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LECTURE NOTES 4

Insight into the Heart: Cardiac Energetics

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In memory of Yechiel Gad Zajdman (Sideman) born in Lukow, Poland—1906, immigrated to Israel—1924, died in Haifa, Israel—1952.

Contents

Preface

The cardiac system consists of, and depends on, an array of micro and macro physiological entities and numerous functional characteristics, ranging from molecular reactions and genetic signaling to global ventricular interacting functions. These include diffusion, active ionic transport, membrane channels and receptors, cellular metabolism, intra- and inter-cellular interactions, energy consumption and power generation, electrical excitation, fiber mechanics, fiber orientation and 3D ventricular mechanics, systemic coronary flow circulation, microcirculation and, not least important, pathological factors affecting the various cardiac functions. Understanding this complex multi-parameter, multi-level system, denoted as the *Cardionome*, or *Cardiome*, requires in depth study of the interactions between the various physiological and physical parameters and utilizing integrated models and/or sophisticated experimental procedures.

The lecture series presented in August 2004 in the Institute of Fundamental Technological Research of Polish Academy of Sciences in Warsaw aimed to introduce the Cardiome, report experimental and theoretical studies and analyses of this highly complex system from different points of view, and attempt to relate and quantify the effects of some major interacting parameters on the cardiac function.

The analyses presented related micro scale phenomena (molecular, cellular, etc.) to the macro scale organ performance. The presentations highlighted analytical physiological models of cardiac contraction dealing with organ level parameter interactions as well as the intra-cellular mechanisms controlling cardiac function and energetics in response to loads. Finally, the molecular motors, which play a crucial part in the performance of the cardiac system and the transformation of chemical energy to mechanical work, were introduced. These molecular motors serve as a baseline for the fast-growing international interest in Nanomedicine and Nanotechnology and relate to our dreams to do better than God...

A salient feature of the seminar series was its integrated presentation of interdisciplinary topics: cell biology concepts and analytic and kinetic modeling were coupled with mechanistic biological and engineering perspectives. The succinct presentations, rich in information and structured in organization, are valuable tools for future research efforts. The lectures distilled the essence of the complex cardiac system and highlighted pertinent approaches for better understanding and improved insight into the molecular mechanisms of cardiac contraction.

The present text is by necessity limited by time and space and concentrates on the most recent analysis of the cardiac contraction and the identification of the intracellular control mechanisms of energy consumption and energetic efficiency of the contracting muscle.

I gratefully thank all those who helped transform this seminar series from vision to reality. Special thanks go to Prof. Tomasz A. Kowalewski, who initiated and facilitated our visit to Warsaw. He vigorously helped to make the visit a success. Special thanks are also due to Prof. Joachim Telega, scientific coordinator of the Centre of Excellence ABIOMED, for inviting me to the IPPT and gently prompting me to put my lectures on paper. I am particularly grateful to Dr. Amir Landesberg, MD, my ex PhD student and present colleague and friend, whose originality, ingenuity and patience allowed me to venture into the exciting world of the control of intracellular contraction. His help in preparing this monograph is extremely valuable. The biblical scholars who coined the phrase "I have learned much from all my teachers, but gained more (knowledge) from my students" must have had him in mind! Sincere personal thanks go to Mrs. and Mr. Jack George of Los Angeles, California, USA, for their trust and support and Dr. Martin Kellner, who inspired me by proving that one can get better with age... Finally, warmest thanks go to my wife Naomi Sideman whose continued support, and particularly during the preparation and presentation of these Warsaw seminar notes, guided me to a better performance.

Sam Sideman

Chapter 1

Cardiology and Technology: an Introduction

1.1. Medicine and Engineering Science

Two monumental phenomena have shaped science and medicine in the last century and practically changed the world we live in. The first relates to the intimate interaction between Engineering Science and Medicine, e.g. Technology and Cardiology. The second relates to the globalization of science, the proliferation of the Internet, and the unprecedented international interaction, communication and scientific cooperation in facing difficult multi-disciplined challenges. The beneficial combination of these two mega-phenomena, accompanied by significant environmental and sanitary changes, has greatly improved the quality of life and increased longevity around the globe. Here we relate only to the cardiac system, which is one of the greatest beneficiaries of modern technology, thus bringing about a most significant decrease in number of early deaths in the modern world.

The inherent complexity of the cardiac system and the wide discipline fragmentation within Engineering and Science are the largest barriers to the cardiac system analysis. It is, however, to be noted that large engineering and biological systems, e.g. the cardiac system, have in common high levels of complexity, hence great robustness. This property of the large complex systems allows adaptation and homeostatic regulation, thus preserving particular unique characteristics of the system despite uncertainties and imposed changes of their components by the environment. Furthermore, the robustness of the cardiac system under study allows the application of reasonable approximations and assumptions in theoretical models, which are generated to gain insight and better understanding of this highly complex system. It

seems that the complex robust system is not too sensitive to "logical perturbations", and thus allows us to penetrate into the system and gain insight into its functional characteristics.

Both engineering and medicine are down to earth disciplines. Both grow by responding to practical needs and thrive on the progress of science and technology. Most noticeable is the huge effect that modern technology, associated with new macro and micro imaging techniques and molecular scale diagnostic technologies, had on expanding the frontiers of medical science in general and cardiology in particular. Utilizing better diagnostic tools and more effective therapeutic modalities had a very profound effect on our lives. The developments in science in the last 40 years or so have catalyzed a transformation from the classical macro-scale studies of the living organisms and whole organs to the micro/nano world of elementary entities, such as the organelles, cells, molecules and genes. In our world of cardiology, we have seen and nurtured the evolution of cardiovascular research from physiology and rheology to a new era of dynamic studies based on molecular biology and cellular and genetic engineering, all aimed at preventing heart failure and *repairing* the ailing heart. However, much is still awaiting our conceptual understanding and quantitative interpretation of observed or imagined phenomena.

1.2. Life and the Cardiac System

Life is defined by the Webster dictionary as an organistic state characterized by the capacity for metabolism, growth, reaction to stimuli and reproduction. The cardiac system, which mobilizes the metabolic substances to the cells of all living multi-cell organisms, is, therefore, critical to the maintenance of life.

All living organisms have only one objective: surviving by preserving constant conditions of life in their internal environment. Living organisms require continuous supply of energy to construct and maintain local regions of ordered specialization in a chaotic universe. All living systems actively exchange substances with the environment. Note that living organisms are not at equilibrium with their surroundings. In fact, equilibrium is a sign of death. Life demands maintaining steady state gradients and depends on metabolic energy which must be derived from the world outside the organisms. The organisms regulate the exchange rate. The sensitivity and specificity of the

regulatory exchange mechanism increases with the complexity of the organism. Any compound away from equilibrium may conceptually serve as an energy source.

As stated, all living organisms require continuous supply of energy to maintain local regions of order and specialization and escape equilibrium with their surroundings. Sustaining life demands energy to maintain the needed steady state and the associated electrical and mechanical activity. All this energy must be derived from the world outside the organisms. The energy sources are sunlight for plants and metabolic oxidation reactions of organic compounds for most living organisms. Metabolism denotes biological energy conversion of external energy sources to the particular energy forms required for sustaining and propagating the particular living organism.

All living organisms are composed of cells and all cells are basically similar in their chemical construction. New cells are created by division. All the cells in the particular organism demonstrate simultaneous integrated activity. Excitable cells alter the electric characteristics of their membranes when properly simulated ("action potential"). The lipid cellular and intercellular membranes have "voltage sensors" protein "gates" controlling the flow of ions (Na^+, Ca^{2+}, K^+) through "channels" in their membranes. There are fast gates, e.g. Na^+ (<1 ms) and slow gates, e.g. for Ca^{2+}, (~ 3 ms). Metabolic energy is required to maintain the active gates.

Living systems exhibit defense responses against internal environmental changes. These include homeostasis, which maintains steady state processes; immune response—resists changes due to outside invasion; stress response—to a variety of stimuli, e.g. "fight or flight"; chemical response—to epinephrine, glucose etc.

Life depends on transport phenomena: *to the cells*—electrical impulse, mass transfer; *in the cells*—chemical reaction, mass transfer, heat transfer; *between the cells*—mass transfer, heat transfer, blood flow; *of the cells*—convection, fertilization. The transport phenomena into and within the cells are driven by concentration gradients (diffusion or convection) and/or by active transport facilitated by specific ionic or molecular pumps, carriers or receptors via the gated transmembrane channels (ligand gates, mechanical gates or voltage gates) or by vesicular protein transport, either directly or via the Golgi apparatus and the endoplasmic reticulum within the cell, or by linear molecular motors, i.e. the kinesins and the dyneins of the tubular tubulin family.

1.3. Some Characteristics of the Human Heart

The human heart is an ingeniously constructed organ. It is unique in structure and its outstanding performance: with a weight of approx. 300 g., it beats some 3 billion times in a normal life span and assures that all the cells in the various organs in the body are continuously nourished by blood carrying oxygen and metabolites. The main cardiac tasks and characteristics are:

- O_2 supply, CO_2 removal,
- heat convection,
- energy and metabolic supply,
- pumping—5 l/min $\approx$18 500 m^3/70 years, or 6 olympic swimming pools in 70 years,
- left ventricular work $\approx 3.3 \times 10^8$ kG m, or 33 ton up the Everest/70 yrs.,
- right ventricular work $\approx 1/4$ Work_{LV}; $\text{Flow}_{\text{RV}} = \text{Flow}_{\text{LV}}$,
- dynamic flow range = 5–30 l/min.

Myocardial metabolism at rest is based on:

- Free Fatty Acid (FFA)—supplies 70–80% of myocardial energy required, utilizing 75% of the cardiac oxygen consumption,
- glucose, lactate, pyrovate—provide the balance of energy required,
- glucose is converted: 35% to pyrovate, 15% to lactate and 50% to glycogen,
- metabolistic energetics: glucose yields 5.0 kcal/dm^3 O_2; FFA yields 4.7 kcal/dm^3 O_2,
- oxygen needs of the myocardium (at rest): 8–10 ml/min/100 g. compared to the skeletal muscle (at rest) 0.15 ml/min/100 g.,
- oxygen extraction: myocardium—75%, systemic—20–25%,
- percent oxygen saturation: arteries 95%; veins 40% and the coronary veins 20%.

Chapter 2

Cardiac System

2.1. Cardiome

The *cardiome* is a one-organ component of the *physiome*, the desired integrated system that will eventually be developed to describe the physiology of the whole body and the interaction between the numerous elements constructing it [4]. The term "cardiome" encompasses the multi-dimensional complexity of the cardiac system and the various parameters that affect the cardiac function. Thus, starting with the whole *organ*, e.g. the left ventricle (LV), we note that the major elements of interest can be grouped [4] by structure (fibers, directionalities, physical properties, composition, etc.), state (contraction, relaxation, patho-physiology, etc.), kinetics (rates of deformations, ejection, outputs, excitation, pressures, volumes, etc.) and functions (activation, contraction, relaxation, etc.). The next level of interest brings us to the *tissues*, again defined by structure, state, kinetics and function. Going a level 'deeper' we address the *cells*, and then the *organelles*, and finally the *molecular* level, each defined by its structure, state, kinetics and function. The inherent complexity of the cardiome is thus established as a multi-level, multi-parameter description of the cardiac system. Clearly, an awesome array of interacting parameters!

2.1.1. Cardiac Cell and Sarcomere

The characteristic feature of all living organisms is that they are made of cells. *Bioenergetics*, which deals with energy transformation within living systems, is expressed and manifested within the cells. There are basically

three types of biological energy consuming processes: 1) chemical (biosynthesis for growth and maintenance; production of small and large organic molecules), 2) transport of ions and molecules in and out of cells (related to concentration gradients, osmotic and electrical work), and 3) Mechanical work (contractile processes, motility propulsion). The cardiac "cell factory" contains the elements required to sustain life and to generate new life by cell division, so as to assure the survival of the species.

Unlike man-made mechanical power generators, the living organisms convert chemical energy into mechanical energy directly at relatively high efficiency. Adenosine three-phosphate (ATP) is the intracellular biological fuel, which fuels practically all energy-consuming activities in the living organisms.

