The Myocardial Cell: New Concepts for the Clinical Cardiologist

The growing body of research into the ultrastructure of the myocardial cell and the function of subcellular systems in the heart has reached proportions that make it both useful and pertinent to bring important new concepts in this area to the attention of the clinical cardiologist. Work on myocardial ultrastructure is no longer the province of the cellular anatomist and physiologist; increasingly, the clinical literature contains studies describing the cellular anatomy of both normal human myocardium and that of diseased hearts. Invariably, of course, such studies draw inferences about the mechanism of myocardial dysfunction on the basis of the ultrastructural changes observed.

Preservation of Tissue
Myocardium, like all contractile tissue, shortens in response to the stimulus of a sudden fall in environmental temperature. Maintenance of the resting length of the tissue prior to immersion in iced preservative, therefore, must be routine. When this is not done, the muscle shortens vigorously on making contact with the cold solution. As a consequence, all intracellular dimensions are distorted and certain ultrastructural features are even obliterated. If tissue is in contracture, no sarcomeric I bands will be visible in the section. The lumina of intracellular tubular structures will appear dilated, mitochondrial numbers will appear increased per unit area of the cell, and the cell membrane will be thrown up into folds, as will the nuclear envelope.

If pieces of tissue placed in preservative are not sufficiently small to insure rapid and uniform penetration of the fixative, or if tissue is not preserved within minutes of the death of the experimental animal, sarcomeric Z bands will appear fuzzy and thickened and mitochondria will appear vacuolated. The nuclear contents will be largely obliterated, with peripheral clumping of chromatin just under the nuclear membrane. These features, if present, do not reflect pathology, then, but are artifacts and, clearly, cannot be interpreted to be the consequence of disease. Several workers have outlined the changes that occur in the cell simply as a consequence of postmortem changes.¹ ²

Normal Anatomy of the Myofiber and the Function of Subcellular Systems in the Cell

The myofiber has, by virtue of the fact that it is a striated muscle cell, certain characteristic physiologic properties. It is capable of excitation and, indeed, depending on the type of cell, can spontaneously depolarize in a periodic fashion, i.e., it has pacemaking ability. The process of excitation and, if present, spontaneous depolarization depends on the properties of the cell membrane or sarcolemma and its derivatives in the cell, the transverse tubular system, and the intercalated disc.

Excitation has a definite mechanical consequence in the normal myofiber, i.e., it engenders a contractile event. In other words, excitation is coupled to contraction so that the cell shortens in response to stimulation. Contraction is achieved by the shortening of the contractile unit (or sarcomeres) that fill the cell in orderly lines called myofibrils and which make up the bulk of the volume of the myofiber. The contraction of individual sarcomeres occurs when the calcium concentration in the area of the myofilaments making
up the contractile units is raised to a certain critical level (above 10^{-5} M). The coupling of excitation to contraction, then, is mediated through calcium, which is released in response to excitation and presented to the area of the sarcomeric myofilaments. To achieve relaxation of the muscle cell, the calcium must be removed from the area of the myofilaments and restored to an inactive site in the cell. The phenomenon of relaxation is almost certainly the consequence of the operation of the sarcoplasmic reticular (SR) network, a sleeve of intricately branching tubules that surrounds each individual sarcomere and has a well-demonstrated ability to pump calcium out of the environment against a concentration gradient. The calcium is then restored to the specialized modifications of the SR tubular network called the lateral sacs. These sacs, as has been well demonstrated in both cardiac and skeletal muscle, are storage depots for large quantities of calcium ion.3, 4

Each of the functional processes in the muscle cell is postulated to be the consequence, then, of the activity of a certain specific subcellular system in the myofiber. The identity of the system is not entirely clear in some cases, however, and the mechanism whereby the system operates, even if it has been identified with certainty, is not always understood. It is in these areas that fundamental investigation is still ongoing, and many problems are still unsolved.

**Current Problems in Subcellular Function**

(1). *Excitation-Contraction Coupling*. One of the most important points currently under investigation in cardiac cell physiology is the mechanism by which excitation is coupled to contraction in the myofiber. The sarcolemma, or myocardial cell membrane, has two components: a thin, electron-dense plasma membrane and a much thicker, amorphous coating which surrounds the entire cell and is its outermost layer. This substance, which is called the basement membrane or perimembrane, is a mucopolysaccharide and has a multiplicity of negative charges by virtue of which it can bind cations.

