Mechanisms in Cell Physiology

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By

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This book is dedicated to Edoardo, Louis, Giovanni and Arnaldo

TABLE OF CONTENTS

PREFACE

With the advent of new technologies, decisively supported by the computerization of scientific instruments, biological research in general and the study of physiology in particular have made enormous progress in the last 20 years. Technologies alone, however, cannot explain the successes that scientific research continues to achieve. Achieving certain goals requires comprehensive science policy with specific objectives, common efforts and exceptional economic support. Three examples of dedicated international programs bear witness to this. First, the Human Genome Project, coordinated by the National Institutes of Health (NIH), launched in 1990, concluded in 2003, and with an initial allocation of more than $250 million, led to the decoding of the entire human genetic make-up. A second example is the decade dedicated to the brain: 1990-2000 saw enormous resources concentrated on research on the function of the central nervous system. Last is The Human Brain Project (HBP) launched by the European Commission's Future and Emerging Technologies (FET) scheme in October 2013. The HBP has the following main objective to create and operate a European Scientific Research Infrastructure for brain research, cognitive neuroscience, and other brain-inspired science.

There have been several factors contributing to the particular attention paid to scientific research – the opportunity for huge gains, the idea of defeating diseases, the desire to study the biology of humans to satisfy the timeless search for immortality. However, the prevailing reason for the success of biological investigation, we believe, and we hope, is rooted in the human tendency to discover the unknown, in the thirst for knowledge, in the idea of exploring inner nature of the human being in human beings beyond wretched drifts and dangerously unrealizable ideas. It is the combination and convergence of different events that determine the global success of a particular developmental trend.

In the years following the end of the Second World War, a policy of supremacy was pushed by the two superpowers, the United States and the

Soviet Union. The challenge concerned the control of territory, waters and skies. The "frontier" was represented by space conquests, with an enormous economic effort on the part of the two main players. The space enterprises raced each other with exceptional peaks of accomplishment, such as the first man in space in 1961 and the first man's walk on the moon in 1969. However, a sequence of events beginning in the 1980s, including the collapse of the Soviet empire, but also the oil and economic crisis, and the realization that the conquest of space did not give the expected results in economic terms, led to a downsizing of this strategic project. Even the idea of mass commercialization of interplanetary travel and exploitation of extraterrestrial resources has gradually died out under the relentless blows of the prohibitive costs and technologies that this type of conquest requires, which are still probably barriers today.

Therefore, almost naturally, instead of the new frontier being the extremely large, to satisfy the thirst for knowledge, human curiosity and the innate egocentricity of humans, it turned out to be the infinitely small. The investigations into the origin of living beings, the attempt to unveil the secrets of biological "machines" and, above all, the idea of generating and controlling a process central to life have an attraction and an intellectual fascination that few researchers have been able to or wanted to resist.

We believe that for a student who approaches the world of biology and scientific research, it is important to know, in addition to experimental theory and practice, the history of the evolution of biological thought and the consequent technological evolutions that have led to such great success. There are many publications dealing with this topic and we think that some should be obligatory reading for the training of future scientists. In addition, in order to fully understand the physiology of organs, cells and sub-cellular compartments, it is important to have a clear understanding of the fundamental principles of biochemistry, biophysics and molecular biology. Based on these principles, it is possible to understand well the totality of physiological processes that we know today and the hypotheses developed to explain those that are still obscure.

Biological research is a relatively young field of investigation compared to physics, chemistry and medicine for several reasons. The most important factor of all, we believe, is the fact that even in the most basic experiments, there is a need for adequate technology. Beyond some brilliant intuition that we see in the monk Gregor Mendel, in the most sensational example, research on the origin and function of living organisms has gone hand in hand with technology development.

There is also an equally important reason of an almost philosophical nature: biological research is a mixture of chemistry, physics and mathematics. For this reason, the birth of schools of biology had to wait for the concept of a researcher in the field of biology to mature. The process was slow until 1970, when it underwent an acceleration whose push continues today. Biology courses have changed at all levels. In primary and secondary school, the biology program has changed topic from a descriptive observation of living being towards up to date experimental biology capable of interfering with the natural processes (genetic engineer for example). University biology curricula have been enriched with new, more and more thorough courses. Biological knowledge has become increasingly socially, culturally and ethically relevant.