The chemical-to-mechanical energy transformation in the muscle involves three intracellular organelles, Figs. 2.1, 2.2. 1) The *mitochondria*, the chemical factory that metabolizes external nutrients to form the high-energy adenosine tri-phosphate (ATP) by utilizing nano-scale rotary motors, the ATPsynthase enzyme. 2) The *sarcomeres*, made of actin-myosin protein filaments, whose interactions generate the power which mobilizes muscular contraction. Hydrolysis of one high energy phosphate bond of the ATP by the enzyme ATPase yields adenosine di-phosphate (ADP) plus phosphate (P) and liberates the energy ($\Delta H = 30.6\,\mathrm{kJ/mol}$) required for generating the contraction. 3) The *sarcoplasmic reticulum* (SR), which serves as a dynamic store for calcium ions required for the control of the excitation-contraction coupling. Here we relate only to the sarcomeres, which convert chemical energy to mechanical work and generate muscle contraction

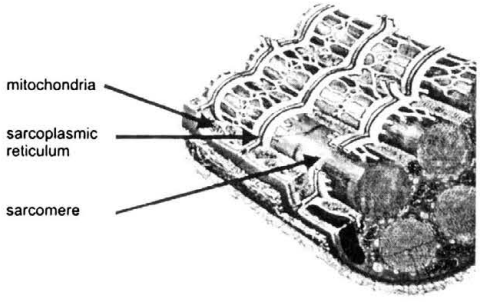

FIGURE 2.1. Main intracellular elements affecting cardiac contraction: the mitochondria, the sarcoplasmic reticulum and the sarcomere. (Netter's Atlas of Human Physiol. Icon Learning Publ. permission requested)

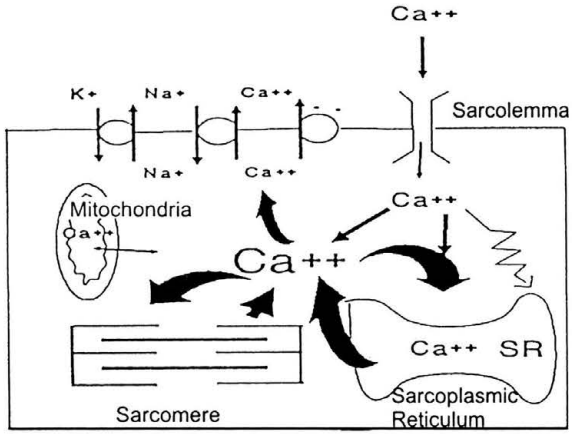

FIGURE 2.2. Schematic presentation of a cardiac muscle cell, highlighting the intracellular organelles that participate in energy transduction and regulation of the excitation-contraction coupling. (permission requested)

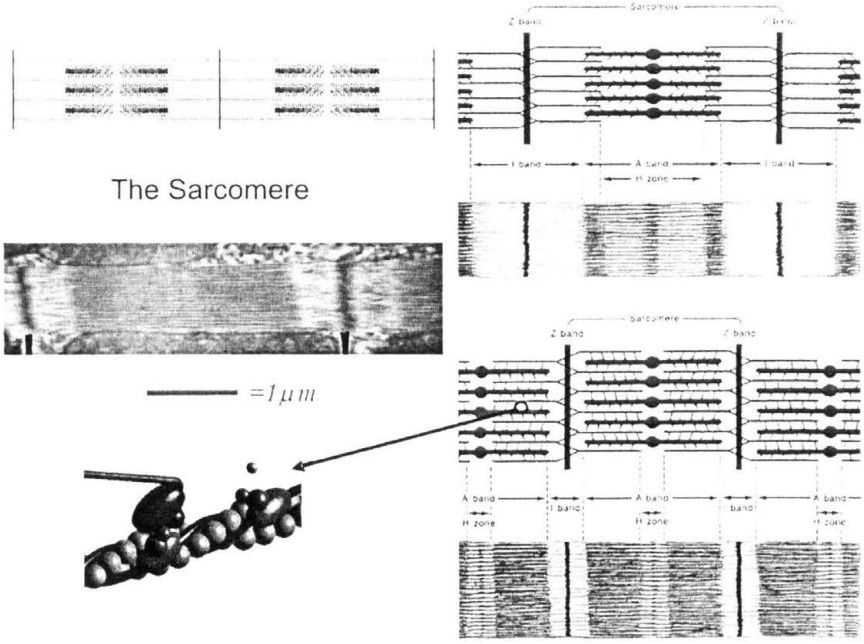

FIGURE 2.3. Right: schematic description of the contraction and relaxation of the sarcomere's actin (thin) myosin (thick) filaments; Left, bottom: cartoon of the myosin cross bridge (Xb) between the filaments, (borrowed from the Internet). Based on Netter's Atlas of Human Physiol. Icon Learning Publ. (permission requested))

The tissue of the cardiac muscle, as all other muscles, is made of many thousands of fibers, which are constructed of structural elements, mostly cells, and contain enzymes, e.g. the ATPase, which modulates the cellular metabolic processes and the ionic transport across of the inner and outer membranes of the cell. A fiber contains some 10^3 fibrils, each composed of some 4,000 sarcomeres per cm length of a fibril [12].

The sarcomere is the basic contractile motoric unit of the muscle cell and is made of a comb-like interlocking mesh of thin actin and thick myosin filaments, cf. Fig. 2.3. The thin filament is made of two bead-like strands of some 500 single actin molecules. The thick myosin filament is made of some 350 molecules of myosin arranged in longitudinal bundles. The oar-like ends of the myosin molecules are arranged in six longitudinal rows, which project out of the myosin bundle.

2.2. Deciphering the Cardiome: Interactions and Integration

The Technion's Heart System Research Center, established in 1982, set its goals to: 1) foster interdisciplinary interaction, so as to identify missing knowledge and catalyze new research ideas; 2) relate basic micro scale, molecular and sub-cellular phenomena to the global, clinically manifested, cardiac performance; 3) apply conceptual modeling and quantitative analyses to better explore, describe, and understand cardiac physiology; 4) interpret available clinical data and design new revealing experiments, and 5) enhance international cooperation in the endless search for a better understanding of the various interacting variables which govern the heart's functions and their implications in cardiac pathophysiology.

Understanding the micro- and macro-scale cardiac characteristics and physiological function requires the study of the interactions between the major governing parameters by utilizing integrated models and experimental verification. This long range program aimed initially at an integrated model of the interrelated macro-scale parameters of hemodynamics, coronary circulation, cardiac mechanics, and electrocardiography in the 3-dimensional (3D) ventricle (Fig. 2.4) by using imaging and other related techniques. This involved the development of computerized 3D dynamic models of the heart [52], and attempts to incorporate the mechanical properties and orientation of the fibers, the electrical activation, the coronary circulation and the metabolic

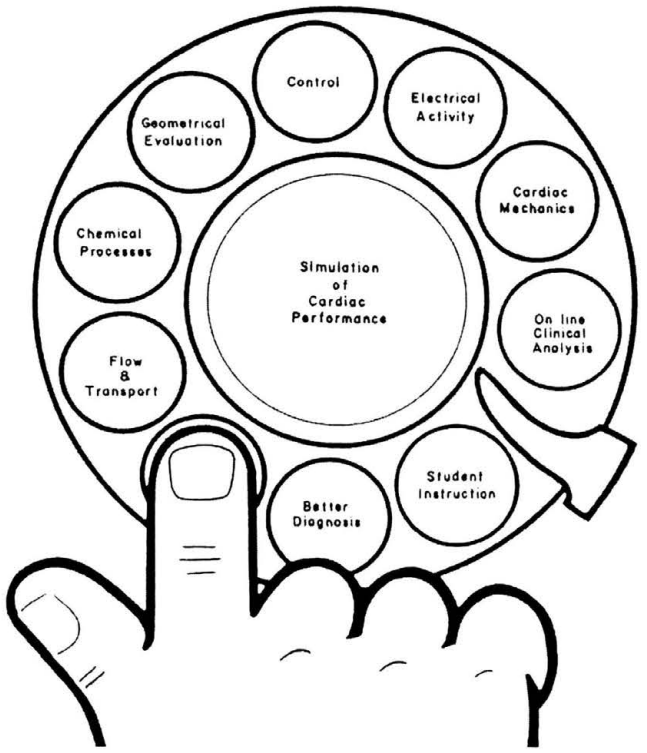

Figure 2.4. Symbolic presentation of the integrated cardiac parameters

energy balance in the analysis of the cardiac muscle. Thus, early studies explored the interactions of organ-scale mechanics, electrical activation, perfusion and metabolism, emphasizing the importance of 3D imaging in the clinical environment. Other studies demonstrated a slant towards the control aspects, [53] and highlighted the transformation of the micro-scale activation phenomena to macroscale activity and performance, relating electrophysiology, energy metabolism and cardiac mechanics, [54]. Efforts followed to elucidate the various parameters affecting cardiac performance, with emphasis on the ischemic heart [55] and the effect of the non-homogeneity of the cardiac muscle on its performance was reported, [56]. The studies included the basic micro-level phenomena that affect the cardiac system [57], with particular emphasis on the electrical activation, followed by the analysis of the interactions between these phenomena and the application of cardiac research to clinical practice [58].

The ventricular studies were based on real and assumed geometries and on electric and hemodynamic data obtained from animal studies and human patients. Aiming for better insight, the multifunctional organ approach was then related to the physiology of the muscle's fibers, the physical structure of the 3D heart and the systemic parameters of cardiac circulation, the electrical activation signals and the electrical characteristics of the cardiac function. Cardiac mechanics and cardiac energetics were related to the mechanical parameters and the instantaneous myocardial perfusion, which satisfies the metabolic needs of cardiac function. The overall goal was to achieve an integrated picture of the interacting parameters of muscle and vessel mechanics, blood circulation and myocardial perfusion, oxygen consumption and energy metabolism, electrical activation and heart rate, thus providing insight into the physiological and patho-physiological characteristics of the heart. New imaging techniques, which allow insight into the local and global cardiac performance [59], and new procedures for extracting engineering and clinical data by modern imaging and visualization techniques, were also pursued [60].

In-depth studies on the molecular and subcellular aspects of the cardiac system [61] were followed by analytical and quantitative investigations relating basic genetics to cardiac function, aiming to highlight the interface between structure and function at all levels of the cardiac system [62, 63]. The contributions of the micro-scale elements to the structure and function of the cardiovascular systems involved in-depth investigation of basic elements: i.e. cells, organelles, genes, molecules and ions [63], as well as the control mechanisms affecting the various interactions and cardiac functions [63]. *The challenge is to relate modeling, system analysis approaches, engineering principles and clinical physiology to the basic molecular and/or genetic factors and stimuli that determine the macroscale interactions and cardiac function.*

Chapter 3

Cardiac Energetics

Muscle contraction is accompanied by a simultaneous increase in its metabolic rate, which reflects the energetic costs of the processes activating the muscle, i.e. mobilizing intracellular Ca^{2+} for binding to the troponine/tropomyosin complex, so as to allow crossbridge (Xb) attachment of myosin to actin, as well as generating force and/or muscle shortening. The force generation accounts for 70–75% of the metabolic consumption, while the associated processes account for the rest [15].

Two levels of control mechanisms operate simultaneously to assure organ viability:

1. the essentially macro-scale maintenance of homeostasis,
2. the basically micro-scale dynamic control and response to transient demands.

3.1. Macro Scale Organ Analysis

The physiological and pharmacological determinants of whole heart energetics have been elucidated in the past 70–80 years, so that we can now quantify the energetic importance of the four major parameters of cardiac output, namely the heart rate, preload (end-diastolic pressure), afterload (mean systolic pressure) and contractility.

A number of mechanical indices were developed from the early 1960's. The most outstanding is Suga's, [68] model of the time varying elastance of the heart. Suga et. al, [64] reported the experimental result that the *total* mechanical energy of the heart, i.e. work plus potential energy, measured as

the pressure-volume-area, (PVA), in the pressure-volume plane, is linearly related to the myocardial O_2 consumption, mVO_2.

Suga's finding is consistent with the earlier observations of Fenn, [14] and Mommaerts, [42]. Fenn, [14] reported that the excess energy due to shortening is very nearly equal to the work done. Mommaerts, [42] noted that a muscle doing work mobilizes energy over and above that needed for activation and maintenance of tension, energy accounting for work and for the dissipation of energy accompanying the work process.

The PVA/time varying elastance model has dominated the field of cardiac energetics, [72], even though the ejection fraction remains the clinical contractility index. However, while Suga's elastance model contributed significantly to the understanding and quantification of cardiac contraction, this experimentally based model could not resolve a great number of physiological ambiguities. Some of the most intriguing phenomena included the explanations to Starling's Law, the Fenn Effect [14, 45], and the experimentally obtained linearity between power generation and energy consumption. For a recent review of past and present state of the art in cardiac research, see [15].

3.2. Micro Scale Analysis

Here we relate to the micro-scale intracellular phenomena of the cardiac muscle contraction.

As stated above, the cell is the common denominator of all living entities. Life is sustained by the continuous steady transport to and from the cells and the intracellular conversion of biochemical to mechanical energy and heat. The conversion involves molecular translocation and reversible biochemical reactions, modulated by complex nano-scale protein motoric machines. This cellular exchange of substances with their surroundings is achieved by diffusion and convection, and facilitated by active transport through the cells' membranes. The energy needed for the active transport as well as for motion and contractility is obtained by the chemo-mechanical transformation of chemical metabolic energy, and usually involves linear and rotary molecular motors utilizing the intracellular high-energy phosphate cycle of ATP→ADP+P and proton transfer, respectively.

Great progress has been achieved in our understanding of cardiac performance and the role of the cardiac cell and intracellular phenomena by

gaining insight into the intracellular characteristics of the cardiac calcium cycle, [5] and the Xb cycle, [23]. Clearly, knowledge of energy regulation at the molecular cellular level is needed in order to understand how the energy supply and demand are matched in different physiological situations, [3].

3.2.1. Intracellular Phenomena

As schematically presented in Fig. 2.2, and detailed in Sec. 4.1, the electrical stimulation of the cardiac muscle affects a transient response of calcium concentration in the SR and a consequent elevation of the intracellular concentration of calcium, which binds to the regulatory troponin protein units, activates the actomysin ATPase and facilitates Xb cycling. This cellular mechanism determines the consequent heart beat and the cyclic cardiac contraction in response to different loads [69, 76]. However, this open loop excitation-contracting coupling cannot explain the linear relationship between PVA and mVO_2, the energy consumption and mechanical energy production, and how the loading conditions affect the energy consumption.

As early as 1978, Bernard Katz, the Noble Laureate, suggested that there is some internal feedback in the active muscle, whereby the total energy liberation is regulated by the mechanical condition, [45]. Obviously, the muscle possesses a fundamental property that enables it to adjust its energy loss to prevailing mechanical constraints, [72].

