Ionic transfer in cardiac muscle. An explanation of cardiac electrical activity

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The transmembrane potential

To understand the sequence of changes in ionic concentration which explain electrical activity, one must first be familiar with records that represent transmembrane action potentials. With the perfection of the ultramicroelectrode, it became possible to measure the difference in potential between the tip of this electrode located intracellularly and some extracellular point, usually nearby, during both the resting phase and during excitation of the cardiac fiber. These two measurements are called the "transmembrane resting potential" and "action potential," respectively. Such a record is seen in Fig. 1, recorded from a left ventricular fiber of the dog heart. During the resting state the interior of the fiber is −90 mv., as can be seen at the left of the recording. This is followed by activation, a swiftly rising deflection which passes through zero and reverses its sign so that at its peak the cell interior is +30 mv. It is during this rapid upstroke that the QRS complex of the standard electrocardiogram is written. Recovery is defined as the return of this reversed potential difference to the resting level again. It takes place in three distinct phases: (1) an initial rapid phase, (2) a plateau, (3) and a final rapid phase.[1] It is during the latter that most of the T deflection of the electrocardiogram is recorded.

The transmembrane resting potential and the spike of the action potential have been carefully studied, and much is known of their genesis. The three phases of recovery are of great interest, but their origins remain speculative.

Basic to an understanding of the problem is the knowledge that, at the cellular membrane level, electrical current is synonymous with the movement of ions. The laws which apply to the literal physical transfer of ions across cardiac membranes are different from the laws which apply to electrical current in the rest of the body. These ions may be transported by traveling down a concentration gradient, propelled in the other direction by a potential gradient,

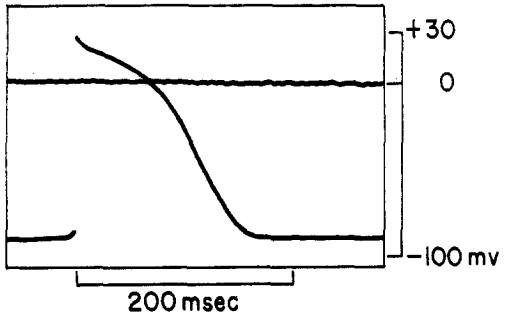

Fig. 1. Transmembrane potential from dog ventricle. (Reproduced by permission from C. McC. Brooks, et al., *Excitability of the Heart*, Figure 9B, New York, 1955, Grune & Stratton, Inc.)

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pulled by solvent drag forces, or may combine with another membrane constituent and be transferred by external metabolic work. The first method is illustrated by the junction potential produced at the interface of two solutions containing different concentrations of the same ions. If, for example, a concentrated solution of hydrochloric acid touches a dilute solution, both hydrogen and chloride ions tend to diffuse from the concentrated solution into the more dilute solution. The hydrogen ion moves faster and thus the dilute solution soon becomes positively charged because of an excess of positive hydrogen ions. The more concentrated solution is left with an excess of negative chloride ions and thus acquires a negative charge. With the passage of time, the dilute solution accumulates an excess of positive electricity which retards the velocity of the hydrogen ions and accelerates the velocity of the chloride ions, so that ultimately the two ions move with the same average velocity. As the two solutions acquire the same concentration, no difference in potential will exist between the two solutions for there are an equal number of positive and negative ions present in each compartment, with no tendency for a net improvement in either direction; thus, at equilibrium, electrical neutrality exists.

A useful rule is that the difference in potential produced at the junction of two solutions of different concentration is caused by the rates of migration of the ions present: the more dilute solution acquires a charge corresponding to that of the faster-moving ion. This is expressed in the following equation:

$$E_j = \frac{u_a - u_c}{u_a + u_c} \frac{R\ T}{nF} \ln \frac{c_2}{c_1}$$

where c_2 is the activity of the electrolyte in the concentrated solution, and c_1 is the activity in the dilute solution; and u_c and u_a are the migration velocities of the cation and anion, respectively (Table I). R, T, and F are the gas constant, absolute temperature, and Faraday, respectively, and n is the valence. See Table III for values of these constants. Activity may be defined here as effective concentration and is similar to p^H measurements of hydrogen ion concentration. Since the calculations always involve ratios of activity, the actual activity of the ion is of small importance if the activity ratio is proportional to the actual concentration ratio.

Table I. Absolute ionic velocities at 18°C. under a potential gradient of 1 volt

H^+ =	3.2×10^{-3} cm./sec.
Cl^- =	$.69 \times 10^{-3}$ cm./sec.
K^+ =	$.66 \times 10^{-3}$ cm./sec.
Na^+ =	$.45 \times 10^{-3}$ cm./sec.

