ELECTROPHYSIOLOGY OF THE HEART

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Electrophysiology of the Heart

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To
Joseph Erlanger
J. A. E. Eyster
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FOREWORD

I consider it quite a privilege and an honor to be asked to write the foreword for this book by Brian Hoffman and Paul Cranefield. My qualifications for this task may properly be questioned, since I have had no personal experience with the exacting technique required for the registration of the transmembrane potentials from single fibers of heart muscle and this book is almost entirely concerned with presentation and discussion of records obtained by this method. Perhaps, however, my long experience in clinical and experimental electrocardiography may entitle me to express an opinion about work like the authors are doing with microelectrodes.

The action potentials recorded by a microelectrode placed within single cells in the muscle anywhere in the heart may be regarded as the basic sources from which the electrocardiogram, recorded by any type of surface lead, arises. The situation in the heart is extremely complicated because so many cells, having action potentials of different duration and form, are involved. Nevertheless, a clear understanding of the different kinds of potentials that exist normally in the SA and AV nodes, in the His bundle and interconnected special conducting pathways, and in ordinary atrial and ventricular muscle fibers, together with accurate information regarding the effects that many factors (changes in rate, temperature, electrolytes, oxygen supply, or presence of physiologic agents like acetylcholine or ephinephrine and drugs like digitalis or quinidine) have on these action potentials is essential before many features of normal and abnormal electrocardiograms can be further clarified.

One of the fields of greatest ignorance in clinical electrocardiography concerns the T waves. Why do some apparently healthy individuals have flat or inverted T waves in leads where these waves are "normally" upright? To put the question in even more fundamental form, why do subjects with normal hearts have upright

T waves in leads I, II, and in the left precordial leads, where the chief deflections of the QRS complexes are also upright? Dr. F. N. Wilson provided a partial answer to this question many years ago by pointing out the existence of the "ventricular gradient." The complete answer, however, must lie in differences in the duration or form of transmembrane action potentials during repolarization, especially in phases 2 and 3, that exist normally in ventricular muscle.

The problem of the T waves is only one of many puzzles that may eventually be solved by the systematic use of techniques for registration of action potentials from single fibers in the heart. Much light has already been thrown on the reasons why cells in the SA node and occasionally elsewhere serve as pacemakers, and information pertaining to AV conduction and excitability of various types of heart muscle fibers which may prove to be the key to the genesis of many of the cardiac arrhythmias is rapidly being collected. Need more be said to emphasize the potential value and importance of the matters discussed in this book?

In Chapter 1 the reader will find a discussion of the technique for obtaining action potentials from single cardiac fibers, a survey of the different types of records to be found in various kinds of fibers, and some discussion of the relationships between these records and those obtained by external leads. Chapter 2 is devoted to the electrical properties of excitable cells and a summary of the facts known regarding the ionic basis for the resting membrane potential and the action potential. Chapters 3, 4, 5, 6, and 7 are concerned with detailed discussions of the action potentials obtained from muscle cells in the atrium, ventricle, sinoatrial node, atrioventricular node, and Purkinje fibers, respectively. These chapters not only point out the variations in the records found normally in different species but summarize available data concerning the effects of many factors such as variation in rate or temperature, changes in sodium or potassium content of external medium, or presence of acetylcholine or epinephrine on the action potentials obtained from the various cells. Although much of the material presented (some of it previously unpublished) was obtained by the authors or by others in their laboratories, a great deal of work done by others is presented and discussed. When differences in results or interpretations have appeared, these discrepancies or divergent opinions have been

clearly stated and the need for further and perhaps better studies to settle the questions involved has been pointed out. I was greatly pleased to see that the authors have not failed to appreciate that some of the conclusions arising from recent work by themselves and others had been predicted from much earlier studies by that great physiologist Joseph Erlanger (see Chap. 6).

Chapter 8 is devoted to a discussion of the excitability of heart muscle cells using both cathodal and anodal stimuli. The results of these studies are very interesting, particularly those obtained by anodal stimuli, since they appear to have direct bearing on the "supernormal phase" of conduction occasionally seen in humans in high-grade AV heart block and in other arrhythmias. Much of Chapter 9 is concerned with summary and electrophysiologic interpretation of material presented earlier in the book. This procedure is excellent and helps greatly to emphasize and fix in the reader's mind many of the important matters presented previously. This chapter concludes with a discussion of a possible ionic mechanism for repolarization. I am not in a position to say more than that this proposal seems to be a logical and reasonable one.