The study of Physiology had, like all disciplines, its golden age. The years between 1940 and 1970 saw a flourishing of schools that took into account the mechanisms underpinning the functions of biological events. Names like Hodgkin, Huxley, Fen, Margaria, and many others, should be known to today's students for the immense contribution of ideas and experiences that they have left us. They not only had the ability to create schools of thought: we believe that their genius was to predict the physical mechanism hidden among the layers of membranes, clusters of proteins, strands of filaments and fluids of all kinds that we call living matter. They knew how it was likely to work, before embarking on any kind of study of the biological element. They studied it from the outside, acquired data on the relationships of the phenomenon with the surrounding world, hypothesized a probable mechanism and only at the end proceeded by opening, dissecting and dividing the object of their research to find support for their hypothesis. Everything was the result of an in-depth logical discussion on the most probable mechanism underlying a certain physiological phenomenon. **Logic was their most powerful weapon of investigation**.

The discussion with their collaborators and other scholars, the responses to their hypotheses and the possibility to modify them allowed them to acquire new elements and reach theoretical conclusions. This prelude to the laboratory work not only gave excellent results, but led to the growth of generations of scholars, first, and researchers, then, which have allowed the great development of research in the field of biology to the present day. We are purposefully talking about ‘scholars’ and not ‘students’. We tend to associate the term ‘student’ more with the young high school pupil who, with few exceptions, acquires information, metabolizes it and retains by heart what is most useful. The scholar is an actor, who does not only learn. The scholar discusses, proposes, enters into contradiction with the tutor and is able to very often modify the final thought and theoretical conclusions that lead to the choice of one experimental path over another.

A university professor loved to repeat often: "To do good research you have to have good teachers, but above all good students". One cannot exist without the other. Several years ago, the old Mr. Honda, the founder of the Japanese multinational company of the same name, declared publicly: "I do not understand my young engineers. I do not understand their ideas and their way of thinking. I am particularly happy about this fact: if I understood their ideas, it would mean that there has been no progress". This is a lesson that everyone should hold very dear, because we believe it is the basis for good research and the most intelligent way to interact with others.

Today our laboratories are full of sophisticated tools and machines that are used far below their potential. More and more often, we find ourselves chasing technology to adapt our ideas to it. We believe that this technological prevalence, which has had a very important function, should be reduced. Today what is missing are the new ideas: the reason lies in the fact that there are no longer the "schools", those aggregations that were used to propose new ideas. There are scientific problems, such as the mechanism of the basis of human thought, which require and will require innovative mental efforts that must be produced by competent and prepared minds, but with a strong sense of scientific integrity. Of course it is a great challenge to study the mechanism of thought and behavior using the same tool, the brain, which generates both thought and behavior. Some people think that we will never succeed, that understanding ourselves is unachievable.

This book has had a difficult "birth". Difficult because there are many good books on Physiology. Difficult because the idea matured over several years of teaching, in an attempt to write something different. Difficult because this book focuses a great deal on the logic of thought. We have observed for years horrified groups of students, facing the study of Physiology, absorb hundreds of sometimes very complex concepts, pouring them out more or less politely in front of exam boards and then purging them from their brains as soon as they passed the exam. This book is an attempt to reduce this disorder. We believe that for a basic Physiology course, the size of the program or the number of systems that are listed is not so important. We believe that with a solid foundation in cytology, biochemistry and biophysics a student has everything needed to understand the concept of dynamic processes in biology. Those who work with Physiology must have clear concepts of structural biology and energy. **WHERE**, **HOW** and **WHY** are essential. However, what discriminates physiology from other biological disciplines and its unifying element is certainly **WHEN**. The concept of time – which very few students grasp – becomes essential for the study of the dynamics of biological processes, which usually requires a physical-mathematical description of phenomena. If the elements that contribute to the biological phenomenon are clear, the mathematical description will be not only easy but also very helpful. Physics and mathematics that can be used by students of Biological Sciences could represent another great goal: not as pure science, but as language.

