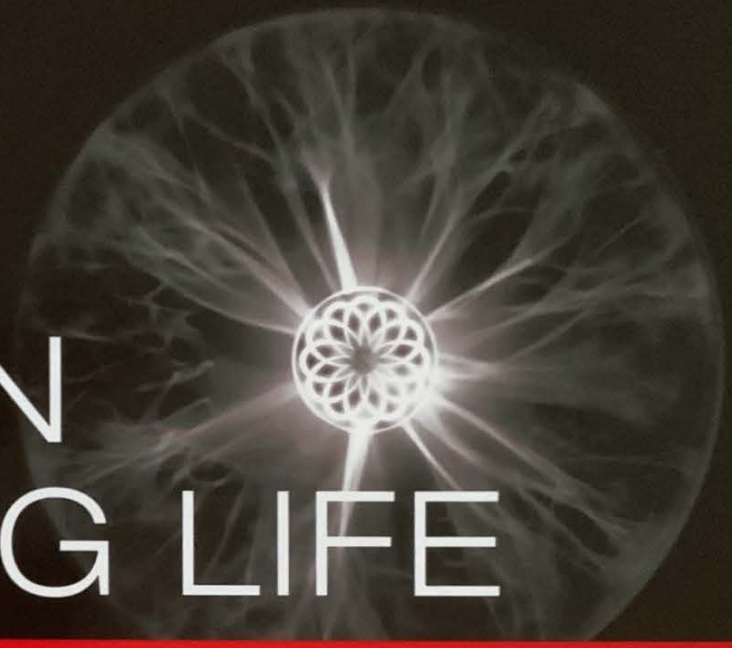
ROELAND VAN WIJK



LIGHT IN SHAPING LIFE

BIOPHOTONS IN BIOLOGY AND MEDICINE

· PERSPECTIVES BY JOHN M. ACKERMAN ·

Light in Shaping Life

Biophotons in Biology and Medicine

An Interdisciplinary Textbook

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Preface

Welcome to the study of the *photonics of life*. As you venture into this exciting field, you will discover many wonderful things.

Photonics is an all-encompassing light-based optical technology that is being hailed as one of the dominant technologies for this new millennium. Photonics utilizes photons instead of electrons to transmit, process, and store information and thus provides a tremendous gain in speed in information technology. *Biophotonics* is an extension of photonics dealing with the interaction between light and biological matter. In nature, one recognizes beautiful examples of biophotonics at work in basic principles of life such as harnessing photons in photosynthesis and conversion of photons in molecular structures.

Conversely, biology is also dealing with the endogenous production of *ultra-weak photon emission* (also indicated as *biophotons*) in and from cells, organs and organisms. This light emission is endogenously formed and characteristic for alive organisms. Its sources, interactions with biological matter, and its temporal/spatial emission are increasingly investigated with the use of highly sensitive photomultiplier tubes (PMT) and charge-coupled device (CCD) imaging systems.

The connection between endogenous photon emission and free radical reactions has been known by biologists and clinicians for many years. However, such interest has been amplified by recent discoveries of a.) the importance of free radical reactions in normal body chemistry as well as b.) novel developments in whole (human and animal) body photon counting and imaging technology.

Unfortunately, any expanding field attracts the charlatan (i.e., those who make money out of proposing that endogenous photon fields are effectively changed by their "therapeutic" methods and "will make you live forever" or, at least, will enhance your health). In evaluating these and other less obviously silly claims, it is useful to understand that the basics of ultra-weak biophoton emission need to be scientifically discussed.

Although several books and journals exist that cover selective aspects of biophotonics, there is a void that could provide a space for a unified synthesis of biophoton research. This book provides such an overview which is intended for multi-disciplinary readership that aids research integrating endogenous light and the shaping of biological systems. It focuses on historical biological background of the biophoton (field) concept and its

recent progress plus potential benefits to medicine. It encompasses the fundamentals of light, photonics, biophotons, and biology. In short, you'll begin a journey of discovery about how ultra-weak light makes life possible.