This book summarizes the important information available and adds much new material in this very interesting and rapidly developing field. It should be of great value to physiologists or physicians seriously interested in electrophysiology.

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PREFACE

The electrocardiographic record of the electrical activity of the whole heart depends upon the shape of the action potentials of the various cells of the heart and upon the sequence of activation of those cells. The development of the method of microelectrode recording has made possible a new degree of precision in our knowledge of the action potentials of single cardiac cells. The technique of single-cell recording is only ten years old, but a great deal of work has been done on the heart in that time. It is our hope that the presentation of the results of that work, which we have undertaken to give in this book, will be of use not only to physiologists but also to those interested in electrocardiography. We have also touched very briefly upon results obtained by new methods of studying the sequence of activation of the heart. It is not too much to hope that in the next five or ten years new studies of the sequence of activation will be combined with the results of single-cell studies to provide electrocardiography with a more systematic foundation in experimental electrophysiology than it has previously had.

We are grateful to all our friends and colleagues who have helped and encouraged us in this work. Our indebtedness to the men to whom this book is dedicated is twofold. We have found their published papers valuable and stimulating, and one of us (P. F. C.) studied for some years with Dr. Eyster and Dr. Meek. We have had many valuable discussions with Dr. Silvio Weidmann of Bern and with Dr. Antonio Paes de Carvalho of Rio de Janeiro, each of whom has also been most generous about sending us unpublished material. We have also benefited greatly from our association with Dr. Kojiro Matsuda, Dr. Walmor Carlos de Mello, Dr. Morris Kleinfeld, Dr. Jackson Stuckey, and Dr. John J. Kelly, all of whom either have helped us in our research or have assisted us by discussion and by supplying unpublished information.

We both owe our interest in cardiac excitability to the studies of that subject which were initiated in the Department of Physiology of the State University of New York, Downstate Medical Center by Drs. Oscar Orias and C. McC. Brooks. We are further indebted to Dr. Brooks for his general encouragement of our studies of cardiac electrophysiology.

Much previously unpublished research from our laboratory is reported in this book. That research was supported by grants from The American Heart Association, The New York Heart Association, and The National Heart Institute of the United States Public Health Service (U.S.P.H. Grant H-3916). The manuscript was completed and submitted to the publisher on July 15, 1959.

Brian F. Hoffman

Paul F. Cranefield

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CONTENTS

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RECORDING TECHNIQUES

Three quite different techniques are used to record electrical activity directly from cardiac muscle: One employs conventional unipolar or bipolar surface electrodes in close proximity to uninjured tissue; the second employs one lead in contact with injured muscle and another which may be either close to or distant from the site of injury; in the third and most recent technique one intracellular microelectrode is paired with an extracellular lead to record the transmembrane potential of a single cardiac fiber. Although we have attempted to treat most of the subject matter of this book in terms of results obtained by means of the intracellular microelectrode, certain important aspects of cardiac electrophysiology have as yet been studied only by other methods. This chapter describes those methods and attempts to show which of them is appropriate in the study of certain problems.

TRANSMEMBRANE POTENTIALS OF SINGLE CARDIAC FIBERS

Recording the transmembrane potential of single fibers by means of an intracellular microelectrode provides an accurate and sensitive index of the electrical changes associated with activity of excitable tissues. One limitation of this method is that only local changes in the single cell or part of a cell can be detected with a single electrode. Moreover, the delicate glass microelectrode is easily broken or dislodged from the cell under study. Many special precautions are necessary in terms of the properties of amplifiers and recording equipment in order to avoid artifactual distortion of the records.

However, this technique permits the most accurate determination of the magnitude and time course of the transmembrane action potential and also succeeds, where other methods fail, in demonstrating the peculiarities of the excitatory process in the sinoatrial and atrioventricular node. An understanding of microelectrode methods is thus essential for any comprehension of modern cardiac electrophysiology.

Microelectrode Methods

It is possible to record the electrical activity of a single cell or of a single unit of the cardiac syncytium by using a microelectrode of the type introduced by Ling and Gerard (1949). Such a microelectrode is a fine glass capillary pulled to a tip diameter of less than 1 μ and filled with a concentrated electrolyte. The electrolyte acts as a conductor which is insulated, except at the open tip, by the surrounding glass capillary. The outside diameter of the microelectrode tip is critical. Ling and Gerard showed that, in order to record the transmembrane potential of single fibers of frog sartorius muscle, the tip of the electrode must be 1 μ or less in diameter; larger electrodes damaged the fiber membrane and gave erroneously low values. Somewhat similar estimates of size were obtained by Woodbury, Hecht, and Christopherson (1951) for frog heart and by Nastuk and Hodgkin (1950) for frog sartorius. In general these estimates of the critical size can be accepted. To record potentials from certain very small cells such as the fibers of the sinoatrial node and parts of the atrioventricular node or from small nerve fibers and cell bodies it is necessary to employ electrodes that are considerably less than $\frac{1}{2}$ μ in diameter. For studies of large cells such as the giant axon of squid it is likely that a somewhat larger electrode tip is permissible.