One cannot conclude a book introduction today without mentioning the relationship with the internet. The internet offers an infinite support to knowledge and therefore an opportunity that must be seized. However, it offers nothing for understanding. Unfortunately, in the last few years, a degeneration of knowledge has taken hold, in which users mistake an informational "warehouse", where anyone can put anything, for a "truth provider". We are witnessing a worrying “leveling” of knowledge, where it seems that all have become experts in any given field. Watching the sea from a jet ski speeding across the surface is not the same as going down in a bathyscaphe in the Mariana Trench. Everyone knows how to use a jet ski. Going down into the Mariana Trench not only takes courage, it takes knowledge, skills and experience.

The internet, with a huge amount of totally uncontrolled data, has a lot of valuable information, but precisely because its access is free to all, it contains an "avalanche of stupidity", sometimes harmless but very often harmful or even dangerous. More authoritative sources than us say that the quantity of false information on the web is far greater than that controlled by experienced staff. The huge success of the "do it yourself" aspect offered by the web lies in the extreme ease of use, in the immediacy of the response, in the lack of contradiction and, no less important, in the possibility of always finding the answer that we like best.

This hint of discussion on the validity of education via the web, on the topicality of printed books and all the way to the role of professors in face-to-face lessons is addressed in an exemplary way by the book "The Death of Expertise: The Campaign Against Established Knowledge and Why it Matters (2017, Oxford University Press). We believe that today this is essential reading for teachers and students. What is attempted is the interruption of the decline in higher education taking place today more than ever. The task is arduous but we hope that our effort is understood for what it is and not for what it lacks.

I would like to express all my gratitude to my “Maestri” of Life and Science: Edoardo Mazzanti, Giovanni Cavagna, Louis DeFelice and Arnaldo Ferroni. My English language knowledge is not enough good to find the proper words to express my gratefulness. Raffaella Tonini who always supported me with love and hate. A special thanks goes to the undergraduate students of the General Physiology Course of the Degree in Biological Science, of the University of Roma and Milan, who were used as guinea pigs without their knowledge and on whom the logic with which this book was conceived was tested. I hope that they received sufficient compensation during my lectures. I am in debt with my colleague and friend Enzo Mancinelli who did most of the work. Without his help this book was not possible. A special thanks to what were more than simply colleagues: Henry Malter, Berth Smith, Steve Ralph, Raffaele DeFrancesco, Antonio Torroni, Aurelio Galli, Federica Bertaso, Laura Bianchi, Gaia Novarino, Marco Foiani, Andrea Locarno, Anna Moroni. Thanks also to my young collaborators Ivan Verduci, Francesca Cianci, Gaetano Cannavale and Federico Brandalise. In one way or in another they all help me physically and psychologically during these trouble years.

M. Mazzanti

CHAPTER 1

Introduction

1.1 The concept of living matter in Physiology
1.2 The concept of separation
1.3 The concept of energy
1.4 The concept of work

The study and understanding of Physiology is based on three fundamental concepts: the possibility of creating different but not isolated environments, the ability of these environments to accumulate energy and the ability to use this energy with maximum efficiency through appropriate structures that transform it into work. The development of even the simplest systems with these characteristics took an enormous amount of time, so much that the oldest example likely able to perform these functions was found in a 1.9-billion-year-old Precambrian flint. This seems to be the oldest organism morphologically comparable with some modern iron bacteria (Schopf et al., 1965). The process that led to this ancient system and later to today's cellular forms was possible because of three important factors. The first is the enormous amount of time that has passed from the origin of the Earth to the appearance of the first forms of life. The second is the presence in the primordial aqueous environment, formed by the cooling of the Earth's crust and the condensation of water vapor, of chemical components in large quantities, such as chlorides, carbonates and

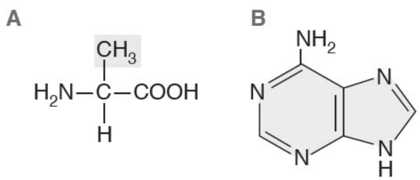

Figure 1.1 Structure formula (A) of alanine, a simple amino acid, and (B) of the nucleotide adenine.

