Molecular Biology

ELEMENTARY PROCESSES OF NERVE CONDUCTION AND MUSCLE CONTRACTION

Edited by DAVID NACHMANSOHN

A symposium sponsored by Columbia University and The Rockefeller Institute; held at The Rockefeller Institute from September 25th to 30th, 1958

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Introductory Remarks

DETLEV W. BRONK

President of The Rockefeller Institute

It is my pleasant privilege to extend the welcome of The Rockefeller Institute and the National Science Foundation to so many of our friends and colleagues from so many other countries, as well as other institutions. Dr. Nachmansohn will, I am sure, wish to extend similar greetings from Columbia University.

We are all of us in this country grateful to you for having come so far to share with us your friendship and your knowledge. If we can make your visit intellectually profitable and socially pleasant, it will be a very small symbol of our great gratitude for your hospitality and friendship of other years in other places, for the inspiration of ideals that we hold in common, for the benefit of shared knowledge. This company in a very personal way evokes for me happy memories spread over three happy decades. They began just thirty years ago this year in the laboratories and in the company of Adrian and Hill. And for me it is a very special and a moving privilege to have them here in this institution which is beginning a new era. Through them I came to know Sherrington, Lapicque and many others who are known to you and who have meant so much to us in the past development of our science. To them I am also deeply indebted for the opportunity they provided to begin the building of a scientific life which has included the friendship and association with most of you who are here assembled. I speak of this not as a prelude to the history of our subject which you and they and their predecessors have built. Those achievements will be implicit in the communications which follow. Our motives, our unity of effort and the significance of our achievements are, however, worthy of further interpretation which I would like to take just a few minutes to develop even though it may seem to have no place in a discussion which is to be informal and focused on a very special field of science. But I think that a gathering of friends such as this has a special significance in these times of trial, tribulation, and potential triumph of the human mind, which is, however, greatly threatened. I think if there was ever a time in the history of the modern world when there is need for friendship, it is now, and I think that such a gathering as this illustrates in a very meaningful way the capacity of men to live together in sincerity and to work for a better way of life which includes the creative efforts rather than the destruction of so much that is good that has come before. And so, as I say, I know that you will forgive me if I try to give a broader interpretation than the mere scientific development of our ideas, if I speak of what seems to me to be so meaningful a thing as the gathering together of those of us who have come together here and who have spent so much time in the past working and playing together without regard for national boundaries or limited ideologies. I wish I had the power to put in adequately expressive words the especial reasons why the friendly associations of international gatherings such as this give to those of us who are Americans so much pleasure. It is in part, I think, because we are so recently come from your native lands. Our nation has been made by those who are brothers of your fathers. Your ancestors were our ancestors too. Our feeling of friendly kinship is due in part, I further think, to our common cultural heritage. The first visit of an American to your several countries is a remarkable experience. Before it one has the bewildering feeling, if I can interpret your feelings in terms of mine, the bewildering feeling that one has seen it all before. And so one has, through the eyes and words of those who wrote the literature of your country which was the literature of our fathers on which we, too, were reared. Another deeply rooted reason for our feeling of close association with you is the recent growth of science in this country from the seeds brought by our teachers who first studied in your lands, as we who had the benefit of working with you in your laboratories are trying to plant the seeds in the minds and spirit of our students and younger colleagues. It is significant to remember at a time when diplomats are not always motivated by the friendly spirit of true scientists and at a time when science is increasingly being used as an instrument of political propaganda and conflict between nations that Benjamin Franklin, our first great diplomat was encouraged by election to the Royal Society of London, the Royal Academy of Sciences at Goettingen, academies of science at Paris, Padua, and St. Petersburg. From those days of Franklin until now we have a deep debt of gratitude to you for first nurturing those who then developed science here. And so I say our warm welcome has natural roots and traditions and brotherly associations which we hope you of our mother countries may understand. And so I say again that in these times when some of us cannot escape the daily consciousness of impending catastrophe and, yes, disaster because there are not enough who are willing to associate in friendship as we are that we may take just a moment as we begin these days of friendly meeting to recall that we can do more than further science and apply science as an instrument to a better way of life. We can help to make a more peaceful way of life which will lead to a better way of life. We will represent to many others what we have found it possible to do. In these gatherings then, today, and in the days to come I hope that we may demonstrate the spirit of friendship which is not always present even within our several countries, friendship based on frankness and willingness to discuss our ideas and divergence of opinion. And having said that I go on to a more practical consideration and that is the remark of George Wald just made to me a moment ago in which he said that the real reason for coming together is to have such a body as this meeting together for some days and for frank discussion. He went so far as to say that he hoped that I would encourage you to interrupt the speakers, let alone discuss their papers at the end, if there were points which you do not understand as they proceed. I know that you will do this in orderly fashion and I think that George had an important point in beginning at the beginning to develop the spirit of discussion which is the purpose of this small gathering of those of us who have worked in the various fields in the furtherance of our common field. But it would be better for me to let the aims of the Symposium be described by David Nachmansohn who has been the initiator of this conference, its constant stimulus and to whom we are all indebted.

