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POTENTIAL OF GUINEA-PIG VENTRICLE

Dissertation

Presented in Partial Fulfillment of the
Requirements for the Degree Doctor
of Philosophy in the Graduate School
of the Ohio State University

by

Thomas Michael Nosek, B.S.

*****

The Ohio State University

1973

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INTRODUCTION

The bioelectric activity of mammalian cardiac muscle cells is characterized by a unique action potential configuration. While the action potentials of nerve and striated muscle cells display a state of depolarization lasting approximately one to five milliseconds respectively, the cardiac action potential is distinguished by a duration of a few hundred milliseconds. (Fig. 1) This extended period of depolarization is referred to as the cardiac plateau potential.

Because of this plateau potential, the cardiac action potential serves as more than a trigger for the mechanical response of the myocardial fibers. The excitation-contraction coupling relationship is more complex than that since it has been demonstrated that the duration of the action potential is directly proportional to the duration of the muscle contraction (37) and that a percentage of the plateau potential duration is directly proportional to the magnitude of the muscle contraction (52). The duration of the cardiac action potential also determines the functional refractory period of the muscle cells, i.e. the
Intracellular microelectrode recordings of action potentials from nerve (upper left (32)), striated muscle (upper right (42)), cardiac Purkinje fibers (lower left (17)), and cardiac ventricular muscle (lower right (37)).
period following the beginning of an action potential during which the cell is inexcitable. The normally long duration of the cardiac action potential acts as a deterrent to premature excitation and possible fibrillation of the myocardial fibers.

The duration of the plateau potential plays a central role in determining the values of these parameters, all of which are crucial in determining the pumping efficiency of the heart. The plateau potential is very sensitive to changes in heart rate, ion concentration, temperature, oxygen levels, and various chemical concentrations in the fluids bathing the muscle cells which take place during the functional activity of an organism. Since the performance of the heart is so crucial to the well being of the entire organism and since the duration of the plateau potential is so central to determining the activity of the heart, it is important that there be a thorough understanding of this particular bioelectric potential.

The physical basis for the plateau potential has not been completely elucidated. For this reason it has been the subject of a great deal of scientific investigation. The purpose of this dissertation is to: 1) review the formalisms traditionally used to describe the potential difference found across the surface membranes of living cells and adopt a general formalism which adequately
models this phenomenon; 2) review the scientific literature dealing with the cardiac action potential and in particular the cardiac plateau potential; 3) propose the theory that a transient increase in the active transport of potassium ions into the cell during the action potential contributes to the ionic current responsible for the maintenance of the plateau potential. This theory utilizes the general formalism adopted in 1) and is intended to resolve some of the contradictory data that exists concerning the cardiac plateau potential; 4) report and interpret the results of radioactive tracer experiments which measured the transmembrane fluxes of sodium and potassium ions under specific conditions in order to test the validity of the proposed theory.
LITERATURE REVIEW

Basis for Transmembrane Potential

In order to analyze the bioelectric characteristics of individual cells, a formalism must be adopted to describe this activity. What follows is a chronological review of the formalisms that are presently used to relate ionic fluxes and transmembrane potentials.

An ionic activity gradient across a cell membrane can be viewed as a force which is causally related to a net ionic flux across the membrane. *

\[ M(\gamma,d) = P(\gamma)(A(\gamma,o) - A(\gamma,i)) \]  
Eq. 1

where: \( M(\gamma,d) \) = net flux of ionic species \( \gamma \) due to diffusion
\( A(\gamma,o) \) = extracellular activity of \( \gamma \)
\( A(\gamma,i) \) = intracellular activity of \( \gamma \)
\( P(\gamma) \) = permeability of the membrane to \( \gamma \). This term is defined as the proportionality constant relating \( M(\gamma,d) \) and \( A(\gamma,o) - A(\gamma,i) \).

* The formalism used in the following development is a modified form of that used by Delahayes (15). The development itself has been taken from the following sources; 6, 15, 21, 42, 46, 53, 74, 78.
The permeability term is related to the thickness of the membrane (x) by a diffusion coefficient (D(γ)):

\[ P(\gamma) = \frac{D(\gamma)}{x} \quad \text{Eq. 2} \]

It follows that as \( x \to 0 \):

\[ M(\gamma, d) = -D(\gamma) \frac{dA(\gamma)}{dx} \quad \text{Eq. 3} \]

The negative sign appears because flux resulting from diffusion is directed down the activity gradient.

A charge separation across a cell membrane sets up an electrical potential gradient which can be modeled as a force causally related to a net ionic flux across the membrane:

\[ M(\gamma, e) = -z\mu(\gamma)A(\gamma)\frac{dV}{dx} \quad \text{Eq. 4} \]

where:

- \( M(\gamma, e) \) = net flux of ionic species \( \gamma \) to an electrical potential gradient
- \( \frac{dV}{dx} \) = electrical potential gradient
- \( z \) = valence of ionic species \( \gamma \)
- \( \mu(\gamma) \) = electrical mobility of \( \gamma \) through the membrane
- \( A(\gamma) \) = activity of \( \gamma \) at position \( x \)

Once again the negative sign appears because the flux resulting from the electrical potential gradient is directed down the electrical potential gradient.

The net electrochemical force across a membrane is the sum of the forces resulting from an activity gradient and an electrical potential gradient. The expression for
the net electrochemical flux \( M(\gamma, \text{ec}) \) is given by:

\[
M(\gamma, \text{ec}) = -D(\gamma) \frac{dA(\gamma)}{dx} - \mu(\gamma) zA(\gamma) \frac{dv}{dx} \quad \text{Eq. 5}
\]

Near equilibrium the diffusion coefficient is related to electrical mobility by the following expression:

\[
D(\gamma) = \frac{\mu(\gamma) RT}{F} \quad \text{Eq. 6}
\]

where:
- \( R = \) the gas constant
- \( T = \) absolute temperature
- \( F = \) the Faraday constant

Applying the equality relationship of Eq. 6 to Eq. 5 yields the following expression for the net electrochemical flux across a membrane (64, 81):

\[
M(\gamma, \text{ec}) = - \frac{(\mu(\gamma) RT) dA(\gamma)}{F} - \frac{(zF A(\gamma)) dv}{RT dx} \quad \text{Eq. 7}
\]

This expression is known as the Nernst-Planck equation.

\[
A(\gamma) \frac{d(ln A(\gamma))}{dx} = \frac{dA(\gamma)}{dx} \quad \text{Eq. 8}
\]

Applying the equality of Eq. 8 to Eq. 7 yields:

\[
M(\gamma, \text{ec}) = - \frac{\mu(\gamma) RT A(\gamma)}{F} \frac{dlnA(\gamma)}{dx} - \frac{zF}{RT} \frac{dv}{dx} \quad \text{Eq. 9}
\]

Rearranging Eq. 9 and integrating from the outside of the membrane to the inside gives:

\[
M(\gamma, \text{ec}) \int_{0}^{i} \frac{dx}{(z\mu(\gamma)A(\gamma))} = -\frac{RT}{zF} \int_{0}^{i} dlnA(\gamma) - \int_{0}^{i} dv \quad \text{Eq. 10}
\]

\[
M(\gamma, \text{ec}) \int_{0}^{i} \frac{dx}{(z\mu(\gamma)A(\gamma))} = \frac{RT}{zF} \ln \left[ \frac{A(\gamma, 0)}{A(\gamma, i)} \right] - V(M) \quad \text{Eq. 11}
\]

where \( V(M) = V(i) - V(o) \) is the potential difference across the membrane. When a cell is in equilibrium, the flux resulting from diffusion is equal and opposite
to the flux resulting from the electrical potential gradient. We have:

\[
M(\gamma,ec) = 0 = \frac{RT}{2F} \ln \left[ \frac{A(\gamma,0)}{A(\gamma,1)} \right] - V(M) \quad \text{Eq. 12}
\]

\[
V(M) = \frac{RT}{2F} \ln \left[ \frac{A(\gamma,0)}{A(\gamma,1)} \right] = E(\gamma) \quad \text{Eq. 13}
\]

where \( E(\gamma) \) is the equilibrium or Nernst potential for the particular ionic species \( \gamma \).