A number of different substances have been employed to fill microelectrodes. Concentrated potassium chloride is used most frequently, although other electrolytes and even metals are sometimes substituted (see Shanes, 1958). Theoretical considerations of the generation of junction potentials at the electrode tip, as well as the results of actual experimentation (Nastuk and Hodgkin, 1950; Adrian, 1956; Shanes, 1958) have led most workers to use a 3-molar solution of KCl. Filling is most easily accomplished by boiling the electrodes, held on some suitable mount, until all the

air is replaced by the KCl; other techniques involve filling in a vacuum, filling first with alcohol and then KCl, and filling by capillarity. In our hands the first of these methods has proved simple and reliable for work in which the microelectrode is employed to record the transmembrane potential. When the electrode is used for other purposes, the composition of the electrolyte used for filling will differ. Thus, for studies of the pharmacology of the postsynaptic membrane of the myoneural junction, electrodes may be filled with acetylcholine, curare, or other substances. If a current of the appropriate polarity is passed through the electrode, calculable quantities of the electrolyte can be ejected from the tip (Nastuk, 1953). This technique can be used when the electrode is either extracellular or intracellular in position; in the latter case, however, the nature of the ionic species which carry current across the cell membrane is unknown, and this factor must be considered when the purpose of the injection is an alteration of the ionic composition of the intracellular fluid.

The electrode is commonly mounted directly on a micromanipulator, and the electrode tip is introduced into the cell during direct observation through a microscope. If the tissue under study moves vigorously, this method may fail, in which case it is possible to suspend a short microelectrode from a very fine, flexible wire (Woodbury and Brady, 1956); the electrode tip is lowered against the surface and allowed to penetrate under the influence of the tissue movement. Although this latter method has many disadvantages, it does permit records to be obtained from moving tissue for long periods of time.

Use of the intracellular microelectrode to record the transmembrane potential of a single fiber requires special input circuits, amplifiers, and display apparatus. These have recently been reviewed (Grundfest, 1957) and will be mentioned only in summary. Because of the high electrical resistance of the microelectrode (5 to 50 megohms) and the requirement for minimal grid current, a cathode-follower input is employed. Also, since the large electrode resistance and relatively high capacity tend to give a poor response to high-frequency components of the signal, it is desirable to employ some form of input-capacity neutralization (Amatniek, 1958). Direct-coupled amplifiers of extremely low drift are necessary if measurements of the resting potential over a prolonged period of

time are to be meaningful. Finally, the recording device should have a frequency response capable of following transients up to 30 kc; standard electrocardiograph machines are thus of little value in giving a faithful record of either the rising phase or amplitude of the transmembrane action potential.

The Contour of the Cardiac Action Potential

The Resting Potential. If a microelectrode is advanced slowly through several layers of cardiac cells, sudden sharp shifts of potential are recorded. During these shifts the microelectrode records either no difference of potential or becomes 80 to 90 mv negative with respect to the external reference electrode. These potential changes are thought to represent the appearance and disappearance of the transmembrane resting potential as the electrode enters and leaves individual cells. In general, it is felt that the shifts in potential do in fact represent the resting potential of the individual cells for the following reasons:

1. The potential appears abruptly when the electrode is advanced a very small distance, remains constant presumably while the electrode is advanced farther within the cell, and then disappears abruptly during a further small displacement, presumably as the electrode leaves the cell.

2. If one electrode is inserted in the cell and another one is inserted in the same cell as close as possible to the first, no change in the potential recorded by the first electrode is seen (Draper and Weidmann, 1951); this observation shows that the insertion of an electrode does not in itself alter the resting potential to a measurable extent.

3. The transmembrane potential recorded by an intracellular electrode is altered by all the various agents which are known to alter the demarcation potential (Hodgkin, 1951).

4. The transmembrane resting potential recorded with an intracellular electrode exceeds the demarcation potential by a considerable amount. This would be expected from the shunting of the demarcation current through the extracellular fluid.

5. When the transmembrane potential recorded with an intracellular electrode is excessively low, it is often noted that the electrode is broken and that its tip is large enough to have damaged the cell membrane.