The Aims of the Symposium

DAVID NACHMANSOHN

The eloquent and moving words of welcome by President Bronk have admirably expressed the spirit in which this Symposium was organized and the deep feelings of friendship prevailing at present among the scientists of the Western world, transcending all boundaries. Our ardent hope is, in spite of situations and events that are sometimes frightening, that the scientific community will eventually encompass the whole civilized world.

A few remarks appear appropriate about the aim of this Symposium, the ideas which formed the basis of its organization. The startling developments which have taken place in physics since the turn of this century have had a strong impact on science in general. First, they have deeply affected all scientific thinking and philosophy. Classical physics based on Newton's mechanics led scientists to believe that their concepts were a direct result of observation and experimental data and that the laws proposed were necessarily derived from their findings. Certain concepts and notions were considered as undisputable and final truths; any change was opposed with a rather rigid frame of mind. One of the results of the achievements of modern physics has been the realization, emphasized particularly by Einstein, but also by many others, that the physical scientist arrives at his theory only indirectly by speculative means. Without going into the ever more complex philosophical problems raised by the quantum theory, especially the Heisenberg principle of indeterminacy, it is apparent that we are faced in modern physics with the existence of different closed and coherent sets of systems, concepts with a limited applicability which sometimes result in paradoxical answers to a special question. The analysis and the understanding of experimental data and of new phenomena frequently cannot be achieved by the use of known concepts and established laws, but require entirely new notions and theories. The limited range of applicability of every concept is one of the most important lessons learned.

If facts and experimental data alone are inadequate for formulating a theory or a concept, if our knowledge is based not purely on observation and experiment but depends largely on the underlying theory, it follows that any concept is of necessity bound to be subjected to profound modifications and corrections. The appearance of new evidence incompatible with previous assumptions and theories, no matter how well established they may have appeared to be for long periods of time, makes necessary a readjustment of existing notions. Scientific attitude toward pronouncements of theories and concepts can, therefore, not accept the claim of certainty but only of probability on the basis of present evidence.

Another outcome of the progress of physics and chemistry is the availability of extremely powerful tools for biological sciences. These new tools have made possible extraordinary and fascinating progress in our understanding of mechanisms of living cells in terms of physics and chemistry far beyond anything which was possible to foresee or to imagine at the beginning of this century. In some fields the advances have reached molecular levels. The question raised by many philosophers and scientists in the past, namely whether we are able to explain events in a living cell taking place at a given time and in a given space in terms of physics and chemistry, was discussed by Schroedinger in his essays: "What is Life?". Schroedinger admits that we have not yet reached the stage where we can explain life phenomena on the molecular level, but he considers the progress made as an indication that it will eventually become possible. Although the assumption of "vital" forces still discussed some thirty years ago has been generally abandoned today, the optimistic attitude of Schroedinger is not shared by all. There are certain aspects of living cells for which many scientists would be reluctant to accept the view that physics and chemistry will be able to provide the final answer. This applies for instance to certain functions of the brain such as the working of the human mind: the problems of thinking, of psychology, of emotions, of ethics, and others. These phenomena belong in a different category in the definition of Kant.

