nodal cells of the rabbit heart

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Abstract Transmembrane potentials recorded from the rabbit heart in vitro were displayed as voltage against time (V, t display), and dV/dt against voltage (dV/dt or phase-plane display). Acetylcholine was applied to the recording site by means of a hydraulic system. Results showed that (a) differences in time course of action potential upstroke can be explained in terms of the relative magnitude of fast and slow phases of depolarization; (b) acetylcholine is capable of depressing the slow phase of depolarization as well as the plateau of the action potential; and (c) action potentials from nodal (SA and AV) cells seem to lack the initial fast phase. These results were construed to support a two-component hypothesis for cardiac electrogenesis. The hypothesis states that cardiac action potentials are composed of two distinct and physiologically separable "components" which result from discrete mechanisms. An initial fast component is a sodium spike similar to that of squid nerve. The slow component, which accounts for both a slow depolarization during phase 0 and the plateau, probably is dependent on the properties of a slow inward current having a positive equilibrium potential, coupled to a decrease in the resting potassium conductance. According to the hypothesis, SA and AV nodal action potentials are due entirely or almost entirely to the slow component and can therefore be expected to exhibit unique electrophysiological and pharmacological properties.

Differences in the voltage-time course of transmembrane action potentials recorded from a variety of cell types in the same mammalian heart have been described by several investigators (see Hoffman and Cranefield, 1960). The detailed electrophysiological studies carried out on the right atrial preparation of the rabbit heart showed that action potentials from sinoatrial (SA) and atrioventricular (AV) nodes have a remarkably slow rising phase, lasting up...
to 30 msec, and show no distinct initial spike (Paes de Carvalho et al., 1959; Hoffman et al., 1959; Paes de Carvalho and Almeida, 1960). Yet records from atrial, ventricular, and Purkinje tissue of the same heart show the fast-rising, often “spiked” transmembrane action potential typical of mammalian cardiac tissue. To date, it has not been clear whether the voltage-time course of nodal potentials results from a progressive slowing of the mechanism responsible for the “spike” of other cardiac action potentials, or from the absence of this mechanism in SA and AV nodal cells.

A considerable amount of electrophysiological data obtained from Purkinje fibers supports the view that the initial spike is due to a transitory augmentation of sodium conductance (Draper and Weidmann, 1951; Weidmann, 1955; Deck and Trautwein, 1964). Studies concerned with atrial or ventricular muscle point towards a similar ionic mechanism for the upstroke of the action potential (Delèze, 1959; Hoffman and Cranefield, 1960; Brady and Woodbury, 1960; Paes de Carvalho et al., 1966; Giebisch and Weidmann, 1967; Rougier, Vassort, and Stämpfli, 1968). Many of the properties of nerve find their counterpart in atrial, ventricular, and Purkinje cells (electrical excitability, discrete threshold with all-or-none response, refractoriness, etc.). Such properties of nerve fibers in fact result from the characteristic voltage- and time-dependence of the mechanism controlling sodium permeability (Hodgkin and Huxley, 1952 a–d).

The question of whether the mechanism generating the initial spike is absent from or inoperative in nodal cells is well worth clarifying. To suggest that no such mechanism is operative in nodal cells is to say that their excitability may be quite different from that of adjacent parts of the myocardium. It seemed to us justifiable to inquire further into the matter, particularly when one considers the peculiar electrophysiological and pharmacological behavior of both the SA and AV nodes (Hoffman and Cranefield, 1960; Trautwein, 1963). The results reported below for rabbit heart show that the action potential of the “common” atrial cell (and ventricular and Purkinje fibers as well) is composed of a fast initial component and a slower component (plateau). They show also that the mechanism causing the initial fast component probably is lacking in SA and AV nodal regions.

METHODS

Preparations were dissected from the hearts of young rabbits weighing around 1.5 kg, and consisted of right atrium, interatrial septum, interventricular septum, and right atrial roof, including the sinoatrial and atrioventricular nodal regions. The endocardial surface of these preparations is readily accessible to electrophysiological exploration (Paes de Carvalho, De Mello, and Hoffman, 1959). Fig. 1 shows schematically the experimental arrangement. The tissue (PP) was perfused continuously with modified Tyrode solution (Na, 149 mM; K, 2.7 mM; Ca, 2 mM; Cl, 143 mM; Mg,
0.5 mM; H₂PO₄, 1.8 mM; HCO₃, 12 mM; glucose, 11 mM) equilibrated with a mixture of oxygen (95%) and carbon dioxide (5%) at 36°C.

Acetylcholine hydrochloride (Roche) dissolved in Tyrode solution (30 μg/ml) was topically applied to the surface of the recording site through a small glass micro-pipette (EP, Fig. 1; tip diameter about 30 μ) connected to a simple system of syringe and rigid tubing (SY). The tip of the ejecting pipette was kept immersed in the bath throughout the experiment to permit temperature equilibration. The pipette could be moved to the immediate vicinity of the microelectrode when a test was desired.

![Diagram](https://example.com/diagram.png)

**Figure 1.** Experimental arrangement for recording the transmembrane potential of cardiac cells as a function of time (V, t display) and its first time derivative as a function of membrane voltage (phase-plane or V, t display). PP, muscle; PB, paraffin bed; IN and SUC, inlet and suction of Tyrode solution; COX, O₂–CO₂ gas mixture; SIU, stimulus isolation unit (Bioelectric Instruments, Inc., Hastings on Hudson, N.Y. ISA 100); RM, recording micropipette; A, high input impedance preamplifier (Bioelectric Instruments NFlp); B, Tektronix type 0 plug-in unit main amplifier and type 132 power supply amplification system; C and D, Tektronix type 0 plug-in unit, operational amplifiers with standard compensating adaptors for frequency response control; display system: Tektronix 565 oscilloscope; ITB, internal time base; CA, rectangular pulse generator (Bioelectric Instruments CA5); a and b, recording and calibrating positions of the switch. Small quantities of a modified Tyrode solution could be ejected from a micropipette (EP) onto the recording site by applying pressure to syringe (SY).

The syringe was mounted at the same level as the tissue chamber in order to prevent unwanted gross leakage of fluid between trials. The actual concentration of acetylcholine (ACh) in the extracellular space during ejection was much lower than the concentration in the pipette due to dilution in the continuously flowing ACh-free perfusion medium. A dose of ACh sufficient to cause marked local effects at the site of recording exerted a detectable effect over an area approximately 300 μ in diameter.

The preparation was electrically driven at constant rate (2–3 cycles per sec) throughout an experiment. Stimuli were rectangular pulses isolated from ground (duration, 1 msec; intensity, 1.5 times threshold) applied to the atrial roof through a
pair of stainless steel pins. Only occasionally was the preparation allowed to beat spontaneously or stimulated at sites other than the atrial roof.