The transmembrane potential for cells is not the equilibrium potential for any one ionic species. Cells at rest are not in equilibrium but are rather in a steady state condition in which there is no change in the transmembrane voltage or the ionic activities with time. An expression describing the transmembrane potential for a non-equilibrium steady state in the nonphysiological condition where no metabolic processes are contributing to the transmembrane fluxes of the individual ions, comes out of Eq. 7.

\[
M(\gamma,ec) = - \frac{\mu(\gamma) RT dA(\gamma)}{F} - \frac{zFPA(\gamma)}{RT} \frac{dV}{dx} \quad \text{Eq. 7}
\]

\[
d(A(\gamma) \exp \left[ \frac{2FV}{RT} \right] = \frac{2F}{RT} A(\gamma) \frac{dV}{dx} \exp \left[ \frac{2FV}{RT} \right] + \frac{dA(\gamma)}{dx} \exp \left[ \frac{2FV}{RT} \right] \quad \text{Eq. 14}
\]

Applying the relationships of Eq. 14 to Eq. 7 and rearranging terms yields the following expression for the net electrochemical flux:
\[ M(\gamma,ec) = -\frac{\mu(\gamma)RT\exp\left(-\frac{zFV}{RT}\right)}{\mu(\gamma)RT} \int_0^1 \frac{d\left[A(\gamma)e^{\left(\frac{zFV}{RT}\right)}\right]}{dx} \]  
\text{Eq. 15}

\[ M(\gamma,ec) \frac{F}{\mu(\gamma)RT} \exp\left[\frac{zFV}{RT}\right] = -\frac{d\left[A(\gamma)e^{\left(\frac{zFV}{RT}\right)}\right]}{dx} \]  
\text{Eq. 16}

Integrating Eq. 16 from the outside of the membrane to the inside, where \( M(\gamma,ec) \) is held to be a constant throughout the membrane, yields:

\[ M(\gamma,ec) \frac{F}{\mu(\gamma)RT} \int_0^1 \exp\left[\frac{zFV}{RT}\right] dx = -\int_0^1 \frac{d\left[A(\gamma)e^{\left(\frac{zFV}{RT}\right)}\right]}{dx} \]  
\text{Eq. 17}

It follows that:

\[ M(\gamma,ec) \frac{F}{\mu(\gamma)RT} \int_0^1 \exp\left[\frac{zFV}{RT}\right] dx = -(A(\gamma,i)e^{\left(\frac{zFV(M)}{RT}\right)} - A(\gamma,0)) \]  
\text{Eq. 18}

Since \( \int_0^1 \exp\left[\frac{zFV}{RT}\right] dx \) can not be accurately integrated without some knowledge of the distribution of \( V \) in the membrane, let (53):

\[ \int_0^1 \exp\left[\frac{zFV}{RT}\right] dx = \frac{1}{f(V)} \]  
\text{Eq. 19}

where \( f(V) \) is a function that depends on a particular model for the distribution of the electric field in the membrane.

\[ P(Y) = \frac{\mu(\gamma)RT}{F} \]  
\text{Eq. 20}

It follows that (53):

\[ M(\gamma,ec) = -\frac{\mu(\gamma)RT}{F} f(V) \left[A(\gamma,i)e^{\left(\frac{zFV(M)}{RT}\right)} - A(\gamma,0)\right] \]  
\text{Eq. 21}
If we consider a living cell with extracellular ionic activities equal to the intracellular ionic activities, metabolic energy will be used to transport potassium ions into the cell and sodium ions out of the cell. For simplicity let us first assume that one potassium ion is actively transported for every sodium ion actively transported. This active transport process will produce activity gradients across the membrane for both sodium and potassium ions. Sodium will tend to diffuse back into the cell and potassium out of the cell. Since $P(K)$ is much greater than $P(Na)$ under resting conditions, more potassium will diffuse out of the cell than sodium into the cell. This imbalance in the diffusion fluxes will create a charge separation and an electrical potential gradient across the membrane, the inside of the cell becoming negative with respect to the outside. This potential difference ($V(M)$) is so directed as to oppose the efflux of potassium and to enhance the influx of sodium. As the pump continues to pump sodium out of the cell and potassium into the cell, the rate of potassium efflux will exceed the rate of sodium influx until $V(M)$ is large enough to set up a steady state condition where:

$$M(Na,ec) = - M(K,ec)$$

Eq. 22

When this condition is satisfied, the transmembrane potential will be a constant (74).
If we assume that only sodium and potassium ions determine membrane potential in the steady state, by applying the boundary conditions of Eq. 22 to Eq. 21 we have:

$$P(\text{Na})f(V)A(\text{Na},i)\exp\left[\frac{zFV(M)}{RT}\right] - A(\text{Na},o) + P(\text{K})f(V)A(\text{K},i)\exp\left[\frac{zFV(M)}{RT}\right] - A(\text{K},o) = 0$$

Eq. 23

Rearranging Eq. 23 yields:

$$\left( P(\text{Na})A(\text{Na},i) + P(\text{K})A(\text{K},i) \right)\exp\left[\frac{zFV(M)}{RT}\right] = P(\text{K})A(\text{K},o) + P(\text{Na})A(\text{Na},o)$$

Eq. 24

Solving for $V(M)$ gives:

$$V(M) = \frac{RT\ln\left[ P(\text{K})A(\text{K},o) + P(\text{Na})A(\text{Na},o) \right]}{zF}$$

Eq. 25

This is the steady state Goldman-Hodgkin-Katz equation which describes the dependence of the transmembrane potential on the passive electrochemical fluxes of sodium and potassium ions. If other ions such as chloride or calcium contribute to the transmembrane potential they can be incorporated into Eq. 25.

Besides these passive electrochemical fluxes, chemical reactions within the membrane are capable of using metabolic energy to transport ions across the membrane and produce a net ionic flux ($M(\gamma,A)$). The formalism of Eq. 25 must be modified to account for the effect of these active fluxes on the transmembrane potential.
In the steady state the active and passive fluxes must balance. If only sodium and potassium ions are actively transported we have:

\[ M_{(Na,ec)} = - M_{(Na,A)} \quad \text{Eq. 26} \]
\[ M_{(K,ec)} = - M_{(K,A)} \quad \text{Eq. 27} \]

If \( r \) is defined as:

\[ r = - \frac{M_{(Na,A)}}{M_{(K,A)}} \quad \text{Eq. 28} \]

it follows from Eqs. 26 and 27 that:

\[ M_{(Na,ec)} = - rM_{(K,ec)} \quad \text{Eq. 29} \]

Substituting Eq. 29 for Eq. 22 above and solving for \( V(M) \) yields the following relationship (53):

\[ V(M) = \frac{RT \ln \left[ \frac{P_{(Na)}A_{(Na,o)}}{P_{(Na)}A_{(Na,i)}} + rP_{(K)}A_{(K,o)}}{P_{(Na)}A_{(Na,i)} + rP_{(K)}A_{(K,i)}} \right]}{2F} \quad \text{Eq. 30} \]

**Equivalent Circuit Analog**

For steady state conditions Eq. 30 has proven accurate in describing the transmembrane potential generated by both passive and active fluxes. However, this equation does not provide a satisfactory description of the membrane response to excitation (8). In order to describe the excitatory process, an equivalent circuit analog has been adopted. The equivalent circuit analog can be developed out of the basic flux equations (21, 79).

The ionic flux, \( M_{(\gamma,ec)} \), is related to the ionic current, \( I(\gamma) \), by the Faraday constant:
If membrane chord conductance to a specific ion, $g(\gamma)$, is defined as:

$$g(\gamma) = \frac{1}{n} \frac{k}{(\mu(\gamma)FA(\gamma))}$$  \hspace{1cm} Eq. 32

$$M(\gamma,ec) \int_0^1 \frac{dx}{(\mu(\gamma)A(\gamma))} = E(\gamma) - V(M)$$  \hspace{1cm} Eq. 11

From Eq. 11 it follows that:

$$I(\gamma) = g(\gamma)(V(M) - E(\gamma))$$  \hspace{1cm} Eq. 33

$$g(\gamma) = \frac{I(\gamma)}{V(M) - E(\gamma)}$$  \hspace{1cm} Eq. 34

$$V(M) = \frac{\sum I(\gamma) + \sum g(\gamma)E(\gamma)}{\sum g(\gamma)}$$  \hspace{1cm} Eq. 35

This electrical analog can be described by the equivalent circuit diagrammed in Figure 2.