Data from F. H. Getman and F. Daniels, *Outlines of Theoretical Chemistry*, ed. 6, N. Y., 1937, John Wiley & Sons, Inc.

The concentration cell

If the two solutions are separated by a semipermeable barrier which effectively blocks one ion, then this particular ion makes no contribution to the difference in potential between the two compartments, and any observed difference in potential must be due to the ions which can penetrate the barrier. The presence of a partial barrier in the form of a membrane or cell wall makes the two solutions act somewhat differently from a junction potential, in that certain ions are no longer free to move, whereas others appear to penetrate the membrane with no difficulty. The model presented by the cardiac fiber is that of a membrane separating the intracellular and extracellular spaces with changing permeabilities to each ion specie with time. The changes in permeability are least during the resting state and greatest during the rising phase of the action potential.

There are large differences in concentration across this membrane interface, and therefore a tendency for ions which are freely permeable to move down their respective concentration gradients. But, as in the case of H^+ ion movements in the HCl concentration cell, forces exist to oppose or slow down these movements. This situation is best illustrated by the potassium ion, which is highly concentrated inside the cell and tends to move outward, but which is opposed by the attractive force of anions within the cell and repelled by cations, including potassium, outside the cell. Thus, K^+ tends

to move outward because of its concentration gradient, and inward because of a potential gradient. When these two forces are equal, a state of equilibrium exists. Unlike the HCl concentration cell, this equilibrium is not at the point of equal concentration. The relation between the ionic concentration (activity) ratio, temperature, and transmembrane potential for zero flux of the ion specie (equilibrium) is called the "Nernst equation":

$$E = -\frac{RT}{nF} \ln \frac{(a) \text{ inside}}{(a) \text{ outside}}$$

Since R, T, n, and F are constants at constant temperature of 37°C. (310° absolute), and including factors for converting natural logarithms to base 10 and for converting volts to millivolts, this may be more simply stated:

$$E = -61.5 \log_{10} \frac{(a) \text{ inside}}{(a) \text{ outside}}$$

When the transmembrane potential equals the equilibrium potential, there should be no net flux of this particular ion specie across the membrane. Any change in transmembrane potential away from the equilibrium potential constitutes a driving force to change the ionic concentration gradient in the appropriate direction until a new equilibrium is established. If the membrane potential is greater than the equilibrium potential of the ion, the movement of the ion will be against its concentration gradient; and if the membrane potential is less than the equilibrium potential of the ion, then the ion will move down its concentration gradient. Thus, the transmembrane potential which may be the result of movements of both positive and negative ions in either direction will determine at equilibrium the concentration of ions on either side of the membrane.

Transport forces and permeability

The following are the general rules for the net transport of ions across cell membranes. There are only four known kinds of transfer forces involved in the movement of ions. These are: (1) differences in concentration, (2) differences in electrical potential between phases in contact with the cell membranes, (3) differences in activity coefficients, and (4) solvent drag force arising from the passage of solvents through the membrane. There are clear instances when the movement of ions cannot be explained by one of the above-mentioned processes, and it is customary to reserve the term "active transport" for these instances. In no case has the nature of active transport been clarified, but a specific chemical binding with membrane constituents appears to be essential. It is known that the transport in these instances cannot be explained in terms of the aforementioned physical forces. The transfer of sodium from inside the cell to the outside is thought to be an active transport.

Prior to 1941, it was generally accepted that the muscle membrane is permeable to potassium and essentially impermeable to anions and sodium. It was then that Boyle and Conway[2] introduced a new hypothesis that the muscle membrane is permeable to potassium and cations of the same or smaller diameter in aqueous solutions and also to the smaller anions, such as chloride. It was further stated that the critical size for passage of cations is at the potassium level (hydrated ion) or between it and the sodium ion, and that the critical size for the anions is at or near the dimensions of the chloride ion. In short, the critical diameter for free entrance of cations or anions is 8 angströms (hydrated ion). Thus, while K, Rb, and Cs ions can enter the cell at appreciable rates over short periods, Na and Li ions are virtually excluded; and whereas Cl, Br, and NO_3 ions diffuse only slowly, SO_4 ions are practically excluded. Also existing is a mechanism for the slow extrusion of sodium ions which may be continuously functioning, but the net entrance rate of sodium ions in vitro is vanishingly small.