Gibbs and Chapman [16, 10] have studied the chemo-mechanical transduction in the cardiac muscle and stated that a "stoichiometric slippage", which is essentially built into the models of Huxley [23] and Eisenberg and Hill [13], predicts no linearity between ATP consumption and "the manifestation of the pressure-volume (PV) or force-length potential energy". Following the 1994 model of Landesberg and Sideman, which includes two feedback mechanisms and explains the experimentally observed linearity [29], Gibbs, [15] has noted that "to explain the cardiac data there must be some autoregulatory mechanism at work in the myofilaments". Furthermore, Gibbs showed that there was no linear relation between the force-length areas (FLA) in the force-length plane in Suga's elastance theory "because too much extra energy was liberated when the (skeletal) muscles shorten" [15].

Suga [69, 76] modified Huxley's Xb model, but did not explain the desired linearity between energy consumption and power generation. This is not surprising, since the classical Huxley's model (Fig. 3.1) assumes that the

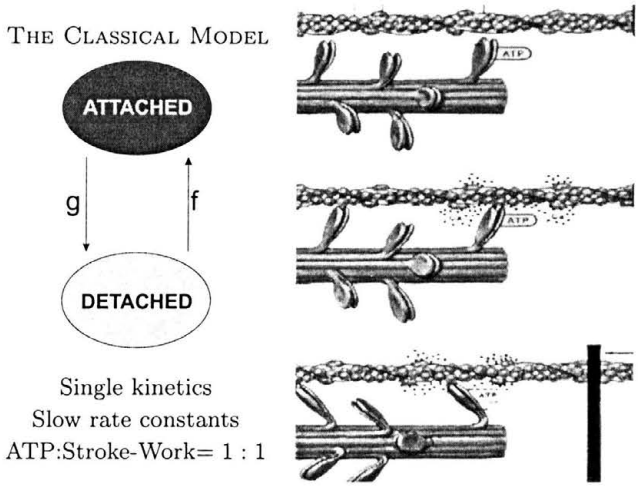

FIGURE 3.1. Left, the classical Huxley's model of a single kinetic rate for Xb attachment and detachment, $f = g$. Right, a schematic presentation of the attaching-detaching of the myosin Xbs and the actin filaments. Based on Netter's Atlas of Human Physiol. Icon learning pub. (permission requested)

chemical kinetics of calcium binding and ATP consumption is essentially the same as the mechanical kinetics of the attachment-detachment of the Xbs.

As shown by Landesberg and Sideman [29, 30] and discussed in detail in Chapter 4, these two kinetic rates, i.e. the chemical and the mechanical ones, f and g respectively in Fig. 3.1, are quite different in the four-state Landesberg-Sideman model, [29]. This new model, which includes a calcium-unbound but power generating state, predicts a linear relationship between the total energy consumed, i.e. consumed ATP (or MVO_2), and the energy produced, and resolves the other long standing enigmas. Furthermore, unlike Huxley's model, this new four-state model allows multiple work steps per ATP hydrolyzed. Also, the rate of Xb attachment-detachment in Huxley's model is a linear function of the displacement of the Xb from its equilibrium position, whereas the rate of Xb change from the strong, power producing state to the weak state in the four state model is a linear function of the shortening velocity.

Chapter 4

Intracellular Energetics: Consumption and Generation

4.1. Major Parameters Involved

Whereas man-made power generators transform the chemical energy of hydrocarbons into mechanical energy by utilizing heat or electrical energy, the muscles utilize chemical energy directly by transforming the chemical energy of ATP into mechanical energy. ATP, a common reactant in all living cells, links endergonic and exergonic processes and has three high-energy phosphate bonds. Normally, hydrolysis of one high energy bond by the hydrolytic enzyme ATPase yields adenosine diphosphate (ADP) plus phosphate (P) and liberates energy ($\Delta H = 30.6\,\mathrm{kJ/mol}$). ATP is then re-synthesized by ATPase from ADP and a new phosphate group, which is obtained from the hydrolysis of creatine phosphate, another highly exergenic reaction ($\Delta H = 35\,\mathrm{kJ/mol}$), [73]. This elementary biochemical cycle is nature's main energy source for generating work, affecting movement and driving secretion, electric conduction, ion pumps, and other energy demanding tasks in most living cells. Here we relate only to the contractile work of muscle contraction in the heart.

Contraction of the muscle occurs when the myosin heads attach to the actin molecules and form the actomyosin Xb [12, 9]. The motion of the Xbs causes the actin-myosin filaments to slide in opposite directions, thus shortening and contracting the sarcomere, Fig. 2.3. This parallel sliding of the thick and thin filaments is mobilized by the cyclic oar-like motion of the attachment and detachment of the Xbs, which are energized by the hydrolysis of ATP and activated by the ATPase enzyme. The Xbs contain the AT-

Pase enzyme, which modulates the biochemical activity of the Xb. Note that a single attachment-detachment cycle shortens the single sarcomere by approximately 5–8 nm (less than 1% of its length), while the fibrils shorten by about 15% of their 2 μm length during the contraction cycle. Consequently, the Xbs attachment-detachment cycle must be repeated many times during a single contraction cycle, so as to affect the full contraction range of 0.6 to 1.0 μm per beat. One ATP hydrolysis affects one Xb turnover of the contraction cycle [13]. The activity of the Xbs is thus energetically sustained by ATP hydrolysis and, as discussed below, this activity is regulated by the free calcium ions (Ca^{+2}) within the cell.

The Ca^{+2} enters cell's fluid media, the cytoplasm, through the external cell's membrane channels during repolarization. The Ca^{+2} in the cell is stored in the sarcoplasmic reticulum (SR), a dense intracellular membranous network that plays a key role in excitation-contracting coupling by its ability to regulate the intracellular Ca^{+2} cycling, [24]. The membrane of the SR contains an active transport system—ion pumps—for the uptake of Ca^{+2} from the cell's cytoplasm. The Ca^{+2} is then available for release into the cytoplasm at subsequent contractions. The intracellular Ca^{+2} in the skeletal muscle is mobilized from the SR by the action potential and its concentration is independent of the extracellular Ca^{+2} concentration. In contrast, the intracellular Ca^{+2} concentration in the cardiac muscle depends on the extracellular Ca^{+2} concentration, although some 85% of the Ca^{+2} is supplied by the SR in each cycle, [24]. The rate and extent of myocardial relaxation is determined by the rate and extent of Ca^{+2} uptake from the cytoplasm into the SR; the rate and extent of myocardial contraction is determined by the rate and extent of Ca^{+2} released from the SR into the cytoplasm and available for binding to the troponin complex, the regulatory proteins of the contractile filaments. Clearly, the control of Ca^{+2} transport across the SR membrane is a fine tuning mechanism of regulating muscle relaxation and the heart's response to increased work demand. The mechanisms of Ca^{+2} transients to and from the SR are presented elsewhere, [18, 17].

A linear relationship exists between the energy consumption, measured by the oxygen consumption, and the mechanical energy generated by the left ventricle (LV) of the heart measured by the pressure-volume area (PVA), [48, 65]. A similar relationship was found between oxygen consumption and energy production in the isolated cardiac fibers, [20, 41], as measured by the force-length area, (FLA). This linearity phenomenon and the underly-

ing mechanism of cardiac mechanics [6, 8] and cardiac energetics [20] have recently been elucidated to be based on Xb dynamics and the intracellular control of contraction, [26, 27, 29–32]. As discussed below, this linear relationship is attributed to the intracellular power generating elements of the muscle, the actin-myosin Xbs, which are the motoric units of the muscle.

Having briefly discussed the major parameters involved in the contraction-relaxation cycle of the cardiac muscle, we proceed to explore the mechanisms that control the contraction phenomena and address the quantitative characteristics of the basic phenomena of cardiac contraction. These include the relationship between the energy consumed by the Xbs and the work they generate; the efficiency of chemical to mechanical energy conversion by the Xb, and muscle economy, i.e. the energetic conditions under which the myocardium develops force. Deciphering the regulation of the conversion of biochemical to mechanical energy by the Xbs is clearly of great importance in the understanding of the performance of the normal and failing hearts.

4.2. Physiological Model

4.2.1. Actomyosin-Troponin Complex

Figure 4.1 represents the schematic structure of the contractile actomyosin complex, formed by the thin actin and thick myosin filaments and the oar-like Xb. The troponin-tropomyosin complex regulates Xb cycling, [44], Fig. 2.3. The tropomyosin winds around the whole actin filament, and is part of the on/off switching mechanism of the muscle. Troponin is a protein with three sub-units C, I and T. TN-C has the calcium binding sites, TN-I acts

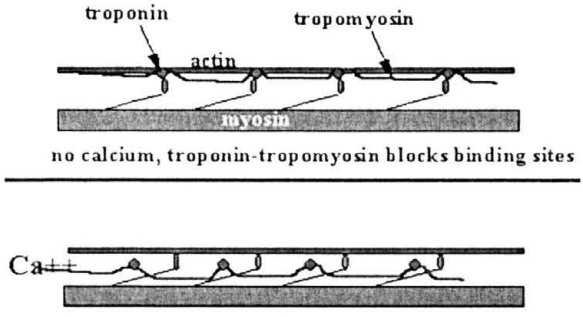

FIGURE 4.1. Schematic presentation of the actomyosin-troponin complex. Taken from the Internet. Author unknown.

as a guard against the attachments of the Xbs and is inactivated only when TN-C is saturated by Ca^{+2}. TN-T binds to tropomyosin.

4.2.2. The Four-State Model of the Actomyosin-Troponin Complex

The analysis of cardiac mechanics, and the ability to simulate the cardiac muscle dynamics and myocardial energetics, depends on a coherent description of the complex relation between calcium kinetics and Xb cycling in the sarcomere. Our four-state model (Fig. 4.2) of the actomyosin-troponin complex is based on coupling the kinetics of calcium binding to troponin with the regulation of Xb turnover. Since the troponin complex plays a key role in the regulation of Xb cycling, we define the regulatory unit of the sarcomere as a single troponin complex, which includes the adjacent Xb and actin binding sites. The different troponin regulatory units are divided into four groups of states [29, 30] by relating to two criteria: 1) Calcium is either bound or unbound to the low-affinity sites of the troponin, and 2) the Xbs are either in the strong, power generating, conformation or in the weak, not power generating, conformation. Hence, the four states in Fig. 4.2 provide the probability to find the regulatory units at any state.

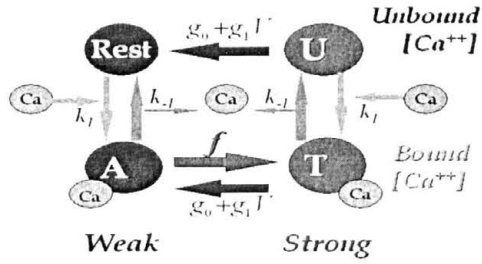

FIGURE 4.2. The four states model relating Ca^2 kinetics to cross-bridge (Xb) dynamics [29]

State R represents the rest state wherein the Xbs are in the weak conformation and no calcium is bound to the troponin. State A denotes the regulatory units already 'activated' by calcium binding to troponin (hence the notation 'A'), but the adjacent Xbs are still in the weak conformation: Thus, state A represents the number of Xbs that can turn from the weak to the strong force generation conformation. Xb cycling leads to state T, where calcium is bound to the low-affinity sites and the Xbs are now in the strong, tension-generating conformation (hence the notation 'T'). Calcium

dissociation at state T leads to the unbound calcium state U in which the Xbs are in the strong conformation, but without bound calcium (hence the notation 'U').

The transitions between the states are defined by calcium kinetics, Xb cycling and the filaments sliding velocity. Note, that the transition from U to R is unidirectional, corresponding to Assumption 3 below.

4.2.3. Some Basic Assumptions

The basic assumptions underlying the model are detailed elsewhere, [29, 30] and the most relevant ones are briefly listed here for coherency and convenience:

1. The Xb cycles in a repeated oar-like motion between the weak non-force generating conformation and the strong force generating conformation, due to nucleotide binding and release, [44]. The Xb turnover from the weak to the strong conformations requires the hydrolysis of one molecule of ATp. Energy consumption is thus proportional to the total amount of Xbs turning over from the weak to the strong conformation.
2. Calcium binding to the troponin regulatory sites regulates ATP hydrolysis by actomyosin ATPase, which is required for the transition of the Xbs to the strong conformation, [9]. Thus, calcium binding regulates Xb recruitment and the energy consumption by the sarcomere.
3. Calcium can dissociate from the troponin before the Xbs turnover to the weak conformation; Xbs can exist in the strong conformation without having bound calcium on the adjacent troponin, [44].
4. The affinity of troponin to calcium depends on the number of Xbs in the strong conformation. This mechanism is denoted as the *positive cooperativity feedback* mechanism, [29, 31].
5. The rate of Xb turnover from the strong to the weak conformation is affected by the Xb strain rate and thus by the filament sliding velocity, [13]. This feedback loop is denoted as the *negative mechanical feedback*, [27, 31]. The linear dependence of the weakening rate on the shortening velocity V is given by: $g = g_0 + g_1 V$ where g_0 is the rate of Xb weakening in the isometric regime and g_1 is the mechanical feedback coefficient.
6. The individual Xbs act like Newtonian viscoelastic elements, [70]: the average force-velocity relationship (FVR) of a single Xb is linear [32].

