SIMPLE TECHNIQUES SUITABLE FOR STUDENT USE TO RECORD ACTION POTENTIALS FROM THE FROG HEART

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Demonstrating action potentials during class experiments is very educational for science students. It is not easy, however, to obtain a stable intracellular recording of action potentials from the conventionally used skeletal muscle cells, because the tip of a glass microelectrode often comes out or breaks due to muscle contraction. Here, I present a much simpler recording method using a flexible polyethylene electrode with a wide orifice (~1 mm) for a bullfrog heart beating on automaticity. Extracellular recordings of action potentials (electrocardiogram) can be obtained by placing an electrode on the cardiac surface, and transmembrane potentials can be obtained by rupturing the membrane with negative pressure, i.e., whole cell configuration. Once attached to the heart by suction, the polyethylene electrode does not easily come off during contraction of the heart. Perfusion of the heart via the postcaval vein offers us opportunities for observing the effects of either changing ionic compositions of solutions or applying drugs. The techniques shown here provide a simple and convenient way to perform a variety of class experiments.


Key words: cardiac muscle; extracellular recording; whole cell recording

It is important to observe and understand the properties of action potentials, which support excitability of nerve and muscle cells, as class experiments. Extracellular recordings of action potentials can be easily obtained from frog sciatic nerves or from human muscles [electromyogram (EMG)] or human heart [electrocardiogram (ECG) or the German word elektrokardiogram (EKG)]. In contrast, it is quite difficult to find a suitable material and method to monitor transmembrane potentials for student experiments. Frog sartorius muscle is often used for intracellular recording with glass capillaries microelectrodes (15).

However, stable recording of action potentials is not easy, because a glass microelectrode often comes out from a skeletal muscle during the contraction induced by an action potential. In addition, sophisticated apparatuses are necessary for the intracellular recording method, including micropipette pullers, micromanipulators, stimulators, stereomicroscopes, microelectrode illumination systems, vibration-free tables, and Faraday cages.

Here, I present a much simpler method for stable recording of transmembrane potentials that does not
need the sophisticated devices mentioned above. The method uses a polyethylene tube that can be used repeatedly, instead of an easily breakable glass microelectrode. The suction-electrode method (9) is employed for recording transmembrane potentials under whole cell configuration (3). Also, because further studies on cardiac function can be achieved by changing the ionic compositions of the solutions used or by the application of drugs, the present techniques are suitable for undergraduate science students to perform a variety of experiments.

**METHODS**

**Preparation of the frog heart.** Adult bullfrogs of either sex were anesthetized by cooling in icewater for 30–50 min. After the vertebral canal was disconnected at the neck or decapitation, the spinal cord and the brain were destroyed with a wire. Then the frog was placed in a plate in a supine position. The thorax was opened with a pair of scissors for the skin and muscles and with another pair of bone scissors for thoracotomy. The covering pericardium was removed with forceps to expose the heart and the branches of the arterial trunk originating from the bulbus cordis (Fig. 1A). The heart was turned over to expose the postcaval vein, and the connective tissue was cut along both sides of the vein, freeing the vein without damaging it (Fig. 1B). A thread was put through under the postcaval vein, and it was ligated at the peripheral point to stop blood entering the heart (Fig. 2B). To make an outlet for perfusate, the right branch of the arterial trunk was ligated, and a window was cut open in its left branch (Figs. 2A and 3). Do not cut off the left branch; otherwise, the heart becomes unstable for performing experiments. A winged needle of a drip-infusion system was inserted into the postcaval vein (Figs. 2B and 3), and the needle was fixed by its wings with a tape or a string around the body (Fig. 3). The tube of the infusion system is connected to a container (e.g., 50-ml plastic syringe) for perfusate (Fig. 3a). A drip chamber of the infusion system (Fig. 3b) is useful for monitoring the speed of perfusion. Fast drug application can be achieved with a small-volume syringe (5–10 ml) through a three-way stopcock that is placed near the winged needle (Fig. 3c).