Concentration gradient of cardiac fibers

Cardiac fibers like skeletal muscle are low in sodium and high in potassium content. The actual values determined from dry cat heart muscle and expressed in milliequivalents per kilogram of fiber or serum water are shown in Table II.[3]

Calcium is present in the serum in low concentration and probably exists intra-

Table II. *Resting cell concentrations (mEq./Kg. of fiber or serum water)*

Intracellular	*Extracellular*
Na^+ 6.5	Na^+ 159
K^+ 151	K^+ 4.8
Cl^- 5 (estimate)	Cl^- 127

From Robertson and Dunihue: Water and Electrolyte Distribution in Cardiac Muscle, *American Journal of Physiology* 177:292, 1954.

cellularly in bound form. There is good evidence to suggest that calcium enters the cell during excitation and may play a role in excitation-contraction coupling,[4] but the role it plays in producing either the resting or action potential is small.

There are about 158 mEq. per kilogram of cations accounted for in fiber water. It is fairly obvious that there are an equal number of anions present to establish electrical neutrality within the cell, but the nature of these anions is still unknown. They are not diffusible through the membrane, except for the small quantity of chloride present. The following anions have been found: chloride, phosphates, and dicarboxylic amino acids.[5] But these total only a few milliequivalents each, so that the residual intracellular anions still remain a mystery.

An explanation of the resting potential on the basis of a concentration gradient

Hodgkin and Horowicz[6,7] have furnished strong arguments that the resting potential is produced by the potassium and chloride concentration gradients across the cell membrane. It is easy to see if this is true why the inside of the cell is negative with respect to the outside, for the high concentration of the positive ion, potassium, exists intracellularly and would tend to migrate outward as a positively charged ion, whereas chloride, a negatively charged ion, is highly concentrated in the extracellular space and would tend to move inward. Both movements would tend to make the intracellular space more negative. To analyze the problem quantitatively requires certain assumptions. The first of these is that the concentration ratio of potassium ($[K^+]$ inside/$[K^+]$ outside) and chloride ($[Cl^-]$ outside/$[Cl^-]$ inside) found is equal to their activity ratios.

If the resting potential is to be explained solely on the basis of differences in concentration of potassium and chloride, then the second assumption must be made that the membrane is relatively impermeable to other ions, but especially to sodium, which is present in high concentration extracellularly. That these assumptions are approximately correct is shown by the apparent agreement between the calculated differences in potential and the experimentally measured value of −90 mv., as shown in Table III. At constant temperature the term RT/F becomes a constant, so that the resting potential should vary with the log of the potassium or chloride concentration ratio at a time when there is no net ion flux. Burgen and Terroux[8] were able to show that this is the case by comparing the measured resting potential while changing the extracellular potassium concentration in cat atrium. For extracellular potassium concentrations above 10 mM. a plot of resting potential against log potassium concentration gave a straight line relationship,

Table III. *Equilibrium potentials for potassium and chloride concentration cells*

$$V_K = -\frac{RT}{F} \ln \frac{(K^+)_i}{(K^+)_o} = -92 \text{ mv. (inside positive)}$$

$$V_{Cl} = -\frac{RT}{F} \ln \frac{(Cl^-)_o}{(Cl^-)_i} = -86 \text{ mv. (inside positive)}$$

R = 8.316 (gas constant), T = 310° (absolute temperature equivalent to 37°C.), F = 96,494 (Faraday constant), $(K^+)_i$ refers to concentration in millimoles per liter inside or outside the cell. Data taken from Table II.

and, significantly, the action potential was not altered for potassium concentrations between 2.8 to 8.4 mM. Potassium-free Ringer's solution abolished all activity, as did raising the concentration above 11.2 mM. To reduce unwanted intracellular ionic shifts as much as possible, all measurements were made within 10 to 20 minutes. This work clearly showed that, in the case of heart muscle, the movements of potassium could account for the resting potential within a wide range. The one difficulty of the unknown intracellular ionic change was overcome in experiments by Hodgkin and Horowicz.[7] They noted that the Nernst equation applied to both potassium and chloride, and, furthermore, that if the external potassium and chloride concentration were varied so that the ion product $(K^+)_o(Cl^-)_o$ is kept constant, there should be no movement of KCl across the membrane in accord with the properties of a Donnan membrane. If, for example $(K^+)_o$ is doubled and $(Cl^-)_o$ is cut in half, the ionic equilibrium will not be altered, provided that the membrane potential responds rapidly and reversibly to this alteration of K and Cl in accord with Equation 1, which is simply a rewritten form of the equation in Table III.

$$\frac{(K+)_o}{(K+)_i} = \frac{(Cl-)_i}{(Cl-)_o} = e^{(VF/RT)} \qquad \textit{Eqn. 1.}$$

Using a value for $(K^+)_i$ of 140 mM., a plot of measured potentials from frog sartorius muscle against calculated values is shown in Fig. 2. This shows excellent agreement for potassium concentration above 10 mM. This result obtained in frog skeletal muscle is in agreement with Burgen and Terroux' work with heart muscle and constitutes good evidence that the membrane resting potential conforms to the model represented by a concentration cell of potassium and chloride ions.