Transmembrane potentials were recorded between an intracellular microelectrode and a distant indifferent electrode (RM and IE, Fig. 1). The KCl-filled microelectrode was connected to an Ag, AgCl/3 M KCl half-cell. DC resistance of these electrodes was 15–20 Mohms. Tip diameters in this resistance range were shown by electron microscopy to lie around 0.25 μm for the type of glass capillary and filling procedure used (Oliveira Castro, 1968). The indifferent electrode was a similar half-cell connected to the tissue bath through a cotton wick and located at the end of the chamber from which perfusion fluid was removed by suction. The microelectrode was connected to a high input impedance preamplifier (A, Fig. 1) provided with input capacity neutralization. The membrane potential was displayed as a function of time (V, t display) on the upper channel of a two-beam cathode ray oscilloscope. The first time derivative of the action potential (dV/dt or V') was obtained with the aid of an appropriate operational amplifier (C, Fig. 1) and displayed in the lower channel of the oscilloscope as a function of membrane voltage (V', V display). Photographic recording of the oscilloscope display required the lower beam to be intensified proportionally to dV/dt. This was achieved by feeding the differentiated signal to the appropriate CRT cathode through a suitable phase-inverter and amplifier system, and superimposing a short square pulse to overcome nonlinearity in the relationship between Z-input voltage and trace brightness.

The recording system was calibrated as follows. Voltage and time calibration for the V, t display were derived from a low impedance source capable of delivering rectangular pulses of known amplitude and duration (CA, Fig. 1). The pulse was applied between the ground and the indifferent electrode (IE) with the switch in position a (Fig. 1). The V', V display was calibrated by means of a ramp pulse resulting from the integration of the rectangular pulse by the operational amplifier, D, and fed to the indifferent electrode with the switch in position b. The slope of the ramp generated by the integrator was determined by dividing its recorded amplitude in the V', t display by the known duration of the rectangular pulse. Fig. 2 C shows the V', t and V', V displays of a ramp waveform of 50 mv and 0.5 msec duration (100 v/sec). The slow potential drift which precedes and follows the ramp was due to AC coupling of the integrator output; this drift did not alter significantly the linearity of the ramp pulse observed. The V', V display shows an open rectangle. These records were used to adjust the input capacitance neutralization of amplifier A and set the frequency response of the differentiator to obtain a satisfactory compromise between fidelity and noise. The perfection of the circle obtained by adjusting the amplitude of V' was used to check for the existence of undesirable distortions of the V', V display due to phase shift in different parts of the recording system. Such distortion was minimal for values of dV/dt up to 1,500 v/sec. Fig. 2 A shows one of the useful properties of V', V display. Namely, it yields a straight line for any process in which V changes with time along a simple exponential time course. Furthermore, the time constant of the original exponential change can be determined from the slope of the straight line. Any process with an S-shaped time course (such as an action potential upstroke) in which both
the beginning and the end of the potential change can be fitted approximately by simple exponentials will yield a $V', t$ display of roughly triangular contour and in which the two straight segments represent the two exponentials (Fig. 2 D). A sudden change in the time course of one of the exponential parts of the action potential is easily recognized in the $V', t$ display. Fig. 2 E shows a record in which the time course of the final portion of the upstroke undergoes a marked slowing which is clearly visible in the $V', t$ as well as in the phase-plane display. Fig. 2 F demonstrates this same phenomenon for a case in which detection of a departure from an exponential time course at the end of the upstroke would be nearly impossible by inspection of either the $V', t$ or $V, t$ displays. This and other properties of the phase-plane will be used in the present paper to detect inflections in the action potential upstroke.

**Definitions**

*Phase of the action potential* refers to a segment of the $V, t$ display during the action potential (not to be confused with the phase-plane display of the action potential). The word "phase" has been used in this sense by Weidmann (1956), and is generally used in dividing the transmembrane potential record into phases 0, 1, 2, 3, and 4. In the present paper reference is made to fast and slow phases of the upstroke of the action potential.

*Component* refers to the membrane voltage expression of a distinct electrogenic entity consisting of depolarization and repolarization. It is the contention of the present paper that the "standard" cardiac action potential is formed by the concomitant activation of a fast and a slow voltage component associated as shown in Fig. 5.
Mechanism refers to the actual permeability (or other) mechanisms controlling the ionic current underlying a given voltage component. A mechanism may involve variations of one or several ionic conductances.

RESULTS

Transmembrane Potentials Recorded from the Rabbit Heart: V, t and \( V_t \), V Displays of Electrical Activity

Fig. 3 shows the electrical activity recorded from various cells in an isolated rabbit heart. Transmembrane potentials displayed as a function of time (\( V, t \) display) are recorded simultaneously with their first time derivative taken as function of membrane voltage (\( V, V_t \) display). The key to coordinates and calibrations is given in the insert. The \( V, t \) displays shown are similar to those reported previously for such cells (Paes de Carvalho et al., 1959; Hoffman et al., 1959). Cells from atrial muscle of the crista terminalis (Fig. 3 D) and right bundle branch (Fig. 3 I) generate reasonably smooth, S-shaped upstrokes as the membrane voltage courses from the resting level to the peak of the action potential. This is better seen in the phase-plane display, where the straight segments of the ascending and descending limbs show that, during these times, voltage changes at the membrane can be described by simple exponentials. Records of transmembrane action potentials obtained from the septal branch of the sinoatrial ring bundle (Paes de Carvalho et al., 1959), from the Purkinje system, and many parts of atrium and ventricle show similar simple S-shaped upstrokes. However, several other types of cells exhibit a variety of notches and inflections in the upstroke of their transmembrane action potentials (Fig. 3). These notches and inflections are brought out clearly in the phase-plane displays and can be considered in two separate groups: first, alterations of the ascending limb of the phase-plane display during the depolarization; and second, alterations of the descending limb of the phase-plane display during depolarization.

Phase-Plane Display of the Upstroke: the Ascending Limb and Its Variations

The ascending limb of the phase-plane display corresponds to the foot of the action potential. Its time course results from the discharge of membrane capacitance by the local longitudinal currents which precede the advancing wavefront (Tasaki and Hagiwara, 1957; Jenerick, 1963, 1964). If cable behavior, constant action potential shape, and constant propagation velocity are assumed, it can be shown that the slope \( k \) of the rectilinear segment of the ascending limb of the phase-plane display equals \( 2R_iC_0/\Theta /a \), where \( R_i \) is the resistivity of the intracellular environment, \( C \) is the specific membrane capacitance, \( \Theta \) is conduction velocity, and \( a \) is the fiber radius (Jenerick, 1964). Although the applicability of these concepts to the myocardium is debatable because of its geometry, a number of considerations warrant its tentative use...
Figure 3. $V$, $t$ (upper tracing) and $\dot{V}$, $V$ (lower loop) display of transmembrane recordings from the isolated rabbit heart. Most phase-plane displays show only the action potential upstroke. Tyrode solution, 36°C. A, spontaneously beating sinus node. B, same as A, driven electrically from the atrium. C, sinoatrial transitional fiber (SARB, Paes de Carvalho et al., 1959). D, crista terminalis. E, atrioventricular node ($N$ region, Paes de Carvalho and Almeida, 1960). F, lower AV node ($NH$ region). G, transition between lower AV node and His bundle. H, upper His bundle. I, right bundle branch; J and K, ventricular cells in the septum. All records except A obtained from hearts driven from the atrial roof. Key to coordinates and calibrations in the insert at lower right corner. Numbers in the $\dot{V}$ calibrations represent v/sec. All other calibrations as shown in insert. Rectangular pulse seen in most $V$, $t$ recordings was used to calibrate for voltage and time.
(see Discussion). Fig. 4 shows a slope, \( k = 7.5 \text{ msec}^{-1} \), for the ascending limb of the phase-plane display of an action potential recorded from a muscle fiber in the atrial roof. When values of 80 cm/sec for \( \Theta \) (Paes de Carvalho et al., 1959) are taken, \( 4 \times 10^{-4} \text{ cm} \) for \( a \) (Almeida, 1961), and 104 ohm cm for \( R_i \) (Weidmann, 1952), the value of the "fast" portion of \( C \) is approximately \( 1.3 \ \mu \text{F/cm}^2 \). A value of \( 2.4 \ \mu \text{F/cm}^2 \) for cardiac Purkinje fibers was reported by Fozzard (1966).