However, Eq. 35 does not take into account the ionic currents due to the active transport processes within the membrane. In order to incorporate them in the formalism, Eq. 31 should be revised to read:

$$(M(\gamma,ec) + M(\gamma,A))F = I(\gamma)$$ \hspace{1cm} Eq. 36

If: $M(\gamma,A)F = J(\gamma)$ \hspace{1cm} Eq. 37

it follows that:

$$I(\gamma) = g(\gamma)(V(M) - E(\gamma)) + J(\gamma)$$ \hspace{1cm} Eq. 38

$$g(\gamma) = \frac{I(\gamma)}{V(M) - E(\gamma)} - \frac{J(\gamma)}{V(M) - E(\gamma)}$$ \hspace{1cm} Eq. 39

$$V(M) = \frac{\sum I(\gamma) - \sum J(\gamma) + \sum (g(\gamma)E(\gamma))}{\sum g(\gamma)}$$ \hspace{1cm} Eq. 40
Figure 2

Equivalent circuit analog of Hodgkin and Huxley (33 - 36). C(M) represents the membrane capacitance, g(l) the leakage conductance primarily resulting from chloride ions, E(l) the leakage equilibrium potential. See text for a detailed description.
These equations were used by Kornacker (45) to describe the transmembrane potential in terms of an electrical analog in which \( J(\gamma) \) is modeled as an ideal current generator. This electrical analog is described by the equivalent circuit diagrammed in Figure 3.

**Description of Nerve Action Potential**

Hodgkin and Huxley (33 - 36) used Eq. 33 as the basic relationship between current and voltage and described the squid axon action potential by analytically solving for \( g(\text{Na}) \) and \( g(\text{K}) \). They found that these conductances are functions of voltage and time and are responsible for the changes in the transmembrane potential during the action potential. Hodgkin and Huxley were able to make an accurate description of the squid action potential using Eq. 33 rather than Eq. 38 to relate transmembrane current and voltage because in their preparation, \( \Sigma J(\gamma) \) was constant and approximately equal to zero. The technique they used in their voltage clamp experiments to separate the sodium and potassium currents, removing extracellular sodium to eliminate the sodium component of the total ionic current measured, did not affect the \( \Sigma J(\gamma) \). As a result they were able to measure the passive ionic fluxes and compute accurate passive ionic conductance
Figure 3

Equivalent circuit analog of Kornacker. (45) All parameters are defined as in the Hodgkin-Huxley analog except for the sodium and potassium active transport currents which are represented as $J(\text{Na})$ and $J(\text{K})$ respectively. See text for a detailed description.
values. However, in other preparations where \( \gamma J(\gamma) \) is not necessarily always equal to zero, more caution must be exercised when computing conductance values. From Eq. 39 it can be seen that passive conductance is defined as a relationship between transmembrane voltage and passive current, i.e. the total measured current minus any active transport current. If this active transport current is not taken into account, a change in the transmembrane voltage could be attributed to a change in the passive membrane conductance when in fact a change in the active transport current was the cause of the change in the transmembrane voltage as well as the erroneous measurement of conductance (67).

The Hodgkin-Huxley voltage clamp analysis of \( g(Na) \) and \( g(K) \) gave the following picture of the squid action potential: upon depolarization of the axon to a threshold voltage, the \( g(Na) \) rapidly increases causing depolarizing sodium ions to enter the cell and produce the spike of the action potential. This increased \( g(Na) \) is immediately inactivated and soon returns to its resting value. After a delay from the onset of depolarization, \( g(K) \) increases (delayed rectification) causing potassium ions to flow out of the cell and returning the cell to its resting potential.
Analysis of Cardiac Action Potential:

Rapid Depolarization

The success of Hodgkin and Huxley in describing the squid axon action potential in the formalism of Eq. 33 has led other investigators to adopt this basic relationship between current and voltage. The Hodgkin-Huxley axon has served as the basis for the analysis of action potentials in tissues other than nerve.

The rapid depolarization phase of the cardiac action potential has been shown to be dependent on the external sodium ion concentration (17) and to function with amplitudes and rates very similar to those determined for the nerve action potential (13, 19, 20). Applying the voltage clamp technique to cardiac tissue in order to accurately determine the kinetics of the sodium excitatory current has proven to be more difficult than it was for nerve. A large proportion of the cardiac membrane capacitance appears to be in series with a resistive element. As a result the fiber is clamped to the desired voltage with a time constant that is too large to accurately dissect the initial sodium current from the initial capacitive current. (20) Dudel and Rudel (20) have attempted to surmount this problem by cooling the preparation so that the development and decay of the excitatory current is slowed down.
One problem with this technique is that it is difficult to maintain normal sodium and potassium gradients. The conditions under which their measurements are made are nowhere near physiological. Johnson and Lieberman (41) have also criticized the cardiac voltage clamp data on the grounds that spatial homogeneity of the voltage clamp is not complete even when the single (77) and double (72) sucrose gap techniques for voltage clamping are employed.

**Analysis of the Cardiac Action Potential:**

**Plateau and Repolarization Phases**

As mentioned in the introduction, one of the unique properties of the cardiac action potential is its characteristic plateau potential. FitzHugh (22) and George and Johnson (23) attempted to explain this phenomenon in terms of simple modifications of the sodium and potassium conductance expressions used by Hodgkin and Huxley to model the action potential of the squid axon. A plateau potential would result if the speed of onset of delayed potassium rectification is decreased and if the increased sodium conductance is not completely turned off after rapid depolarization. Armstrong (2) has demonstrated that squid axons can display a rectangular shaped action potential qualitatively similar to that found in heart cells when the nerve fibers are treated with 27 mM TEA,
an agent which specifically acts to hinder the outward potassium current. However, in cardiac muscle an entirely different mechanism is responsible for the plateau potential. Weidmann (85) has measured the slope resistance during the course of an action potential by passing small current pulses through the cells and observing the effect on the transmembrane voltage. The results of these experiments demonstrate that the slope conductance during the plateau falls from the high levels found during the spike to conductance levels comparable to those measured during diastole. This finding contradicts a necessary condition of the FitzHugh model, that the slope conductance during the plateau be greater than during diastole.

The basis for the small slope conductance during the plateau phase of cardiac action potentials is an anomalous rectification for potassium ions. As described by Adrian and Freygong (1), anomalous rectification is a fall in the membrane conductance when the membrane current flows from the inside to the outside of the cell and a rise in the conductance when the current is in the opposite direction. For heart cells, this means that when the cell becomes depolarized and more potassium tries to flow out of the cell, the potassium conductance decreases. Much less outward potassium current flows than would be expected if the potassium conductance remains unchanged. A consequence of this is that less inward current is
required to maintain the state of depolarization at the plateau level (58).

These facts led Noble (56) to model the bioelectric characteristics of heart cells with the equivalent circuit found in Figure 4. This model is basically the Hodgkin-Huxley equivalent circuit with the exception that the properties of the potassium conductance are described by two rectifiers, \( g(K,1) \) and \( g(K,2) \). \( g(K,1) \) represents the anomalous potassium conductance described above. It is a function of \( V(M) \), decreasing when the membrane is depolarized. \( g(K,2) \) is the delayed potassium rectifier, analogous to the delayed rectification found in nerve. \( g(K,2) \) slowly rises in response to depolarization and is responsible for repolarization of the membrane in this equivalent circuit model.

As mentioned in the preceding section, it is difficult to voltage clamp cardiac preparations adequately. At this time there is no technique which will allow for adequate control of the transmembrane voltage during the flow of the initial inward current. However, there is good control of the membrane potential during the flow of the slow currents that follow the initial current transient (16). Although there is yet a question as to the homogeneity of the clamp along the entire fiber length, voltage clamp experiments on cardiac preparations have
Figure 4

Equivalent circuit analog of Noble. (56) See text for a detailed description.
contributed to the understanding of the currents that flow during the plateau phase of the action potential.

In their voltage clamp experiments on Purkinje fibers, Peper and Trautwein (63) reported that a depolarizing step produced three transient responses: 1) a positive capacitive current; 2) a negative excitatory sodium current; 3) a slower positive transient which reached its maximum twenty msec. after the onset of the depolarizing step and then declined to a steady state level within fifty msec. This third current was termed the positive dynamic current. When the Purkinje preparation was repolarized to the holding potential, a large negative capacitive current flowed followed by a second negative current which declined to zero within fifty msec. This latter negative current was termed the negative dynamic current. The reversal potential for these dynamic currents was found to be between -20 and -30 mV, suggesting that they are both generated by the same conductance change within the membrane. Peper and Trautwein interpreted the role of the dynamic currents in the following way: upon depolarization, the positive dynamic current is activated, resulting in rapid repolarization to -25 mV. This rapid repolarization phase of the action potential is a characteristic unique to Purkinje fibers. Neither it nor the positive dynamic current have been found in other
cardiac preparations (84). Since -25 mV is the reversal potential for the dynamic currents, the negative dynamic current begins to flow as the membrane potential falls below this level producing a depolarizing effect on the cell. The time course of the plateau is determined by the time course of the negative dynamic current. As it declines with time, the cell repolarizes toward its resting potential.