The actual amount of current (I) carried by each ion is a product of its conductance and the difference between the transmembrane potential (V) and the equilibrium potential (V, for the ion). For the potassium ion this would be expressed:

$$I_K = g_k\,(V - V_K).$$

Hodgkin and Horowicz[7] have used this relationship to obtain data for potassium and chloride conductance and permeability in frog skeletal muscle by assuming that the membrane is impermeable to other ions, and, therefore, that the two ions carry all of the current during the resting state. They further used a value for intracellular chloride concentration obtained by substituting external concentration and the potential gradient into the Nernst equation. These data suggest that when $(V - V_K)$ is positive and potassium ions are moving outward, potassium permeability falls to a low value of 0.05×10^{-6} cm./sec.; and when $(V - V_K)$ is negative and potassium ions are moving inward, potassium permeability rises to 8×10^{-6} cm./sec. The same data obtained for chloride suggest that chloride is not greatly influenced by changes in potential or concentration and has a constant permeability coefficient of about 4×10^{-6}cm./sec.

This information suggests that for skeletal muscle the relative contribution of

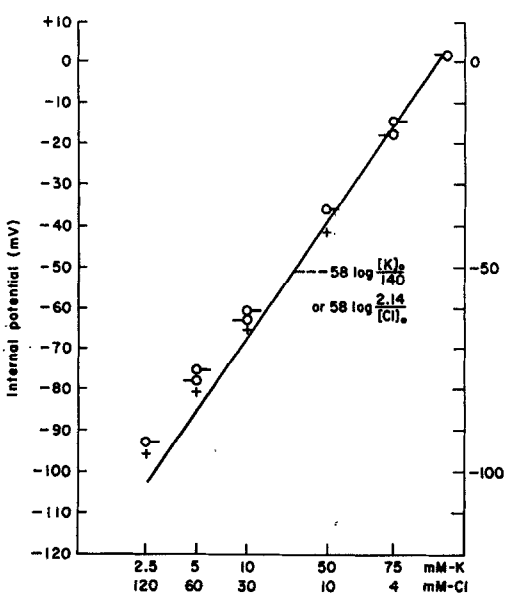

Fig. 2. The relationship of resting potential and the log of the transmembrane concentration gradient of K^+ and Cl^- for solutions with (K_o) $(Cl)_o = 300$ mM.[2]. Crosses (+) are potentials after equilibrium is established for 10 to 60 minutes; circles (O) are potentials measured 20 to 60 seconds after a sudden change in concentration. −O after increase in, and O− after decrease in (K_o). (Reproduced by permission of Paul Horowicz: Influence of Ions on the Membrane Potential of Muscle Fibers *from* Biophysics of Physiological and Pharmacological Actions, Publication No. 69, American Association for the Advancement of Science, 1961.)

K^+ or Cl^- to the membrane potential depends on the direction in which potassium ions are moving, for its permeability is fairly high for inward and fairly low for outward current movements, whereas that of chloride is fairly constant.

The amount of current carried by the chloride ion during the resting phase may quite different in cardiac muscle than in skeletal muscle. Recent studies by Hutter and Noble[9,10] show that, in the resting phase, chloride ions account for 68 per cent of the total membrane conductance in skeletal muscle, but only a small contribution in cardiac muscle.

The action potential

There is good evidence that the rising phase of the transmembrane action potential is the result of a transient increase in membrane permeability to sodium ions and to the passage of these ions across the cardiac membrane. This information is based on the fact that membrane resistance in Purkinje fibers undergoes a profound decrease[11] during the rising phase of the action potential, which suggests an increase in membrane permeability and the fact that the action potential of heart muscle, as well as of most other excitable tissues, disappears completely when external sodium is replaced by sucrose or choline. Overton[12] first noted loss of excitability of frog muscle fibers when external sodium concentraton was reduced below 10 per cent of that present in Ringer's solution. This observation led to several quantitative studies which showed that the height of the action potential was related to the extracellular sodium concentration.[13,14] Increasing the extracellular sodium above normal levels causes higher voltages of the action potential than are normally found, and a progressive decrease in the extracellular sodium concentration progressively decreases the height of the action potential. When the extracellular sodium concentration falls below 10 per cent of the normal concentration, excitability of the fibers disappears.

