(− 60 to − 33 mv), where I_{Na} is largest, the contractions are weak and are not influenced much by the membrane potential. Thus steady-state contraction seems to depend mainly on the flow of the second inward current. The ionic requirements for the second inward current, its dependence on the membrane potential, and its relation to activation of contraction are the subjects of another report (*10*).

Thus our experiments suggest that in dog ventricular myocardium the flow of sodium current is mainly responsible for the rapid depolarization during the action potential. Some of the kinetics of I_{Na} are quite similar to those in other excitable tissues.

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Calcium Current and Activation of Contraction in Ventricular Myocardial Fibers

Abstract. *In thin bundles of dog ventricular myocardium, a slow inward current (distinct from the sodium inward current) could be recorded under voltage-clamp conditions. This inward current was influenced by changes in external calcium concentration, but it was not dependent on external sodium concentration. Therefore, this current which contributes an appreciable amount of charge transfer during the plateau of the action potential, is carried by calcium ions. In sodium-free solution, the flow of calcium ions into the fiber is directly related to activation of contraction. In sodium-containing solution, however, calcium inward current serves primarily to fill up some intracellular stores from which calcium can be released by moderate depolarization.*

In cardiac Purkinje fibers, calcium ions contribute to the total membrane current during rather strong depolarizations (*1*). Evidence has been obtained that, during depolarizations in sodium-free solution, calcium ions carry a charge across the membrane of ventricular myocardial fibers isolated from sheep and calf hearts. Under these conditions, activation of contraction seemed to be directly related to the inward movement of calcium ions (*2*). In order to get more information about the importance of calcium inward current for the action potential as well as for excitation-contraction coupling in mammalian cardiac muscle, we performed voltage-clamp experiments and simultaneously measured contraction in thin trabeculae and papillary muscles excised from dog hearts.

We used the method already described (*3*). In that report, apart from excitatory sodium current (I_{Na}), a much smaller and slower net inward current was described as flowing in dog ventricular fibers during depolarization to potential levels between −30 and −20 mv. Steady-state contraction became large in the same potential range. In the presence of I_{Na}, the slow inward current could not be accurately resolved, but I_{Na} could be completely inactivated with a conditioning depolarization of the membrane to −45 mv. Therefore, we investigated the second inward current in greater detail after the sodium system had been inactivated.

Double-step voltage clamps recorded from a dog ventricular trabecula soaked in Tyrode's solution are seen in Fig. 1. The duration of the first depolarization step, V_1, from −77 mv (resting potential) to −38 mv was 420 msec; that of the second, superimposed step, V_2, was 210 msec. During the first step, which was always of constant amplitude, there was a transient inward sodium current (*3*). The V_1 was sufficiently large and long to inactivate I_{Na} completely at V_2. The second step of depolarization, V_2, was varied in amplitude. A small depolarization, from −38 to −33 mv, produced a slight increase in outward current. Stronger depolarization produced a flow of slow inward current that was maximum in this preparation at −7 mv. Net inward current could be recorded at potentials up to +7 mv. A precise determination of the equilibrium potential for this current was unattainable, because during depolarizations to potentials beyond +30 mv the outward current increased steeply, making it impossible to separate the slow inward current with sufficient accuracy.