Jenerick (1964) also has shown that the phase-plane display can be used to compute the membrane ionic current, \( I_i \), during the action potential, provided \( C \) is known. At any instant the ratio, \( I_i:C \), equals \( [(m/k) - 1]V \), where \( m \) is the slope of the phase-plane display at the instant considered. The plot in the lower part of Fig. 4 represents this instantaneous \( I_i \) as a function of membrane voltage using \( 1.5 \ \mu \text{F/cm}^2 \) as a conservative value for the fast membrane capacitance (it should be kept in mind that \( C \) acts only as a scaling factor for \( I_i \)). It can be seen from the \( I_i \), \( V \) plot that membrane ionic current is virtually zero during the ascending limb of the phase-plane display, at least as long as \( k = m \). Therefore, depolarization at the foot of the action potential is due to local circuit flow that draws capacitive current from the recording site. The departure from the original slope \( k \) towards the end of the ascending limb is due to the progressive and marked increase in ionic inward current as the threshold potential (estimated \( V_T = -62 \ \text{mv} \) ) is reached.
Departures from a rectilinear contour for the ascending limb of the phase-plane display are expected to result from a variety of causes. Upward concavity observed during a propagating action potential indicates generally that the membrane ionic currents beyond threshold are larger than those needed to obtain phase configurations of the type seen in Fig. 4. This can result from two possible situations. First, the passive depolarization at the foot of the action potential may be diminished due to geometrical factors (e.g., branching of cell strands). Second, the active membrane current density is in excess of that generated by the preceding segments of a pathway. In this instance, propagation will be accelerated, as is the case for the lower nodal cells in Fig. 3 F and G.

Upward concavity also is observed in the presence of prominent diastolic (phase 4) depolarization (Fig. 3 A and B). In the special case of a cell group undergoing a slow and synchronous phase 4 depolarization, the voltage-time course should be identical to that of a “membrane” action potential, where \( I_+ = CV \). The similarity of form between the record of Fig. 3 A and the \( I_+ \) plot of Fig. 4 suggests that this situation may be quite closely approached within the pacemaking group of SA nodal cells.

An upward convexity may be expected in regions in which the ionic current is small compared to the local capacitive currents drawn by local circuit flow (e.g., convergence of cell strands or deceleration of propagation due to a local weakening of the depolarizing current).

**Phase-Plane Display of the Upstroke: Descending Limb and Its Variations**

The approach used by Jenerick (1964) for skeletal muscle assumes that the membrane potential during the later portion of the upstroke moves exponentially towards an equilibrium potential. The conductance changes responsible for the depolarizing current attain a maximum, stable value during the rectilinear descending limb of the phase-plane display. Even though this may not be always the case (See Discussion), it can be taken for granted in the case of atrial muscle cells such as the one studied in Fig. 4. It is therefore possible to determine an equilibrium potential, \( E_P \), by merely prolonging the straight segment of the descending limb until it intersects the \( V \)-axis. The slope of this rectilinear segment of the phase-plane display is proportional to the total ionic chord conductance. The equivalent slope in the \( I_+ \), \( V \) plot is actually equal to \( g_s/C \), where \( g_s \) is the sum of individual ionic conductances. If the sodium conductance markedly predominates during this part of the atrial action potential, one would expect to find \( E_{Na} = +30 \) mv and maximal \( g_{Na} = 18 \) mmhos/cm². The membrane potential, \( V \), often does not quite reach \( E_P \), probably because inactivation sets in before the peak of the action potential is reached.

Deviations from the standard type of descending limb of the phase-plane display appear as notches or inflections (Fig. 3 C, G, and H), or upward concavity (Fig. 3 F and K). The interpretation proposed is that these findings
are due to the coexistence of two distinct electrogenic mechanisms, which differ in current capability and in final equilibrium potential. The mechanism that has a high current capability is in all likelihood a Na-mechanism (Paes de Carvalho et al., 1966). The ionic nature of the mechanism with low current capability has not been determined by us, although some concrete possibilities exist (see Discussion). An inflection in the descending limb (Fig. 3 C and H) will be observed whenever the equilibrium potential of the low current (slow) mechanism is more positive than that of the high current (fast) mechanism. No inflection will be seen if both equilibria have the same value, or if the equilibrium potential of the slow mechanism is less positive than the other; no inflection will be seen, either, if the slow or the fast mechanisms appear in isolation (Fig. 3 E).

Identification of Fast and Slow Phases in the Upstroke

Inspection of the phase-plane display of a typical atrial cell (Fig. 3 D) shows a roughly triangular contour with straight segments in the ascending and descending limbs. The maximum rate of rise is 250 v/sec and it occurs approximately at a potential $V_m = -27$ mv (Fig. 4). The estimated threshold potential, $V_T$, is $-62$ mv and the equilibrium potential, $E_r$, for the descending limb is $+30$ mv. Inspection of Fig. 3 E, which depicts records obtained from an AV nodal cell (N region, see Paes de Carvalho and Almeida, 1960), also shows a roughly triangular contour. The inhomogeneity of the ascending limb prevents a clear determination of threshold but a value of $V_T$ around $-40$ mv can be assumed. The maximum rate of depolarization is obtained at a potential, $V_m = -25$ mv, and its value is only 13 v/sec. The peak voltage of the action potential is $V_p = +17$ mv. In view of these data alone it would be impossible to state that the upstrokes of Fig. 3 D and E are due to different mechanisms. In fact, a sensible hypothesis would be to state that the fast sodium mechanism responsible for depolarization in the atrium is “weakened” in nodal cells, so that net inward current becomes much smaller. However, observations on transitional cells interposed between “standard” nodal cells (Fig. 3 E) and “standard” fast-rising cells (Fig. 3 I) show that the difference between them cannot be accounted for solely by a progressive acceleration of the upstroke. In the transitional cell shown in Fig. 3 F (NH region of AV node, see Paes de Carvalho and Almeida, 1960) the maximum rate of rise reaches over 50 v/sec. The inflection in the descending limb of the phase-plane display suggests that such an increase in $V$ is due to the superposition of a fast mechanism, which, in spite of its higher current capacity, would by itself provide a peak voltage, $V_p$, considerably lower than that attained by the action potential. In this case, the observed amplitude of the action potential is due to the persistence of a slow mechanism similar to that seen in the nodal record of Fig. 3 E. It is worthwhile noting that the inflection in
Fig. 3 F occurs approximately at \( V = 0 \), when \( \dot{V} = 13 \text{ v/sec} \). At the same voltage, the phase-plane display in Fig. 3 E shows \( V = 10 \text{ v/sec} \). This observation is, therefore, in keeping with the interpretation provided above.