4.2.4. Intracellular Controlling Mechanisms

The two intracellular feedback mechanisms, noted in Assumptions 4 and 5 and depicted in Fig. 4.3, regulate the energy consumption and the mechanical energy generation, [30, 27]. These mechanisms have been substantiated at the isolated cardiac fiber (trabeculae) level, [36, 37], and in-vivo at the whole heart level, [38] and were recently further validated experimentally and analytically, (see Sec. 4.5.9 below).

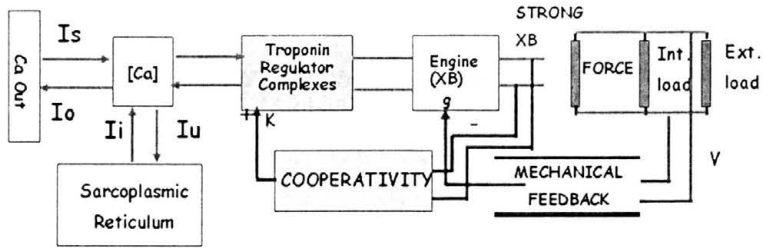

FIGURE 4.3. Schematics of the physiological model depicting the positive and negative feedback mechanisms and calcium release and uptake from the sarcoplasmic reticulum (SR)

The positive cooperativity feedback mechanism regulates the kinetics of Ca^{+2} binding to troponin. It defines the dependence of the affinity of the troponin-C for Ca^{+2} on the number of Xbs in the strong, force-generating, conformation. Consequently, it regulates ATP consumption by the Xb, Xb recruitment and the force generation. The cooperativity mechanism explains the reported length-dependent calcium sensitivity, [26], the related force-length relationship (FLR) and the related Frank-Starling Law, [29, 26, 27]. Lengthening the sarcomere increases the number of force-generating Xbs along the thin actin filament and thus elevates the affinity of troponin for calcium through the cooperativity feedback. Since the cooperativity mechanism determines the amount of bound calcium, it regulates the energy consumption by the sarcomere [29, 30, 27, 31].

The negative mechanical feedback describes the effect of the sliding motion, i.e. sarcomere shortening, on the biochemical rate of Xb turnover from the strong to the weak conformation ('weakening'). This concept was inspired [29, 30] by biochemical studies, [13], which suggest that an increase in the filament sliding velocity increases the rate of Xb 'weakening', i.e. the Xb turnover from the strong to the weak conformation. An increase in the shortening velocity decreases the time over which the Xbs stay in strong con-

formation and consequently decreases the number of force generating Xbs. The mechanical feedback determines the force-velocity relation (FVR) in the fiber as well as in the whole heart, and regulates the generation of mechanical power. Moreover, the mechanical feedback provides the linear relationship between energy consumption by the sarcomere and the generated mechanical energy, [27, 32].

As noted here, the cooperativity mechanism determines the amount of free energy available for consumption, while the mechanical feedback determines its conversion to mechanical energy. Consequently, cardiac muscle performance, and its so-called 'contractility' are determined by the interplay between the loading conditions and these two intracellular feedback control mechanisms.

It is noteworthy that Xb dynamics is characterized by two distinct kinetic phenomena: 1) a relatively slow Xb turnover cycle, which relates to the biochemical reactions of nucleotide binding and dissociation, and 2) a very fast attachment/detachment cycle, which relates to the viscoelastic properties of the sarcomere, [27, 32]. Clearly, multiple work-producing strokes (i.e. attachment/detachment cycles) must occur per hydrolysis of a single ATP molecule [7], so that the Xbs can affect the full range of the sarcomere shortening in the contraction/relaxation cycle. The present study relates only to the 'slow' Xb turnover between the four states and the energetics associated with this cycle.

4.3. Mathematical Model

4.3.1. Interaction between the State Variables

We define $\bar{R}$, $\bar{A}$, $\bar{T}$, $\bar{U}$, as the densities (i.e. number of troponin units per unit length in the relevant state) of the four troponin states existing within the overlap region between the actin and myosin filaments. The transitions between the density state variables (Figs. 4.2, 4.3) are given by [29, 27]:

$$\begin{bmatrix} \dot{\bar{R}} \\ \dot{\bar{A}} \\ \dot{\bar{T}} \\ \dot{\bar{U}} \end{bmatrix} = \begin{bmatrix} -k_l[\mathrm{Ca}] & k_{-l} & 0 & g_0 + g_1 V \\ k_l[\mathrm{Ca}] & -f - k_{-l} & g_0 + g_1 V & 0 \\ 0 & f & -(g_0+g_1) - k_{-l} & k_l[\mathrm{Ca}] \\ 0 & 0 & k_{-l} & -(g_0+g_1 V) - k_l[\mathrm{Ca}] \end{bmatrix} \cdot \begin{bmatrix} \bar{R} \\ \bar{A} \\ \bar{T} \\ \bar{U} \end{bmatrix} \tag{4.1}$$

where [Ca] denotes the free calcium ion concentration. The rate coefficients k_l and k_{-l} represent the rate constants of calcium binding to, and calcium

dissociation from, the low affinity sites of troponin. Note that the rate coefficient k_{-l} is not constant, since the cooperativity mechanism dictates the dependence of this coefficient on the state variables, [29, 31]; Xb cycling is described by f, g_0 and g_1. The rate of Xb turnover from the weak to the strong conformation is denoted by f and g_0 is the rate of Xb weakening (transition from strong to weak conformation) in the isometric regime; g_1 is the mechanical feedback coefficient, which describes the effect of the filament shortening velocity on the rate of Xb weakening (units of 1/m); V is the sarcomere shortening velocity.

4.4. Energy Consumption and Force Generation

4.4.1. Energy Requirement for Xb Turnover

As stated above, we consider here only the rate limiting processes in Xb dynamics, which relate to Xb cycling between the weak and the strong conformation rather than to the attachment/detachment phenomena, [30, 7].

The transition from state A to state T describes the Xb turnover from the weak to the strong conformation, which requires the hydrolysis of one ATP molecule (and one phosphate release) for each Xb turnover, [13]. Thus, the rate of energy consumption, $\dot{E}(t)$, i.e. the rate of ATP hydrolysis by the actomyosin, is determined by the number of Xbs available in state A that change state to T and by the weak-to-strong turnover rate, f, and is given by

$$\dot{E}(t) = \bar{E}_{\mathrm{ATP}} f L \bar{A}(t) \tag{4.2}$$

where L is the length of the overlapping actin-myosin filaments. All the Xbs along the overlapping myosin are at the same level of activation, $\bar{A}(t)$, at any given time; $\bar{E}_{\mathrm{ATP}}$ is the free energy of hydrolysis of a single ATP molecule. The energy consumption, E, of the actomyosin filament during the whole twitch cycle of duration, θ, is thus given by

$$E = \bar{E}_{\mathrm{ATP}} f L \int_0^{\theta} \bar{A}(t) dt. \tag{4.3}$$

4.4.2. Force Generation by the Sarcomere

The force F generated by the sarcomere is the product of the density of force generating Xbs, $(\bar{T} + \bar{U})$, the overlap length L and the unitary aver-

age force $\bar{F}$, generated by each Xb. Neglecting the internal load, [26], the generated force is given by [27]:

$$F = L(\bar{T} + \bar{U})(\bar{F} - \eta V) \tag{4.4}$$

where, according to assumption 6, η represents the viscous property of the Xb, [70]. The change in the number of force generating Xbs, $(\bar{T} + \bar{U})$, is derived from Eq. (4.1), which gives:

$$\frac{d(\bar{T} + \bar{U})}{dt} = f\bar{A} - (g_0 + g_1 V)(\bar{T} + \bar{U}) \tag{4.5}$$

The relationship between the rate of force generation and the rate of change in the sarcomere shortening velocity is derived from Eqs. (4.4) and (4.5), and is given by:

$$\frac{dF}{dT} = \left(LfA\bar{F} - g_0 F\right) - \left(\left(g_1 + L^{-1}\right) F + LfA\eta\right) V - \eta \frac{F}{\bar{F} - \eta V} \frac{dV}{dt}. \tag{4.6}$$

At peak isometric force $F = F_M$, $dF/dt = 0$, $dV/dt = V = 0$ and Eq. (4.6) reduces to:

$$LfA\bar{F} = g_0 F_M. \tag{4.7}$$

Note that Eq. (4.7) allows to evaluate state A experimentally. Utilizing Eq. (4.7) in Eq. (4.6) yields:

$$\frac{dF}{dT} = g_0(F_M - F) - \left(\left(g_1 + L^{-1}\right) F + \frac{g_0 F_M}{V_U}\right) V - \eta \frac{F}{\bar{F} - \eta V} \frac{dV}{dt} \tag{4.8}$$

where $V_U = \bar{F}/\eta$ is the unloaded shortening velocity, [30].

The shortening velocity, which is obtained from Eq. (4.8) at steady state FVR (i.e. $dF/dt = 0$; $dV/dt = 0$) yields the molecular-based expression for the well established, experimentally derived, Hill's equation for the FVR, [30, 21].

$$V_{\text{HILL}} = b_H \frac{F_M - F_{\text{HILL}}}{F_{\text{HILL}} + a_H} \quad \text{or} \quad F_{\text{HILL}} = \frac{b_H F_H - a_H V_{\text{HILL}}}{b_H + V_{\text{HILL}}} \tag{4.9}$$

where V_{HILL} and F_{HILL} denote the steady state velocity and force, respectively. Hill's experimental constants, a_H and b_H are now given by:

$$a_H = \frac{g_0}{g_1 + L^{-1}} \frac{F_M}{V_U} \approx b_H \frac{F_M}{V_U}, \quad b_H = \frac{g_0}{g_1 + L^{-1}} \approx \frac{g_0}{g_1}. \tag{4.10}$$

4.4.3. Efficiency of Energy Conversion

We now address the general case of energy conversion. Substituting the number of regulatory units in the strong conformation from Eq. (4.4), $(\bar{T} + \bar{U}) = F/L(\bar{F} - \eta V)$ into Eq. (4.5) and integrating over the twitch duration from zero to θ yields, [32]

$$0 = \int_0^\theta Lf\bar{A}(t)dt - \int_0^\theta (g_0 + g_1 V)\frac{Fdt}{\bar{F} - \eta V} \tag{4.11}$$

where $V = V(t)$ and $F = F(t)$. Note that the integrated left side of Eq. (4.5) is zero as both $\bar{T}$ and $\bar{U}$ return to their initial values at $t = \theta$, the end of the twitch. Utilizing Eq. (4.2), Eq. (4.11) gives:

$$\frac{E}{\bar{E}_{\text{ATP}}} = \int_0^\theta (g_0 + g_1 V)F\frac{dt}{1 - V/V_U}. \tag{4.12}$$

Using the equality: $1/(1-x) = 1 + x + x^2/(1-x)$ gives:

$$\frac{E}{\bar{E}_{\text{ATP}}} = \int_0^\theta (g_0 + g_1 V)F\left(1 + \frac{V}{V_U} + \frac{(V/V_U)^2}{1 - (V/V_U)}\right)dt. \tag{4.13}$$

Rearrangement provides the general equation for energy conversion:

$$\frac{E}{\bar{E}_{\text{ATP}}} = \frac{g_0}{\bar{F}}\int_0^\theta Fdt + \frac{1}{\bar{F}}(g_1 + g_0/V_U)\left(\int_0^\theta FVdt + \int_0^\theta \frac{FV^2}{V_U - V}dt\right). \tag{4.14}$$

Dividing both sides of Eq. (4.14) by $(g_1 + g_0/V_U)/\bar{F}$, gives:

$$\rho E = W + E_{\text{pp}} + Q_\eta \tag{4.15}$$

where:

$$\begin{aligned} \rho &= \frac{\bar{F}V_U}{\bar{E}_{\text{ATP}}}\frac{1}{g_0 + g_1 V_U}, \qquad W = \int_0^\theta FVdt, \\ E_{\text{pp}} &= \frac{g_0 V_U}{g_0 + g_1 V_U}\int_0^\theta Fdt, \qquad Q_\eta = \int_0^\theta \frac{FV^2}{V_U - V}dt = \eta\int_0^\theta \frac{FV^2}{\bar{F} - \eta V}dt. \end{aligned} \tag{4.16}$$

Here ρ is the conversion efficiency of the biochemical to mechanical energy. $E_{\rm pp}$ is the pseudo-potential energy, and is proportional to the force-time integral, FTI; Q_η represents energy dissipation due to the viscoelastic property of the Xb since it is proportional to η and is given by the integral over the force multiplied by the square of the velocity, (and by even higher degree of the velocity) since

$$\frac{x^2}{1-x} = x^2 + x^3 + x^4 + \ldots, \quad x = V/V_{\max}. \tag{4.17}$$

Note that the viscoelastic contribution to the mechanical energy generation of the Xb is negligible at slow shortening velocities, where $V < V_{\rm U}$, and Eq. (4.14) reduces to:

$$\frac{E}{\bar{E}_{\rm ATP}} \approx \frac{g_1}{\bar{F}} W + \frac{g_0}{\bar{F}} \,\mathrm{FTI} \tag{4.18}$$

or

$$\rho^0 E = W + E^0_{\rm pp} \tag{4.19}$$

where

$$\rho^0 = \frac{\bar{F}}{\bar{E}_{\rm ATP} g_1} \quad \text{and} \quad E^0_{\rm pp} = \frac{g_0}{g_1} \,\mathrm{FTI} \tag{4.20}$$

are first approximations for the conversion efficiency and the pseudo-potential energy, respectively.

