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ELECTROPHYSIOLOGY OF THE HEART

This book contains the Proceedings of an International Symposium on the Electrophysiology of the Heart, held in Milan on October 11–13, 1963, under the sponsorship of the "Istituto di Cardiologia Sperimentale".

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FOREWORD

THE first International Conference on the Electrophysiology of the Heart, sponsored by the New York Academy of Sciences, was held in February 1956, in New York City, with Prof. Hans H. Hecht in the President's chair. The authoritative reputation of the participants, in conjunction with the number, originality and importance of the new data and novel ideas presented, made this meeting a memorable event in the scientific world.

The Acts of the Conference were for years a valuable source of basic information for physiologists and cardiologists desirous of widening their knowledge or undertaking new research in cardiac electrophysiology. The volume contained the latest data on the electrical activity of the single myocardial fibres, on the spread of excitation through the heart and on recovery of the resting condition. The most advanced theories on the distribution of cardiac potentials in volume conductors, as well as the principal methods of electrocardiogram and vectorcardiogram analysis were exhaustively discussed.

In the years that have elapsed since the New York Conference, most of the chapters making up the Electrophysiology of the Heart have undergone a radical change, and it became evident that many of the conceptions set forth in the Acts of the first Symposium needed to be re-assessed and rediscussed in the light of more advanced knowledge.

Preliminary contacts between the scientific staff of the Milan Institute of Experimental Cardiology and distinguished researchers in several countries definitely confirmed that a new meeting would fill a widely recognized need.

The scientific programme of the proposed convention was thoroughly discussed in numerous conversations, the most frequent being with the President elect, Prof. Pierre Rijlant of Brussels, and Prof. Silvio Weidmann of Berne, who for a whole year during the preparation of the Conference generously offered us their invaluable advice. Other important exchanges of ideas took place with all the future participants at the Symposium and with members of the Italian academic world.

In choosing the subjects of discussion, pride of place was given to those fields of research where concepts had evolved most rapidly over the last few years: the physiology and morphology of the cell membrane, the intracellular tubular systems, new theories on the intracellular pathway of action currents, ion fluxes in the various phases of the heart cycle; the coupling of excitation and contraction; the non-syncytial structure of the myocardium and the transmission of excitation through the intercalated discs; the anatomy of the conduction system and the spread of excitation through the myocardium; the

correlation between intracardiac electrical events and extracardiac distribution of currents and potentials; analysis of the electrical field on the body surface at the different moments of the heart cycle; the biophysical bases of modern quantitative vectorcardiography and the use of electronic computers to analyse electrophysiological tracings.

The interest aroused by the meeting among physicians and researchers both in Italy and other countries was clearly evidenced by the throng that attended sessions in the Aula Magna of the Milan University and in the Conference Hall at the Institute during the Symposium, and by the lively discussions that ensued. Of these, however, only the interventions submitted by the authors before the Conference are printed in this volume.

The Organizers of the Symposium will always remember it as one of the most significant and outstanding recollections of their scientific careers; and wish once more to tender their sincere thanks to all those who, with their assistance, their advice and their personal attendance, deserve most of the credit for the success of the meeting.

B. TACCARDI
G. MARCHETTI

OPENING ADDRESS

G. E. GHIRARDI

President of the Istituto di Cardiologia Sperimentale

As president of the Institute of Experimental Cardiology I have the honour, and pleasure, of extending a welcome to you, distinguished scientists in electrophysiology, who have so kindly accepted our invitation to attend this Symposium.

My goal in founding the Institute of Experimental Cardiology was to promote basic and applied research in cardiovascular physiopathology, with the dual scope of pursuing an extensive programme of experimental research and of fostering scientific interchange among researchers all over the world. This part of the programme is implemented by the organization of international meetings at a high scientific level, of which this Symposium is undoubtedly a most outstanding example.

Scientific research has today become of such enormous importance in the cultural and economic development of the modern community; and the demands of research are so manifold, that it behoves all enlightened minds to make a factual contribution, whether they belong to public institutions or work for private enterprise.

It is in this context that emerges the full significance of the hearty thanks I wish to tender to all those who have contributed to the success of this meeting, in particular:

—Professor Pierre Rijlant, Director of the Solvay Institute of Physiology of the Brussels University, who accepted to act as President of the Symposium, and has on many occasions offered us his invaluable experience and the benefit of his authority;

—Professor C. M. Cattabeni, Rector Magnificus of the Milan University, who gave our initiative his greatly appreciated support and most generously allowed us to hold this solemn inaugural ceremony in the Aula Magna of the University;

—the many members of the Academic Body who have given their support to our Meeting;

—the distinguished lecturers and all the participants, who have come to Milan from Belgium, Czechoslovakia, France, Germany, United Kingdom, Italy, New Zealand, Netherlands, United States, Sweden and Switzerland.