Noble and Tsien (57) have carried out a series of voltage clamp experiments on sheep Purkinje fibers. Their interpretation of this data is that repolarization results from a delayed efflux of potassium ions rather than to a decrease in the negative dynamic current (31). By looking at their current voltage curves as a function of time (Figure 5) one cannot distinguish between the two mechanisms. The current responsible for the shift in the curve along the current axis could result from either an increased outward current or a decreased inward current. However, the increase in potassium conductance with time proposed by Noble and Tsien has neither been observed in the experiments of Dudel (18) nor Mascher (50). Recent voltage clamp experiments on sheep ventricular fibers by Giebisch and Weidmann (24) show that the inactivation of the inward current becomes more rapid as the membrane repolarizes and thereby links this inactivation with the
Figure 5

Reconstruction of the repolarization process from the data of Noble and Tsien. (57) I vs V curves calculated at 100 msec. intervals after the initiation of depolarization.
repolarization process (rather than delayed potassium rectification). Coraboeuf, Delahayes, and Sjostrand (11) have reported no increase in the level of potassium efflux from guinea-pig ventricle during the course of an action potential. However, they did see a significant increase in potassium efflux from rat and frog atrium during repolarization. These findings point to the possibility that the process of repolarization is accomplished by different mechanisms in different species and in different preparations (31).

Properties of the Negative Dynamic Current

The finding of Coraboeuf, Delahayes, and Sjostrand (11) that the guinea-pig ventricle does not display an increase in the level of potassium efflux during the course of the action potential leads to the conclusion that the negative dynamic current is primarily responsible for the plateau potential and the repolarization process in this mammalian cardiac preparation. Since this is the preparation in which I have chosen to study the properties of the plateau potential, it is necessary to characterize the negative dynamic current as completely as possible.

Vitek and Trautwein (84) have carried out a series of voltage clamp experiments intended to elucidate the
ionic nature of the currents flowing during the plateau phase of the action potential and contributing to the negative dynamic current. They have demonstrated that the negative dynamic current is not a continuation of the rapid sodium influx responsible for the spike potential. It is activated after depolarization. Manganese, an inhibitor of passive calcium flux into the cell (30), was found to inhibit the slow inward current but not to affect the overshoot of the action potential. These findings point to the fact that the negative dynamic current and the initial sodium current are distinct. Their manganese data suggests the possibility that the negative dynamic current results from the passive diffusion of calcium ions into the cell during the plateau phase of the action potential.

Early models of the cardiac action potential assumed that sodium ions are the sole carriers of depolarizing current. Subsequent experiments, recently reviewed by Reuter (69), have demonstrated that other ions are capable of contributing a depolarizing current during activity. The plateau phase of the action potential was found to be less sensitive to external sodium ion concentration than the initial height of the action potential and the associated rate of depolarization (54) implying that ions other than sodium contribute to the plateau potential.
Pappano (62) in guinea-pig atria, Delahayes (14) in frog atria, and Carmeliet and Vereecke (7) in cow Purkinje fibers have shown that the catecholamines are able to restore action potentials to preparations made inexcitable by increased potassium or reduced sodium in the extracellular milieu. The height and duration of these action potentials were dependent on the extracellular calcium concentration. They were insensitive to TTX, a specific inhibitor of the rapid sodium conductance increase responsible for the rapid depolarization associated with the spike of the action potential. Manganese suppressed the effect of the catecholamines. Slow action potentials with similar properties can be induced when all sodium has been removed from the extracellular bathing medium. Vereecke and Carmeliet (83) have demonstrated them in medium containing large strontium concentrations and Aronson and Cranefield (3) have shown them in rich calcium solutions. These slow responses in fibers in which the fast response has been abolished have properties different from the slow response seen in normal cardiac action potentials. They display summation and the absence of a total refractory period. The normal action potential can not be regarded as the fast upstroke followed by this isolated slow response. The fast upstroke continues to exert an influence throughout the entire course of the
action potential (12). However, these results demonstrate that, under certain specific conditions, the membrane conductance to divalent ions can be altered so as to affect the transmembrane potential. Divalent ions, particularly calcium under physiological conditions, are capable of contributing a depolarizing current. It is possible that they do so during the course of the cardiac action potential and contribute to the current flow associated with the negative dynamic current.

The proposal that calcium ions contribute to the negative dynamic current is supported by the work of Mascher and Peper (50) which demonstrated a negative dynamic current in sodium free bathing solutions only when calcium ions were present above a concentration of 1.8 mM/l. Beeler and Reuter (4) have reported that the negative dynamic current in dog myocardium disappears in calcium free solutions. Using calcium-45, Niedergerke demonstrated that during excitation there is an increased influx of calcium in frog ventricles (55). In a number of hearts (sheep (50), cow (7), rat (87), and rabbit (87)) manganese was found to shorten the action potential duration and decrease the level of the plateau potential as would be expected if calcium ions contribute to the negative dynamic current in these preparations.

It has been proposed that the negative dynamic current
is entirely carried by calcium ions (50). However, there is evidence which points to the fact that the negative dynamic current is more complicated than that. Peper and Trautwein (63) have reported that, although the amplitude of the dynamic currents were affected by changes in the external chloride, sodium, calcium and potassium concentrations, the reversal potential was not affected by these changes in the external milieu. Such a result would not be expected for a passive current carried by a single ionic species. Rougier (73) has proposed that a part of the negative dynamic current is carried by sodium ions but through a channel distinct from the sodium channel responsible for the rapid sodium influx. He was led to this conclusion by the discovery of a sodium sensitive, TTX insensitive component of the negative dynamic current when calcium is removed from the external environment. Dudel (18) has reported a slow inward current in his voltage clamp experiments on Purkinje fibers which is neither due to sodium nor calcium.

Ochi (61) has reported finding that in guinea-pig preparations, a slow inward current can be measured in calcium and sodium free solutions but only when manganese is present. These findings suggest that when manganese is added to the extracellular environment, it can make
a contribution to the negative dynamic current. In support of this interpretation is the work of Coraboeuf and Vassort (10) and Yanaga and Holland (87). They have found that manganese prolongs the plateau phase of the guinea-pig action potential. If manganese acts to suppress the passive influx of calcium, this finding would cast doubt on the contribution of calcium to the negative dynamic current and support the possible contribution of manganese ions. Under physiological conditions manganese is present only as a trace element. The importance of its contribution to the negative dynamic current is only of interest when the ion is introduced for the purpose of producing one of its other effects, for example, inhibiting the passive calcium influx.

If a part of the negative dynamic current is carried by calcium ions, a first order approximation would predict that an increase in the external calcium ion concentration would increase the driving force on the calcium ions, increase the calcium current during the plateau, and thereby increase the action potential duration, or amplitude, or both. The increase in the action potential and plateau potential magnitudes with increased extracellular calcium has been consistently observed (14, 59). However, Beeler (4) and Temte (80) in dog ventricle and Purkinje
fibers, Delahayes and Bozler (16) in frog atria, and Nosek and Hollander (59) in guinea-pig ventricle, have reported that an increased external calcium ion concentration shortens the plateau phase of the action potential. Delahayes and Bozler (16) have also shown that this depression of the plateau phase can be at least partially reversed by TEA and Rb, agents known to inhibit the delayed rectification of potassium. These findings suggest that the effects of calcium on the action potential may be mediated by changes in the potassium and, or, sodium permeability of the membrane (16, 54).