**Electrophysiology.** The suction-electrode method described by Irisawa and Kobayashi (9) was employed for recording electrical signals from the bullfrog heart. To make a recording electrode like the one illustrated in Fig. 4, a polyethylene tube (3–5 mm in diameter) was warmed by a small fire, then removed from the fire, and drawn out by hands when the tube had started to cool down. A thin part of the tube was cut so that the diameter of the stump was ~1 mm. This polyethylene tube (Fig. 4a) was connected to a plastic tube with a branch (Fig. 4b). Chloride-coated silver wire (Fig. 4c) was soldered to the output pin (Fig. 4d), and it was inserted into the tube near the orifice for collecting electrical signals. ECG waves can be obtained by using commercially available pipette tips (100, 200, or 1,000 μl in volume) as the tip of the electrode (Fig. 4a). However, flexible polyethylene tubes are better than stiff pipette tips to record transmembrane potentials by suction.
Commercially available microelectrode amplifiers, usually designed to also provide stimulation, are sufficient to monitor membrane potentials. For recordings from the automatically beating heart, however, only a head stage of such a conventional amplifier is necessary. Therefore, it is recommended that you construct your own amplifiers (voltage followers) to reduce costs, because each group of students will need at least two amplifiers for their experiments. A reasonably priced analog FET-input operational amplifier (op-amp; e.g., LF356, National Semiconductor) is appropriate for recording membrane potentials, because the direct current resistance of polyethylene electrodes is <20 kΩ when filled with standard solution. In contrast, high-resistance glass microelectrodes (usually larger than 10 MΩ) require expensive extremely high-input impedance op-amps. A Faraday cage was not necessary, because the noise level was

![Image of a schematic diagram of the electrophysiological setup. Intracardiac perfusion is achieved with an infusion system for humans. The winged needle was fixed with tape around the body. The wing is shown in dotted lines beneath the tape. a: Container for perfusion. b: Drip chamber. c: 3-Way stopcock for rapid application of drugs. d: Box of an amplifier. e: Rod attached to the box. f: Polyethylene electrode. g: 3-Way stopcock for introducing intrapipette solution and for maintaining negative pressure. h: Syringe for applying suction. i: Reference electrode. j: Output signals from the amplifier to be fed to an oscilloscope. For further explanation, see text. Note that the drawing is not to scale.]

**FIG. 3.**

A schematic diagram of the electrophysiological setup. Intracardiac perfusion is achieved with an infusion system for humans. The winged needle was fixed with tape around the body. The wing is shown in dotted lines beneath the tape. a: Container for perfusion. b: Drip chamber. c: 3-Way stopcock for rapid application of drugs. d: Box of an amplifier. e: Rod attached to the box. f: Polyethylene electrode. g: 3-Way stopcock for introducing intrapipette solution and for maintaining negative pressure. h: Syringe for applying suction. i: Reference electrode. j: Output signals from the amplifier to be fed to an oscilloscope. For further explanation, see text. Note that the drawing is not to scale.

**FIG. 4.**

Recording electrode for the suction-electrode method. A polyethylene tube with a wide orifice of ~1 mm (a) is connected to a plastic tube with a branch (b). A chloride-coated silver wire (c), for collecting electrical activities of the heart, is soldered to the pin insulated with Teflon (d).
low, owing to the low resistance of the polyethylene electrode.

Figure 5 displays an example of a circuit diagram of a voltage follower suitable for student use for recording action potentials from the frog heart. An FET-input operational amplifier (op-amp) LF356 (National Semiconductor) is used in this case. A feedback resistor of 10 kΩ is used to prevent oscillation of the op-amp. A 25 kΩ variable resistor inserted between the offset-null pins #1 and #5 and a negative supply voltage of +15 V are for trimming the input offset voltage to 0.

Figure 5 displays an example of a circuit diagram of a voltage follower. A feedback resistor (10 kΩ) inserted between the inverting input and the output is for preventing oscillation of the op-amp, and a variable resistor (also called a potentiometer or a trimmer) is for nulling the offset voltage. Refer to the instructions that come with your op-amp for canceling the offset, because the method differs from op-amp to op-amp.

To eliminate noise, a Teflon terminal should be used for insulation to convey electric signals to an op-amp. Also, the noninverting input pin of the op-amp must be directly soldered to a Teflon terminal that accepts a metal pin of the polyethylene electrode connected to a chloride-coated silver wire (Fig. 4d). For further details of the electronic construction techniques, refer to appropriate books on electronics (7).