In the ordinary working ventricular cell, the sarcolemma sends penetrating fingerlike invaginations downward into and throughout the entire substance of the myofiber to create an intricate pattern of tubules which are so abundant that each sarcomere has its own portion of this sarcolemmal derivative, known collectively as the transverse tubular system. Both portions of the sarcolemma, the perimembrane as well as the plasma membrane, participate in the formation of the T system, and the amorphous granular substance of the perimembrane fills the transverse tubular lumen.

The transverse tubule comes into intimate contact with the lateral sacs of the sarcoplasmic reticulum. This proximity of the transverse tubular system to the calcium storage depots of the sarcoplasmic reticulum led to the general acceptance of the idea that excitation was initiated at the sarcolemma at the surface of the cell and was rapidly propagated throughout the substance of the myofiber over the transverse tubular system. At the level of each sarcomere in the myofiber, it was thought, the excitatory impulse prompted the release of calcium ion from the lateral sacs to the underlying myofilaments in the contractile unit, thus initiating contraction.

As investigation of myocardial cell ultrastructure progressed, however, it became apparent that there were significant portions of the myocardial cell population which had no transverse tubular system, at all, specifically the atrial and Purkinje cells.5 Even more disconcerting, it is now becoming clear that the ventricular cells of embryonic or neonatal animals, which have well-developed transverse tubular systems in the adult, have no T systems at birth.6, 7

The transverse tubular system, at least in some species, develops at some time in postnatal life, probably as a consequence of the pattern of growth of the cell, which occurs primarily at the periphery of the myofiber. It is clearly not essential, then, as has been previously postulated, either for the rapid propagation of excitation throughout the cell, or for the coupling of excitation to contraction.
The function of the T system, then, is no longer clear; it may be simply a vehicle for the modified extension of the extracellular compartment from the cell surface downward throughout the body of the cell. The negatively charged mucopolysaccharide of the perimembrane, which fills the lumen of the T tubule, probably binds and therefore does not allow for the unmodified exchange and rapid equilibration of charged particles in the lumen of the T system with the extracellular compartment at the surface of the myofiber. This may account for some of the specialized properties of the ventricular myofiber, such as the phenomenon of the afterpotential generated as a consequence of repeated stimuli. The potassium exiting from the cell with each successive depolarization is bound to the substance of the perimembrane. It cannot, therefore, diffuse outward to the surface of the myofiber, and the potential across the cell membrane is thus altered.

How then is calcium release to the area of the myofilaments achieved in those cells which either never have a transverse tubular system or develop it only in postnatal life? The question is unanswered, but current evidence points to the sarcolemmal perimembrane as the source of excitation-contraction coupling in the myofiber. The calcium which is presented to the area of the contractile filaments is released from the superficial cell membrane (possibly by being displaced by sodium ion) and travels inward to the area of the sarcomeres by a simple process of diffusion. Calcium ion diffuses in myoplasm at a rate of 1 \( \mu \)/msec; the average diameter of the cardiac cell is about 10 \( \mu \). The time between excitation and the beginning of the contractile event is about 20 msec in cardiac tissue. There is ample time, then, for calcium to be distributed throughout the cell by simple diffusion in the initiation of contraction. The previous postulate that the exciting impulse released calcium from the lateral sacs at the level of each sarcomere almost simultaneously is not necessary in view of the relatively long time that elapses between the inscription of the action potential and the contraction in the cardiac myofiber.

It follows from the previous discussion that dilatation or disruption of the transverse tubular system in diseased tissue ought not to be interpreted to mean that the mechanism of excitation-contraction coupling in such hearts is faulty and that this is the reason for decompensation in patients whose myocardia show this change.

(2). The Sarcomere: The Mechanism of the Contractile Event, and the Mode of Sarcomeric Proliferation in the Growing or Hypertrophying Myofiber. The effective work of the cardiac cell is achieved by the contractile unit or sarcomere. The sarcomere is delimited at either end by an electron-dense line called the Z band. The Z bands parenthesize the two populations of filaments which make up the sarcomeric unit; it is the interaction between these two types of filaments that achieves sarcomeric shortening. The central portion of the sarcomere is occupied by the so-called thick filaments, which are actually aggregations of the protein myosin. The myosin molecule has a long stalk which terminates in a globular head. The molecules aggregate in such a way that these globular heads are clustered in an orderly sequence at either end of the thick filament, while the stalks of the myosin molecules occupy the central portion of the filament.