Fig. 3 G shows records obtained from a transitional His bundle cell quite near the AV node. The maximum rate of rise has mounted to a value of 93 v/sec and the inflection between fast and slow portions of the descending limb of the phase-plane display is quite evident. Again, it should be noted that what appears to be the contribution of the slow mechanism during depolarization has a rate of rise comparable to that of Fig. 3 E at the same voltage (approximately 12 v/sec at \( V = 0 \)). The existence of a notch in the descending limb in Fig. 3 G instead of a smooth transition (Fig. 3 F) is due only to the time lag between the activation of fast and slow mechanisms. The nearby His bundle cell of Fig. 3 H shows a fast component which is still better developed (maximum \( \dot{V} = 105 \text{ v/sec} \)) with only a small tail due to the slow mechanism. The apparent equilibrium potential for the fast mechanism is near zero. In the right bundle branch cell of Fig. 3 I the fast mechanism is responsible for nearly all of the upstroke, its maximum \( \dot{V} \) being 264 v/sec.

The same type of transitional configurations described above can be observed in the transition between SA node and atrium (Fig. 3 C). Cells in some regions that are apparently not transitional will also show a slower segment in their upstrokes. This is the case with the ventricular cell of Fig. 3 K and is interpreted to mean that such cells have a “normally weak” fast mechanism in comparison to others.

The Two Component Hypothesis

The well-known finding of inflections in the upstroke of the cardiac action potential (Hoffman and Cranefield, 1960) has been construed in the above sections to mean that at least two ionic mechanisms can generate an action potential upstroke in cardiac cells: a fast, high current mechanism and a slow, low current mechanism. Fig. 5 represents diagrammatically a hypothesis that is somewhat broader than this basic assumption (see Discussion for comments). The hypothesis states that the action potential of cardiac muscle has two voltage components which are distinct and separable (Fig. 5 A). The initial fast component consists of a rapid depolarization (provided by the “fast ionic mechanism” referred to above) and has a short duration. The slow component consists of a slow depolarization (provided by the “slow ionic mechanism” referred to above) but its duration is much longer than that of the other component. In a cell with electrical activity of the type shown in Fig. 5 B, the fast component contributes the “initial spike” (phase 0 and 1 of Weidmann, 1956), and the slow component provides the voltage-time course of plateau and repolarization (phases 2 and 3 of Weidmann). The phase-plane display during the upstroke shows no isolated slow segment
which can be ascribed to the slow component (S, Fig. 5 B). In cells of the type illustrated in Fig. 5 C, the fast component contributes only part of the upstroke (up to the arrow). The remainder of the upstroke, as well as plateau and repolarization, is dependent upon the slow component. Accordingly, the phase-plane display of the upstroke exhibits a tail due to the slow component. Fig. 5 D is the diagrammatic representation of recordings from a cell that shows no detectable fast component. The whole action potential and the phase-plane display of the upstroke can be attributed to the slow component.

The hypothesis states, in summary, that variations in voltage-time course of the cardiac action potential are due to a variable contribution of its two voltage components to the final configuration. It also implies that nodal cells exhibit a transmembrane action potential caused by a pure (or almost pure) slow component whose ionic mechanisms may be quite different from those responsible for the fast component of the standard atrial action potential.

The Effect of Local Application of Acetylcholine on the $V_t$ and $V$ Displays

Topical application of acetylcholine (ACh) to the recording site by means of a small micropipette exerts a pronounced effect on all cells in the rabbit atrial preparation from the sinus node through the N region of the atroventricular node. The technique of topical application permits observation of the onset of and recovery from the effect of ACh in the course of a few cycles during maintained impalement of a single cell. This provides a marked advantage where prolonged impalement is difficult to sustain. The technique has the additional advantage of allowing invasion of the area influenced by ACh (about 300 $\mu$ diameter) by a "normal" propagated action potential. Lack of

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\text{Figure 5. A, diagrammatic representation of the two-component hypothesis. B, C,} \\
\text{and D contribution of fast (F) and slow (S) components to the $V_t$, $V$, $V$ displays} \\
\text{of the three action potential types recorded from the rabbit heart (B, atrium, ventricle,} \\
\text{or His-Purkinje system; D, SA or AV nodes; C, transitional regions). Arrow indicates} \\
\text{peak voltage of fast component. Dotted lines in the $V_t$ record mark hypothetical time} \\
\text{course which would be observed if the slow component were absent. Traced lines in} \\
\text{the $V$, $V$ display represent contribution of the slow component. The $V$, $V$ diagrams are not} \\
\text{drawn to scale. Question mark in D indicates uncertainty as to the possible existence of a} \\
\text{residual fast component. Note that the "plus" sign in A is used only to indicate that} \\
\text{the two components contribute to the voltage-time course. This representation should not} \\
\text{be taken to mean that the action potential is due to the algebraic sum of its components.}
\end{array}
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precise control of the quantity ejected and of final extracellular ACh concentration is the disadvantages of the method.

Fig. 6 shows the results of such an experiment. Records are from atrial (A–B) and transitional (C–D and E–F) cells in which impalement was main-

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Effect of topical application of acetylcholine through a micropipette. Preparation driven from the atrium. First column, control records from three different cells. Second column, effect of acetylcholine during impalement of the same three cells. Third column, superimposed tracings of control and acetylcholine records in the same row. First row (A, B), interatrial septum. Second row (C, D) and third row (E, F), two different cells in the sinoatrial transitional region. Coordinates and calibrations as in Fig. 3. Rectangular pulse in $V$, $t$ records has an amplitude of $50 \text{ mv}$ and a duration of $20 \text{ msec}$.

tained throughout application of ACh and recovery from its effects. Records labeled A, C, E show controls in three different cells before the test. Records shown in B, D, F, from the same three cells, were obtained during the action of ACh. Diagrams at the right side of each row represent superimposed records for the same row.
The atrial action potential in Fig. 5 A has a pure “fast” upstroke and a “plateau.” Its maximum is of the order of 300 v/sec. According to the working hypothesis presented above, “fast” and “slow” components combine here in the manner depicted in Fig. 5 B. ACh caused only negligible hyperpolarization of this cell. Shortening of the action potential was marked. The plateau (slow component) was markedly depressed and the fast component was practically unchanged. There is a slight decrease in the peak voltage of the action potential, but this was not a constant feature in such records. Other atrial cells with similar action potentials undergo hyperpolarization and their upstrokes show a concomitant increase in the maximum rate of rise while peak voltage remains the same. This behavior would be expected if the depolarizing current were augmented by the resting hyperpolarization without appreciable change in the final equilibrium potential of the fast component (V intersect of the rectilinear portion in descending limb of V, t display).

The records obtained from a transitional cell (Fig. 6 C and D) show that ACh abolishes the slow phase of the upstroke simultaneously with depression of the plateau. In accord with the scheme presented in Fig. 5 C, both these portions of the action potential result from the slow component. Hyperpolarization is present and the fast phase of the action potential upstroke (fast component) is enhanced. It should be noted, however, that the final equilibrium potential of the fast phase (which can be estimated by prolonging to the V}

Figure 7. Effect of local application of acetylcholine on a single SA nodal cell. Preparation driven from the atrium. A, control. B and C, onset of acetylcholine action. D, E, and F, recovery. Coordinates and calibrations as in Fig. 3. V, t calibration, 50 mv and 100 msec. V, V calibration, 10 v/sec and 50 mv. The small spike observed in D and the shape of the phase-plane display suggest that there may be a residual fast component which is unable to propagate in the presence of acetylcholine due to the weakness of its current-generating mechanism.
axis the rectilinear segment of the descending limb in the phase-plane display) is not much changed by ACh. Much the same is observed in Fig. 6 E and F recorded from a transitional cell in which the fast component reached a potential of only $-20$ mV. The irregularity of the initial part of the upstroke in Fig. 6 F is suggestive of a local conduction disturbance caused by ACh (see Discussion).