phosphates of sodium, potassium and calcium, formed by the reaction between the pulverized rocks and methane generated by volcanic eruptions. The third is the presence of enormous amounts of energy in the form of electric discharges, visible and ultraviolet light, ionizing radiation and heat sources, in some cases very high, with consequent generation of water vapor. All of these factors contributed to the random synthesis of thousands and thousands of different molecules and more or less complex aggregates, until dynamic molecular systems capable of breaking down and reforming depending on energy conditions and material availability were formed. Thus, complex molecules that are characteristic of living matter, such as amino acids and nucleotides (Figure 1.1), phospholipids, nucleic acids and increasingly complex proteins (Figure 1.2), have been formed in a completely random way.

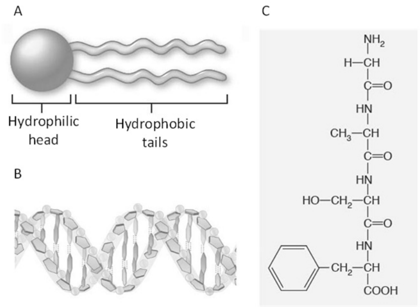

Figure 1.2 Structure formula (A) of alanine, a simple amino acid, and (B) of the nucleotide adenine.

The formation of phospholipids was as important for physiology as the aggregation of nucleic acids was for genetics. The principle on which all physiological processes are based is the presence of aqueous environments with different compositions of solutes. The aggregation of phospholipid molecules in a double layer (Figure 1.3A), due to their characteristic of having a hydrophilic head and a hydrophobic tail, leads to an energetically stable structure that has allowed an evolutionary leap of great importance: the formation of biological membranes. The ability of biological membranes to be in contact with a hydrophilic solute, such as water, through the polar heads, combined with their impermeability due to hydrophobic tail juxtaposition, has given them a fundamental role as universal separators in the biological world. All membranes, whether cellular or intracellular, of cytoplasmic organelles or the nuclear envelope, have an identical basic

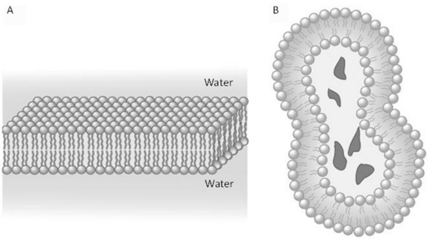

Figure 1.3 A) In water, phospholipid molecules spontaneously arrange themselves in double layer. B) The double layers thus formed can grow three-dimensionally until they close on themselves and form a separate environment from the outside.

structure of phospholipids in a double layer: their primary function is to form separate environments with different compositions of solutes.

It is important to understand that the formation of membranes that delimit a restricted three-dimensional space, defined as in the example of Figure 1.3B, has a functional aspect that, in addition to providing different environments, allows interactions between molecules that would be otherwise highly unlikely.

All of the reactions that occur in the cellular and subcellular world are the result of random impacts due to thermal agitation. Concentrating molecules in confined spaces decreases the degrees of freedom of the system and greatly increases the probability of interaction. Space management is therefore a means of modulating the reactions between molecules that occur both inside and outside the space delimited by the double phospholipid layer.

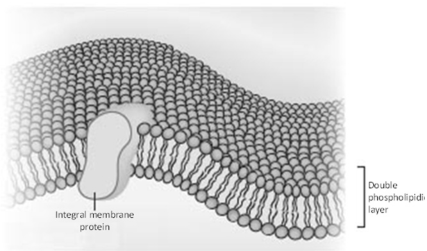

Figure 1.4 Schematic representation of an integral membrane protein. Double phospholipidic layer Integral membrane protein.

While it is true that all biological membranes have the same characteristics, their specificity is ensured by the protein structures that are adjacent to the membrane on both sides or that pass through it (Figure 1.4). Some of these proteins are specialized and are responsible for the passage of material between one environment and another.