But many aspects of living cells are open to physicochemical analysis. However, the extraordinary complexity of living cells presents formidable obstacles. The last few decades have seen great advances in the investigations of structure and ultrastructure, of physical manifestations and chemical processes underlying cellular function. But perhaps due to the rapidity of the progress in special fields the integration of the new knowledge has become difficult and is far from satisfactory. Here the danger of rigidly maintaining traditional concepts becomes obvious. New fresh ideas, on the other hand, may open new ways and greatly stimulate an entire field. If it is true that even in physics no definitely established concepts and notions exist; if, for instance, the theory of relativity has changed such fundamental concepts as space and time and other seemingly solidly established philosophical and scientific notions, then all the more should the biologist be aware of how uncertain his concepts must be at present. Clearly, it is absolutely essential for real progress to keep the mind open for entirely new ideas and concepts in a field that, due to its complexity, is still far behind physics and chemistry as to the level of precision and insight.

The conference differs from the usual pattern of bringing together active and competent investigators of a special field and providing them with an opportunity to present and review the available information. The aim of this Symposium is that of emphasizing the discussion of fundamental concepts and notions by a group of scientists of a widely different background but all interested in the basic problem of what is widely referred to as Molecular Biology. As James B. Conant stressed in his lectures on "Modern Science and Modern Man," the essential element in the advance of modern science has been the interplay between theoretical notion and the experiment of the specialist. "The history of science," he writes, "demonstrates beyond doubt that the really revolutionary and significant advances came not from empiricism alone but from new theories. The development of these theories, in turn, has depended on free discussion of their consequences." Indeed, many examples may be quoted where most fruitful developments took place when different types of thinking met.

It was felt that new types of approach and new ideas might be stimulated and certain notions and concepts clarified by bringing together physicists and chemists with a group of biologists working on different lines and with different methods, i.e. either on ultrastructure or on the chemistry or on the physical events of living cells. Two topics were selected about which much information has accumulated as to the underlying elementary processes: muscular contraction and nerve impulse conduction. Only a relatively small number of papers will be presented in order to formulate problems and to provide a factual basis for a fruitful discussion.

In order to promote a really informal, intensive and fruitful exchange of ideas the Organizing Committee considered it imperative to limit the participants to a small number. The Committee, therefore, had the unhappy duty of making a somewhat arbitrary choice in selecting participants among the great number of investigators who are competent and interested in these problems.

Although the material presented covers only a few aspects of a variety of fields, the high quality of the papers and the wealth of important new information and new ideas made it appear desirable to combine them in a special volume.

The hospitality of President Bronk who made available the facilities of the Aldrich Hall of the Rockefeller Institute with its gracious atmosphere was greatly appreciated by all participants. The Editor would like to express his personal indebtedness to the President and his associates for their active participation and valuable advice and help in organizing this Symposium. The conference was made possible by a generous grant from the National Science Foundation.

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The Structure of Striated Muscle

Hugh E. Huxley

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The phenomenon of muscular contraction has been studied in great detail, and with considerable success, by the classic disciplines of physiology and biochemistry, the one concerned with the macroscopic properties and behavior of muscles, the other with the chemical reactions taking place during the various phases of muscular activity. A well-established picture of many aspects of the contractile process, as seen from these two points of view, is now available, but it is a picture with a very large gap in it, for the causal link between the chemical reactions and the contraction to which they give rise is not known. That is, we do not yet know what exactly are the structural changes at the molecular level which occur as a consequence or an accompaniment of the chemical reactions taking place during contraction, and the process by which those molecular changes are summated to produce macroscopic shortening is only partially understood. However, information concerning the submicroscopic structure of striated muscle offers the possibility of establishing definite relationships between the two different approaches to the subject, so that eventually the detailed physiological observations can be translated into their implications in terms of events at the molecular level, and the physiological consequences of particular chemical events can become calculable. Let us see how far we can take this process at present.

The Development of the "Sliding Filament" Hypothesis

The structural evidence seems to favor very strongly the "interdigitating filament" or "double array" model of striated muscle (Hanson and H. E. Huxley, 1953), and contraction seems to be brought about by a process in which the two arrays of filaments in each sarcomere are made to slide past each other (A. F. Huxley and Niedergerke, 1954; H. E. Huxley and Hanson, 1954). I will describe the main pieces of evidence which have led to this view, and then discuss very briefly some relationships between various phenomena in muscle on which the structural information throws a little new light.