The action potential of nodal cells is depressed \textit{in toto} by ACh (Fig. 7). As expected, hyperpolarization and depression of slow diastolic (phase 4) depolarization are clearly seen. These action potentials show no identifiable separate ACh-resistant fast phase in their upstrokes and would therefore be tentatively classified as pure slow component action potentials (Fig. 5 D). Again, ACh shows its effectiveness in depressing the low current slow component.

It will be recognized at once that ACh causes conduction block in both SA and AV nodes. Such an idea is commonplace in the case of the AV node (Cranefield et al., 1959; Paes de Carvalho et al., 1966). It has not been so clearly appreciated in the case of the SA node since the action of ACh on slow diastolic depolarization results in the cessation of automatic firing. Cells below the middle portion of the AV node (below the N region) are all quite insensitive to ACh. It is felt, however, that cells in the atrial muscle provide reasonably good examples of action potentials composed of both fast and slow components (see Discussion), as do the action potentials of cells located below the AV node.

**DISCUSSION**

1. \textit{Recording Membrane Potentials}

Much of the present argument is based on the assumption that the records obtained are representative of voltage changes which actually take place at the membrane of the cardiac cell. To be sure, irregularities in the upstroke might possibly be caused by the interference of extracellular potential changes due to the advancing front of excitation. This interference would be expected to have a biphasic waveshape in the $V$, $t$ display similar to that of a local electrogram. It would, if strong enough, cause an indentation or step in the upstroke of the action potential which would be clearly seen in the $V$, $V$ display and might be mistaken for the onset of a slow phase of depolarization.

There are two ways in which extracellular currents can influence an intracellular recording. First, the extracellular potential changes might be directly picked up or sensed by the recording system. Second, current flow due to activity in neighboring sites might be strong enough to cause a noticeable IR drop across the cell membrane at the recording site. The first possibility often has been debated; its reality has been recently pointed out by Niedergerke and Orkand (1966) who were able to abolish most of the indentation observed in
the upstroke of the frog ventricular action potential by using a close extracellular microelectrode as a voltage reference. In our experience it has been observed that: (a) microelectrodes that yield such distorted upstrokes in a "unipolar" recording are always able to pick up a sizeable biphasic extracellular potential if their tip is positioned just outside the cell; (b) the larger the microelectrode (as judged from dc resistance) the greater is the interference from the extracellular field; (c) even with large electrodes, direct extracellular interference is sensed at the millivolt level only in regions where the advancing wavefront is sharp and/or the mass of tissue relatively large; (d) with 15–20 Mohm electrodes we recorded only negligible biphasic extracellular potentials; and (e) notches and steps on action potential upstrokes recorded through these electrodes cannot be abolished by recording against a similar and very closely placed extracellular reference microelectrode. On the strength of these observations, it was felt that the voltage data faithfully represent changes occurring at the cell membrane. The second possibility, i.e., that the records were influenced by a local IR drop caused by extracellular currents entering or leaving the cell, must be discussed in relation to membrane characteristics. Interference from extracellular currents will be negligible if membrane resistance is low as is the case during the fast phase of depolarization (see below). However, such interference can and does occur when the polarization resistance of the membrane is high, provided the extracellular current is strong enough. An example of this is provided by the small biphasic potential change often seen during the plateau of the transmembrane action potential recorded from right bundle branch cells in the rabbit's interventricular septum (Fig. 3 I). This is recorded simultaneously with activation of the underlying mass of ventricular muscle and cannot be abolished by use of a close extracellular reference microelectrode. Such voltage displacements are recorded by the microelectrode because they actually represent changes in membrane voltage.

Also important is electronic interaction between neighboring interconnected cells. As noted above, such interaction is minimized while membrane resistance remains low. This is the case during the fast phase of depolarization. On the other hand, electrotonic interaction is favored when membrane resistance is relatively high. The subthreshold potential change caused by local currents which lead an advancing wavefront along a uniform path has been thoroughly studied (Tasaki and Hagiwara, 1957; Jenerick, 1964). The effect of these currents is predictable. They do not lead to notching in regions of uniform propagation (see Results). It remains to be examined whether or not the slow portion of the upstroke of action potentials of transitional cells may be only a sign of electrotonic interaction due to larger action potentials with rapid upstrokes in neighboring cells. If this were the case, most of the evidence presented to demonstrate the slow component could be discarded. However, this
possibility is ruled out by the following considerations: (a) the SA nodal action potential (slow component) is the first electrical activity to make its appearance and, therefore, its voltage-time course cannot be due to electrotonic interaction; (b) AV nodal action potentials (slow component) propagate with a low but finite velocity while keeping an appreciable overshoot, a finding which is difficult to explain in terms of simple electrotonic interaction; (c) even though electrotonic interaction may occur during the plateau, the slow component cannot be considered as such because a plateau appears in preparations small enough to yield a membrane action potential upon excitation (Deck and Trautwein, 1964); and (d) although transitional action potentials gain an initial fast component as they propagate out of nodal areas and lose it as propagation moves in the reverse direction, there is no concomitant decrement in amplitude of the slow component.

2. Phase-Plane, Capacitance, and Ionic Conductances

The method devised by Jeneriek (1963, 1964) to analyze the phase-plane display of action potentials recorded from frog skeletal muscle is based on formulation of the cable equation for a propagating action potential (Hodgkin and Huxley, 1952 d). This formulation assumes: (a) applicability of cable analysis to the giant axon; (b) conformity with the assumption of an equivalent circuit model with independent ionic channels in parallel with a capacitance; and (c) negligible extracellular resistance. Cable behavior is neither observed nor expected in atrium when a polarizing current is fed through a microelectrode (Woodbury and Crill, 1961). However, cable behavior is very nearly approached in the case of a flat, extensive propagating wavefront. The assumption of independent ionic channels is taken as valid by most investigators in the field (Weidmann, 1956; Hoffman and Cranefield, 1960; Noble, 1966). As for extracellular resistance, even though it may be considered low for surface fibers of frog skeletal muscle, it is certainly not negligible in cardiac muscle. Extracellular resistance will introduce a constant factor equal to \( r_e/(r_i + r_e) \) in estimates of membrane capacitance derived from the foot of the action potential, \( r_i \) and \( r_e \) being the intracellular and extracellular resistance per unit length of fiber (Weidmann, 1969). This would result in capacitance values equal to approximately 75% of those calculated from Jenerick's (1964) formulation, provided one takes \( R_i = 104 \, \text{ohms cm} \), \( R_e = 53 \, \text{ohms cm} \), and an extracellular space occupying 40% of the cross-sectional area of a muscle bundle. Another uncertainty is related to the actual value of \( R_i \). Data for Purkinje fibers range from 104 ohms cm (Weidmann, 1952) to 154 ohms cm (Coraboeuf and Weidmann, 1954). Recent estimates (Weidmann, 1969) of \( R_i \) in ventricular trabeculae of ungulate hearts point to a value of 470 ohms cm. Membrane capacitance in these experiments was found to be 0.81 \( \mu \text{F/cm}^2 \). If the same \( R_i \) were valid for rabbit atrium, mem-
brane capacitance should be considerably less than 1.5 μF/cm². However, as pointed out before, C enters merely as a constant factor in the calculation of ionic currents from phase-plane data and its exact value is not important for the present discussion. The shape of the phase-plane display and the critical V-axis intersections would remain the same for any constant value of capacitance.