Evidence for Electrogenie Active Transport During the Cardiac Action Potential

The experiments reviewed above have produced evidence pointing to the contribution of calcium and sodium ions to the negative dynamic current. These studies have restricted themselves in assuming that a change in conductance is the only mechanism by which the transmembrane voltage can be influenced during the course of an action potential. Eq. 40 demonstrates that the transmembrane potential will not only be influenced by changes in conductance but also by an alteration in the sum of the active transport currents. The classic work of Hodgkin
and Huxley was carried out in a preparation where $J(\gamma)$ did equal zero at rest as well as during activity. As a result it has become almost automatic for investigators to begin a study on other preparations with the assumption that the active transport system is electoneutral and does not contribute to the transmembrane voltage either at rest or during activity. Evidence is accumulating (71, 82) that $J(\gamma)$ is not always zero and that active transport plays an active role in determining the transmembrane potential under various conditions. The possibility exists that the $J(\gamma)$ is not equal to zero during the cardiac action potential and that it does exert an influence on the transmembrane potential during the plateau phase of the action potential. There is evidence which suggests that $J(\gamma)$ makes a contribution to the negative dynamic current.

As was found in nerve, Glitsch (25) discovered that the hyperpolarization following hypothermia in cardiac muscle is due to an electrogenic pump, i.e. under these conditions where the cells become loaded with sodium the active efflux of sodium ions is greater than the active influx of potassium ions ($\beta = |J(K)|/|J(Na)| < 1$). McDonald (51), in a study on guinea-pig ventricle, found that the resting potential was maintained during anoxia even though the value of $|E(K)|$ dropped. The maintenance
of the resting potential was attributed to an electrogenic pump. These studies, along with a quantitative comparison of potassium inward and sodium outward transport in frog myocardium by Haas (29), suggest that the ratio of the active fluxes of sodium and potassium are not tightly coupled and that $\beta$ will vary under various conditions. If $\beta < 1$, the active transport system will generate a net current which will act to hyperpolarize the cells. Since this condition can be satisfied by either an increase in $|J(\text{Na})|$ or a decrease in $|J(\text{K})|$, the above experiments give no direct evidence as to which component of the pump is changing.

However, Lieberman and Lundegard (47) have demonstrated that the electrogenicicy of the cation pump in their nerve preparation, accounting for up to twenty percent of the resting membrane potential, is modulated by changes in the potassium rather than the sodium coupling to the pumping system. Brindley and Mullins (5), also in nerve, have shown that the sodium component of the Na-K pump is independent of membrane potential. In McDonald's experiments on guinea-pig ventricle it was demonstrated that during anoxia $|E(\text{K})|$ dropped while the resting potential was maintained. These findings would suggest that $|J(\text{K})|$ is the most sensitive component of the Na-K pump in the guinea-pig cardiac preparation and
that the condition where $\beta < 1$ is brought about by a
decrease in $|J(K)|$ rather than by an increase in $|J(Na)|$.

McDonald (51) also reported that during anoxia with
low levels of glucose in the bathing solution, the dura-
tion of the action potential was decreased to fifteen
percent of that seen in fresh muscle. Since the conditions
under which these experiments were run would decrease
the metabolic activity of the cell, they show a direct
relationship between the level of metabolic activity and
the duration of the action potential. Prasad and
Callaghan (66) reported for human papillary muscle a
decrease in the duration of the action potential in
KCl-free solutions. It is known for guinea-pig that a
potassium free solution inhibits ATPase activity (66)
and thereby metabolic activity. These findings also
point to a direct relationship between the level of
metabolic activity and the action potential duration.
More supportive evidence that the length of the action
potential in guinea-pig ventricular muscle results from
an energy dependent process comes from the drug studies
of MacLeod (48). He has shown that the action potential
duration is directly proportional to the amount of ATP
available for utilization which is, in turn, directly
proportional to the amount of glucose metabolized.

From the above facts it may be concluded that the
action potential duration is proportional to the amount of ATP available which is proportional to the level of metabolic activity which is in turn proportional to the level of active transport. It is known that $|J(K)|$ increases with increased metabolic activity (65). It was indicated above that changes in $|J(K)|$ may be the main factor determining the value of $\beta$. If this is the case, it follows that $|J(K)|$ is directly proportional to the action potential duration. Assuming that a cause and effect relationship exists between the level of active transport of potassium ions and the action potential duration, the following theory is proposed to explain this relationship.
A transient electrogenic active transport current makes a contribution to the negative dynamic current which has been shown to be responsible for the plateau potential and the process of repolarization in guinea-pig ventricle. During the action potential of this tissue, the following sequence of events is theorized. During diastole, the ratio between the active transport of potassium into the cell and sodium out of the cell is close to one and the active transport system makes very little, if any, contribution to the resting potential under normal conditions (Eq. 40). After the initial fast depolarization phase of the action potential, any increases that take place in the passive membrane conductance values for sodium and calcium ions leading to a net flux of positive ions into the cell, are complemented by a transient electrogenic active transport current that is also directed into the cell. The rapid depolarization of the membrane triggers an increase in the active influx of potassium ion, possibly through a voltage dependent property of the active transport system. The simultaneous
active efflux of sodium ions remains constant. $\beta$ becomes greater than one under these conditions. This particular decoupling of the pumping of potassium and sodium ions results in a net inward flux of positive ions that is part of the negative dynamic current. The transient electrogenic active transport current follows the same time course that has been described for the negative dynamic current. As the increased $|J(K)|$ returns to its resting level so does $\beta$. The resultant decrease in the influx of positive ions contributes to the process of repolarization.

An equivalent circuit model that takes into account all the proposed factors that influence the transmembrane potential is diagrammed in Figure 6.
Figure 6

Proposed equivalent circuit for guinea-pig ventricle containing all factors proposed to influence the transmembrane potential. \( g(\text{Na},1) \) is the sodium channel responsible for rapid depolarization. \( g(\text{Na},2) \) is the sodium channel that is activated during the plateau. All other parameters are the same as defined previously. See text for a detailed description.
EXPERIMENTAL RATIONALE

Polimeni and Vassalle (65) have performed a series of radioactive tracer flux experiments on guinea-pig ventricles to determine the difference between potassium influx during rest and activity. They found a six percent increase in the potassium influx during activity over that found in resting fibers. The theory proposed in the preceding section would predict such a result. If \(|J(K)|\) is increased above its resting level during the plateau phase of the action potential, an increased influx of potassium would accompany activity. An alternative explanation for this increased potassium influx with activity is as follows. A net influx of sodium ions accompanies the generation of an action potential (44). In order to maintain a steady state, the active transport system has been shown to increase the active efflux of sodium ions as the intracellular concentration of sodium is increased (27). If there is a direct coupling between the active transport of sodium and potassium ions, the increase in potassium influx observed by Polimeni and
Vassalle with activity would result from this coupling factor rather than from any increase in influx taking place specifically during the plateau potential.

In order to experimentally test the validity of the proposed theory, it is necessary to determine how the levels of active potassium influx and active sodium efflux are affected by changes in amplitude and duration of the plateau phase of the action potential. The most direct means of measuring changes in these fluxes is by the use of radioactive potassium and sodium.

Since it was necessary to determine how the active fluxes of sodium and potassium change with changes in the plateau phase of the action potential, it was necessary to adopt a method of changing the amplitude and duration of the plateau potential. Ouabain is known to decrease the level of Na-K ATPase activity (70), the active influx of potassium (75, 76), and the amplitude and duration of the cardiac action potential (40). Anoxia has also been shown to decrease the duration of the plateau (51). These two agents were used in combination to depress the amplitude and duration of the plateau potential. The active fluxes of sodium and potassium were determined under normal conditions and when the plateau region of the action potential was depressed. These values were compared in stimulated and resting preparations. The
proposed theory predicts that under normal conditions the potassium influx will be greater during activity than during rest. As the plateau potential is suppressed the ratio is expected to decrease. The theory predicts no difference between the ratio of sodium efflux during rest to sodium efflux during activity, determined under normal conditions and when the plateau potential is depressed.
APPARATUS

Figure 7 is a diagram of apparatus I which was used to load all preparations with radioactive tracers. Apparatus I was also used to bathe the loaded preparations in non-radioactive medium when the tracer efflux rate constant was determined by comparing the whole body radiation levels of the preparation over extended time intervals. Figure 8 is a diagram of apparatus II which was used to hold the loaded preparation in the well of the scintillation counter so that the whole body radiation level could be monitored and the efflux rate constant determined continuously. Figures 9 and 10 are detailed diagrams of the plexiglas chambers which held the muscle preparations in apparatus I and II respectively. These apparatuses were designed to: 1) hold the muscle preparation under a given tension and length; 2) stimulate the preparation with an electrical pulse of pre-determined form and frequency; 3) immerse the preparation in the desired medium.