It is, I feel, appropriate to stress the approach adopted in designing the

scientific programme of the Symposium, for it testifies to a definite directive in research policy.

Applied research, which in medicine aims at defining increasingly accurate methods of diagnosis and ever more efficacious therapy, will have no chance of lasting success unless it is accompanied and aided by such basic research, as is performed in laboratories of anatomy, physiology, biochemistry, biophysics and even mathematics. Here it is that our knowledge of cell structure and function, of the organs, of the various apparatus and systems, of whole organisms, is extended and refined. Without basic research we should never have learned about the existence of pathogenic bacteria, of viruses, of penicillin, of chemotherapeutic agents, of X-rays, of vitamins and hormones; and —more relevantly to our meeting—we would not have the electrocardiogram, that invaluable tool for diagnosis, whose theoretical and practical development has in recent years been such a decisive aid to the advancement of clinical cardiology.

This is why, in designing the scientific programme of the meeting, the Organizing Committee has given pride of place to the basic sciences.

The scientific papers, all of which bring new, original and often decisive data to the solution of controversial issues, deal with the macro- and microscopic anatomy of the heart, with the physiology of the cell membrane and the relationships between structure and function in each individual cell; then with the organization of activity at the level of more complex structures and lastly with the extracardiac manifestations of the heart's electrical activity.

New challenges now await the Institute of Experimental Cardiology, which will pursue the same directives that have informed its activities over the past years. I have a very definite idea of the programme to be followed which will, I hope, further new progress in the most advanced research techniques that will, in the future, offer a powerful, practical aid to cardiovascular diagnosis.

I trust this hope will prove an incentive to the Symposium, to your future research and to the activities of the Institute that is honoured to welcome you.

CONTENTS

ELECTRON MICROSCOPY OF THE HEART

(WITH SPECIAL REFERENCE TO STRUCTURES INVOLVED IN REGULATION OF FREQUENCY, FORMATION AND CONDUCTION OF EXCITATION, TRIGGERING OF CONTRACTION AND RELAXATION)

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ABOUT ten years ago, electron microscopists began to describe morphologic features of the heart muscle which were of special interest to the physiologists.[1-11] Review publications[12, 13] and international symposia[14-16] have made the results of their investigations so commonly known that it is impossible to give an introduction to the micromorphology of the heart without repeating known facts. I will try, therefore, to reduce repetitions by emphasizing lesser known findings.

The special interest of the present symposium is directed toward cardiac electrophysiology. With admitted simplifications one may say[17] that cell potentials and their alterations are bound to membranes, that the shortening of the muscle is due to the myofilaments, and the aerobic metabolism to the mitochondria. Furthermore, it is known that glycolysis takes place in the ground-plasm of the cell, and that different nucleotid-phosphatases are distributed among filaments and membranes.

Tissue culture, development of the heart and the aneural hearts of cyclostoma have all provided evidence that the activation of the heart muscle is independent of a nervous impulse. Activity normally starts by spontaneous depolarization of muscular tissue in the pacemaker area. In hearts supplied with nerves the frequency of impulses may, however, be decreased or increased by specific substances released from autonomic fibres. Beginning with the neural influence and probably not ending before relaxation in the muscle cells, electrophysiological phenomena are coupled with the activity of the heart.

It is my task, therefore to demonstrate and discuss those structures thought to be related to frequency modulation, to the formation and conduction of excitation, to the triggering of contraction and to relaxation. All these structures which conduct and influence something expand over characteristic distances from macroscopic to submicroscopic size. Between the conducting membranous parts are discontinuities, where excitation jumps from one membrane to a similar membrane or to a different one.

As far as nerves are concerned, the discontinuities are synapses. Between the cardiac muscle cells they are intercalated discs, desmosomes, or maybe just adjoining cell membranes. Inside the muscle cells they are probably the so-called triads of the transversal or *T*-system of the sarcoplasmic reticulum. All these structures can easily be demonstrated in the plane of sectioning, but their precise three-dimensional arrangements are insufficiently known.

With the light microscope, myelinated and unmyelinated nerve fibers, as well as dentrites and telodendria between nerve cells, may be followed in thick specimens for more than 100 micra or more. In the monograph of Stöhr jr.[18] many illustrations show such dimensions of fibers within the ganglia of the heart. Furthermore one recognizes several types of nerve cells, their cytoplasmic processes, satellite cells and in some places what appear to be synaptic junctions. The muscle tissue itself is interwoven with nerve fibers of different morphology for it contains not only efferent but also afferent fibers (see H. H. Jansen[13]).