Transmembrane proteins contain aqueous pores that determine the selectivity of the membrane itself. There are high-resistance membranes, usually with low protein content, membranes that manage to change their selectivity over time, passively or through energy consumption and, finally, membranes that only perform containment. In general, membranes that enable the movement of solutes from one environment to another must have integral proteins belonging to the category of transporters, specific molecules for this very important function.

The specialization of the phospholipid double layer as a dynamic structure, i.e., able to change its permeability, has created the conditions for membranes to be the main structure responsible for the accumulation and storage of energy. Accumulators are systems that can increase their potential energy and maintain it in a dynamic balance. Potential energy is the fastest form of energy that can be used to perform work. From the point of view of physiological functions, the reactions responsible for maintaining biological systems away from static equilibrium use chemical energy such as adenosine triphosphate (ATP). ATP-controlled reactions are continuously active to ensure a constant accumulation of potential energy. Electrical potential across membranes, ionic accumulations in specific compartments and reserves of complex molecules are the most common ways through which the energy level of biological structures is increased. Systems are constantly maintained at a high level of potential energy in dynamic equilibria based on mechanisms that depend on the hydrolysis of ATP combined with passive events. When the biological system needs to do

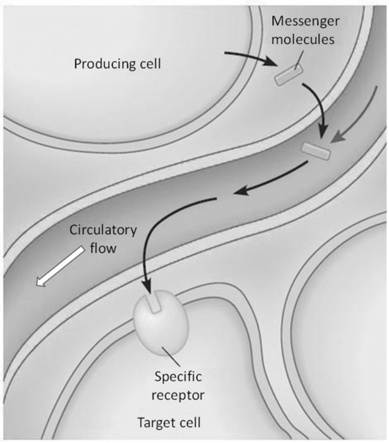

Figure 1.5 Molecular messengers, produced by specialized cells, are carried by circulatory flow (white arrow) near specific receptors of the target cells (black arrows).

some work, the dynamic equilibrium is momentarily disturbed. This occurs when a perturbation of the environment on the biological system removes the constraints that keep the system at a high energy level. This allows the system to "run" spontaneously towards different balances at lower and lower energy levels, rapidly releasing the energy that, from potential, is converted into other forms capable of doing different forms of work. At the end of these processes, active mechanisms, which directly use chemical energy, will restore the dynamic balance of the system with a high potential energy level. However, maximum efficiency is achieved when the dynamic equilibrium can be maintained and re-established in a short period with passive mechanisms and a minimum contribution of chemical energy.

As long as organisms were made up of one or a few cells, cytoplasmic or intracellular communication could be left to passive phenomena such as the diffusion of compounds in the cytoplasm or from cell to cell. The

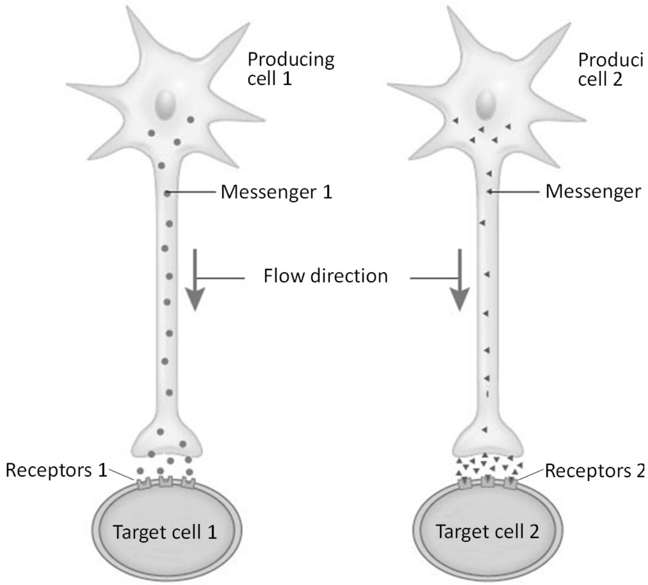

Figure 1.6 Each messenger producing cell makes contact with the target cell that has the specific receptor.