In both apparatuses, one end of the muscle preparation was in contact with a tungsten wire which served as
Apparatus I. Key to letter code: a) chamber I with holder; b) beaker containing bathing medium; c) water to affect thermal contact between water bath and bathing medium; d) water bath; e) temperature controlled circulator; f) thermister; g) telethermometer; h) stimulating electrodes; i) isolated stimulator; j) surgical thread holding preparation; k) strain gauge; l) micro-manipulator; m) stand supporting manipulator and chamber; n) strain gauge calibration resistor; o) Wheatstone bridge circuit and amplifier; p) oscilloscope; q) fritted disk for airation of bathing medium; r) gas cylinder; s) ventricle strip.
Figure 8

**Apparatus II.** Key to letter code: a) chamber II with holder; b) well of scintillation counter; c) spectrometer; d) stimulating electrodes; e) isolated stimulator; f) surgical thread holding preparation; g) strain gauge; h) micromanipulator; i) stand supporting manipulator and chamber; j) calibration resistor; k) Wheatstone bridge and amplifier; l) oscilloscope; m) bathing medium efflux tube; n) bathing medium influx tube; o) water bath; p) thermister; q) telethermometer; r) plasma bottle and administration set; s) gas cylinder; t) temperature controlled circulator; u) ventricle strip.
Chamber 1. Key to letter code: a) stimulating electrodes; b) surgical thread leading to strain gauge; c) tungsten rings; d) ventricle strip; e) plexiglas chamber.
Figure 10

Chamber II. Key to letter code: a) stimulating electrodes; b) surgical thread leading to strain gauge; c) tungsten rings; d) ventricle strip; e) plexiglas chamber; f) influx tube; g) efflux tube.
one of the stimulating electrodes. Another tungsten wire in contact with the medium bathing the preparation functioned as the second stimulating electrode. A Bioelectric Isolator (Model 1S2B-2.5 1014) was used to electrically decouple the stimulus pulse from ground and stimulate the muscle preparation. This isolator was driven by a pulse generating system consisting of a Tektronix #160 series Power Supply, Waveform Generator, and Pulse Generator which enabled the experimenter to apply a square wave electrical stimulus of desired amplitude, duration, and frequency. The other end of the muscle preparation was attached via a tungsten hook and surgical thread to the arm of an isometric strain gauge (Statham Model # G10B-0.15-350) which served as one arm of a Wheatstone Bridge. The remaining bridge circuitry and the amplification of the DC output voltage of the unbalanced bridge was provided by a Statham Precision Readout (Model UR4). The output voltage of the readout was applied to the vertical amplifier of a Tektronix 502 Dual Beam Oscilloscope and continuously monitored. The magnitude of the developed tension (isometric contraction) developed by the muscle preparation in response to an electrical stimulus was calibrated by means of a calibration resistor which could be injected between the strain gauge and the Statham Readout. The strain gauge
was mounted on a micromanipulator which was used to set the muscle length and tension.

All media bathing the muscle preparation were temperature controlled through a water bath by a Lauda K-2R refrigerated circulator (Brinkmann Instruments). Thermisters in thermal contact with the water baths and coupled to a tele-thermometer (Yellow Springs Instrument CO., Inc.) allowed the temperature of the media to be continually monitored.

Chamber I of apparatus I was suspended in a beaker of medium in thermal contact with the temperature controlled water bath. The medium was airated with compressed gas of desired composition through a small fritted disk placed in the beaker. This airation process also provided vigorous agitation of the bathing medium.

Apparatus II was designed to fit into the well of a spectrometer (Packard Tri-Carb Liquid Scintillation Spectrometer, 1-3/4" NaI crystal) which was used to measure the whole body radiation level of the muscle preparation. In order to keep the voltage to the photomultiplier tube of the spectrometer as steady as possible, the line voltage was regulated by a Solatron Line Voltage Regulator. A plexiglas cap fit around the lower end of chamber II providing a 0.25 ml. bath surrounding the preparation. Continuously airated medium flowed by
gravity at a rate of 2.0 ml./min. from a plasma bottle suspended above the apparatus, through a Plexitron solution administration set and polyethylene tubing of 0.023 in. ID X 0.038 in. OD, into the cap of chamber II. A section of the polyethylene tubing was in thermal contact with the temperature controlled water bath. Medium was removed from the chamber by means of aspiration through another polyethylene tube positioned near the top of the cap.
PROCEDURE

Ventricle Strip Preparation

Male guinea-pigs (*Cavia porcellus*), weighing between 200 and 400 gm., were used in all experiments. After a guinea-pig was rendered unconscious by a blow to the head, the chest cavity was opened and the heart removed. The beating heart was placed in a beaker of Krebs-Henseleit medium (Table 1) for approximately two minutes to allow the heart to pump itself free of blood. The right ventricle was removed from the whole heart according to the technique of Hollander (38) and a muscle strip, averaging 7 mm. in length and 2 mm. in width was cut from that section of the ventricle which had been adjacent to the coronary artery. Two small tungsten rings were tied to either end of the ventricle strip with surgical thread to provide a means for mounting the preparation in the experimental chambers.
## Table 1

**Composition of normal Krebs-Henseleit medium** which was used in all experiments. pH = 7.4 ± 0.2

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<table>
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Sodium and Potassium Efflux Measurements

Two experimental protocols, type I and II were followed in order to measure the rates of sodium-24 and potassium-42 efflux from the ventricle muscle preparation. In both protocols the freshly dissected ventricle strip was initially loaded with radioactive ions by being mounted in chamber I of apparatus I and bathed for a given period of time in Krebs-Henseleit medium containing 25 - 5 μCi of sodium-24 or 10 - 5 μCi of potassium-42 per ml. of medium (New England Nuclear, 575 Albany St., Boston, Mass. 02118). The radioactive isotopes were received in the form of $^{24}\text{NaCl}$ and $^{42}\text{KCl}$ in water. The total sodium, potassium, and chloride ion concentrations in the radioactive media were maintained at normal levels. The loading medium was continuously airated with either 95%O$_2$ - 5%CO$_2$ or 95%N$_2$ - 5%CO$_2$ depending upon the requirements of the experiment. During the loading period the preparation was either in a resting state or stimulated with a threshold electrical stimulus of 2.0 msec. duration at a rate of one Hz. The length of the muscle preparation was set at the start and held constant throughout the course of the experiment. This length, as measured by the metric scale on the micromanipulator holding the strain gauge, was determined
by the following procedure: 1) while stimulated, the muscle was stretched until a developed tension was visible on the oscilloscope; 2) the length of the preparation was increased until a final diastolic tension of 900 mg. above that recorded in step 1 was obtained. The preparation was held at this length for the duration of the experiment.

In the type I protocol, the muscle preparation was removed from apparatus I after the loading period, re-mounted in apparatus II, washed with non-radioactive Krebs-Henseleit medium, and the whole body radiation level of the preparation determined as a function of time. All activity levels were corrected for isotope decay. The sodium and potassium effluxes were expressed in terms of the rate constant $k$ where (39):

$$k = \frac{d \log Q}{dt}$$

and where $Q$ is the level of radioactivity in the muscle preparation expressed in terms of counts per minute. A Hewlett-Packard calculator was used to determine the rate constants for these effluxes by the method of least squares. This loading and efflux procedure was repeated when it was necessary to measure the efflux in the same preparation under varying conditions.

In the type II protocol, the effluxes of sodium and potassium were determined in the following way. After
loading the preparation, the radioactive loading medium was removed from the thermal bath of apparatus I and replaced by non-radioactive medium. The muscle preparation was washed in this vigorously agitated medium for a given period of time after which it was removed from chamber I, placed in a plexiglas cap containing 0.2 ml. of medium, positioned in the well of the spectrometer, and its whole body radiation level measured. Following measurement, the preparation was remounted in chamber I and washed in non-radioactive medium for a given period of time. At the end of this time interval the whole body radiation level of the preparation was again determined in the manner described above. \( k = -\Delta \log \frac{Q}{\Delta t} \) was determined for this time interval and taken as an indication of the rate of efflux at that time. In this way the rates of sodium and potassium efflux could be determined at various times after loading and under various conditions.