The statement was made that a rectilinear segment in the descending limb of the upstroke in the phase-plane display was assumed to indicate constant membrane ionic conductance. This is not necessarily true, for certain exponential variations in one or more conductances also will yield straight segments in the phase-plane display. A constant ionic conductance is, nevertheless, a fair assumption, since activation of "excitable" ionic conductances is thought to be complete at these voltages (Hodgkin and Huxley, 1952 a-d). Therefore, we interpreted the straight segment of the descending limb as meaning constant ionic conductance and showing, by its V-axis intersect, the actual equilibrium potential during that phase. The slope of this straight segment in the I-V plot is equal to the ratio g:\( \frac{C}{C} \), where g is the sum of ionic conductances. For practical purposes, when a fast component is present it is probable that g = gNa + gK. It is therefore impossible to ascertain the value of ENa in transitional cells, where peak gNa is low. However, it should be pointed out that even if peak gNa is as low as 5 mmhos/cm² it would still be from 10 to about 50 times greater than resting gK (Weidmann, 1952; Weidmann, 1969) and the membrane equilibrium potential should be at most 10-15 mv negative with respect to ENa during the straight segment of the descending limb. When peak gNa is 15 mmhos/cm², the active membrane equilibrium potential should be removed from the Na equilibrium potential by no more than 5 mv even if gK remains at its resting value. Another point that warrants consideration is whether a swing in gK from its assumed resting value of 0.5 mmho/cm² to a low value of 0.1 mmho/cm² during the slow component would be enough to explain the shift of equilibrium potential to the more positive values observed at the end of the descending limb of the phase-plane display for transitional cells when the assumed peak gNa = 5 mmhos/cm². Taking ENa = +30 mv and EK = -100 mv, the equilibrium potential at peak gNa and resting gK would be:

\[ E_r = \frac{g_{Na} E_{Na} + g_K E_K}{g_{Na} + g_K} = +9.1 \text{ mv} \]

while the equilibrium potential after fall of gK to its low value would be +27.4 mv. This calculation apparently is consistent with the possibility that the change from fast to slow phase in the upstroke of transitional cells is due only to the onset of a decrease in gK. However, in order for the above conclusion to hold true, it would be necessary that a gNa of 5 mmhos/cm² be main-
tained at least until the peak of the slow component is reached. This does not conform to the finding of high polarization resistance of these cells during the slow phase of their action potential upstroke (B. F. Hoffman and A. Paes de Carvalho, unpublished observations). The effect of ACh on the slow phase of depolarization is also suggestive of a low conductance in the inward current channel at that moment. Furthermore, it would be difficult to explain by this mechanism the amplitude and overshoot of nodal action potentials, since peak $g_{Na}$ must be extremely low (if the fast $g_{Na}$ mechanism is activated at all). It seems possible, therefore, that the slow component has its own unique ionic mechanism for providing depolarization and that its equilibrium potential is in the region of or even beyond the Na equilibrium potential.

Another objection that could be raised against the above interpretation is related to the actual equivalent circuit of the cardiac cell membrane. Fozzard (1966), using subthreshold pulses, showed that about 80% of the total membrane capacitance of Purkinje fibers is in series with a resistance. If this capacitance corresponds to inactive membrane, its charge during the action potential could divert ionic current from the other 20% of the membrane capacitance. The result would be to decrease peak potential for the fast component. If inactivation of Na$^+$ current is slow a clear slow component would be obtained, with a peak voltage higher than the apparent equilibrium potential for the fast component. Even though the possibility presents an apparently attractive physical explanation for fast and slow components it does not explain satisfactorily the observed results. First, a final slow phase of depolarization would only be obtained from this model if $g_{Na}$ remained high during the slow component. This does not seem to be the case (see above). Second, the action of ACh (resulting in an increase in $g_K$) would not abolish the slow component in the model. The slow component might in fact be enhanced if peak $g_{Na}$ were to be increased by hyperpolarization. Neither of these two predictions is in agreement with experimental findings. In fact, one may wonder why Fozzard's series capacitance-resistance branch does not affect more obviously the voltage records during activity in the present experiments on rabbit atrium. It is well to keep in mind that the "tubular" membrane which would correspond to such a capacitance may itself be active during the action potential, thus obviating or at least decreasing the effect of current diverted from the fast surface membrane capacitance.

Furthermore, it seems unwise to speculate about the contribution to the fast and slow components of the upstroke by the fast and slow components of the membrane capacity since we know so little about the geometry of the membranes under discussion. Several authors (Johnson and Sommer, 1967; Bencosme et al., 1969) have demonstrated that T tubules are not present in Purkinje fibers. Further, it has been shown (Johnson and Sommer, 1967; McAllister, 1969) that nexuses could have the same effect as the T tubules
and provide fast and slow components of membrane capacity. However, since the nexuses connect active membrane of adjacent fibers, the likelihood that this would provide a fast and slow component of the action potential upstroke is minimized. Finally, electron microscopic observations on nodal cells have suggested that T tubules and tight junctions of the nexus type are sparse or absent (Kawamura, 1961; James and Sherf, 1968).

3. Reality of the Two-Component Concept

Differences between the behavior of the membrane during phase 0 and during the remainder of the action potential in atrial, ventricular, and Purkinje fibers have been emphasized by several authors (Weidmann, 1956; Hoffman and Cranefield, 1960). It has been made quite clear that the ionic mechanism underlying the initial fast depolarization (phase 0) differs from that responsible for the plateau irrespective of what the mechanism for the latter might be (Weidmann, 1956; Cranefield and Hoffman, 1958; Shanes, 1958; Brady and Woodbury, 1960; Deck and Trautwein, 1964; Noble, 1966). However, no attempt was made by these investigators to explain the mechanisms responsible for action potentials that changed slope abruptly during the upstroke; and no thorough studies are available to warrant serious consideration of possible ionic mechanisms for nodal action potentials.