increased complexity of organisms necessarily posed the problem of communication between and control of the various locations within the multicellular system in formation (Figure 1.5). Controlling the different parts as well as other interacting systems, decoding signals from the surrounding environment, and generating instructions to coordinate a better survival strategy represents a further great evolutionary leap. The synthesis and release by cells, in response to an adequate stimulus, of diffusible molecular messengers was probably the primordial extracellular form of a communication system. This system, however, presents a fair degree of complexity because since there is a diffusible molecular messenger, there must also be the ability to intercept the message by means of specific receptors present on target cells. To increase the probability that the message meets its receptor, there are several structures that can be functionalized. First, releasing a diffusible molecule could be a slow process depending on the distance of the target. A solution is represented by the circulatory systems: a preferential method that uses a liquid carrier to move a great variety of substances from the manufacture site to structures with specific receptors. A communication system of this type, consisting of a sufficiently high number of molecules and capable of interacting with all cells that have specific receptors on their membrane, can be, for example, the control system for the growth and harmonious functioning of a multi-cellular organism. A specialization of this communication system is represented in Figure 1.6. A cell produces, at the level of the cell body and in response to an adequate stimulus, a molecular messenger, which is carried using molecular engines near the target cell, which in turn has the specific receptor for the messenger on the membrane.

With further specialization and with clear functional advantages, the messenger can be synthesized by the producing cell directly near the target cell. This cellular organization allows direct communication between two cells and has two major advantages: the accuracy of the message, as it establishes a direct and precise relationship between a producing cell and a target cell, and the control and speed of the response, as there is no need for circulatory flows and large areas of dispersion. Rather, the diffusion takes place in extremely small spaces.

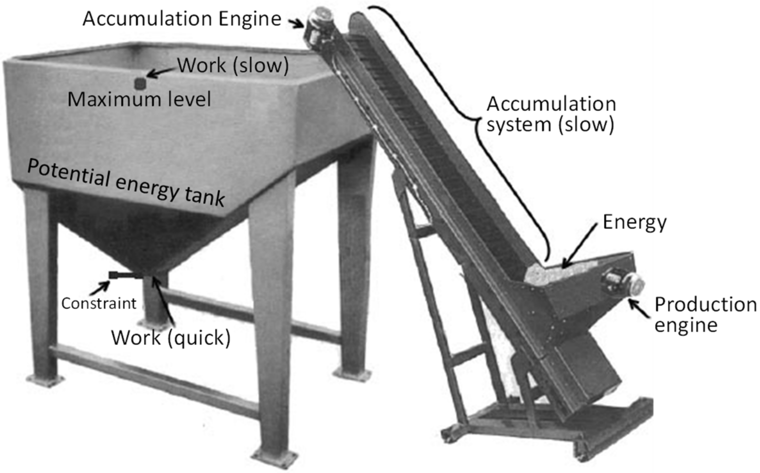

Figure 1.7 The diagram represents the mechanisms of production, storage and use of potential energy. Slow work maintains the body's homeostasis, while fast work responds to a stimulus inside or outside the body and requires the removal of the constraint.

The different communication systems just described have been adopted by biological organisms, either only one or all of them. Depending on the complexity of the organism, these communication systems are adaptable to various situations. Apart from direct contact between cells, the paradigm used by living organisms to communicate is always composed of a stimulator apparatus, which produces and releases messenger molecules, and a receptor apparatus. The organism is able to use the potential energy released by the appropriate stimulus to transform it into kinetic, chemical or mechanical energy and produce work. In this case, the apparatus that converts potential energy into work is, in fact, transformers. There homeostasis are many systems capable of accumulating potential energy and transforming it, directly or indirectly, into work. These systems can be very different from one another. In particular, the most important axis of diversity concerns the factor of time, which impacts the primary use of chemical energy and/or the interval to perform given work.