The earliest electron microscope studies on muscle (Hall et al., 1946; Draper and Hodge, 1949) had shown that the myofibrils of striated muscle contained longitudinal filaments about 100 A or so in diameter, which remained relatively straight and well oriented even after contraction. They also showed clearly that the characteristic band pattern arose from a well-defined variation in density along the length of the fibrils. The precise way in which the filaments were arranged *in vivo*, and in particular their lateral arrangement, could not readily be ascertained at that time, for the only technique available was one in which isolated myofibrils, prepared by mechanically fragmenting the whole muscle, were dried down into the electron microscope grids.

X-Ray Observations

The first indications that the contractile material of striated muscle was built up from two sets of filaments came from low-angle X-ray diffraction studies on muscle examined in the wet state, both under normal physiological conditions and in a state of rigor (H. E. Huxley, 1952, 1953a). Such muscles give a characteristic and well-defined X-ray pattern; the equatorial reflections reveal the presence of a regular hexagonal array of filaments spaced out about 450 A apart and obviously corresponding to the filaments seen in the earlier micrographs. It became clear that these filaments formed a more or less continuous array across each myofibril and were not located around the periphery alone, as had sometimes been supposed. The space between the filaments would of course be occupied by sarcoplasm.

In muscle in rigor, the hexagonal array still appeared to be present, but the intensities of the X-ray reflection differed from those given by living muscle in a manner which suggested that a secondary array of filaments had now become fixed in position at the trigonal points of the lattice, i.e., at the points lying symmetrically between three of the original "primary" filaments.

As the onset of rigor was known to be associated with the disappearance of adenosine triphosphate (ATP) from the muscle, and as the absence of ATP would allow combination between actin and myosin to take place, it was suggested that these two principal structural proteins were localized in the two separate sets of filaments; that the secondary filaments were fairly randomly arranged among the array of primary ones in living muscle containing ATP; and that in rigor the secondary filaments became attached at specific sites in the array by the formation of actin-myosin linkages, thus giving rise to the observed changes in X-ray pattern. The available evidence did not make it possible to decide which protein was contained in which set of filaments, nor did it of course provide any evidence or indication about localization of those proteins in particular regions of the sarcomere, except that it required them to be present together in some regions at least. However, it was found that living muscle gave a very sharp set of axial reflections corresponding to a 415 A axial periodicity, which remained unaltered when the muscle was stretched and which, so far as could be judged from the rather poor patterns given by contracted muscle, was not changed by shortening either.

CONFIRMATION OF THE "DOUBLE ARRAY" THEORY

The development of the technique of ultrathin sectioning of tissue for the electron microscope made it possible to take the structural studies several stages further. It was confirmed that the myofilaments were arranged in hexagonal fashion across the myofibrils (Bennett and Porter, 1953; Hodge et al., 1954), a result also indicated by some very early observations on thin sections (Morgan *et al.*, 1950); and the presence of the double array of filaments was confirmed by more detailed studies of cross sections of muscle taken through various bands of the sarcomere (H. E. Huxley, 1953b). These showed, in the A-bands, a very regular hexagonal array of thicker filaments about 100 A in diameter, with thinner filaments, about 50 A in diameter, lying symmetrically at the expected positions in between them (see Fig. 1). No such double array could be detected in the

I-bands however, which at that time were usually rather badly preserved but which only seemed to show thin filaments.

This was a puzzling result at first, but the reason for it became apparent when it was found that the high optical density and birefringence of the A-bands disappeared when muscle was treated with solvents (high ionic strength salt solutions plus ATP or pyrophosphate) known to dissolve out myosin selectively (Hanson and H. E. Huxley, 1953; Hasselbach, 1953).