A reduction in extracellular sodium causes a small increase in resting potential, but the main change is a decrease in the height of the action potential. In both situations the inside potential becomes more negative, but the effect on the action potential is quantitatively greater. It is significant that varying external potassium concentration, although it greatly affects the resting potential, causes only a slight change in the amplitude of the action potential until the potassium concentration reaches higher levels than those seen in physiologic preparations.

Using the technique of the "voltage clamp" devised by Marmont,[15] Cole[16] and Hodgkin and associates[17-20] have obtained direct information that the rising phase of the action potential coincides with the entry of sodium into the squid giant axon. This result was obtained by inserting two electrodes through the length of the cell. One was used to measure intracellular potential, and the other was made to hold the intracellular potential constant or to change it from one value, such as at the resting potential level, to a second value equivalent to depolarizing the cell at constant voltage. The fiber was immersed in a bath divided into compartments, with a pair of electrodes oriented perpendicularly to the fiber, and, therefore, sensitive to movements of current in or out of the fiber. Thus, with a known driving force, the net flux of ions in either direction could be measured. The movements of single ions, such as sodium, could be studied by reducing the concentration of this one ion in the bath, and by regulating the clamp voltage so that the sodium equilibrium potential was reproduced, so that the net sodium current would be zero.

For example, when the membrane potential was lowered by 65 mv. from the resting level, there was a transient inward movement of current. This current could be traced to the movement of sodium ions by replacing sodium ion with choline in the external bathing fluid, in which case the inward current did not occur. In both cases, there was a delayed, long-lasting, outward current attributed to potassium ions. When the electrical potential was regulated so that it approached the equilibrium potential for sodium, the inward current disappeared, but so long as the polarity of the membrane potential was in the same direction as the resting potential (negative), this inward current was observed. Thus, the net current carried

by the positive charge of the sodium ions is inward, unless the membrane potential is made sufficiently large and positive inside to overcome the effect of the difference in concentration. The critical value of membrane potential at which the fluxes are equal and the net sodium current zero closely approximated the sodium equilibrium potential calculated by the Nernst equation. The delayed and long-lasting outward current which is attributed to potassium ion flow is unaffected by changes in the external sodium concentration.[19] Changes in external potassium concentration affect it in an unpredictable manner.

With the entry of sodium into the cell, strong forces exist in the form of both a potential gradient and a concentration gradient to cause the exit of potassium from the cell. Wilde and Obrien[21] have clearly shown that there is a pulsatile outflow of potassium with each electrical systole in the turtle ventricle. From this point on there is little direct evidence, but it is apparent that a mechanism exists for removing sodium from the cell, and, also, that potassium must re-enter to re-establish the pre-existing ionic gradient. There is some evidence that the active sodium transport system in frog skeletal muscle at least is linked to potassium influx. This rests on the data of Keynes[22,23] which show that sodium efflux varies fairly promptly and reversibly with extracellular potassium concentration, and that net sodium extrusion from sodium-loaded muscle cannot be accomplished in a potassium-free media.

Summary

The evidence has been reviewed which suggests that the upstroke of the action potential in heart muscle is due to the entry of sodium ions. This conclusion is based on the failure of the upstroke to occur if 90 per cent of the sodium is replaced by sucrose, and the demonstration of a reduction in amplitude of the rising phase of the action potential with each decrement in extracellular sodium concentration or an increase in amplitude with increasing extracellular sodium concentration. In addition, the demonstration of a change in membrane resistance of one-hundred-fold at the time of the rising phase suggests increased permeability of the membrane at this time.

The voltage-clamp studies in the squid giant axon clearly show an inward movement of current during the rising phase, which disappears when choline replaces sodium in the perfusing bath.

The resting membrane potential resembles the model of a potassium and chloride concentration cell, since calculations based on measured concentrations across the membrane agree fairly closely with measured potentials. Furthermore, the membrane resting potential is altered in a predictable manner by changed extracellular potassium and chloride concentration, but is not appreciably affected by changing sodium concentration. Since the skeletal muscle membrane appears to be freely permeable to chloride, and only sparingly so to potassium, and since potassium permeability is selectively altered during the electrical cycle, the chloride ionic concentration gradient is probably dependent on the transmembrane potential, and, therefore, is passive. The current carried by the chloride ion in cardiac fibers is small.

Little is known of the factors which alter membrane permeability or affect the transfer rates during recovery, but it is apparent that sodium is removed from the cell after the rising phase and is replaced by potassium to restore membrane resting potential.

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