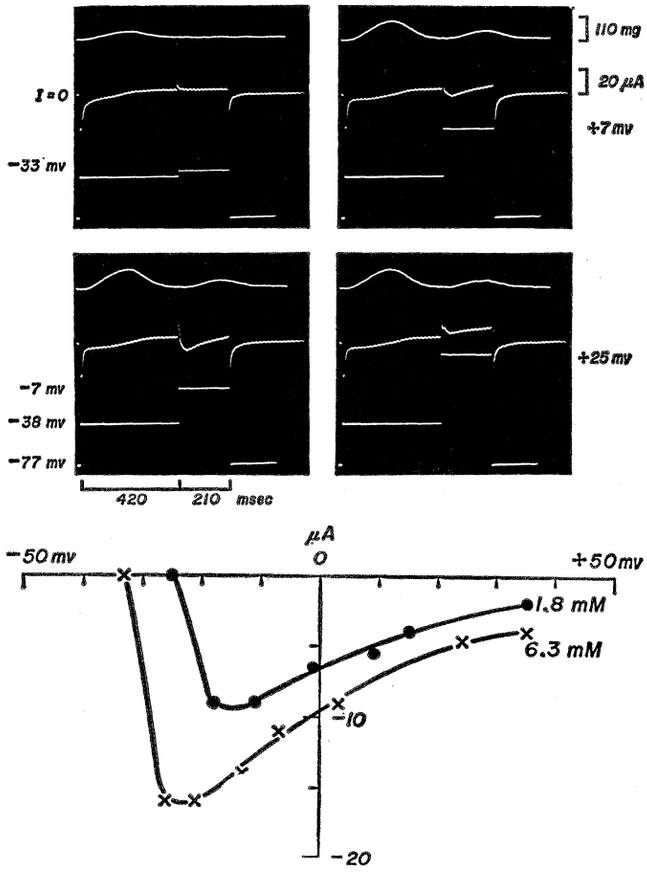
The slow inward current was influenced by the external calcium concentration $[Ca]_o$. It became extremely small when $[Ca]_o$ was low (0.1 mmole/liter), and its amplitude increased with increasing $[Ca]_o$ up to 7.2 mmole/liter. The difference between the initiation and the maximum of the slow inward current (after correction for the capacitative outward current) was considered to represent an estimate of the total inward current and was plotted against membrane potential (Fig. 2). The slow inward current in $[Ca]_o$ of 1.8 mmole/liter increases steeply at potentials from −25 to −18 mv, and decreases again during depolarizing clamp steps above −10 mv. When $[Ca]_o$ was increased to 6.3 mmole/liter, the threshold for the slow inward current was shifted negatively by 8 mv and the amplitude of the current was larger, attaining its maximum at about −25 mv. In positive-potential regions, the two current-voltage relations are almost parallel, the separation between the curves being about 18 mv. Although there are uncertainties in this method of estimation, this tends to agree with a theoretical 16-mv shift of the equilibrium potential calculated from the Nernst equation under the assumption that the current is carried solely by calcium ions.

This assumption is strongly supported by the finding that the slow inward current was not much affected by the complete absence of sodium in the bathing solution, and also that it was sensitive to alterations of $[Ca]_o$ in sodium-free medium. The main difference between the two conditions seemed to be a shift of the threshold for the slow inward current by 10 to 20 mv toward more

negative potentials in sodium-free solution. Figure 3 shows a series of voltage-clamp steps, corresponding membrane currents, and contractions measured in sodium-free solution. Here, the slow inward current could be recorded even though the initial fast sodium current was completely abolished. The inward current had its threshold at −45 mv in this preparation. Its amplitude increased smoothly with stronger depolarization, the maximum being attained at −20 mv. The steady-state current-voltage relation obtained in sodium-free solution showed inward going (anomalous) rectification with negative slope conductance in the potential range between −50 and −20 mv, much the same as in sodium-containing solution (*3*). With $[Ca]_o$ of 1.8 to 7.2 mmole/liter, appreciable net inward currents could be recorded in several experiments, regardless of whether sodium was replaced by sucrose, choline, or tris(hydroxymethyl)-aminomethane (tris buffer).

These experiments confirm the finding that the application of constant current pulses to ventricular trabeculae of sheep and calf hearts in sodium-free solution can produce calcium-dependent regenerative depolarizations (*2*). From our experiments, we conclude that calcium ions carry positive charge through the membrane of ventricular myocardial fibers at potentials beyond −30 mv and thus contribute appreciably to the total membrane current during the plateau phase of action potentials.

We studied the influence of the calcium inward current on contraction of the myocardial fibers. The time course and voltage dependence of the degree of activation of contraction were different in solutions with and without sodium. In sodium-free solution, the degree of activation of contraction during any depolarization was directly dependent on the size of the calcium inward current I_{Ca} during that depolarization. The steady-state contraction at any given membrane potential was attained immediately upon the first depolarization, provided that I_{Ca} was switched on (Fig. 3). Moreover, at different potentials, the time constants for activation of contraction and activation of I_{Ca} were identical; that is, the contraction was maximum if I_{Ca} was fully activated, and decreased when the clamp duration was shortened so that I_{Ca} was only partially activated. The threshold for contraction as well as for I_{Ca} in sodium-free solution was typically about −50 mv, the potential at which the slope of the current-voltage relation became negative. Maximum contraction generally was attained at about −10 mv. Thus, we conclude that, in sodium-free solution, contractions of mammalian myocardial fibers are direct-