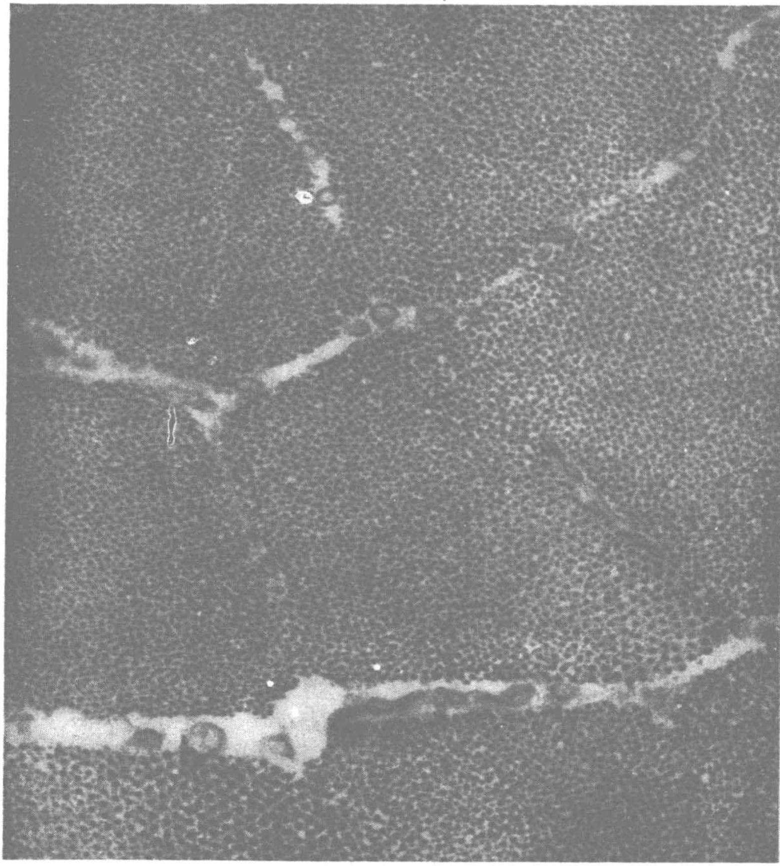

FIG. 1. Transverse section through the A-bands of a number of fibrils of rabbit psoas muscle showing double hexagonal array of large and small filaments, cut end-on. Magnification: $\times$ 50,000.

Moreover, electron micrographs of muscle which had been treated in this fashion showed that the larger filaments were no longer present, and that only thin filaments remained; the distinction between A- and I-bands had disappeared.

It was, therefore, natural to suggest that the myosin in the muscle was contained in the array of thicker filaments in the A-bands (each filament being continuous from one end of the A-band to the other), and that the presence of this array of myosin filaments was responsible for the high density and birefringence of the A-bands. The protein actin, on the other hand, was assigned to the thinner filaments, extending from the Z-lines, through the I-bands, into the A-bands, where they lay in between the myosin filaments, up to the H-zone, where they terminated. No thin filaments could be seen in cross sections through the H-zone (Fig. 2), although the thicker ones were still

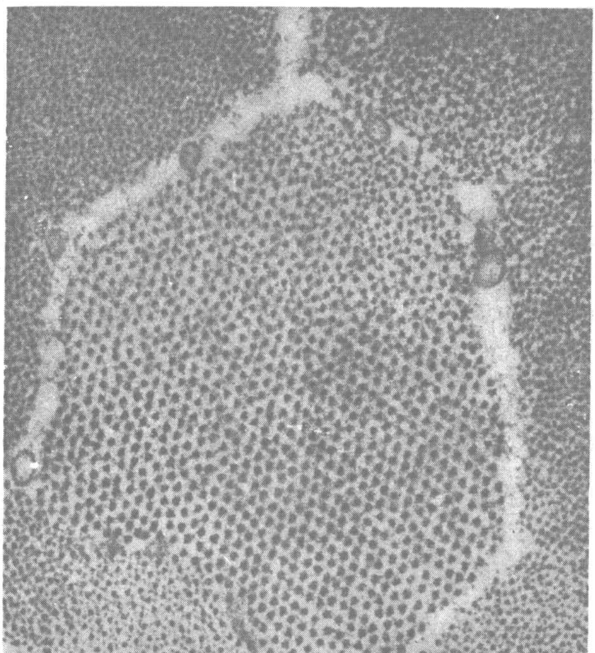

FIG. 2. Transverse section through the H-zone of a psoas fibril, showing simple hexagonal array of large filaments.