A metal rod (Fig. 3e) was attached to the box of the amplifier (Fig. 3d) so that it could be held with a clamp instead of with a sophisticated and expensive micromanipulator. Positions of the stand and the clamp can be adjusted by hand. A reference electrode (chloride-coated silver wire or pellet; Fig. 3f) was placed at an appropriate place (e.g., at the neck or in the abdomen) where it was relatively unaffected by electrical activities of the heart muscle. A vibration-free table was not necessary for ensuring stable recording because the polyethylene tube (Fig. 3f) sticks to the heart by suction, and it does not easily come off. A three-way stopcock (Fig. 3g) was used for maintaining negative pressure after the cell membrane was ruptured by suction. The same stopcock was also used for introducing solution into the polyethylene electrode to immerse the silver wire so that signals can be obtained without noise. The electrical signals picked up by an amplifier were fed to an oscilloscope with a bayonet naval connector cable (Fig. 3f), and the data were printed out with a plotter for later analysis and discussion. Reasonably priced sets of a digital oscilloscope (Hitachi Denshi, model VC-6523, Tokyo, Japan) and an x-y plotter (Hitachi Denshi, model 68-XA) are used in my classes. Data stored on the display of the oscilloscope can be immediately drawn by the plotter. If possible, though, data should be processed by a computer and be stored on appropriate media such as zips, magnet-optical disks, magnetic tapes, etc., for later analysis. All the data shown in the present work (Figs. 6-14) were obtained at room temperature (20–24°C).

**Solutions.** The composition of the standard extracellular solution (frog Ringer solution) used both for preparation and experiments was (in mM) 111.2 NaCl, 1.8 KCl, 1.08 CaCl₂, 0.08 NaH₂PO₄, and 2.38 NaHCO₃ (adjusted to pH 7.4 with HCl). The same solution was used as a pipette solution for extracellular recordings. As for the whole cell recording of action potentials, the pipette solution (i.e., intracellular)

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**FIG. 6.** Extracellular recording of action potentials from the bullfrog ventricle (unipolar cardiac electrogram). Both records were obtained simultaneously using the 2 sets of recording devices. The tip of the polyethylene electrode was gently placed on the surface of the middle part of the ventricle (top trace) and another electrode at the apex or the lower part of the ventricle (bottom trace).
lar solution) consisted of (in mM) 115 KCl, 1 EGTA, and 10 HEPES (adjusted to pH 7.2 with KOH). It was found, however, that no significant difference was observed between the records obtained with this intracellular solution and those with the extracellular solution in the pipette, probably because the diffusion of the pipette solution into cardiac muscle cells was very slow due to maintained negative pressure. For convenience, therefore, it is recommended to use the standard extracellular solution all the time inside the pipette. Then, we do not need to change the intrapipette solution when we transfer from extracellular recording to whole cell recording.

EXPERIMENTS

Extracellular recording of action potentials. When the tip of the recording electrode was gently placed on the surface of the ventricle, electrical activities similar to those of human ECG (or EKG), were observed (Fig. 6). Precisely, it is a unipolar cardiac ECG because the recording electrode is directly

A: relationship between the electrocardiogram (ECG; top trace) and the transmembrane potential (bottom trace) recorded simultaneously from the bullfrog ventricle. The action potential consists of 5 phases (0–4). B: enlarged display of the initial part of the simultaneously recorded action potential (top trace) and ECG (bottom trace). The traces were obtained from a different frog.