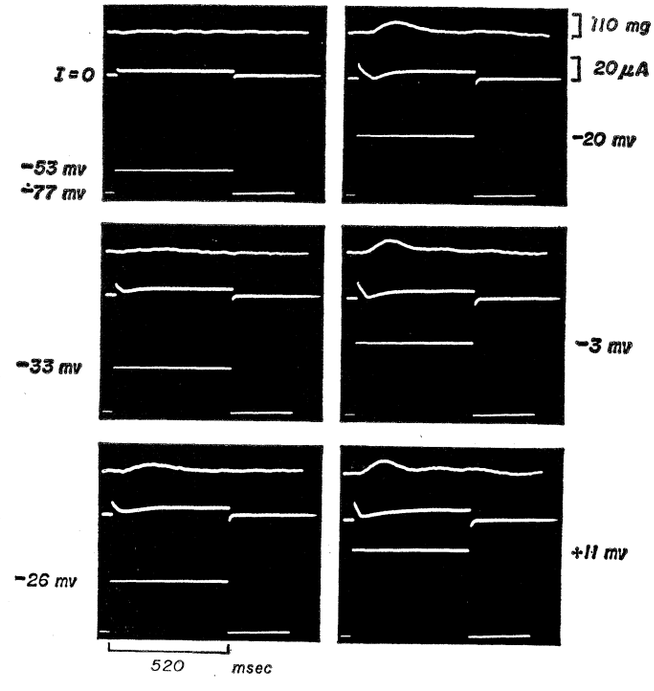


Fig. 1 (top left). Double-step voltage clamps of ventricular trabecula from dog heart in Tyrode's solution; upper traces, isometric contraction; middle traces, membrane current (upward deflection from $I = 0$ indicating outward current, downward deflection inward current); lower traces, displacement of membrane potential from its resting level, −77 mv, in two steps. First step, V_1, was always to −38 mv ;and second step, V_2, was variable in amplitude (the figures beside the record indicate membrane potential level during V_2). Contraction during V_1 increased after slow inward current at V_2 had been activated during preceding depolarizations (steady-state conditions). Fig. 2 (bottom left). Current-voltage relations for slow inward currents measured in dog ventricular fiber by double-step voltage clamps; ordinate is difference between initiation and maximum of inward current in microamperes; abscissa is membrane potential during second voltage-clamp step in millivolts. First voltage-clamp step, V_1, was always from −80 mv (resting potential) to −34 mv. Currents were measured in Tyrode's solution with 1.8 mmole/liter (●) and 6.3 mmole/liter (X) of $CaCl_2$. Fig. 3 (top right). Voltage clamps of a ventricular trabecula from dog heart in sodium-free solution of following composition (mmole/liter): sucrose, 274; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.05; glucose, 5; tris(hydroxymethyl)-aminomethane buffer plus HCl to *p*H 7.4. Traces as in Fig. 1.

ly activated by Ca ions flowing into the fibers during depolarization (*2*).

In sodium-containing solution, the flow of calcium ions into the fiber again is essential in order to obtain maximum activation of steady-state contraction, but this relation is not direct and instantaneous. In the potential range between the threshold for sodium current (−65 mv) and the threshold for calcium current (−30 mv), only a small steady-state contraction could be activated; and this did not depend much on the membrane potential. Beyond the threshold for I_{Ca}, the steady-state contraction increased gradually at each potential. This steady state was reached, however, only after five or six equal depolarizations, even though I_{Ca} was fully activated during the first depolarization. Maximum steady-state contractions were attained at about +20 mv. The time constants of activation of I_{Ca} and of activation of steady-state contractions were very different in sodium-containing solution, maximum activation being attained only with depolarizations lasting 200 to 300 msec (*4*).

During double-step voltage clamps (Fig. 1), the first step of depolarization (to −38 mv) activated only a small contraction if I_{Ca} was not previously switched on at V_2 (−33 mv). When I_{Ca} was fully activated at V_2, however, the steady-state contraction at V_1 was greatly increased and a second, smaller contraction was observed at V_2. In order for the positive inotropic response at V_1 to reach steady state, I_{Ca} had to flow at V_2 during five or six identical depolarizations. Moreover, if the clamp step V_2 was suddenly omitted (not shown in Fig. 1), the first depolarization to −38 mv still activated a large contraction; but this early contraction decreased to a much smaller value, within two or three depolarizations, to −38 mv.