4.4.4. Economy of Force Generation

Under isometric conditions, whereby $V = 0$, $W = 0$, and $Q_n = 0$, Eq. (4.15) reduces to

$$\rho_{V=0} = \frac{E_{\rm pp}}{E}. \tag{4.21}$$

Equation (4.21), which is derived from the analysis of Xb dynamics and calcium kinetics, provides insight into the economy of power generation, as it represents the ratio of the FTI and the energy consumption by the Xbs, per beat. Moreover, it highlights the functional importance of the FTI in the contraction cycle.

Consistent with Eq. (4.21), the commonly used practical definition of muscle economy, ε, is an experimentally determined quantity based on measurements of the heat and force generated by the stimulated muscle under isometric conditions, [43]. In this experimental definition, the economy represents the ratio of the FTI to the tension-dependent-heat, TDH associated with the

myosin Xbs cycling in the isometric state. Thus, TDH corresponds to E, the energy consumption by the Xbs in the present analysis. Note that tension generation is the major determinant of energy demand during contraction, [11], as compared to the tension independent heat, TIH associated with ionic uptake, (see discussion below).

Referring to Eq. (4.18), with $W = 0$ and $E = \text{TDH}$ in the isometric contraction, we obtain the biochemically based expression for the experimentally defined economy

$$\varepsilon \equiv \frac{\text{FTI}}{\text{TDH}} = \frac{\text{FTI}}{E} = \frac{\bar{F}}{\bar{E}_{\text{ATO}}} \frac{1}{g_0} \tag{4.22}$$

indicating that the economy is inversely related to g_0. Obviously, since $\bar{F}/\bar{E}_{\text{ATP}}$ is a known quantity, Eq. (4.22) allows to determine g_0, the rate of Xb weakening under isometric conditions.

Returning to Eq. (4.17), we note that the rate constants g_0 and g_1 define the rate of energy conversion to heat and work, respectively.

Finally, the relationship between muscle economy (Eq. (4.21)) and muscle efficiency (Eq. (4.16)) is given by:

$$\frac{\varepsilon}{\rho} = \frac{1}{V_U} + \frac{g_1}{g_0}. \tag{4.23}$$

4.4.5. Simulating Isometric Contraction and Energy Consumption

The utility of Eq. (4.1) can be demonstrated by simulating the Xb system characteristics involved in the isometric contraction of the sarcomere. To that end, we need five state variables: the free calcium concentration [Ca], the three regulatory troponin states: $\bar{A}$, $\bar{T}$, $\bar{U}$, and the calcium bound to the troponin. Here we distinguish between calcium binding to the high-affinity troponin site (TR_{BH}), which plays a major role in calcium buffer binding (negligible here), and to the low-affinity sites (TR_{BL}), which regulate the actomyosin ATPase activity, [9] required for Xb transition to the strong conformation, [13]. Other variables used in the calculation are derived from these variables. For example, the troponin regulatory state $\bar{R}$ is given by:

$$\bar{R} = \text{TR}_0 - \bar{A} - \bar{T} - \bar{U} \tag{4.24}$$

where TR_0 is the total troponin concentration per unit length of filament. The calcium bound to the troponin low-affinity sites, TR_{BL}, is given by $(\bar{A} + \bar{T})$.

The isometric concentration of the immobile system is described by the following set of five ordinary differential equations, [31]:

$$\begin{cases} \dfrac{d[\mathrm{Ca}]_f}{dt} = I_i + I_s - I_u - I_o - \dfrac{d[\mathrm{TR_{BH}}]}{dt} - \dfrac{d(A+T)}{dt}, & \text{(a)} \\ \dfrac{d[\mathrm{TR_{BH}}]}{dt} = (2\mathrm{TR}_o - \mathrm{TR_{BH}})[\mathrm{Ca}]k_h - \mathrm{TR_{BH}}k_{-h}, & \text{(b)} \\ \dfrac{d\bar{A}}{dt} = k_l[\mathrm{Ca}]\bar{R} - (f + k_{-l})\bar{A} + g_0\bar{T}, & \text{(c)} \\ \dfrac{d\bar{T}}{dt} = f\bar{A} - (g_0 - k_{-h})\bar{T} + k_h[\mathrm{Ca}]\bar{U}, & \text{(d)} \\ \dfrac{d\bar{U}}{dt} = -k_{-l}\bar{T} - (g_0 + k_h[\mathrm{Ca}])\bar{U}. & \text{(e)} \end{cases} \tag{4.25}$$

As seen in Fig. 4.3, the free calcium transient is determined by the calcium release rate from the SR, (I_i); the calcium inward current through the sarcolemma, (I_S); the efflux through the sarcolemma, (I_0); the calcium uptake rate by the SR, (I_u); the calcium binding rate to the troponin high-affinity sites, $d\mathrm{TR_{BH}}/dt$, and the calcium binding rate to the low-affinity troponin regulatory sites, $d\mathrm{TR_{BL}}/dt = d(A+T)/dt$. The simple phenomenological model used here for the description of the sarcolemmal and the SR calcium transients was described elsewhere, [29]. Calcium binding to the troponin high-affinity sites is described by the second differential equation, where k_l and k_{-l} are the calcium association-dissociation rate constants. The last three differential equations in Eq. (4.25) are obtained from Eq. (4.1) and describe the distribution of the troponin regulatory units between the four states.

Regulation of energy consumption. The rate of Xb recruitment and energy consumption is determined by State A, the activation level. The rate of change of the activation level is derived from Eq. (4.25)(c). It is instructive to note that in the absence of a cooperativity feedback mechanism, the coefficients k_l, k_{-l}, f, and g_0 in Eq. (4.25)(c) are constants. The right-hand side of Eq. (4.25)(c) is, therefore, length-dependent, suggesting that the activation level A and the consequent energy expenditure calculated without a cooperativity mechanism, are independent of the sarcomere length. However, the isometric force depends on the sarcomere length. Hence, if all the coefficients are constant, there is dissociation between energy consumption

and force generation: the energy expenditure is constant and independent of the muscle length, whereas the force is length dependent. Consequently, the experimentally observed linear relationship between energy consumption and the generated mechanical energy cannot be analytically reconstructed in the absence of a cooperativity mechanism wherein f, g_0, k_l and k_{-l} are assumed to be constant. Thus, the analytical expressions for energy consumption Eq. (4.2), force generation Eq. (4.4), and the rate of change in the activation level Eq. (4.25)(c) imply that at least one of these coefficients is not constant. Since the rate of Xb cycling (f and g_0) is length independent in the isometric regime and the rate of calcium binding to troponin k_l is diffusion limited, the varying coefficient is k_{-l}, the rate of calcium dissociation from troponin, consistent with our suggested cooperativity mechanism.

The cooperativity mechanism implies that the affinity of the regulatory site for calcium ($K_{[\mathrm{Ca}]}$) is determined by the number of force-generating Xbs, which is defined by the number of troponin units in the strong conformation, $(\bar{T}+\bar{U})$, in the actin-myosin overlap region. Based on our previous studies [29, 30, 26], $K_{[\mathrm{Ca}]}$ is a monotonic function of $(\bar{T}+\bar{U})$, and can be described by a simple polynomial function:

$$K\,[\mathrm{Ca}] = \frac{k_k}{k_{-l}} = K_0 + K_1(\bar{T}+\bar{U}) + K_2(\bar{T}+\bar{U})^2 \tag{1.26}$$

where K_0, K_1 and K_2 are constants.

The system of the ordinary differential equations (4.25) has been integrated by the fourth order Runga-Kutta method, utilizing 'Matlab' subroutine: (`ode45.m`) on a PC. The chosen tolerance was 10^{-8} for the integration steps. The values of the coefficients used for the simulation are taken from [29]: $L = 1.4\,\mu\mathrm{m}$, $k_h = 10^8\,\mu\mathrm{M}^{-1}\mathrm{s}^{-1}$, $k_{-h} = 0.33\,\mathrm{s}^{-1}$, $K[\mathrm{Ca}] = 2\times10^6\,\mu\ \mathrm{M}^{-1}$, $k_l = 4\times10^7\,\mu\mathrm{M}^{-1}\mathrm{s}^{-1}$, $f = 40\,\mathrm{s}^{-1}$, $g_0 = 12\,\mathrm{s}^{-1}$, $g_1 = 10\,\mu\mathrm{n}^{-1}$.

These simulated isometric contractions at various sarcomere lengths (SL) include, [28]: A) time course of force development; B) effect of SL on energy consumption and peak force generation; C) force-length area (FLA) and the force-time integral (FTI) as related to the total energy consumption by the Xbs. The simulation [28], demonstrates the linearity, and the similarity, of these two characteristic properties, FLA and FTI, which is sustained by the cooperativity feedback mechanism.

4.5. Discussion

4.5.1. Ventricular and Xb Energetics

The basic hypothesis underlying the present study is that the performance of the LV is based on the intracellular control of contraction, i.e. calcium kinetics and Xb cycling. The analysis demonstrates that energy consumption and power generation by the sarcomere are intrinsic properties of the muscle cells. Furthermore, as seen by Eq. (4.15) the kinetic relationship between energy consumption and the generated mechanical energy is determined by the mechanical feedback at the sarcomeres' Xb level and, consequently, in the tissue and the whole cardiac muscle. This conclusion is consistent with the well-known experiments of Suga et al. [48, 65] at the whole LV level, demonstrating that a linear relationship exists between the oxygen consumption, V_{O_2}, and the generated mechanical energy. The latter is quantified by measuring the PVA of one full beat on the P-V plane. Thus, [65]

$$V_{O_2} = a\text{PVA} + b \tag{4.27}$$

where a and b are constants. Note that a is the slope of the V_{O_2} vs. PVA curve and represents the O_2 consumption per unit PVA. Consequently, $1/a$ is the ventricular analog of the sarcomere contraction efficiency defined by Eq. (4.18). The constant b represents the energy required for Ca^{+2} uptake, the Na/K exchange across the various cellular membranes and the basal metabolic consumption.

The mechanical energy generated by the LV, as given by the PVA, corresponds to the energy generated by the Xbs from ATP hydrolysis by the actomyosin ATPase. PVA represents the sum of the external work W done by the LV and the mechanical potential energy, PE. Thus, [48, 65]

$$\text{PVA} = \text{W} + \text{PE}. \tag{4.28}$$

Eq. (4.28) is consistent with Eq. (4.15) since the term Q_η, which describes the energy requirement due to the viscoelastic property of the XL, is negligible, especially for small shortening velocities ($V < V_u$). Thus, V_{O_2} is linearly related to the ATP consumption expressed in terms of E, the energy consumed by the contractile filaments per beat. PVA (= W + PE) corresponds to the sum of the work and potential energy generated by the contractile filaments as given by ($\text{W} + \text{E}_{\text{pp}}$) in Eq. (4.18). Note, however, that Eq. (4.27) was developed experimentally for the whole LV and includes the constant b, which accounts for the energy consumed for basal metabolism and the ion pumps.

In practice, the total energy utilization is quantified by measuring the cardiac oxygen consumption V_{O_2} and its biochemical equivalence is 1 ml O_2 = 20 J ($\pm 5\%$), depending on the metabolic substance [65]. The relationship between heat output and the isometric force can be put in molecular terms by calculating the FTI of a single Xb (FTI_{Xb}). Muscle economy, ε, is defined as the ratio of the isometric FTI and the TDH. The muscle FTI is the sum of all FTI_{Xb} developed by each Xb cycle (in half a sarcomere), i.e. FTI = $N_{Xb}FTI_{Xb}$ where N_{Xb} is the number of Xbs cycles that were used during the twitch. Utilizing the TDH and the enthalpy of hydrolysis of one high energy phosphate bond in each Xb cycle (34 kJ/mole) one can calculate N_{Xb}, the number of Xbs involved in the development of isometric FTI, and hence the value of FTI_{Xb}. For example, the calculated average normal rabbit FTI= 0.36 ± 0.06 pN sec, and about half of this value in the thyrotoxic hypertropic rabbit heart [1]. The FTI_{Xb} is inversely proportional to the isometric rate of Xb weakening. Hence, the cardiac muscle can modulate its economy by modulating g_o: the lower the isometric weakening rate, the higher is the economy.

4.5.2. Pseudo-Potential Energy

The potential energy (PE) term in Eq. (4.28) originates in Suga's elastance model [65] which assumes that the energy consumed during the isometric contractions is stored in the LV wall as elastic potential energy. The PE in the elastance model suggests that the contraction phenomenon represents a physical conservation field as an ideal spring, and that the potential energy can be fully recovered. This is evidently not the case. As seen from Eq. (4.15), the energy consumption in the isometric contraction ($W = 0$) is proportional to FTI, the force-time-integral. E describes the energy used for Xb recruitment during contraction, but only part of it can be utilized to generate external work. Hence, the energy is stored in the Xbs. However, not all of them are active at any given point of time during the twitch. The Xbs have a limited duty cycle, and the energy used by the Xb cannot be reutilized if the Xb has turned back to the weak state. Consequently, we suggest, [31, 32], that the pseudo-potential energy concept, which relates to the FTI, is a more appropriate term than the classical PE concept based on the elastance model of cardiac contraction and can better describe cardiac mechanics and energetics.