The mechanisms that the cell has selected to produce and store energy are represented schematically in Figure 1.7. The organism uses a "motor" that produces potential energy through a slow and continuous process, in greater quantity during periods of inactivity, and with chemical energy consumption mainly represented by ATP. The energy produced is stored as potential energy in a "reservoir" with an equally slow process, again by a "motor" that consumes ATP. The overproduction of potential energy is usually sufficient to maintain the basic functions of a living organism. In the presence of an external stimulus, the stored energy can be released. The system uses the stimulus energy to remove the constraints implemented to maintain a high potential energy level. From a physiological point of view, a biological organism is considered "alive" when it reaches a state of dynamic equilibrium with a high potential energy level. When an immediate response is required, such as a voluntary action or a response to an external stimulus, whether useful or harmful, the system is able to respond with a passive mechanism transiently perturbing the dynamic equilibrium. The combination of morphological and functional arrangements allows the reestablishment of equilibrium. In the process, the potential energy is transformed into several other forms (electrical, chemical or kinetic), sustaining the performance of all of the cellular process. The mechanism reaches its maximum efficiency when the potential energy lost in the process is easily and quickly restored when the transient system imbalance is passed.

The sequence of events begins when the voluntary or external stimulus has enough intensity to remove the constraint that keeps the system in dynamic equilibrium with high potential energy. Without constraints, the system would tend towards static equilibrium, dispersing all the energy in the surrounding environment. In biological systems, the achievement and stabilization of this form of equilibrium represents an irreversible condition that decrees the end of the system itself. For a system to be compatible with biological life, the transformation of potential energy must take place in a controlled and reversible way. In order for the system to respond quickly, efficiently, and, above all, continuously, the mechanism must have a very low energy dispersion. The timing of chemical energy generation is incompatible with the response to a stimulus. Thus, long-term recharging mechanisms are useful only to accumulate potential energy. Therefore, to respond to stimuli for a sufficiently long time, the system must increase its efficiency.

There are several artifices put into practice by biological systems to increase efficiency. The energy used to start any physiological function is an infinitesimal fraction of the stored potential energy. Secondly, the potential energy transformed to perform a function must be partly used to restore the initial conditions. Biological systems, in this case, have achieved impressive processes for energy recovery by exploiting a combination of structure and functional relationships that are still in many cases inimitable today because of their efficiency and complexity.

The work that is produced by energy conversion serves very important relationships of the organism with both the internal and external environments. In the internal environment, most of the work is used for communication between the various departments of the organism and is therefore essentially a coordination task; this task becomes increasingly important the more complex the organism.

The transformation of potential energy allows different uses for the relationship of the organism with the external environment. The energy is used to receive and respond to various stimuli that characterize an active biological organism. In order to perform the function of receiving external stimuli, specialized systems are needed that can pick up signals of different natures. Sensors are used for this task, highly specialized cellular structures that perform the function of interfacing between the organism and the surrounding environment. The responses that are then generated depend on the stimulus and are highly varied, from actions related to survival, such as the supply of nutrients, to the reaction to harmful stimuli.

In the following chapters, we will define and analyze the mechanisms by which the elements just described are able to perform and control the main functions of living organisms. Knowing the basic principles of physiological processes can help to understand each mechanism, from the simplest to the most complex. More importantly, it can help to understand still-obscure or only-partially-explored physiological processes. This path is fundamental for the training of biological research operators, who aim to study physiological processes whose function is not yet clear.

1.1 The concept of living matter in Physiology

Explaining what is meant by a living organism is extremely complex and understandably delicate, as it risks slipping into fields of knowledge that are outside the topics to be dealt with in the present context. We will limit ourselves, therefore, to the elaboration of concepts that are supported by scientific data and considerations that specifically concern the scientific field of physiological processes.

A definition that can be valid for all biological disciplines is that life occurs when an organism, however simple it may be, has the ability to reproduce itself. For disciplines such as Biochemistry, Genetics and Physiology, which deal with molecular processes, there are fundamental stages that underpin such a complex event as reproduction. These fundamental stages must be acquired by an organism to establish its "living being". From a physiological point of view, a living organism is one that is able to accumulate energy and transform this energy into work to feed, grow, defend and, ultimately, reproduce itself. From the evolutionary point of view, a biological organism reaches the highest chance of survival once it has reached the highest degree of adaptation to the surrounding environment.