Effects of elevating extracellular K⁺ concentration on the resting potential and the action potential. The concentration of K⁺ was increased by 50 mM by simply adding 1 M KCl solution to the standard solution. The high-K⁺ solution was introduced into the heart via a 3-way stopcock for rapid perfusion (Fig. 3c). Perfusion was started immediately before this illustration. Inconsistent peak values of the action potential peaks are due to the slow sampling time (see text).
placed on the heart, but it will be called ECG in the present work for convenience. The amplitude of the QRS complex of the human ECG is usually less than a few millivolts, because recording electrodes are placed on the body surface and the electrical activities of the heart are picked up through the body, which is a volume conductor. In contrast, the size of the QRS complex was often larger than 10 mV in the present experiments, because recording electrodes were directly placed on the heart (Figs. 6–8). If the recording is impaired by noise, the thickness of the trace widens; if so, check for the presence of air bubbles at the tip of the polyethylene electrode. Noises can be eliminated by filling the recording tip with solution, i.e., by expelling air bubbles by pushing the piston of the syringe (Fig. 3h). The top trace of Fig. 6 was obtained from the midportion of the ventricle, and it consists of a positive (R wave) and negative (S wave) of similar size. The bottom trace, simultaneously recorded with another amplifier from the apex of the ventricle, depicts the large R wave followed by the small s wave. Notice that large waves are described in upper case. It should be remembered that the frog has two atria and one ventricle. The P wave (atrial depolarization) is invisible, proba-

FIG. 11.
Blocking effect of tetrodotoxin (TTX) on the action potential of the ventricular muscle cells. Introduction of perfusate containing $10^{-7}$ M TTX (indicated by the horizontal bar with arrows) from the 3-way stopcock (Fig. 3c) gradually inhibited action potentials without affecting the resting potential.

FIG. 12.
Suppression of action potentials by lowering the concentration of Na$^+$ to $1/3$ that of the standard solution. Traces obtained in standard and $1/3$ Na$^+$ solutions are superimposed for comparison.

FIG. 13.
Reduction of a part of the upstroke and the plateau of the action potential induced by high-Mg$^{2+}$ (30 mM) solution. Records obtained in standard and high-Mg$^{2+}$ solutions are superimposed.

FIG. 14.
Relationship between electrical and mechanical events. Action potential (top trace) and muscle tension (bottom trace) were recorded simultaneously from the frog heart. Tension curve was obtained with a force transducer.
bly because the electrical activities of the atria are too small to be recorded at the ventricle. The T wave (ventricular repolarization) is clear in both records. The heart rate is quite low because of the body temperature, which has not recovered from being immersed in icewater.

Transition from extracellular recording to transmembrane potential recording. A prominent characteristic of cardiac muscle cells is that they are connected to each other via intercalated discs through gap junctions (1, 11), forming a functional network. The heart, therefore, can be regarded as a large muscle cell or an electrical syncytium, and this offers a great advantage for carrying out whole cell recordings on the frog heart with a polyethylene electrode having a huge tip diameter.

To record transmembrane potentials under whole cell configuration, the membrane under the polyethylene tube (recording electrode) should be broken by applying negative pressure to the recording electrode (Fig. 3f) with a syringe (Fig. 3b). A syringe of 10 ml in volume is useful for this purpose, and suction of 3–6 ml is sufficient to break the membrane. A sudden pull of a syringe piston is more efficient than a gradual pull to obtain large and stable recordings. After membrane ruptures, negative pressure should be maintained with a three-way stopcock (Fig. 3g) attached to the syringe. As indicated by an arrow in Fig. 7, electrical access to the cell’s interior by suction is shown by a shift of the membrane potential in a negative direction (i.e., resting membrane potential) followed by repetitive action potentials. For convenience, the recording electrode contained the standard extracellular solution for monitoring ECG and transmembrane potentials in succession (see METHODS). The positive part of the action potential is called overshoot. Note that action potentials obey the all-or-none law or principle, i.e., action potentials are always the same amplitude and shape when produced. The average resting membrane potential obtained with a polyethylene electrode was \(-33.48 \pm 6.47\) mV (mean \(\pm\) SD; \(n = 25\)).

Relationship between electrocardiogram and transmembrane potential. To understand the meanings of the ECG waves, the relationship between the ECG and the action potential should be presented to students. This can be achieved by simultaneous application of the unipolar ECG and the suction-electrode techniques to the heart muscle. Traces shown in Fig. 8A were obtained from the middle portion of the ventricle with two amplifiers. The top trace shows the ECG with the QRS complex and the T wave. The P wave, which indicates atrial depolarization, is virtually invisible. The bottom trace illustrates the action potential consisting of five phases: phases 0 (rapid upstroke), 1 (partial repolarization, better seen in Fig. 8B), 2 (plateau), 3 (rapid repolarization), and 4 (resting potential). It is to be noted that the QRS complex is synchronous with the rapid upstroke (phase 0) and the T wave with the repolarization (phase 3). In other words, the QRS complex and the T wave reflect excitation and repolarization of the heart muscle, respectively. The relationship is shown in more detail in Fig. 8B. The ECG was recorded from the apex, thus showing a prominent R wave in ECG (bottom trace), and the action potential was obtained from the midventricle. The traces indicate that the major part of the QRS complex corresponds to the phase 0 of the action potential and to some part of phase 1.