This book is aimed to lead the reader as painlessly as possible into an understanding of what biophotons are, how they are generated, and how they are involved in life. Having established this basis, the role of biophotons in health and disease is critically evaluated in the hope that their importance will become more widely utilized. I have tried to maintain a relaxed and conversational prose in the book without being excessively colloquial. This style makes it easier to convey to the reader my own excitement and enthusiasm for the beauty, intellectual challenge, and fundamental unity of biophotons within biophotonics. I hope that I have succeeded.

Before beginning, I would like to offer a few words of advice:
—Be prepared to learn a multi-disciplinary vocabulary. An understanding of biophoton facts requires that you learn a little biological, biochemical, physics, photonics and biomedical vocabulary. As with any newly studied discipline, the more familiar you are with the vocabulary, the more easily you can learn and appreciate the discipline's potential. To help the student from getting lost in what may seem like a blizzard of detail, I have introduced *Boxed Essays* to aid in the understanding of particular important, intriguing, or basic aspects of biology and physics.
—Each chapter begins with an introduction describing what a reader will discover. Each chapter ends with a take home message. The book emphasizes referencing. Specific referencing is more difficult than it seems because many of the statements made are distilled from several published papers and interpreted through the scientific experiences (or prejudices) of the author. Each major statement in the text is now provided with some references placed at the end of each chapter under the corresponding header. We hope that experts will forgive us if they find their pet paper not cited.
—Please tell me what you think! I welcome communications from anyone, especially concerning errors or deficiencies. I have spent much time and effort writing the book. In turn, you can help me by letting me know (by email or regular mail) what still needs to be improved! Your comments will be greatly appreciated.

In order to author a book such as this covering a very broad range of topics, I received help from a large number of professors, associated professors and senior scientists, cooperating scientifically vis-à-vis the International Institute for Biophysics (and Biophotons). Such assistance consisted of gathering technical content and illustrations kindly shared at the Summer schools (2000-2010) of the Institute. I owe a special debt of gratitude to: Rajendra P. Bajpai (North Eastern Hill University, Shillong,

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I also acknowledge the individuals whose broad-based support has been of paramount value in completing the book. I am much indebted to John M. Ackerman, M.D. (Santa Barbara, CA, U.S.A.) for sacrificing so much of his quality time with me to discuss and edit each chapter. It was a pleasure that such cooperation received its final form in our co-authorship of the chapter on "Perspectives". It was also a joy and a privilege to express my gratitude to my son, colleague and researcher, Eduard for his support during writing as well our joint many years of common research addressing human photon emission. I would further like to thank my colleagues Wim Berendsen, Fred A. C. Wiegant, and Jan Souren at Utrecht University (Utrecht, The Netherlands) and Jan van der Greef, Herman van Wietmarschen, and Everine van de Kraats at Leiden University (Leiden, The Netherlands) for their wholehearted support during many helpful discussions. I wish to also express my thanks to Jan Eikelenboom for his careful literature data work. In addition, I am deeply indebted to the publishing professionals who made this venture a reality. Special recognition goes to Kirsten Stekelenburg and Rens Wezelman.

Last but certainly not least, I wish to express my thanks to my wife, who has been a constant source of inspiration, providing support and encouragement for this project, in spite of her own busy schedule. Hey Mar, the book is finished, let us start a new one ☺.