In the electron microscope one visualizes instead of thick layers extremely thin layers so that the course of nerve fibers or other structures can usually be followed only over a few micra or less. The thinner the structures are and the more complicated their arrangements, the shorter the distances they can be followed in a single section. This fact explains the difficulty one encounters in correctly representing three-dimensional pictures of synaptic areas or of the sarcoplasmic reticulum as will be seen later.

Our experiences, which I demonstrate in part with electron micrographs, were gained from sinus venosus specimens of the frog, turtle and rat, from false tendons of the sheep heart, and from the working muscle of the frog and mouse. Most of the specimens were prepared with Mrs. C. Ruska and Dr. B. Rybak in the Institute of Zoophysiology of the University in Caen. Micrographs of Purkinje fibers I received from Dr. R. Caesar, Institute of Pathology in Tübingen.

Near the sinus venosus of the frog heart beneath the epicardium, nerve fibers and small ganglia can be found (Fig. 1). In the lower half of Fig. 1 is a nerve cell with its large nucleus, and on its left side is the nucleus of an adjacent satellite cell. One nucleus (center right) belongs to a Schwann cell of a polyaxonal unmyelinated fiber. Between the above structures are three nuclei of connective tissue cells. The number of myelinated fibers is less than the number of unmyelinated ones.

On many unmyelinated fibers (Fig. 2) it is easy to demonstrate how the axons of nerve cells are enveloped by the Schwann cells, and how mesaxons are formed by the Schwann cell membrane. In Fig. 2 each axon has its own indentation. To pass in the shortest way from one axon to its neighboring axon, four membranes have to be traversed: the axonal membrane, the Schwann cell membrane twice, and again an axonal membrane. Sometimes, several axons are observed to run in a common channel of the Schwann cell. In such

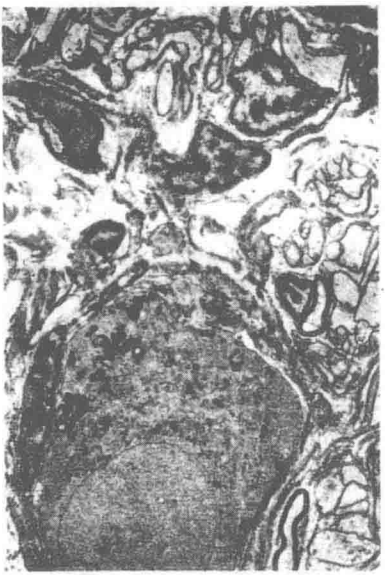

FIG. 1. Nerve cell and nerve fibers from the wall of the sinus venosus of the frog. (× 2000)

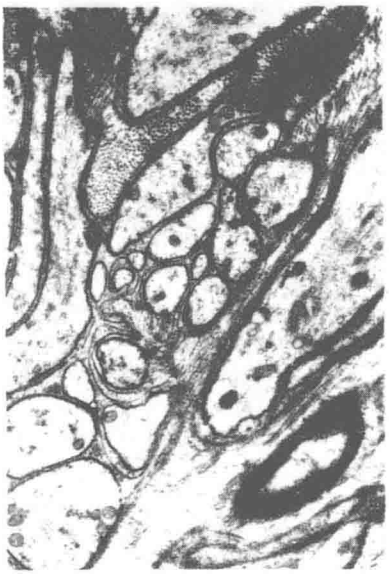

FIG. 2. Longitudinal and cross-sections of axons in unmyelinated fibers. Numerous mesaxons in the central part. Tangential section of a myelin sheath at the lower right corner. (× 10,000)

instances the membranes of several axons contact each other and only the two axon membranes restrict diffusion from the axoplasm of one into the other. In myelinated fibers as is well known a great number of duplications of the Schwann cell membrane forms the myelin sheath.

In the axons of all myelinated fibers (probably vagus in origin) one recognizes neurofilaments and a few small mitochondria (Fig. 3). However, in the

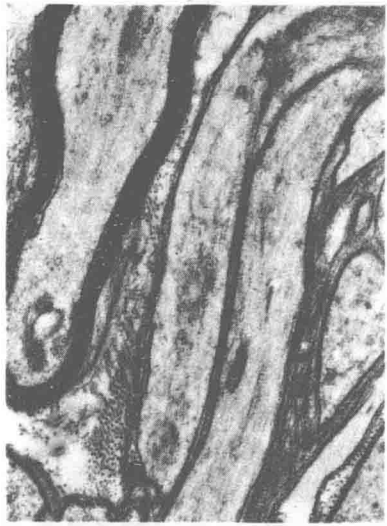

FIG. 3. Longitudinal section of different nerve fibers, containing predominantly filamentous or vesicular structures in their axoplasm. (× 10,000)

unmyelinated fibers the axonal contents are different and vary. They may contain predominantly filaments as in the axon on the right, thin-walled vesicles as the axon in the middle of Fig. 3, or one may find small granulations as in the axon of the lower right side of Fig. 4. These differences may be pursued over several parallel sections, but the origin and termination of the different axons cannot be accurately determined. Some end in synaptic junctions in contact with the dendrites or soma of intramural ganglion cells. One can, however, say if the structures in a limited axon-like area looks essentially like the structures of nerve cell protoplasm, for such areas represent sections through dendrites (Bodian[19]).