As the next step, observe that the atria and the ventricle do not contract simultaneously but alternate. Figure 9 reveals the basis of these contractions. Action potentials show alternate firings of the atrium (top trace, left atrium in this case) and the ventricle (bottom trace). The figure also illustrates that the atrial action potential is considerably different from the ventricular action potential in shape.

Effects of elevating K\(^+\) concentration on the transmembrane potential. The resting membrane potential depends on the permeabilities of the cell membrane, mainly to K\(^+\) and to a certain extent to Na\(^+\), and it shows a depolarizing shift when the extracellular K\(^+\) concentration is elevated (6). This can be demonstrated by perfusing a solution containing a high concentration (50 mM) of K\(^+\) (Fig. 10). The introduction of high-K\(^+\) solution into the frog heart resulted in a gradual increase in the concentration of K\(^+\) inside the ventricle, and it caused a slow depolarizing shift of the resting potential (Fig. 10). In addition, the amplitude of action potentials became smaller with time. Careful examination of the record reveals that the plateau phase became smaller con-
comitant with the reduction of the spike amplitude. These phenomena can be explained by the theory that a larger fraction of all Na\(^+\) channels becomes inactivated as the resting potential shifts to the depolarizing direction (4). Unfortunately, the peaks of action potentials, which should all be at the same level before the resting potential starts to change, are not consistent in Fig. 10. This is simply due to the limitation of the sampling time of the digital oscilloscope that was used for the experiments. Horizontal time display is divided into 1,000 points for any time scale, and the sampling time is as large as 50 ms in the case of Fig. 10.

**Cardiac massage.** Students should be reminded not only to pay attention to the electrical activities of the heart but also to how the contraction of the heart is affected in their experiments. In the case of perfusing high-K\(^+\) solution (Fig. 10), the heartbeat was weakened as action potentials were inhibited, and the heart finally stopped beating (cardiac arrest). When you see the heart begin to be inflated with perfusate, you must immediately stop the perfusion and start a cardiac massage by compressing or pinching the heart. Compressions can be carried out by pushing the atria downward with an index finger followed by compressing the ventricle. As for the pinching method, the heart is compressed with two fingers. Compression or pinching should be strong enough to see content solution coming out from the window opened in the left branch of the arterial trunk (Fig. 3). In either case, the procedure should be repeated until the heart starts to beat on its own. While doing such a cardiac massage, we should pour standard solution flushed from the window. Usually, atria recover first. Therefore, when you see the atria start to beat, stop cardiac massage and wait for ventricular contractions. These resuscitation techniques are always useful when cardiac arrest occurs due to drug applications or alterations in the ionic composition of perfusates as you see in Figs. 11–13.

**Effects of TTX on the transmembrane potential.** Depolarization of cardiac muscle cells is produced by openings of voltage-gated Na\(^+\) and Ca\(^{2+}\) channels, and Na\(^+\) is the primary ion carrier responsible for the greatest part of the upstroke (2, 14). In mammals, cardiac Na\(^+\) channels are much less sensitive to puff-fish toxin or tetrodotoxin (TTX) than Na\(^+\) channels of nerve cells (17). On the other hand, Na\(^+\) channels of the frog heart are as sensitive to TTX as nerve cells and can be inhibited by low doses of TTX (8, 14). In fact, complete suppression of action potentials was recorded (Fig. 11) when a few milliliters of the standard extracellular solution containing 10\(^{-7}\) M TTX were applied to the bullfrog ventricle via the side tube of the infusion system through the three-way stopcock (Fig. 3c). Note, that the resting potential was not affected by TTX (Fig. 11), very different from the depolarizing shift of the membrane potential caused by high-K\(^+\) solution (Fig. 10). A complete recovery of the action potential was obtained when TTX was washed away by cardiac massage (not illustrated). Although this TTX experiment is informative in understanding the ionic mechanism of the action potential, the experiment itself should be carried out by an instructor as a demonstration because of the toxicity of TTX. Gloves must be worn when doing cardiac massage on the TTX-treated heart. Also, it is important that solutions containing TTX should be collected and made alkaline with NaOH or KOH before disposal, as TTX decomposes and loses its toxicity under alkaline conditions.