Irregularities in the upstroke of the action potential of frog ventricle have been construed by Hoshiko, Sperelakis, and collaborators (see Tarr and Sperelakis, 1964) as evidence in favor of special, nonelectrical transmission at intercalated discs. Their evidence and their interpretation of experimental data have been seriously challenged by many who favor Weidmann's conclusion that resistance of the intercalated disc is low enough to permit the transcellular flow of local current at an excitatory wavefront (see Weidmann, 1966). Wright and Ogata (1961) were the first to call attention to the fact that a sudden change in slope often is present in the upstroke of the action potential of frog heart and that such change in slope suggests a transition from one type of membrane behavior to another. They stated clearly the hypothesis of a two-component action potential. Their initial fast component was acetylcholine-insensitive; the slow component was abolished by acetylcholine, much as we have observed. They did not extend their analysis to sinus pacemaker potentials and did not emphasize the possibility that the slow component might be an autonomous electrogenic mechanism. The same can be said of the more recent contribution of Antoni and Delius (1965) who described correctly the fast and slow phases of the action potential upstroke recorded from frog myocardium, calling them phases I and II. They showed that phase II was markedly sensitive to manganese ions but did not clearly state that this phase is part of the same electrogenic process that generates the plateau. Their characterization of a slow component is thus not as clear as that of Wright.
A. PAES DE CARVALHO ET AL. Two Components of Cardiac Action Potential

and Ogata. Niedergerke and Orkand (1966) noted the occurrence of a slow, calcium-sensitive phase in the final part of the action potential upstroke. Notches in the transition between fast and slow phases could be seen in the records, but were mostly discarded as resulting from a summation of intracellular and extracellular potentials, even though experiments using a close reference microelectrode failed to abolish notching completely.

Our own concept of a two-component action potential was derived independently as a result of observations on the regional electrophysiological peculiarities demonstrated by different cell types in the rabbit atrium (Paes de Carvalho et al., 1959; Hoffman et al., 1959; Cranefield et al., 1959; Paes de Carvalho and Almeida, 1960; Paes de Carvalho, 1961, 1964; Paes de Carvalho and Hoffman, 1963; Paes de Carvalho et al., 1963, 1966). Evidence presented in favor of this view is the following: (a) successive recordings from a series of cells located at different points on the pathway between either SA or AV node and the surrounding fast-conducting tissue show that the fast component acquired by the atrial action potential is superimposed upon the preexisting slow component of the nodal action potential; (b) acetylcholine decreases the amplitude of the slow component while leaving the fast component either apparently unchanged or enhanced by hyperpolarization; (c) the action potential of nodal cells appears to be formed by a slow component not accompanied by an initial fast component. Attempts to modify the slope and magnitude of the upstroke of action potentials recorded from cells in the AV node (Hoffman, 1961) have shown that hyperpolarization does not increase and that depolarization does not decrease either the slope of phase 0 or the overshoot in a manner to be expected of the usual voltage-dependent \( g_{Na} \). It is therefore likely that different and independent electrogenic mechanisms underlie the two components. These mechanisms would operate in sequence in a "complete" action potential; they also may occur and propagate independently.

Important evidence for a dual composition of the cardiac action potential comes from data obtained by other investigators. Churney and Ohshima (1963), in a paper in which they suggest that the "fundamental response" of the cardiac cell is a spike, show recordings in which action potential shapes are seen to alternate between a normal contour and a pure spike. Moreover, the action potentials with a normal contour had initial fast phases of amplitude identical to that of the pure spikes (see Figs. 3 and 4, Churney and Ohshima, 1963). These data show that the slow component may fail as a whole and, therefore, can be possibly considered as a physiological entity in cardiac electrogensis. A quite clear temporal separation between fast and slow components can be observed in Purkinje fibers during exposure to and recovery from prolonged exposure to lithium (see Fig. 4 in Carmeliet, 1964). In this case, recovery of a full-sized slow component precedes recovery of the
fast component. A similar point was made by Pillat (1964) who noted that early extrasystolic action potentials recorded from ventricular muscle failed to develop a plateau. These abnormally short action potentials evidently consist of a pure fast component, the slow component having failed as a whole. Prominent separation between spike and plateau in Purkinje fibers has been seen by almost every worker in the field. All these findings strengthen the view that the two components of the cardiac action potential are indeed independent physiological entities.

4. Electrophysiological Behavior and Probable Ionic Mechanism for the Fast Component in Rabbit Heart

The electrophysiological characteristics of atrial, ventricular, and Purkinje fibers are well-known (Hoffman and Cranefield, 1960). These cells are electrically excitable by short pulses and their threshold is low (short chronaxie and low rheobase). Excitation results in a full-blown, all-or-none propagated action potential. Conduction velocity is high (0.5–4 m/sec) when compared to that of nodal tissue. Conduction velocity and maximum depolarization rate are enhanced up to a limit by an increase in resting potential. Decreasing the resting potential has the opposite effect. Absolute and relative refractory periods are present and reexcitation by cathodal pulses of short duration is impossible unless a minimum degree of repolarization is achieved. Propagation seems to be the result of local currents which electrotonically depolarize and bring to (or beyond) threshold the fibers lying ahead of the propagating wavefront.

All these properties can be ascribed to the fast component. It is the fast component that leads the action potential in these fibers. Abolition of the slow component of atrial cells by acetylcholine does not produce any qualitative change in the properties described above.

Evidence has been accumulating since 1950 to show that the cause of what we call the fast component is in many respects similar to the changes in sodium chord conductance ($g_{Na}$) which contribute to the action potential of squid nerve (Hodgkin and Huxley, 1952 a–d). The following observations are particularly relevant: (a) the maximum rate of rise of the fast component changes with $[Na]_o$ according to expectation, as demonstrated by experiments on ungulate Purkinje fibers (Draper and Weidmann, 1951), frog ventricle (Brady and Woodbury, 1960), and rabbit atrium (Hoffman and Cranefield, 1960; Paes de Carvalho et al., 1966); (b) the peak voltage of the action potential changes with log $[Na]_o$ if the peak is achieved during the fast component (Draper and Weidmann, 1951); otherwise the relationship is not obeyed (Coraboeuf and Otsuka, 1956; Défèze, 1959; Paes de Carvalho et al., 1966); and (c) the maximum rate of rise of the fast component, as recorded from Purkinje fibers, shows an S-shaped dependence on the membrane voltage pres-
ent prior to excitation (Weidmann, 1955), depolarization causing a decrease and hyperpolarization an increase in maximum $dV/dt$ (similar findings are obtained from atrium; see Paes de Carvalho et al., 1966); (d) the membrane "impedance" or apparent polarization resistance falls sharply during the upstroke of the fast component (phase 0; Weidmann, 1951) as would be expected to result from a large increase in $g_{Na}$; (e) voltage clamp data from Purkinje fibers (Deck and Trautwein, 1964; Dudel et al., 1967 a) and frog atrium (Rougier, Vassort, and Stämpfli, 1968; Rougier, Vassort, Garnier, Gargouil, and Coraboeuf, 1968) show a clear Na inward current upon depolarization beyond threshold level.

From the considerations mentioned above it seems reasonable to state that the ionic mechanism underlying the fast component is indeed a voltage- and time-dependent change in $g_{Na}$ which is qualitatively similar to that described for squid nerve.

5. Electrophysiological Behavior and Possible Ionic Mechanism for the Slow Component in the Rabbit Heart

The electrophysiological characteristics of the slow component are less well-established than those of the fast component. Judging from recorded behavior of SA and AV nodal fibers, the slow component is less excitable by short current pulses (up to 10 msec long). Our observations (unpublished) show that excitation of the N region of the rabbit heart was impossible to achieve using short pulses applied through intracellular electrodes even when the current was 10 times greater than that which stimulated atrium or the His bundle.

These data are open to criticism due to the likelihood of poor impalement and sealing of the electrode in the AV nodal area. Nodal tissue must be electrically excitable if propagation results from local circuit currents. Conduction in nodal structures progresses very slowly due to the absence of any noticeable fast component. Rates of propagation within the N region of the rabbit AV node have been estimated to be as low as 0.01–0.05 m/sec (Hoffman et al., 1959; Scher et al., 1959; Paes de Carvalho and Almeida, 1960). Similar values of propagation velocity were demonstrated for the sinus region in the same animal (Paes de Carvalho et al., 1959).