**Potassium Influx Measurement**

Potassium influx during a loading period was expressed as \( I = \frac{Q_2 - Q_1}{t_2 - t_1} \) where \( Q_1 \) and \( t_1 \) were measured at the end of the efflux period preceding the loading period and \( Q_2 \) and \( t_2 \) were measured at the beginning of the efflux period following the loading period.
DATA

Sodium Efflux Determination

Initially, protocol I was followed to determine the rate of sodium efflux from the isolated ventricle strip preparation. After an initial loading period of one hour in apparatus I, the tissue was placed in apparatus II and the whole-body radioactivity of the preparation continuously monitored while it was washed with non-radioactive Krebs-Henseleit medium. Figure 11 contains a plot of the data from one such experiment. The first twenty minutes after the preparation was removed from the loading bath was characterized by a rapid rate of efflux followed by a much slower rate. This slower efflux rate eventually fell off to zero while there was still a large number of counts recorded in the apparatus. Upon moving the preparation from apparatus II and measuring the level of activity in the muscle strip alone, it was found that the tissue activity was less than ten percent of the total activity measured in apparatus II. This high background was probably due to the accumulation
Figure 11

Sodium efflux curves. The solid line (Experiment #23) is a record of sodium efflux determined by protocol I. The broken line (Experiment #24) is a record of sodium efflux determined by protocol II. See text for details of the experimental procedure. Both curves are plotted on a semi-log scale, the ordinate expressing the activity in counts per minute and the abscissa expressing time in minutes after the cessation of loading.
of sodium-24 somewhere in the apparatus resulting from the high level of sodium-24 washed out of the preparation during the fast efflux phase. This continuous efflux technique could not yield accurate values for the slow rate of sodium efflux since it was impossible to determine the component of the sodium efflux resulting from an exchange of the activity accumulated in the apparatus. Also, the high background activity caused any changes in the low tissue activity level to become part of the background noise. Therefore, protocol I was replaced by protocol II. Though this technique limited the number of data points that could be taken, the general shape of the sodium efflux curve determined by protocol II was the same as that determined by protocol I. (Figure 11)

Since there is evidence in the literature that both the fast and slow rates of sodium efflux contain a transmembrane flux component (28, 43), data on both the fast and slow components of sodium efflux was desired. The efflux rate constant determined 35 to 55 minutes after cessation of loading, designated k(2), was taken as a measure of the slow sodium efflux component. The efflux rate constant determined 5 to 15 minutes after the cessation of loading, minus k(2), was designated k(1) and taken as a measure of the fast sodium efflux component. In each experiment these values were determined after a
one hour loading of a stimulated preparation with sodium-24. The loading conditions were standardized since it has been demonstrated that the sodium compartments filled depend upon the state of the preparation during loading (60). k(1) and k(2) values were compared with the tissue in each of the following states: 1) electrically stimulated at a rate of one Hz (S); 2) at rest, i.e. not being electrically stimulated (R); 3) electrically stimulated while anoxic and in medium containing 2 x 10^{-7}M ouabain (AOS); 4) at rest while anoxic and in medium containing 2 x 10^{-7}M ouabain (AOR). When anoxic-ouabain preparations were used they were allowed to equilibrate in the anoxic-ouabain containing environment for two hours before any measurements were made. The data from the sodium efflux experiments is recorded in Table 2 in terms of the actual efflux rate constants and in Table 3 in terms of the ratios of stimulated versus non-stimulated efflux for each experiment. Figure 12 graphically summarizes the sodium efflux data.

Potassium Efflux Determination

All muscle preparations were initially loaded with potassium-42 for a period of one hour. Both protocols I and II were used to measure the potassium efflux. In
Table 2

Sodium efflux rate constants. See text for details of experimental technique. When a student t-test was run between the S and R and between the AOS and AOR values for both the fast and slow efflux components, there was found to be no significant difference between the distributions. There was also no significant difference between the \( k(1) \) AS and S and AOR and R conditions. However, the t-test did show a significant difference between the \( k(2) \) values for the AS and S \((p = .01)\) and the AOR and R \((p = 0.1)\) conditions.
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Table 3

Ratios of sodium efflux rate constants recorded in Table 2. A student t-test showed no significant difference between the S/R and AOS/AOR distributions for either the fast or slow efflux components.
Sodium efflux data summary. Bar graphs representing the data contained in Tables 2 and 3.
protocol I, the efflux curves (Figure 13) had both a fast and a slow component. The fast component was assumed to result from the wash out of extracellular potassium (65). The potassium efflux rate constant, $k'$, determined from the curve at least fifteen minutes after the termination of a loading period was taken as a measure of the transmembrane potassium efflux. These rate constants were compared when the muscle preparation was at rest ($k'(R)$) and when electrically stimulated ($k'(S)$) as summarized in Table 4. The background radiation in these experiments was insignificant when compared with the tissue activity and, therefore, had no significant effect on the determination of the efflux rates.

In protocol II, the efflux rate constants were determined between two measurements of the whole body radioactivity of the preparation, one made ten seconds after loading and the other made thirty minutes after loading. This rate constant was taken as a measure of the transmembrane potassium efflux. Efflux rate constants were compared when the tissue was: 1) at rest, not electrically stimulated (R); 2) electrically stimulated at one Hz (S). In a typical experiment, the sequence of measurements illustrated in Figure 14 would be repeated a second time. The efflux rate constants for like conditions were averaged to give the rate constant for
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Table 4

**Potassium efflux values: Protocol I.** See test for details of the experimental technique. A typical experiment is graphed in Figure 13. The ratio of $(\text{Mean } k'(S))/(\text{Mean } k'(R)) = 1.29$. A student t-test showed that the S and R distributions were distinct at the $p = 0.1$ confidence level.
Figure 14
Potassium efflux and influx: Protocol II. Experiment #31
See text for details of the experimental technique.
that condition in that particular experiment. Table 5 summarizes the data from a series of experiments using this technique for measuring potassium efflux.

**Potassium Influx Determination**

Figure 15 is an influx curve for potassium-42 in the ventricle strip preparation. The rate of influx is relatively constant for the period between 30 and 90 minutes from the beginning of the loading period. In order to compare the rate of influx under the various conditions to be investigated, the preparation was held in this linear region by the method illustrated in Figure 14. In a typical experiment, the sequence of measurements illustrated in Figure 14 would be repeated a second time. The influx values for like conditions were averaged to give the influx value for that condition in that particular experiment. Potassium influx was measured under the following conditions: 1) at rest, not stimulated (R); 2) electrically stimulated at one Hz (S); 3) at rest while anoxic and in medium containing \(2 \times 10^{-7}\text{M} \) ouabain (AOR); 4) stimulated while anoxic and in medium containing \(2 \times 10^{-7}\text{M} \) ouabain (AOS). When anoxic-ouabain preparations were used they were allowed to equilibrate in the anoxic-ouabain containing environment
Table 5

Potassium efflux values: Protocol II. For details of the experimental technique see the text. A typical experiment is graphed in Figure 14. The ratio of 

\((\text{Mean } k'(S))/(\text{Mean } k'(R)) = 1.24\). A student t-test showed that the S and R distributions were distinct at the \(p = 0.01\) confidence level.
Figure 15

Potassium influx curve. Experiment #19
for two hours before any measurements were made. All influxes were quantified as a percentage of the R influx value for the non-anoxic non-ouabain preparation and as a percentage of the AOR influx value for the anoxic-ouabain preparations. Table 6 summarizes the data collected from these influx determinations.
Table 6

Potassium influx ratios See text for details of the experimental technique. A student t-test showed that the S/R and AOS/AOR ratios constitute two distinct distributions at the p = 0.01 confidence level.
DISCUSSION

The theory put forth and experimentally tested in this dissertation proposes that a component of the negative dynamic current in guinea-pig ventricular myocardium is composed of a transient electrogenic active transport current which is caused by an increased active transport of potassium ions into the cell during the plateau phase of the action potential. Such a theory requires that the following two conditions be necessarily satisfied: 1) active potassium influx increases during the plateau phase of the action potential; 2) no increase in the active efflux of sodium ions accompanies and offsets the effects of the increased active potassium influx during the plateau. Does the data reported in this dissertation satisfy these necessary conditions and thereby support the theory that has been put forth?