The sarcomeric thin filament contains at least four proteins. It is composed of two linear strands of the protein G (globular) actin, one strand twisted about the other in a double helical arrangement. At each pitch of the helix (located at approximately 400-A intervals) is a so-called active site on the filament, where cross-bridge formation between the thin filaments and the thick filaments occurs. In the resting state, the active sites on the thin filament are blocked by a protein complex of troponin and tropomyosin. When calcium is presented to the area of the thin filament, the configuration of the troponin molecule is changed so that the active site is exposed and cross-bridge formation between the thin filament and the active

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sites on the thick filament (which are the globular heads of the myosin molecules) ensues. It is the sequential making and breaking of cross bridges that achieves the movement of thin filaments from either end of the sarcomere centrally along the stationary thick filaments, with which they interdigitate. Thus, Z to Z distance diminishes and sarcomeric shortening is achieved.

How the myocardial cell achieves normal growth, how it repairs worn or destroyed contractile units, and how the myofiber increases its size in hypertrophy are problems of tremendous interest among cellular anatomists at the moment.

It is apparent that the number of sarcomeric units increases in both normal cell growth and with the augmentation of cell mass that occurs in hypertrophied states. The sarcomeric Z band plays a pivotal role in this process of the generation of new sarcomeric units in the cell, not only in the embryonic and postnatal heart, but also in the adult myofiber which undergoes hypertrophy.6,10

Biopsied specimens of human myocardium with chamber hypertrophy have shown that in such tissue, Z substance, whether at the periphery of the cell or deep within the substance of the myofiber, proliferates and subsequently differentiates into a homogeneous group of filaments (termed by us "primary myofilaments"). These serve as the template upon which fully differentiated thick and thin filaments evolve and are arranged to form new sarcomeric units in the cell. Exactly similar configurations of proliferating and differentiating Z substance were found in the newborn puppy and rat ventricular cells grown in tissue culture, establishing the important principle that, whether the cell is embryonic, engaged in normal postnatal growth, or increasing its mass in the adult situation in response to the pressure or volume overload, the mechanism of sarcomerogenesis is always exactly the same. The growing or hypertrophying cell, then, contains its own internal machinery for sarcomeric replication, and sarcomerogenesis is one of the principal means whereby the myofiber increases its mass.

3. The Role of the Mitochondrion in Cell Growth and Hypertrophy. The other important intracellular component increased in hypertrophied states is the mitochondrion or sarcosome. This is the energy-producing unit of the cell, and it is evident that, if the cell increases the number of contractile units, sarcosomes must also increase to keep pace with increased work in the myofiber. A recent study of the mechanism of mitochondrial reproduction in a population of rat ventricular cells grown in tissue culture indicates that the mitochondrion grows in mass (and in the complexity of its cristal pattern) to a certain crucial point, whereupon it divides by fission.7

Like the nucleus, mitochondria are known to have DNA, and so contain their own genetic information. They direct not only the formation of their own cristae, but impart their genetic code to the two daughter units formed as a consequence of the fission of the parent sarcosome. Studies of the mode of mitochondrial replication in human biopsied tissue have not been done to date; such an investigation seems indicated, however, in the light of the recent work on tissue culture cells.

The role of nucleus in directing the increase in intracellular components in the growing and hypertrophying myofiber is an interesting one to consider; unlike the tissue culture cell, in which there are abundant nuclear pores through which genetic information is being exported to the cytoplasm in the form of ribosomes, there are no nuclear pores in the adult hypertrophying cell. This raises the interesting possibility that it is mitochondrial DNA, which instructs not only sarcomosomal replication, but controls the differentiation of Z substance into primary myofilaments, with the subsequent construction of new sarcomeric units. This concept, at present, is purely speculative, but it serves to illustrate that a careful analysis of myocardial cell ultrastructure, considered in the light of current concepts of the function of subcellular systems, may help to solve important problems.

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The answers to these problems are fundamental to our understanding of the reasons for myocardial malfunction in disease states.

MARIANNE J. LEGATO

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