6. All of the observations on resting potential made with small electrodes which puncture the cell membrane are comparable to those made on the giant axon of the squid with an electrode inserted directly into the axoplasm through the cut end of the fiber (Hodgkin, 1951).

7. The number of cells judged from the number of resting potentials recorded during the penetration of several layers of muscle accords reasonably well with the number of cells determined by histological examination (Creese et al., 1958).

The resting potentials recorded from the heart muscle of various species and from different functional types of cardiac cells range from 50 to 95 mv (Cranefield and Hoffman, 1958*a*). The exact quantitative meaning of the values for the resting potential obtained in this manner has been subject to study and criticism (Adrian, 1956; Shanes, 1958); it is generally agreed that if the electrode is sufficiently small and is filled with 3-molar KCl, the observed value of the potential may be in error by not more than a few millivolts.

The Action Potential. If a microelectrode is inserted into a cardiac cell and the cell is excited, the transmembrane action potential is recorded. The shape and amplitude of the action potential vary considerably from one species to another and from fiber type to fiber type. In general, the action potentials recorded from all cardiac fibers resemble those of other excitable cells in showing an initial rapid depolarization or upstroke. They differ, however, from the action potentials of most other excitable cells in showing a prolonged depolarization and a slow, delayed repolarization. Schematic action potentials of three types are shown in Fig. 1-1. The action potential in Fig. 1-1*A* is typical of many types of ventricular cells: The initial rapid upstroke is labeled phase 0, a phase of early rapid repolarization is labeled phase 1, a prolonged phase of slow repolarization (often called the plateau) is labeled phase 2, the terminal phase of rapid repolarization is labeled phase 3, and the diastolic period is labeled phase 4. An action potential from a spontaneously rhythmic pacemaker fiber is shown in Fig. 1-1*B*. This action potential is characterized by the presence of a slow depolarization during phase 4 which eventually reaches threshold; this results in excitation and in the initiation of the upstroke of the locally arising action potential. In such fibers the upstroke velocity seen in phase 0 is usually somewhat low. The action potential shown

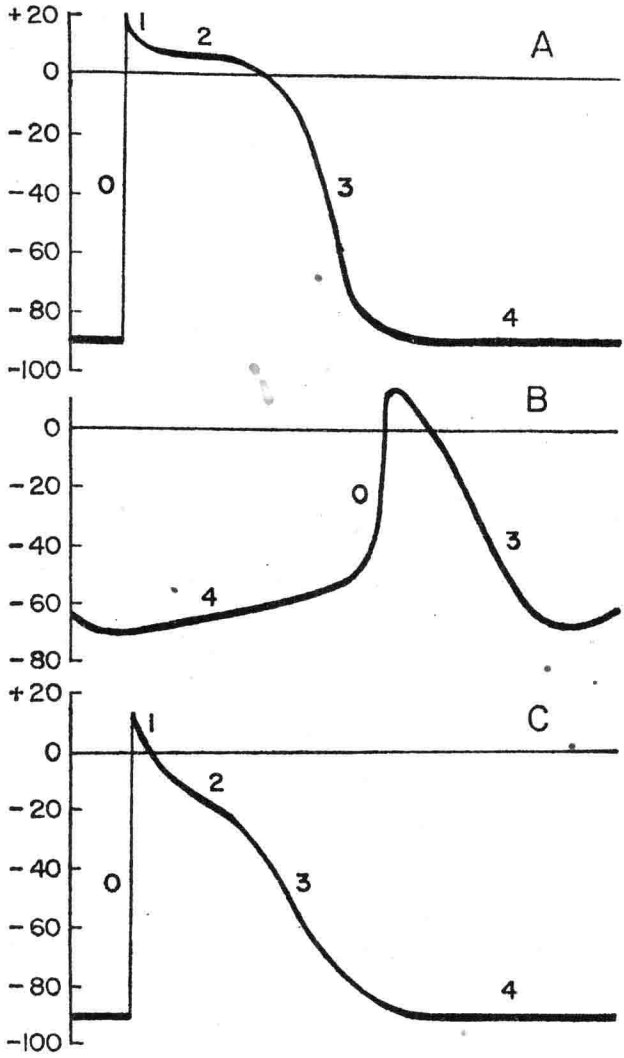

FIG. 1-1. Schematic records of transmembrane action potentials recorded from ventricle (*A*), sinoatrial node (*B*), and atrium (*C*). Sweep velocity in *B* one-half that in *A* and *C*. Ordinate scale in mv. See text for discussion.