4.5.3. Tension Dependent Heat (TDH)

The experimental evaluation of TDH and ε is based on measuring the total activity-related heat production, T_{A}, given by [2]:

$$T_{\mathrm{A}} = \mathrm{Re} + \mathrm{TDH} + \mathrm{TIH} \tag{4.29}$$

where the recovery heat, Re, is the heat output associated with the mitochondrial resynthesis of ATP (from ADP + P), and TIH denotes the tension-independent-heat associated with the excitation-contraction phenomena and describes mainly the energy used for Ca^{+2} uptake. TDH, the tension-dependent heat is associated with Xb cycling, and can be calculated from Eq. (4.29) since the myothermal data yields T_{A}, Re and TIH. Typical values in normal human hearts: TDH $= 3.39 \pm 0.66\,\mathrm{mJ/g\text{-}beat}$ and TIH $= 0.51 \pm 0.13\,\mathrm{mJ/g\text{-}beat}$ for an isometric peak force of $25.9 \pm 3.9\,\mathrm{mN/mm^2}$ and time of peak tension of $189 \pm 9\,\mathrm{msec}$, [2].

4.5.4. Economy vs. Efficiency

The efficiency of biochemical to mechanical energy conversion is never 100%, even when proceeding close to equilibrium. The lost energy appears in the cell as heat, used to maintain the organism at a temperature above that of its surroundings and, not incidentally, to facilitate faster biochemical reactions. The significant difference between the bio-conversion efficiency and the thermodynamic efficiency of the whole contraction process is particularly noteworthy. Taking for example the oxidation and transformation of one mole of glucose to mechanical work, we observe a thermodynamic efficiency of 20–25%. However, the efficiency of the transformation of ATP to mechanical energy by the motor units is close to 70%, which is not surprising in view of the very high efficiency of the actin-myosin molecular motors. Examining this apparent 'discrepancy', we note that close to half of the 'wasted' energy dissipates as heat and the other half is spent on maintaining the numerous control mechanisms that assure the steady function of the cell, i.e. the organism. These control mechanisms include the $Na^{+}K^{+}$ and Ca^{+2} pumps, as well as other elements involved in the continuous maintenance of the living organism.

In practical terms based on the whole LV, the myocardial economy relates the FTI during isometric contraction to the myocardial oxygen (or its ATP equivalent) consumption, [1]. The efficiency index, on the other hand,

describes the utility of the ventricle as a generator of external work [1] The different responses of these two indices to cardiac stress have so far cefied their characterization within a single analytical framework. Two physiological examples are illuminating.

1. The heart muscle employs similar strategies to meet chemically incuced (catecholamine) and shape induced (hypertrophy) changes in demand. Thus, catalcholamine as well as hypertrophy affect an increase in power efficiency and a decrease in economy, [1].
2. Tortoise muscles are slow, have high economy and develop low power, i.e. they work with a low efficiency. In contrast, the frog's muscles are fast, have low economy and high power output. The higher economy of the tortoise is expressed by the higher FTI for a given energy consumption, [19]. The frog/tortoise isometric economy ratio is 0.024 suggesting that the frog develops significantly more power at the expense of decreased economy.

Suga and Taylor, [66] have tried to relate these two indices of cardiac performance analytically, basing on Huxley's 1957 Xb model, [23], but obtained quantitative inconsistencies. Suga et al. [67] concluded that "the mechanism underlying the disproportionate changes of the efficiency and the economy needs to be clarified in the future" and that "the myocardium enjoys the freedom to modify the thermal economy of force generation and maintenance without sacrificing its mechanical work efficiency when it encounters different mechanical demands".

Suga et al. [67] attributed the greater variability of the economy as compared to that of the efficiency to the fact that the efficiency which, according to him, represents energy expended per energy consumed, is constrained by the first law of thermodynamics. The economy, on the other hand, represents the FTI, and reflects the energetic conditions under which the myocardium develops force, including the activity of ATPase, Xb cycling rate and the speed of contraction. Taking for example the case of cardiac muscle hypertrophy with enlarged muscle body, we note that the Xbs cycle more rapidly at the expense of economy, while still maintaining the almost normal efficiency without violating the first law of thermodynamics. Similarly, when working against hypertension (high after-load), the Xbs cycle slowly thus enhancing economy of force generation with a relatively small change in the mechanical work efficiency. As shown by Landesberg et al. [28, 34] (see Sec. 4.5.7),

the energetic efficiency of the sarcomere is maintained constant by the individual Xbs. Suga's observations concerning the less variable efficiency as compared with the economy index in his ventricular studies are generally consistent with our more recent studies based on intercellular energetics. It is reasonable to assume that a different definition and variable experimental conditions may have yielded slightly different efficiency values, but the correct trend is there.

The analysis presented here, leading to Eq. (4.15), provides the basic Xb control mechanism, which consistently leads to the physiological micro-scale definitions of economy and efficiency and quantitatively relates their roles in the metabolic conversion of biochemical energy to mechanical energy. Indeed, Eq. (4.15) provides an integrated framework for these two indices and suggests that they relate to the same basic property of the Xb molecule and although they can be independently determined, they are not mutually exclusive.

4.5.5. Sarcomere Control of Contraction

Two dominant feedback mechanisms (assumptions 4 and 5, Sec. 4.2.3) regulate the sarcomere function:

1. **The cooperativity mechanism**, which defines the dependence of the affinity of troponin to calcium on the number of Xbs in the strong, force generating, confirmation. This mechanism was recently substantiated by analyzing the force response to sinusoidal length oscillations in the tetanized intact cardiac trabeculae, [39, 75]. A recent study of the skinned isolated rat trabeculae, [33], has demonstrated that the dependence of the affinity of troponin for calcium on the number of cycling Xbs is the dominant cooperative mechanism in skinned myocytes. The cooperativity mechanism explains the "length dependence calcium sensitivity", the force-length relationship (FLR), and the related Frank-Starling Law of the LV, and provides the adaptive control of energy consumption to changes in the loading conditions, [34]. Calcium binding to troponin turns the regulatory troponin unit to the strong conformation and enables ATP hydrolysis in the adjacent myosin head. The cooperativity acts as an adaptive mechanism, assuring that the loading conditions affect the number of strong Xbs and determine calcium affinity. An increase in the load increases the number of strong Xbs, and increases energy consumption by increasing the amount of

calcium bound to troponin. The cooperativity mechanism provides the molecular basis for the regulation of cardiac muscle energy consumption. An increase in the afterload increases the affinity of troponin to calcium and increases the bound calcium. An increase in the bound calcium hastens the rate of Xb recruitment and ATP hydrolysis, and vise versa.

2. **The negative mechanical feedback** suggests that the filament shortening velocity affects the rate of Xb transition from the strong conformation to the weak, non-force generating, conformation (Xb 'weakening'), thus maintaining a linear dependence of the rate of Xb weakening on the shortening velocity, The mechanical feedback concept is substantiated by providing the analytical derivation of the force-velocity relationship (FVR) in the cardiac muscle, and the experimentally derived Hill's Equation, [21]. The mechanical feedback regulates the FVR as well as the generated power. Moreover, this mechanism explains the linear relationship between energy consumption by the sarcomere and the generated mechanical energy.

4.5.6. Control of Energy Conversion

The electrical stimulation of the cardiac muscle causes a transient elevation of the intracellular calcium, which binds to the regulatory troponin proteins, activates the actomyosin ATPase and enables Xb cycling. However, this open-loop excitation-contraction coupling cannot explain the linear relationship between energy consumption and mechanical production and how the loading conditions affect the energy consumption. The cooperativity mechanism provides the necessary close-loop feedback mechanism, which quantifies the bound calcium, hence the activity of the actomyosin ATPase, and the rate of energy consumption. The mechanical feedback provides the analytical solution for the linear relationship between energy consumption and power generation by the sarcomere and the oxygen consumption V_{O_2} and the mechanical energy generated, at the LV level, [65] and the isolated fiber level, [20, 11]. The analysis indicates that the amount of hydrolyzed, ATP, i.e. the energy consumption E, is proportional to the sum of three components: the external work W, energy dissipation as heat Q_η due to the viscous property of the Xbs and the pseudo potential energy E_{PE}, [30, 31]. The E_{PE} corresponds to Suga's potential energy, and equals it for isometric contractions. This energy also dissipates as heat.

The cooperativity mechanism explains the ability of the muscle to adapt to the loading conditions, since elevation in the afterload will increase the rate of ATP hydrolysis and afterload reduction will reduce energy expenditure, through the cooperativity mechanism The mechanical feedback determines how much of the biochemical energy stored in the Xbs, as biochemical potential energy, is converted to external work. Hence, the cooperativity mechanism determines the rate of ATP consumption and the mechanical feedback determines the amount that will be converted to external mechanical energy. Clearly, the energy consumption by the muscle and the generated external work are determined by the interplay between these two feedback loops.

4.5.7. Efficiency and Adaptive Control of Energy Consumption

The studies of cardiac energetics at the whole heart level and the isolated fiber level lead to two fundamental conclusions, which are direct consequences of the roles of the two feedback mechanisms [34]:

1. *The cardiac muscle efficiency is constant and load independent.* The analysis yields that the mechanical feedback relates to the dynamic properties of a single Xb and describes how the filament sliding velocity affects the weakening rate of a single Xb. The efficiency is defined by Eq. (4.16). Note that $\bar{F}V_U$ is the hypothetical maximal power of the single Xb. The efficiency depends on the basic intrinsic properties of the individual Xb and is constant and independent of the number of activated Xbs or the loading conditions. Since all the Xbs work independently, the efficiency of the whole muscle is identical to the efficiency of the single Xb.
2. *There is an adaptive control mechanism whereby changes in the loading conditions modulate the energy consumption.* This second conclusion relates to the incorporated cooperativity mechanism suggested here whereby the regulation of energy consumption is determined by the number of the activated Xbs. Note that the linear relation between energy consumption and the generated mechanical energy, is not a trivial manifestation of the First Law of Thermodynamics and suggests that the energy consumption is determined by the loading conditions. Clearly, the cardiac myocytes must have a build-in intracellular adaptive control, which modulates the consumption to accommodate the load, while maintaining the efficiency, as defined above, constant. The

cooperativity mechanism provides this adaptive control of energy consumption whereby the sensors determining the demands are the Xbs themselves. An increase in the number of strong Xbs increases calcium affinity and thus increases energy consumption, and vise versa when the number of Xbs decreases.

As seen here, the two feedback loops work at two different levels. The mechanical feedback operates at the single Xb level and relates to the constant efficiency. The cooperativity mechanism operates at the whole sarcomere level and modulates the response of the energy consumption to the changing load, thus providing the explanation for the adaptive control of energy consumption.

4.5.8. Dominant Cooperativity Mechanism

The existence of the two control mechanisms imbedded in the present model is further substantiated by the successful explanations and descriptions of a wide spectrum of phenomena of cardiac mechanics and energetics, [30, 32]. The dominance of the particular cooperativity mechanism stipulated here as compared to other conceivable mechanisms, [35] has recently been experimentally and analytically established, [39]. The study [39] tested three additional possible feedback mechanisms so as to identify the dominant one that determines the force-SL relationship (FLR). The hypotheses tested were:

1. SL-dependent Ca^{2+} affinity;
2. inter-filament spacing dependent Ca^{2+} sensitivity;
3. Ca^{2+}-dependent Xb kinetics, and
4. force-dependent Ca^{2+} affinity.

The SL-dependent and Ca^{2+} dependent mechanisms (1–3) were unable to explain the measured stress-SL–$[Ca^{2+}]$ relationships. However, the unique experimentally obtained relationship between Ca^{2+} affinity and stress demonstrated that an increase in the generated stress is associated with a proportional increase in the apparent Ca^{2+} affinity. The results strongly suggest that the dependence of Ca^{2+} affinity on the number of force generating Xbs is indeed the dominant mechanism that regulates the Xb recruitment and the generated force in the muscle activation. The model presented in Fig. 4.2 thus provides a fundamental basis for understanding cardiac muscle performance. Furthermore, it provides the parameters that determine the cardiac muscle efficiency and economy [28], i.e. the energetic utility of ATP consumption by the Xbs.

4.5.9. Hystereses Validate the Feedback Mechanisms

The experimental, [39], and analytical, [75], investigations of the force response to large amplitude SL oscillations at various frequencies and constant Ca^{2+} yielded hystereses in the force-length plane and revealed that the generated force depends on the history of contraction. These studies and the analysis of the hystereses in the force-length and force-calcium relationships, [39, 75], provide insight into the feedback mechanisms, and establish the validity of these two feedbacks which control the sarcomere Xb recruitment and cardiac contraction.

The hystereses phenomena seen in Fig. 4.4 are related to the existence of the two feedback mechanisms that regulate the number of force generating "strong" Xbs in the sarcomere:

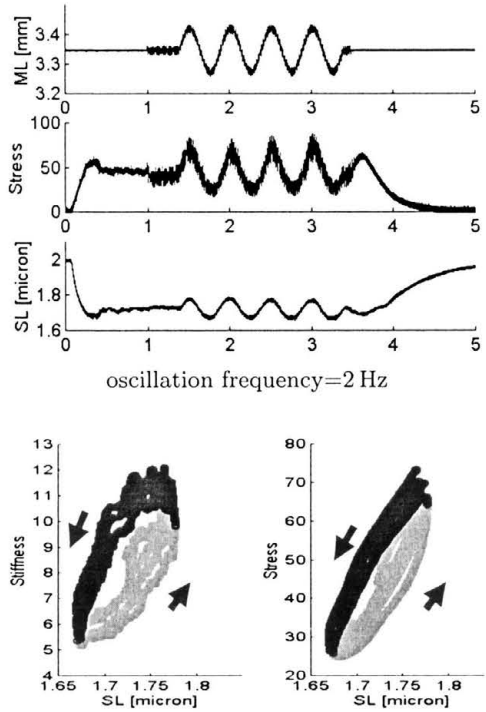

FIGURE 4.4. Effect of SL oscillations (2 Hz) in the Force–Length plane at a constant concentration of Ca^{2+}, [39]. Note that even at this oscillation frequency of 2 Hz the force response still lagged the SL oscillation by 34.64 ± 28.15 ms and the hysteresis remained in the counterclockwise (CCW) direction.