These indirect effects of I_{Ca} on contraction in sodium-containing solution can best be explained if the flow of calcium ions into the fibers is assumed to fill intracellular stores from which calcium can be released by depolarizations below the threshold for I_{Ca}. Owing to the presumed partial depletion of these stores during each depolarization, steady-state levels within the stores are achieved only after I_{Ca} has been activated during five or six identical depolarizations. If these stores have not been filled previously by the flow of I_{Ca}, only a weak steady-state contraction can be activated. In contrast to the situation in sodium-free solution, direct activation of contraction by the flow of calcium ions seems to be only of minor importance in sodium-containing solution. These results could help to explain the recent observations by others of staircase phenomena and potentiation of contraction in mammalian cardiac muscle (*5*).

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Pistillate Papaya Flower: A Morphological Anomaly

Abstract. *The pistillate flower of* Carica papaya *is a pleurogynous unisexual form derived intraspecifically from a perigynous bisexual ancestor. It is a morphological anomaly in that the ovary consists of two cycles of carpels which, in the ancestor, arise on the receptacle at different levels as dissimilar orders of organs. The bulk of the pentamerous ovary and the dorsal carpellary vascular system are derived from a cycle of five stamens in the ancestor. The ventral carpellary system is inherited intact from the ancestor which has normal morphology in the sense that the carpels consist of a single cycle of sporophylls.*

The papaya (*Carica papaya* L.) is a polygamous species of large herbaceous dicotyledonous tropical plants (popularly called trees), cultivated for its edible melon-like fruit. Hermaphroditic trees are characterized by a form of bisexual flower known as "elongata" which has ten epipetalous stamens arranged in two cycles of five each. (Fig. 1A). Superficially, the upper cycle appears to lie opposite the lobes of the pentamerous sympetalous corolla and the lower cycle lies opposite the sinuses between the lobes. The ovary is su-

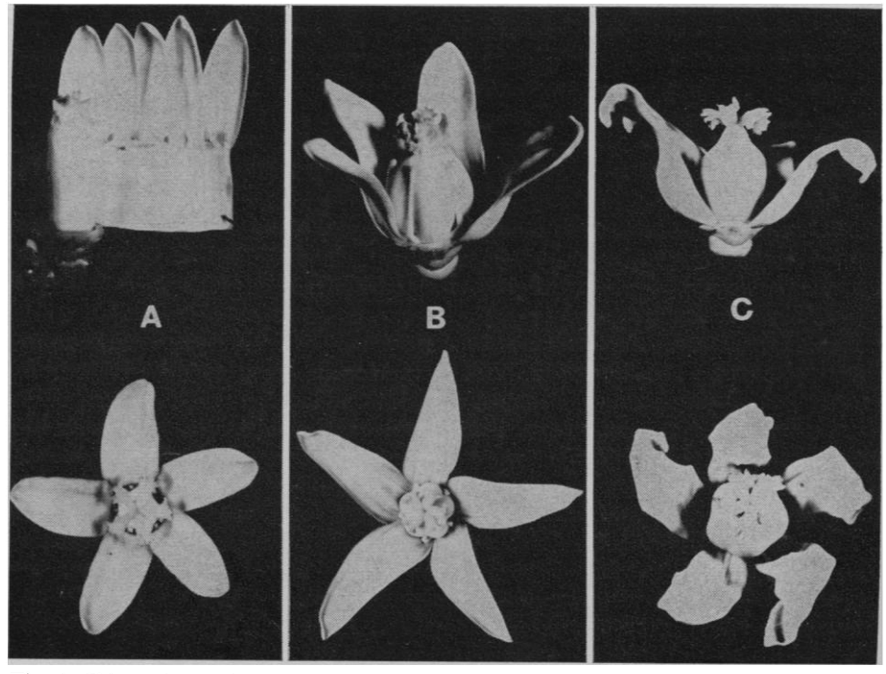

Fig. 1. Side and top views of papaya flower types. (A) Elongata, (B) pentandria, and (C) pistillate.