Table 6 summarizes the experiments run to determine potassium influx under various conditions. Any changes in the potassium influx resulting from action potentials were averaged along with the influx during the quiescent intervals occuring over the influx period. In order to
provide the most favorable conditions for measurement of any changes that take place in the potassium influx during the action potential, the maximum possible rate of stimulation was desired. However, it has been shown that as the frequency of stimulation increases, the magnitude of the dynamic current (63) and the duration of the action potential (37) decrease. A stimulation frequency of one Hz was chosen as an acceptable compromise between these two factors. Upon stimulation (S) the average potassium influx, resulting from both passive and active transport processes, was found to increase by 22% over the average influx determined when the preparation was at rest (R). This result would not be predicted if only the passive potassium influx was changing when the preparation was stimulated. If only passive ionic fluxes are considered, a decrease rather than an increase in the potassium influx would be expected with stimulation. Compared with the resting transmembrane potential, stimulation causes the inside of the cells to become more positive during the course of the action potential and a decrease in the passive influx of potassium ions occurs at that time. The increased potassium influx with stimulation can be explained by an increased active transport of potassium into the cells as a result of stimulation.

This increase in potassium influx with stimulation
was found to be very sensitive to the state of the preparation. A problem encountered in a majority of experiments in which potassium-42 was used was that the muscle strip would, upon stimulation, become spontaneously active. These preparations would often settle down with time. If a measurement of stimulated potassium influx was made after this spontaneously active period had ended, the increased potassium influx was not observed. Only healthy preparations which did not display a tendency to become spontaneously active demonstrated the increased potassium influx with stimulation.

When potassium influx was determined under stimulated and resting conditions in a preparation made anoxic and bathed in medium containing $2 \times 10^{-7}$M ouabain, it was found that the stimulated (AOS) influx was reduced to 81% of the resting (AOR) influx. Under these conditions the active transport processes and the plateau potential are depressed. As explained above, such a ratio between stimulated and resting potassium influxes would be predicted from a consideration of the changes in the passive component of the potassium influx that take place with stimulation. If there is a compensation for the decreased passive potassium influx with stimulation by the active potassium system, its contribution is much less in the anoxic-ouabain preparation than in the normal preparation.
This data demonstrates that the active influx of potassium is increased with stimulation and that this increase is directly proportional to the duration of the plateau potential. These findings provide evidence that the observed increased potassium influx with stimulation takes place during the plateau phase of the cardiac action potential and thereby satisfy necessary condition number one.

Upon stimulation the rate of potassium efflux increased significantly over the resting rate by 24% to 29% depending upon the method used to measure the efflux. This result was also observed in the experiments of Polimeni and Vassalle (65). When an attempt was made to determine the potassium efflux in anoxic-ouabain preparations it was found that the rate constants increased as a function of time rather than as a function of the stimulated or resting condition. An increase in the potassium efflux rate in the anoxic-ouabain preparation is expected since a depolarization of the cells accompanies anoxia (51). This depolarization would increase the passive force on the potassium ions and cause an increased flux down the potassium concentration gradient. However, since the efflux rates did not stabilize with time, no attempt was made to determine the effect of stimulation on their magnitudes.
The values for both the fast \( k(1) \) and slow \( k(2) \) components of sodium efflux, measured under R, S, AOR, and AOS conditions, are summarized in Tables 2 and 3 and in Figure 12. The fast efflux component has been attributed to both extracellular sodium washout (75) and intracellular exchange (43). Haas and Trautwein (28) have reported a frequency of stimulation dependency of this fast efflux component. However, the data on \( k(1) \) herein reported does not show a significant difference between the S and R or the AOS and AOR flux values. The S/R and AOS/AOR ratios are identical. The anoxia-ouabain conditions would be expected to decrease the rate of intracellular sodium exchange. No decrease is observed for the \( k(1) \) values. These findings strongly suggest that the \( k(1) \) component does not reflect the exchange of sodium across the cell membrane.

Haas and Trautwein (28) have reported a slight frequency of stimulation dependency of the slow sodium efflux. During depolarization there is a large increase in sodium conductance which leads to a net sodium influx with each impulse. At the same time there is a large increase in the passive efflux of sodium. Although there are no absolute values available for these fluxes in cardiac tissues, the relationships between these values should not be significantly different from those determined
for nerve since the sodium conductance follows relatively the same time course in both preparations (19). From the nerve data of Keynes (44), the sodium efflux is twenty percent greater during stimulation than at rest. The $k(2)$ values reported herein do not demonstrate a significant increase with stimulation although there is a tendency toward a ten percent increase for both the S and R and AOS and AOR conditions. In light of this data, the objection may be raised that $k(2)$ does not reflect transmembrane sodium exchange. However, there was a significant decrease in $k(2)$ when the preparation was subjected to an anoxic-ouabain containing medium in which the sodium active efflux is expected to be depressed. Therefore, $k(2)$ was accepted as a measure of the active flux of sodium ions. Since the extracellular concentration of sodium is fourteen times the intracellular, it is expected that the $k(2)$ sodium efflux rate constant is in part a result of a continuing extracellular sodium exchange. Such a contaminating factor would limit the sensitivity with which any changes in the transmembrane flux of sodium could be measured. The potassium influx measurements are influenced to a lesser extent by the contribution of the extracellular potassium ions to the total tissue activity since the extracellular to
intracellular potassium ion concentration ratio equals 0.033. As a result the potassium influx measurements are more sensitive to changes in transmembrane flux than are the sodium efflux measurements.

The finding that sodium efflux does not significantly increase with stimulation is surprising since in order to maintain a steady state condition the cell must increase its rate of sodium efflux with stimulation. However, this anomalous behavior of the sodium efflux has been seen before. Coraboeuf, Delahayes, and Sjostrand (11) have reported that there is no increase but rather a decrease in the sodium efflux during the guinea-pig ventricular action potential. They have explained these results by suggesting that an increased amount of radioactive sodium does leave the cell during activity but a substantial proportion of it passively flows back into the cell during the fast depolarizing phase of the action potential. Another interpretation of this data is that a decreased active sodium efflux during the action potential cancels the increased passive sodium efflux during the initial phase of the action potential. If this is the case, and if this decrease extends into the plateau phase of the action potential, it would actually enhance the depolarizing effects of the increased potassium influx occurring at that time. Such a mechanism would help to
explain this anomalous sodium efflux data. However, there is only indirect evidence for such a mechanism in the data that is available.

In any case, the ratio of slow sodium fluxes during stimulated and resting conditions are not significantly different when compared when the normal plateau potential is present and when it has been depressed by an anoxic-ouabain containing medium. These data suggest that there is no significant increase in the level of sodium active transport during the cardiac action potential and if there is any cancellation of the increased potassium influx during the plateau, it is not due to actively transported sodium ions. If, on the other hand, there actually is a decreased active sodium efflux during the action potential, it is not inhibited by the anoxic-ouabain containing medium and is present independent of the amplitude and duration of the plateau potential. In either case, this sodium data satisfies necessary condition number two.

The results of the experiments carried out in this study satisfy the two conditions that necessarily must hold if the active transport system makes a contribution to the negative dynamic current. It is possible that activity initiates other responses in the cell which generate currents that offset this electrogenic active
transport current. For example, intracellular calcium has been shown to exchange with extracellular sodium in cardiac preparations (26, 68). As a net flux of sodium enters the cell during the rapid depolarizing phase of the action potential, it would stimulate the efflux of calcium ions. If this increased level of calcium efflux is extended over the duration of the plateau potential it would produce a hyperpolarizing current offsetting, at least in part, the depolarizing electrogenic active transport current. Also, if there is an active calcium transport out of cardiac cells as is found in red blood cells (86), it too could be stimulated by the increased calcium influx that has been shown to flow with excitation (55) and offset some of the increase in potassium active transport. These are both possible sources of currents which may flow during the cardiac action potential. Their influence on the dynamics of the negative dynamic current has yet to be investigated. However, no matter what other mechanisms contribute to the negative dynamic current, the results of this investigation provide evidence that the Na-K active transport system does influence the composition of the negative dynamic current.
SUMMARY

In guinea-pig ventricular fibers, the time course of the negative dynamic current is responsible for the maintenance of the transmembrane potential during the plateau phase of the action potential as well as for the process of repolarization. The theory was put forth that a transient electrogenic active transport current is developed after the rapid depolarization phase of the action potential and contributes to the negative dynamic current. Radioactive tracer experiments were carried out to measure the fluxes of sodium and potassium across the cell membrane in order to test this theory. The data collected and herein reported suggests that there is an increase in the active transport of potassium ions into the cell during the course of the action potential and that this increased level of potassium influx is not offset by a simultaneous increase in the active efflux of sodium ions. It is therefore concluded that the Na-K active transport system does contribute a negative current to the total negative dynamic current that flows during the plateau phase of guinea-pig ventricular action potentials.
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