For several reasons, it was necessary to repeat the morphology of nervous fibers. In the muscle tissue of the heart the velocities of conduction are relatively high or low, but obviously dependent upon quite different structural principles. Membranes which cross the direction of conduction—the properties of which have to be considered for the conduction in muscle tissue

—are missing in nerve fibers. Nevertheless, the velocity of conduction along axons is not in all cases higher than in specific muscle tissues.

For example, the myelinated, preganglional visceral efferent, *B*-fiber conduction velocities are several meters per second. The unmyelinated postganglionic *C*-fiber velocities are less than 1 m/sec. On the other hand, the conduction

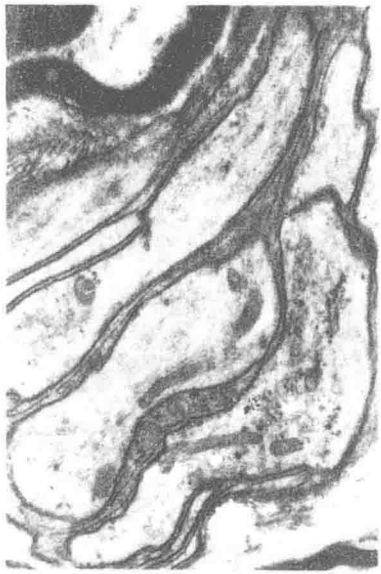

FIG. 4. Axons containing predominantly either filamentous or vesicular or granular axoplasm. (× 10,000)

velocity in the atrioventricular bundle is 3 to 5 m/sec in spite of the transverse cell membranes. In the nervous fibers, not only transverse walls but also special contacts with neighboring fibers are missing. Such missing elements of the axon are believed to play an important role in the high conduction velocity along chains of muscle cells. Eventually, one should think in this connection of the Ranvier rings of myelinated fibers. If we could but see the condition of excitation rush through our electron micrographs, the process would take only a fraction of a microsecond to pass one micron.

The axons can be followed to their contacts with nerve cells, and several synapses with different structural details can be found (Fig. 5). The presynaptic axons contain vesicles as do the cholinergic synapses of skeletal muscle, and they may contain granular structures as well. (For more details on the morphology of synapses, the reader is referred to the work of Whittaker and Gray[20]). Besides such obvious synaptic contacts, one finds between the nerve cells and their satellites very thin axonal fibrils which may be either presynaptic axon terminals or parts of dendritic arborizations of other nerve

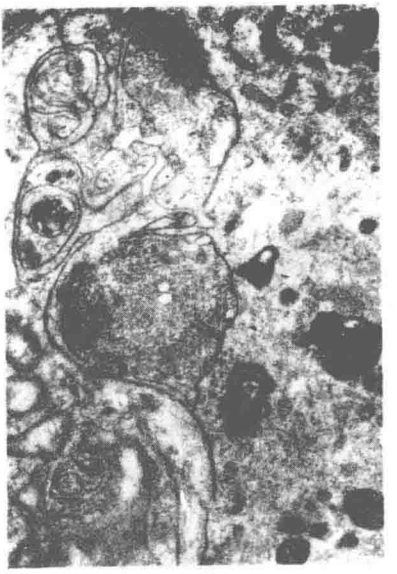

FIG. 5. Synaptic axons and foldings of satellite cells on the surface of a nerve cell of the sinus venosus of the frog. (× 10,000)

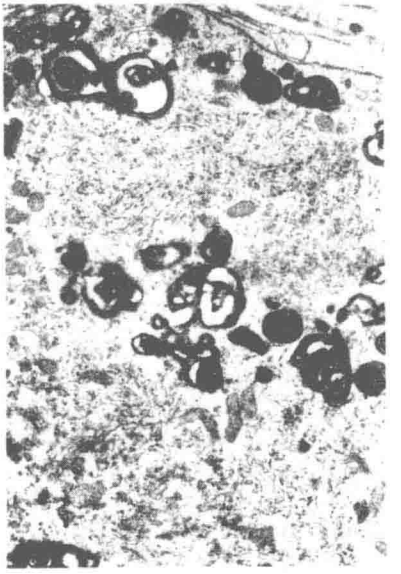

FIG. 6. Nerve cell cytoplasm. Cell border on the upper edge. Large polymorph lipofuscin granules between ergastoplasm (Nissl substance). Golgi field at the lower left. (× 10,000)