1. The cooperativity (positive) feedback, whereby the number of strong Xbs determines calcium affinity and Xbs recruitment.
2. The mechanical (negative) feedback, whereby the sarcomere shortening velocity determines the duration in which the Xbs are at the strong state.

The studies demonstrate the effect of these feedback loops on the hystereses in the force responses to SL oscillations at constant calcium concentration and the force responses to free calcium changes, [39, 35], simulated at constant SL. As seen in Fig. 4.5, counterclockwise (CCW) hystereses are obtained in the force-length plane as the force lags behind the SL when the system is modulated only by the cooperativity feedback. A similar response was found when the Ca^{+2} concentrations were changed at a constant SL. As seen in Fig. 4.6 the force precedes the SL and a clockwise (CW) hysteresis is obtained in the force-length plane when only the mechanical feedback is assumed to exist. The data used in these simulations [9, 70, 22] are presented in Table 4.1.

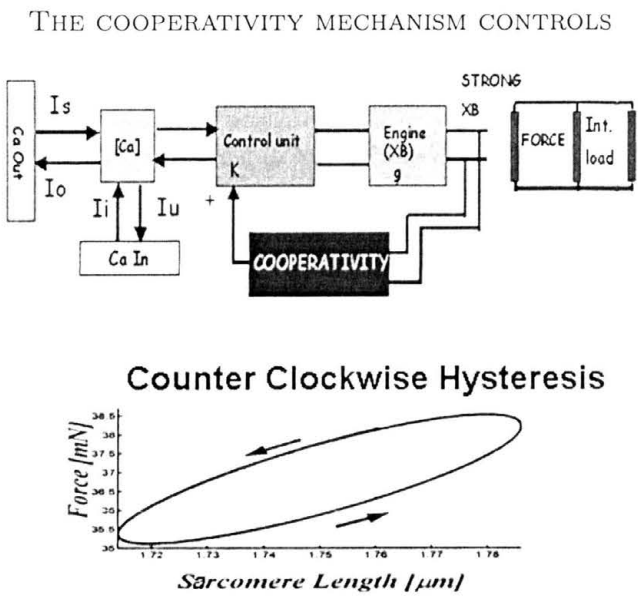

FIGURE 4.5. Effect of SL oscillations on force response in the absence of the mechanical feed back mechanism. Ca^{2+} affinity is determined by the number of active Xbs. Note that the force response lags behind the length oscillations, due to delay in signal transduction from the Xbs to the Troponin complexes.

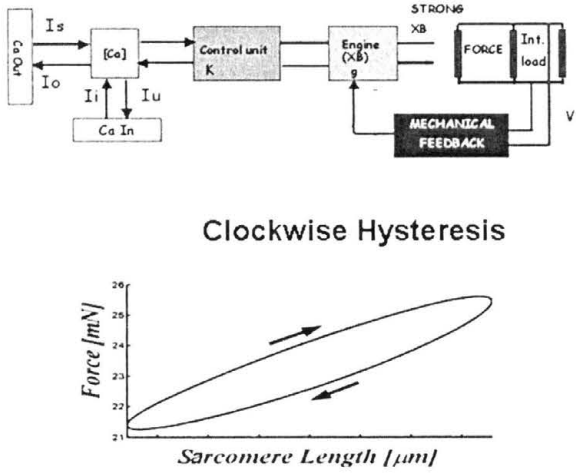

FIGURE 4.6. Force response to SL oscillations in the absence of the cooperativity mechanism. Xb weakening is determined by the load. Note the clockwise direction, as the force response precedes the length changes since the mechanical feedback is a function of the velocity.

TABLE 4.1. Values of the various parameters used in the simulations

Constant	Intact fiber	Skinned fiber	Units	Ref.
k_l	60	60	$\mu M^{-1}s$	[67]
K_0	0.3	0.015	μM^{-1}	–
K_1	3	0.5	μM^{-1}	–
$K_{0.5}$	24	30	μM	–
F	40	12	s^{-1}	[9, 70]
g_0	10	8	s^{-1}	[9, 70]
g_1	2	N/A	μm^{-1}	–
L_0	0.8	0.8	μm	[70]
F_c	2.1×10^5	2.1×10^5	mN/mm^3	–
$V_u\,(\bar{F}/\eta)$	10	10	$\mu m/s$	–
$\bar{F}$	2	2	pN	–
N	3.5	3.5	1	–

Both CW and CCW hystereses exist when both feedbacks co-exist. At low SL oscillation frequencies (<2.5 Hz), the cooperativity mechanism dominates and CCW hystereses are obtained, consistent with Landesberg's recent experimental observations, [39]. At higher frequencies (>2.5 Hz) the negative mechanical feedback dominates and the direction of the hysteresis is reversed.

The cooperativity provides the adaptive control of the cardiac response to short term changes in the load by modulating Xb recruitment. The analysis of the hysteresis phenomena reveals that the cooperativity mechanism allows the muscle to adapt Xb recruitment to the loading conditions and the changeover frequency from CCW to CW hysteresis defines the maximal frequency at which the muscle can accommodate changes in the loading conditions. The mechanical feedback mechanism defines the velocity limit, corresponding to the changeover frequency of 2.5 Hz, where the muscle absorbs rather than generates energy.

The combined effects of these two feedback mechanisms regulate sarcomere's dynamics and the response characteristics.

4.6. Conclusions

The four state model presented here is based on intracellular biochemical processes and has obvious advantages over the 'classical' Huxley's model and Suga's phenomenological elastance model: it provides a more fundamental basis for the understanding of cardiac performance, including the non-linearity observed in the end-systolic curve on the PVA maps, and the dynamic unsteady FVR associated with quick release experiments. Furthermore, it provides a quantitative tool for estimating the actomyosin contraction efficiency and economy, i.e. the energetic utility of ATP consumption. Also, it focuses on Xb dynamics and thus provides a handle to estimate the pathogenesis of the contraction mechanism. Last, the description of the LV function based on the intracellular mechanisms highlights the basic mechanical properties of the cardiac muscle, i.e. the FLR and the FVR, and provides the cellular parameters determining Hill's coefficients, explains the energy utilization dependence on needs and enriches our understanding of the normal and pathological LV function.

The analysis presented leading to Eq. (4.15) indicates that the energy consumed is spent on producing external work, heat dissipation due to the viscoelastic properties of the Xbs, and pseudo-potential, mostly non-recoverable, energy which is determined by the FTI. The linear relationship between oxygen consumption and power generation of the LV originates from the intracellular mechanisms that control and sustain the linearity between the consumption of ATP and the mechanical energy generated by the Xbs. This is facilitated by the two dominant feedback mechanisms. The experimental

and simulation studies, [39, 75] of the force response to large amplitude SL oscillations at a constant calcium concentration confirm the existence of the two simultaneously operating intracellular control mechanisms, reveal the force dependency on the history of contraction, and establish the range of dominance of the two mechanisms: The cooperativity dominates at the normal range of low frequencies and allows the muscle to adapt Xb recruitment to slow changes in the loading conditions. The mechanical feedback dominates at higher frequencies where the muscle absorbs rather than generates energy, and controls muscle function by the velocity of contraction. The efficiency, defined by Eq. (4.16), is determined by the mechanical feedback, reflecting an inherent property of the single Xb. The efficiency is thus independent of the number of strong Xbs, is constant and load-independent, what explains the low cost of driving the systemic circulation. The constant and high contractile efficiency is an intrinsic property of the single Xb due to the mechanical feedback. The Xbs are the myocyte sensors that moderate, via the cooperativity mechanism, Xb recruitment in response to length and load changes.

Finally, the phase of the hystereses depends directly on the stress level and only indirectly on calcium concentration and sarcomere length [40].

Chapter 5

Molecular Motors and Nanotechnology

5.1. Molecular Motors in the Cardiac Cell

A wide range of intracellular motoric activity and cellular motility in living organisms depends on millions of linear and rotary molecular protein motors of nanometer scale, which propel (bacteria, sperms), transport (messengers in neural network, cell division), generate high-energy metabolites (ATPsynthase) and perpetuate motion (muscle shortening). Of particular interest in the cardiac muscle cells are the linear motors of actin-myosin Xbs, which perpetuate motion, i.e. sarcomeres shortening and muscle filaments contraction, and expansion and the mitochondrial rotary ATPsynthase motors, which upgrade the biochemical fuel (ATP), or hydrolize it.

5.1.1. Linear Molecular Motors—Sarcomere

The linear motors, i.e. actin-myosin, kinesin or dynein, are fueled by the hydrolysis of ATP. The linear actin-myosin molecular motors actuate muscle contraction by the relative motion of the actin-myosin filaments sliding one over the other, Fig. 2.3. The molecular structure was determined by single crystal X-ray diffraction [46]. Figure 5.1 represents an electro-microscopic picture of the actin-myosin Xb [47, 50]. The muscle contracts by millions of these actin-myosin nano-scale linear molecular motors. Each cubic mm of muscle tissue contains 40×10^{12} motor units. The Xb is 19 nm long and 5 nm thick and creates a unitary force of ~ 2 pN and a single stroke step of ~ 5 nm, [66]. Image analysis of isolated actin filaments sliding over isolated myosin Xbs reveals two distinct kinetic mechanisms: the actin-myosin Xb

THE MOTOR UNIT—THE CROSSBRIDGE

The S1 fragment:

Weight:	45 kD
Size:	19 × 5 nm
Density:	40 × 10 mm^{-1}
Step:	2–8 nm
Max. force:	2 pN
Max. speed:	6000 nm/s
Max. work:	60 pN×nm
Cycle time:	40 ms
Stiffness:	0.6 pN/nm
Efficiency:	70%

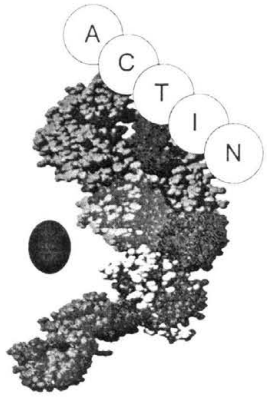

FIGURE 5.1. An electron microscopic presentation of the Xb and the physical properties; The dark spot represents a molecule of ATP, [46, 47, 50]. kD = 1000 Daltons. 1 Dalton represents a unit of molecular mass (= 1.66 × 10^{-27} kg) and equals 1/12 of the mass of carbon 12.

attachment/detachment rate ~ 2000/cycle, compared to ~ 10/s biochemical kinetics of nucleotide binding and dissociation, corresponding to the energy (ATP) consumption rate by the cycling Xbs. Consequently, the Xbs attachment-detachment cycle must be repeated many times during a single contraction cycle of about 0.6–1.0 mm per beat, sustained by the hydrolysis of one ATP mol.

5.1.2. Rotary Molecular Motors

Two kinds of rotary motors are known: the flagellar motors in bacterias and the ATPsynthase motors in the mitochondria. Unlike the linear motors, these rotary motors use transmembrane chemical (pH) or electrochemical gradients to produce rotary motion with a fixed stoichiometry of protons per revolution. Both kinds are equally effective: the protons efficiently (~100%) generate torque regardless of the original energy form. The ATPsynthase motor, Fig. 5.2, consists of an 8 nm rotor and stator and has a proton driven mechanism in F_0, which drives another mechanism (F_1) to catalyze the conversion of ADP to ATP. The same protein motor can work in reverse, utilizing ATP, to pump ions against the electro-chemical gradient. The rotary motor is about 10 nm in diameter and works with a very high efficiency

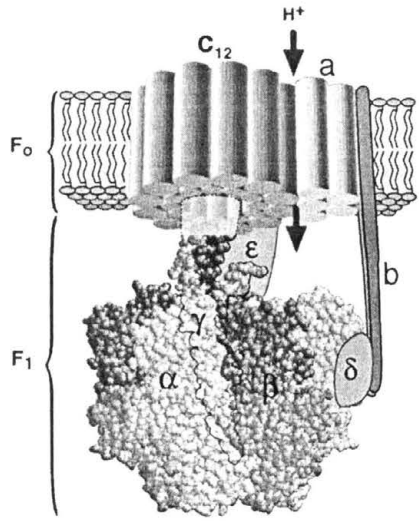

FIGURE 5.2. Schematics of the ATPSynthase rotary molecular motor, [71]

since energy conversion proceeds close to equilibrium [71]. The protons move through a pore in the enzyme complex across the membrane, like water flowing through a turbine converting mechanical energy into electricity. Energy of the proton is trapped in the complex to produce ATP from (ADP+P). ATP hydrolysis releases chemical energy that activates the linear (or radial) molecular motors and generates mechanical work. An excellent description of the energy transduction in the F_1 motor of ATPsynthase is given by Wang S. Oster, [71].

Rotary motors are capable of generating more power than the linear/track motors, and are, therefore, more feasible as power sources in nano-devices by, say, integration with nano-electro-mechanical systems (NEMS).