**Inhibition of the action potential in low Na\(^+\) solution.** As mentioned above, the rapid upstroke (phase 0), which is important for producing the action potential, is dependent on Na\(^+\) influx through Na\(^+\) channels (2, 14) (Fig. 8). Therefore, action potentials recorded from the frog ventricle became smaller when the Na\(^+\) concentration of the perfusate was lowered by replacing NaCl with an equimolar amount of membrane-impermeant substance such as choline chloride. Figure 12 illustrates superimposed traces obtained in standard and in \(\frac{1}{3}\) Na\(^+\) solutions for comparison. Under low Na\(^+\) conditions, the upstroke was not sufficient to trigger the plateau (phase 2), which is dependent on openings of Ca\(^{2+}\) channels. When Na\(^+\) was totally removed from the perfusate, the action potential was completely blocked (not shown). The inhibition of the action potential was easily reversed by returning the perfusate to the standard extracellular solution. These results indicate that the activity of Na\(^+\) channels is important for excitation, as pointed out by Hodgkin and Katz (5) for squid giant axons.
**Inhibition of the plateau by Ca\(^{2+}\) channel blockers.** Influx of Ca\(^{2+}\) contributes to the late part of the upstroke and the plateau of the cardiac action potential (14, 15). This can be proved by applying Ca\(^{2+}\)-channel blockers to the cardiac muscle. As shown in Fig. 13, a part of the upstroke and the plateau of the action potential were reversibly suppressed by adding 30 mM Mg\(^{2+}\) to the perfusate. More potent Ca\(^{2+}\)-channel blockers may be used for inhibition of the plateau. However, it is not preferable, for instance, to use Cd\(^{2+}\) (1 mM) for student experiments because of its hazardous toxicity to humans and the environment (10, 18). Clinically used organic blockers, such as nifedipine (1 \(\mu\)M) or verapamil (1 \(\mu\)M), are alternative choices for inhibiting cardiac Ca\(^{2+}\) channels (12).

**Relationship between action potential and muscle tension.** So far, electrical activities of the heart have been shown (Figs. 6–13). Although it can be observed that QRS complex or action potential corresponds to each heartbeat, the cardiac muscle tension must be monitored to understand that an action potential induces a muscle contraction (16). Figure 14 illustrates a relationship between action potential and muscle tension of the frog heart. To monitor muscle tension, the apex was hooked with a fishhook that was connected to a force transducer. Note that the muscle tension gradually increases during the action potential and that the peak of the tension comes considerably later than the peak of the action potential (Fig. 14).

**DISCUSSION**

The techniques described above seem to be reasonable in recording ECG, transmembrane potentials (resting and action potentials), and muscle tension from the frog heart for class use. The illustrated records (Figs. 6–14) are examples of experiments, and subjects can be changed depending on teaching demands. Some points to be noted will be discussed below.

**Equipment.** The most prominent advantage of this method is the simplicity of the experimental setup. To record transmembrane potentials with glass microelectrodes using the intracellular recording method, a considerable number of sophisticated and expensive devices for electrophysiology are indispensable (see introduction). These devices, however, are not necessary when we use a low-resistance polyethylene electrode (Fig. 4) and a spontaneously beating frog heart. Amplifiers (voltage followers) can be constructed with a minimum number of electric parts (Fig. 5). Force transducers can also be homemade, although not as easily as making voltage followers. A force transducer is made up with an instrumentation amplifier, a bridge circuit, and a strain gauge. I placed a strain gauge on a thin razor blade to monitor the small tension produced by a frog cardiac contraction (Fig. 14). Refer to appropriate books or instruction manuals if you wish to construct these devices (7).