Several conditions must be achieved for an organism to be physiologically viable. Some of these conditions are fundamental:

1. the ability to create separate environments, different from each other in content and composition of solutes, and at the same time to ensure communication strategies between different environments;
2. the physical-chemical characteristics of the interfaces between the different environments must allow the accumulation and maintenance of energy in the form of potential energy;
3. the ability to use electrical, chemical or mechanical potential energy to carry out work.

1.2 The concept of separation

What can be considered a “physiological living organism” must be able to create different environments, to maintain them as they are and with the fundamental characteristic of maintaining communication between them. Of what does this diversity consist? How can we hypothesize a process that leads to the formation of closed structures, such as the one presented in Figure 1.3 B. How is it possible that such structures manage to maintain different conditions between the two environments and at the same time communicate with the outside world without this leading, over time, to the formation of homogeneous environments?

The availability of time in the order of tens or hundreds of thousands of years, large quantities and a variety of materials in the elementary state and energy of various natures in enormous quantities have made the probability of events useful for the formation of complex structures relatively high. Secondly, it is also necessary to remember that there probably have been hundreds of thousands of attempts with different forms of proto-organisms, but that most have not exceeded the level of prototype. The simplest structures known today able to realize a separation of environments are the bacteria, followed by nucleated cells.

From the physiological point of view, the division between prokaryotes and eukaryotes can still be considered valid. In fact, while prokaryotes show only a division between internal and external to the organism, eukaryotes, in agreement with the etymology of the term, "perfect nucleus", have at least two internal environments. These internal environments are the cytoplasm and nucleoplasm, different with respect to the composition of solutes and the distribution of subcellular components. Not only that, but there are also two environments, the interior and the exterior, which differ in the distribution of ions. The sodium ion is abundant outside the cell and is present inside at a concentration ten times lower; on the other hand, the potassium ion is abundant inside the cell and dilute outside of it.

Therefore, in the course of evolution, the roadmap to build separations starts from the creation of two or three environments with different ionic compositions. But what were the conditions that favored this process? The analysis of rocks a few billion years old suggests that in primordial waters, potassium was weakly concentrated, from 10^{-3} to 10^{-9} M/L, while sodium and chlorine ions were at high concentrations, overall about 500 x 10^{-3} M/L. Similarly, if we analyze seawater, an environment where the first forms of life are believed to have evolved, sodium is about 500 x 10^{-3} M/L. Potassium is from 1 to 5 x 10^{-3} M/L. Between the two main ions present in the water, potassium thus represents the most suitable to be accumulated in a separate structure. Accordingly, the randomness of events has preferred potassium as the ion to be concentrated within defined structures to create differences in the concentration compared with the outside environment. Constitution of an environment where a particular ion species has a different concentration compared with the surroundings represent the first step to generate a chemical potential.

The creation of two different environments is more energy efficient if one of them is small compared to the other, as in the case of cells. In addition, if the interface between the two environments allows a mutual exchange of information, it must be equipped with selected structures able to discriminate the solutes that can pass through it. These structures are not simple holes integrated into the separation barrier. In addition to working like a filter, their conformation is in equilibrium between at least two states. If they constitute an aqueous pore spanning the separator, the equilibrium, depending on thermal agitation, can switch between a closed and an open state. The general conditions of the environment, the energy level of the system, several chemical modulators and finally, yet importantly, time are all variables that can modify the probability of being in one state or the other. The filter and the open and closed conditions represent one of the simplest examples of constraints.

The size and the shape of the smaller environment is fundamental from a physiological point of view and the relationship between surface area and volume therefore becomes important. In fact, the increase in size of a spherical organism leads to an increase in membrane surface area according to the square of the radius and an increase in cell volume according to the cube of the radius. The need to exchange information with the surrounding environment follows this principle. Therefore, a progressive increase in size leads to an overload of the exchange surface. From this simple consideration, it can be understood that there is an optimal size limitation for a living organism in order to obtain functional and valid control of internal as well as external communications.

The creation of two or more distinct environments, the presence of physical separators, the optimal size of the organism, the characteristics of the interface and the elements present on the separator to ensure proper functioning of the system are a significant example of the close correlation between structure and function, which in the study of Physiology is of primary importance.