5.2. Nanomedicine and Related Nanotechnology

5.2.1. Specific Goals of Nanomedicine

Nanotechnology, and specifically nanomedicine, is the study of physiology, pharmacy and biosensors at the cellular level, all aimed at manipulating and affecting natural cellular and intracellular phenomena in order to achieve unique useful therapeutic devices. The birth of nanomedicine and nanotechnology is attributed to Maxwell's demons: "Separate hot and cold molecules

and get free energy ...". Richard Feynman followed it up, saying that "if cells can do it ... so can we make a thing very small, which does what we want; arrange the atoms as we want". Eric Drexel talked about "Engine of Creation": "Nanorobots Assemblers" which can reproduce and arrange atoms in any form. Many others followed these ideas with enthusiasm and productive imagination. Specific goals are:

1. maintain and improve human health on the molecular scale by targeted medical procedures,
2. identify and define diseases by genomic analysis,
3. improve diagnosis and therapy, including aging,
4. discover new drugs.

To achieve these and other related goals we must understand the nature of the nano-scale and pico-second intracellular phenomena. The nano-scale is demonstrated in Table 5.1. Consequently, we need to develop some unique capabilities to manipulate this molecular nano-world with precision. This involves the ability to construct objects with 3D positional control of molecular structures, manipulate atoms with atomic scale control, avoid harmful mistakes in pico-second constructions, identify and continuously correct unavoidable errors. Other specific goals include self replication (which is synonymous with creating a new life form!) and the observation of molecular events so as to identify incipient pathology.

TABLE 5.1. How small is small? Note that the small virus is almost invisible to the immune system!

	thickness
dime (coin)	1,000 micron
human egg	100 micron
red blood cell	10 micron
capillaries	$\sim$ 4 micron
nerve axon	1 micron
virus	0.1 micron; 100 nm
cell membrane	0.01 micron; 10 nm
DNA strand	0.02 micron; 2 nm
amino acid	0.7 nm
H_2 atom	0.07 nm
small virus	50 nm

5.2.2. Functional Characteristics of Nanomedicine

Nanomedicine is mainly related to improve our welfare and address the challenges presented by our physiology and the environmental antagonists. The main arena for nanomedicine is the body of a living organism.

1. The body is a *workyard* where molecular machines (proteins) abound in all organs. These molecular machines continuously build and break down molecules for fuel (enzymes), motoric motion (myosin), molecular pumps (nerves), ion pumps (cell membranes).
2. The body is a *construction site*: growing, healing, renewing tissue. Molecular machines in cells are programmed by genes to build and dissemble structures, move whole cells amoeba like. Unfortunately, construction failures increase with age (teeth, skin, eyes and bones) and the molecular machines seem tired.
3. The body is also a *battle field* in medical warfare: attackers (parasitic worms, protozoa, fungi, bacteria, virus) use the body organs to prosper. The defence, that includes molecular machines, e.g. white blood cells, and antibodies direct the attack. Unfortunately, the immune system can fail (tuberculosis, herpes, aids) or overreact (arthritis, lupus, rheumatic fever). Antibiotics (e.g. penicillin) are selective, sticking and jamming molecular machines in the bacteria. Alas, the virus has much less if any such machines...

5.2.3. Nanotechnology Based on Hybrids Utilizing Biological Nanomotors

Potential nanotechnological applications are commonly visioned as hybrids: integration of biological motors with micro and nano-structures. These include:

1. Force generation by molecular motors.
    - Mechanism of force generation by kinesin and nano-actuatoric developments
    - Single molecule enzymology of myosin triphosphatase
    - Chemical energy conversion into mechanical work by using ATP-Synthase
    - Functional biomolecular motor-powered nanomechanical devices

- Surface-mounted dipolar molecular rotors
- Using sunlight to power natural and biomimetic molecular motors
- Directing the translational motion of motor proteins in synthetic environments

2. Engineering molecular shuttles.
   - Cilium as a biological nanomachine
   - Regulation of kinesin motor activity at the level of the microtubule track level
   - Structural changes that drive myosin molecules along actin filaments
3. Bio/synthetic elements using molecular motors.
   - Light-driven synthetic molecular motors
   - Conducting polymer molecular muscles
   - Chemically driven molecular electron pump
4. Biomedical applications of molecular motors
   - Implantable molecular factories as therapeutic delivery vehicles
   - Vehicles for therapeutic agents in human medicine

5.2.4. Potential Developments in Nanotechnology

Nano-Applications in health and medical systems, chemistry, energy, optics, food and the environment involve new production processes and devices, new materials and new production technologies for improved construction, chemicals and surface transport. Future developments include:

1. analytical tools for understanding, anticipating and eventually affecting incipient molecular based normal and pathological nano-scale phenomena,
2. specialized artificial hybrids, e.g. metal-atom-molecular devices, by harnessing the unique electrical properties of the molecular motors,
3. molecular scale robots serving as tiny mechanical operators in the blood-stream, destroying harmful viruses; molecular gears and other components needed for constructing nanometer-scale machines,
4. electronic materials based on carbon nano-tubes, which will have high thermal and electric conductivities.

5.2.5. Future Knowledge-Based Multifunctional Systems

Long-term interdisciplinary research aimed at understanding the nano-scale phenomena, mastering nano-scale processes and developing new research tools include:

- self-organisation and self-assembling,
- artificial or hybrid molecular motors,
- nanostructured materials and interfaces for new applications,
- nano-scale engineering techniques to create materials and components,
- industrially relevant production of nanoparticles,
- "intelligent" biomaterials for tissue repair and regeneration,
- tribology-related surface engineering for multifunctional materials,
- nano-scale handling and control devices and instruments,
- characterisation and/or manipulation of devices and techniques,
- bio-sensing systems for health control,
- other novel applications.

Obviously, these futuristic objectives are stimulating and challenging the present technological world. As such, they attract great human resources, bright innovators and great capital investments in many countries. The exciting race for new nano-scale systems is on, and the winner to be is hopefully all mankind.

Chapter 6

Short View into the Future

6.1. Cardiac Tissue Engineering and Therapy

Unlike conventional clinical therapies of whole organ replacements, cell therapy makes use of the cells replicative ability to repair a failing tissue or organ and thus improve a pathological condition and restore natural function. Philippe Menasch, a heart surgeon at the Bichat Hospital in Paris, seems to have, in 2000, carried out the first autograft of muscle cells in a patient of seventy-two, whose heart had been partly damaged by several heart attacks [51]. Cells extracted from the patient's thigh were put into culture and then injected into his heart in the course of a heart bypass operation. Other types of adult cells known as "immature" or "original cells", are not constantly active. They can, under certain circumstances, adapt to their environment and give rise to functional cells. These adult original cells (found mainly in the bone marrow) are rare and difficult to multiply in culture. Therapeutic cloning, which consists of transferring a cell nucleus taken from the patient into an ovum that has had its own nucleus removed, can become a source of immunologically neutral original cells. "Virgin" cells (with 46 chromosomes) may provide a reasonable source of cells for the practice of tissue engineering. The obvious advantage of the virgin or embryonic stem cells is that they do not trigger immune reaction and do not generate antigenic response.

The huge investments in tissue engineering research is beginning to bear fruits. A very recent development involves implanting embrio-based stem cells into an electrically malfunctioning rat's heart and observing the development of a normally beating pacer, as well as cell-based pacers, [74].

Tissue engineering utilizing cells for cardiac tissue therapy is presently practiced to develop natural blood vessels as substitutes in coronary bypass procedures and cardiac patches to support or replace failed cardiac muscles. In the present race for biomaterials that can work in vascular surgery as arterial prostheses of tissue grafting, research is increasingly moving towards hybrid systems, combining inert polymer materials and living cell matrices. Other sophisticated approaches include transforming growth factor (e.g. TGFβ) to stimulate endothelial cell activity, so as to minimize post catherization re-stenosis. The creation of a whole heart by tissue engineering is, however, a challenging goal for future generations.

6.2. Cardiology and Genetic Engineering

The discovery of the genome sequences has kindled the expectations for the quick development of the ability for gene manipulation and gene therapy. Genetic engineering is essentially the art and science of modulating a particular gene or a group of genes to affect new characteristics in the cell. Much of the recent genetic research efforts are directed to identify the genes that regulate cardiac morphogenesis, by applying molecular genetic techniques to disorders of human cardiac development. For example, linkage analysis and positional cloning technology show that mutations in TBX5 cause Holt-Oram syndrome. Missense mutations appear to directly interfere with DNA binding and have severe phenotypic consequences. Linkage analysis and positional cloning is also used to study the development of the vasculature. As much as 20% of aortic aneurysm disease is hereditary. Positional cloning technology is now used to identify the specific gene mutations, which cause familial aortic aneurysm disease.

The molecular and genetic revolution reached the cardiovascular field sometimes in the nineteen-eighties, with the application of molecular biology in the cardiac field, mainly in physiological phenotyping and developing pathological animal models. Gene therapy, aimed to repair mutations in genes and overcome the deficiencies of defective cardiac cells, is a more recent phase representing an immense source of hope for many people. For example, the genetic forms of dilated cardiomyopathy account for over 25% of idiopathic human cases. Transvascular gene transfer by viral vectors such as the addeno-associated virus is a common and promising approach to improve cardiac function in animal trials.

However, our understanding of this new form of therapy is, for the time being, in its infancy. While gene therapy is still a long way from being able to heal all forms of genetic illnesses, researchers optimistically hope to be able to use it to fight cell-based diseases, e.g. cancer, by integrating a gene in the affected cells so as to make them more receptive to drag therapy. Today, some geneticists are shifting their hopes to "proteomics", which aims to determine the precise functions of the 30,000 genes that characterize each human being. Yet, the functions of only some 5,000 of them have been discovered. Another source of hope involves the messenger RNA, derived from DNA, which is able to switch genes on or off according to the needs of the organism. The aim is to be able to synthesize the messenger RNA for manipulating genetic abnormalities, as well as combating infectious agents and certain genes that are responsible for certain pathologies, e.g. cancer.

6.3. Genetic Challenge

The genome is commonly seen as the entity that creates the organism and as being its ultimate explanation. However, the progress that followed this tremendous achievement is rather disappointing in certain circles, noting that even over ten years after the discovery of the genome sequence of the AIDS virus, no vaccine is yet on the agenda. Criticisms of an epistemological kind are also being made by scientists, who feel that if genetics is not leading to concrete results, it is because its theoretical bases are wrong. The "central dogma" of the genetic discipline asserted that a strand of DNA—a gene—coded one and only one protein. But this is not the case. In reality, the same gene can code several proteins, whilst several genes can contribute to the expression of the same characteristic.

The sequencing of the human genome has confused the issue even further. It has been discovered that a constantly growing number of genes present only a statistical correlation with the characteristic that they are supposed to determine. This means that some of the individuals carrying these genes, present the characteristic (an illness, for example) and others do not. In view of this problem, which complicates the goal of using genes for therapeutic purposes, some geneticists now suggest that the complex influences of numerous genes and environmental factors have to be taken into consideration. As a result, one now tends to speak of genetic "component" rather than genetic "determinism". This seriously calls into question the initial view of the

all-powerful nature of the gene, for a long time considered as the "essence of life".

Proteomics, the cataloging of proteins, can presently only slightly help us to understand this complexity of life, but not much more than the sequencing of the human genome does. To achieve this goal, we have to reach an understanding of the kinds of interactions between the different components of the cell and the laws that govern these interactions, and these laws may not be coded in the genome. To put it bluntly, the genetic engineers opening this Pandora's Box are interfering with the genome of living beings without having a precise vision of their functioning and their structure. It is, for example, impossible to predict the consequences of transferring a gene from one type of organism to another. The transferred gene might in fact mutate within the genome, or even be transferred to another organism, even to another species. No wonder that in order to get out of the epistemological impasse in which genetics finds itself confined today, some scientists propose to apply Darwin's theory to their discipline, breaking radically with the present deterministic model. Accordingly, they propose that it is not the genetic program that structures cell populations, but the competition engaged to get hold of resources that they need in order to survive, [25]. The assembly of molecules would happen in a random way, and a principle of "natural" selection would be at work to retain viable assemblies. Obviously, much is yet to be learned and explored before understanding is gained and consequent abilities to manipulate the genome are achieved.

6.4. Technological Challenge

Ongoing efforts to sequence microbial genomes for commercial applications are already on their way. We optimistically focus on the futuristic interplay between technology and medicine. This requires making great progress in nanotechnology, and particularly in creating hybrid bioconjugates by combining bio-molecules with man made materials, so as to achieve ease of handling and utilization in diagnostics or targeting. These hybrids may include solid gene chips or soluble bio-polymers. For example (suggested by VC Giampapa, UMDNJ, NJ. USA), the basal and long range patterns of gene expression of normal and aging individuals could be evaluated in vitro by utilizing gene chips wherein selected sequences of genes will be imbedded and monitored for their expression under controlled conditions. Thus, we may be able to

follow the effects of various body fluid compositions, impose therapeutic modalities on gene expression in relatively short time, and possibly identify critical developmental characteristics or anti-aging therapies. Another example (suggested by A.S Hoffman, University of Washington, Seattle, WA, USA) is the use of 'smart' polymers to form conjugates with proteins (e.g. enzymes) to control enzyme reactions and assay antigens. Other potential applications are only limited by our imagination. These forthcoming nanotechnology innovations may eventually introduce a new era of medicine wherein we can treat the causes and not the effects of various cardiac (and other) pathologies, including aging. Technology in these future days will no longer be the limiting step in clinical physiological maintenance.

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