**Electrocardiogram.** Understanding the basic principles of an ECG is of great value, especially to medical, comedical, and paramedical students. First, students should learn the naming principles of ECG waves from appropriate textbooks (Figs. 6–8). Moreover, the techniques offer us a good opportunity to observe relationships between the electrode position and the ECG wave pattern (e.g., Fig. 6) and estimate the direction of the excitation spreading through the ventricle. It is known that a wavelike excitation spreads over from cell to cell when the heart is excited, and this wave of excitation consists of a positive region followed by a negative region. The important principles of the QRS complex, which reflects the excitation of cardiac muscle cells, are 1) a negative deflection is recorded when the wave of excitation goes away from the recording electrode (Fig. 15, A and Da) and 2) a positive deflection is recorded when the

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**FIG. 15.** Diagrams showing a principle for interpreting ECG waves. A: negative deflection to be recorded from the ventricle near the atria. B: biphasic (positive and negative) deflection to be obtained at the midventricle. C: positive deflection to be monitored at the apex. D: points a, b, and c indicate the places where the waves shown in A, B, and C are to be recorded, respectively. The arrow shows the direction of the excitation.
excitation wave approaches the electrode (Fig. 15, C and Dc). Thus the trace obtained at the midventricle becomes biphasic, a positive deflection followed by a negative one (Fig. 15, B and Db). The actual recordings can be interpreted on this principle. For example, Fig. 6 suggests that the recording electrode was placed in the middle part of the ventricle for the top trace and that another electrode was positioned near the apex for the bottom trace. Figure 7 suggests that the recording electrode was placed on the ventricle near the atria. Indeed, the electrodes were present at the places as inferred for Figs. 6 and 7. Thus students should be encouraged to use the principles for clinical interpretations of human ECGs, especially chest leads, which more directly reflect the activity of the heart than limb leads.

**Transmembrane potential.** The value of the resting potential using the technique described above (−33.48 ± 6.47 mV, n = 25) is smaller than that obtained by glass microelectrodes from ventricle muscle cells of the frog (between −78 and −88 mV, average −83.8 mV) (14). The reason is that a tight seal or a so-called “gigaseal” (3) cannot be obtained between the network of cardiac muscle cells and the polyethylene electrode used in the present work because of the electrode’s huge orifice. I admit, therefore, that the present techniques are not suitable for accurate research to obtain absolute values of the membrane potential. However, the principles that can be demonstrated are qualitatively accurate and could be used to illustrate many things about heart function. In addition, the simplicity and the convenience of the present techniques give them merit as class experiments.

**Practical applications.** The present techniques can be used as a convenient examination system for checking the effects of altering ionic compositions of solutions and of applying drugs on the cardiac function, because ECG, transmembrane potential (both resting and action potentials), and muscle tension can be easily monitored. In the present report, only a few examples have been shown, i.e., changing extracellular $K^+$, $Na^+$, or $Mg^{2+}$ concentrations (Figs. 10, 12, and 13) and TTX application (Figs. 11). Other possible factors that affect the cardiac function include temperature, pH, extracellular $Ca^{2+}$ concentration, application of epinephrine, caffeine, or other drugs; any one or several of these could be investigated using this technique. The combination of factors to be examined should be decided based on the teaching program being followed.

As for medical, comedical, and paramedical students, the present method offers clues to understand the causes underlying cardiovascular diseases. For instance, progressive hyperkalemia (clinically important state to be treated in which concentration of serum $K^+$ increases) finally causes a cardiac arrest as shown in Fig. 10, and therefore it attracts intensive clinical care. The mechanism of this cardiac disorder has been shown here, i.e., a gradual elevation in $K^+$ induces a depolarizing shift of the resting potential resulting in inactivation of ionic channels or suppression of action potentials (Fig. 10) and of the heart beat (to be observed by eye or by monitoring the muscle tension). Thus the present method seems to be helpful for medical, comedical, and paramedical students to understand the basic principles of ECG, action potentials, and contraction of the heart in humans.

**Concluding remarks.** To my knowledge, this is the simplest, most stable, and least expensive method of recording action potentials from muscle cells for class experiments. The techniques presented here may be revised, and I expect such modified methods to be published for us teachers and students in the future.

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