Sinoatrial (SA) action potentials arise from a marked "slow diastolic depolarization" during phase 4. This slow diastolic depolarization, or pacemaker prepotential, is believed to characterize automatic fibers with pacemaking capabilities (Paes de Carvalho et al., 1959; Hoffman and Cranefield, 1960). The upstroke of the action potential in automatic pacemaking cells arises out of the pacemaker prepotential with a slow, smooth voltage-time course (see Fig. 3 A). Under these conditions, the function of an electrogenic mechanism showing rapidly responding voltage-dependent inactivation (as is
the case for the fast component) should be grossly hindered. On the other hand, the slow component apparently shows no inactivation in this voltage range (Paes de Carvalho et al., 1966). Its rate of rise may even increase with an enhancement of the pacemaker prepotential (Paes de Carvalho, 1968). This statement seems at first glance to be at variance with Weidmann's observation on Purkinje fibers exhibiting phase 4 depolarization (Weidmann, 1955). He found that the rates of rise for extrasystolic action potentials were maximal at the beginning of phase 4 of the preceding beat, when preexcitation membrane potential was highest. Maximum rate of rise decreased at later times as would be expected to result from membrane depolarization. The discrepancy is explained if one realizes that Purkinje fibers exhibit a fast component while nodal fibers apparently do not. It is curious to note that, in Purkinje fibers, both rate of depolarization and overshoot are markedly depressed when true (not latent) pacemaker activity is present. One wonders whether careful scrutiny of the upstroke at this stage still would yield any sizeable fast component. It is also of interest that experimental manipulations that result in a more rapid diastolic depolarization (local warming, epinephrine) often result in an increased rate of propagation across the AV node. The opposite is true for situations known to decrease the steepness of phase 4 depolarization (acetylcholine, cooling). Moreover, an early extrasystole that reaches the AV node before the full development of phase 4 depolarization is conducted at a low rate even if it originates after complete repolarization of the nodal fiber. Decremental conduction and block are often seen in AV nodal cells at high stimulation rates and in the presence of extrasystoles (Paes de Carvalho, 1961, 1966). When the above evidence is considered as a whole, it seems tempting to suggest that the slow component is normally facilitated by the existence of phase 4 depolarization, some associated change in membrane properties, or the passage of time after the onset of the preceding action potential.

A decrease in potassium conductance \( (g_K) \) is believed to occur during the plateau of Purkinje fiber action potentials (Weidmann, 1956; Cranefield and Hoffman, 1958) or whenever its membrane is depolarized (Hutter and Noble, 1960; Carmeliet, 1961; Hall et al., 1963). The exact kinetics of potassium conductance are still being clarified (Vassalle, 1966; McAllister and Noble, 1966, 1967; Dudel et al., 1967; Noble and Tsien, 1968, 1969; Hauswirth, Noble, and Tsien, 1969). Most authors agree that the current-voltage relationship of the potassium channel may show a negative slope in the voltage range where the slow component threshold is thought to lie. A small inward current with a positive equilibrium potential would then create conditions for excitation to occur upon membrane depolarization. Attention is now being given to slow inward currents that are momentarily activated upon depolarization. One early candidate in Purkinje fibers was a slow
Two Components of Cardiac Action Potential

sodium channel (Deck and Trautwein, 1964). More recent data point towards the existence of a calcium channel in Purkinje fibers (Reuter, 1967) or to a combination of both (Reuter, 1968). Evidence from frog atrial muscle clamped in a sucrose-gap arrangement points toward a slow channel that is used by either calcium or sodium (Rougier, Vassort, Garnier, Gargouil, and Coraboeuf, 1968). This would be in keeping with the interpretation of data from frog myocardium (Niedergerke and Orkand, 1966). Any of these mechanisms, if allied to an inward rectifying potassium channel, can explain the electrogenic mechanism that we call “slow component.” At present our results do not permit us to speculate on the nature of such ionic currents in the rabbit atrium.

However, two points should be emphasized. One is the fact that acetylcholine, which causes an increase in $g_K$, depresses the slow component (Burgen and Terroux, 1952; Hutter and Trautwein, 1956; Wright and Ogata, 1961; Paes de Carvalho et al., 1966). It seems, therefore, that a low $g_K$ is essential for the development of the slow component, as has already been pointed out. The second fact is that if the fast component is dominated by Na current ($g_K$ negligible in face of $g_{Na}$), then the equilibrium potential for the current-carrying ion involved in the slow component may at times be more positive than the Na equilibrium potential. This would appear to be the case in transitional cells, where the fast component is seen to terminate well before the crest of the action potential even when maximum $V$ is reasonably high. It is realized (see p. 624) that $E_{Na}$ may be more positive than the potential at which the fast component of the upstroke ends and that further depolarization (the slow component) might result from continuing Na influx in the presence of a decreasing $g_K$. The slow component thus might result from an inward Na current and $E_{Na}$ might be more positive than the peak of the action potential. On the other hand, it seems unlikely that this question can be resolved without measurement of changes in $g_K$ during the action potential of transitional and nodal fibers.

The occurrence of an isolated slow component in nodal fibers shows that it is an independent electrogenic mechanism. Excitation of the slow component can be expected to occur by (a) progressive and prolonged depolarization, as during the pacemaker prepotential, or (b) abrupt and shorter but intense depolarization, as provided by propagating action potentials due to the fast component. The slow component should then follow in the wake of the fast component when this is present (e.g., atrium and transitional atrio-nodal cells, Purkinje fibers, ventricular muscle), progressing at the same rate because it is initiated or renewed at each point. In nodal tissue, since there is no significant fast component, propagation from point to point by local circuits will be possible only if $g_K$ is low enough, as is the case when phase 4 depolarization is well-developed. If this “priming” is not available, the slow
component might be expected to propagate with decrement and finally fail. Decremental conduction is observed as a rule in the AV node of the isolated rabbit heart when atrial activity is rapid (Hoffman et al., 1959; Cranefield et al., 1959; Paes de Carvalho and Almeida, 1960). Decrement easily can be enhanced to produce block, e.g., by increasing the driving rate or interposing extrasystolic beats. The site of block is then the N region (where only the slow component is present).

The same sort of reasoning can be applied to explain the AV nodal block caused by acetylcholine. In addition to the direct effect of ACh on nodal fibers, the loss of action potential amplitude caused by the abolition of the slow phase of the upstroke in transitional AN cells makes them less effective as a stimulus for the N region. The N cells, on the other hand, are less prone to conduct an isolated slow component due to the increase in $g_X$ caused by acetylcholine. In the extreme case, when ACh concentration is high, excitation will not reach the NH region. At lower concentrations, block will occur, if at all, somewhere within the N region, depending on how much the resting $g_X$ and phase 4 depolarization (or associated changes) are affected. Considering these factors, the observed range of first and second degree and complete AV block is to be expected as the concentration of acetylcholine is increased. The dependence of block on heart rate is also to be expected, since slowing will allow phase 4 depolarization or associated changes to progress further and thus "condition" propagation of the slow component (Paes de Carvalho, 1966).

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