Contents

Chapter 1	Prelude	*1*
■ **PART A**	*The Roots of a Field in Biology*	*11*
	Introduction Part A	*13*
Chapter 2	Historical Developments Within the Understanding of Regeneration	*15*
Chapter 3	Further Discoveries Acknowledge a Field that Guides Generation	*35*
Chapter 4	Does a Field Also Guide the Development of Cancer?	*53*
	Conclusion Part A	*73*
■ **PART B**	*Birth and Growth of Biophoton Field Concept*	*77*
	Introduction Part B	*79*
Chapter 5	Early Systems Biology and the Morphogenetic (Ultraviolet) Radiation Field	*81*
Chapter 6	Biology Looks at Physics	*101*
Chapter 7	The Entrance of the Photomultiplier	*113*
Chapter 8	World-wide Expansion of Photon Emission Research	*129*
	Conclusion Part B	*153*
■ **PART C**	*Cancer: Metabolism, Biochemistry and Photon Emission*	*157*
	Introduction Part C	*159*
Chapter 9	Cancer as Model to Understand Energy Metabolism in Relationship to Differentiation vs. a Dedifferentiation Switch.	*161*
Chapter 10	More about Glycolysis and Respiration vis-à-vis Cancer as a Model and its Relationship to Proliferation	*179*
Chapter 11	Beyond Respiration: Free Radicals and Photon Emission	*197*
	Conclusion Part C	*219*

■ **PART D** *From Bioelectromagnetism to the Photon Field Concept* 223

Introduction Part D 225
Chapter 12 Field Theory and Bioelectromagnetism 227
Chapter 13 Bone: Semiconductors as Biophoton Emitters 243
Chapter 14 Electromagnetic Fields and Life: A more Comprehensive View 259
Chapter 15 Revival of the Photon Field Concept: Towards an Integration of Sciences 273
Conclusion Part D 291

■ **PART E** *Improving Understanding and Application of the Photon Field Concept in Health and Disease* 295

Introduction Part E 297
Chapter 16 Photon Storage and Emission in Cancer Cells 299
Chapter 17 Photon Field Aspects in the 1990's 323
Chapter 18 Human Photon Emission in Health and Disease in the 2000's 341
Chapter 19 Imaging Human Photon Emission 361
Chapter 20 A Perspective: Biophotons As a Bridge Between Western and Chinese Medicine? 375

Glossary 385

Author Index 407

Subject Index 409

1

Prelude

During the transition from the twentieth into the twenty-first century, the first amazing images of human body light emission were eventually captured. A Japanese team of researchers were able to develop a novel and sensitive imaging technology that facilitated the recording of photon emission spatial patterns emanating in a completely dark room from anatomical sites of the human body. The emission of the body's light intensity was extremely weak. In order to record the light emission, the recording device (based on photon energy multiplication principles) was positioned very close to (but not touching) the body. Therefore, only light emission from very small body surfaces (such as fingers or a spot on the hand) were targeted. The utilization both of lenses as well as effective data handling improved the detection of signals, facilitating the recording from further distances. Larger surfaces could then be imaged. The light intensity was so weak that it was expressed in single photons per unit of time. It was calculated per square cm of body surface. No more than ten photons or multiples of ten escaped from the body. The energy of such photons was high enough to result in a blueish-green color.

The attention to the phenomenon of light-emitting humans was very limited for several decades both in biology and other sciences because only a small number of researchers and few biological laboratories world-wide had the technology to detect ultra-weak photon emission. However, those who were interested hypothesized that the emission was actually emitted by all living organisms. In the field of biochemistry, attention had been focused on chemiluminescence only as a byproduct of specific enzyme reactions in cells without any special function. In biology then, it was only known that several living organisms do, in fact, emit light (bioluminescence) with an intensity that we, as humans, can visually detect. Although rare in nature, bioluminescence can be observed in some simple as well as complex organisms. It varies from bacteria to more organized multicellular animals (both invertebrates and vertebrates) living terrestrially or in the ocean. Bioluminescence is at least 1000 times more intense than ultra-weak photon emission. Biologists eventually discovered that bioluminescence can play a role in communication such as mating or attracting prey. The communicative role of ultra-weak photon emission was then not easily accepted without clear evidence.

However, one discovery within ultra-weak photon emission research was the fact that emission intensity was not a constant property. It seemed to be related to life style, health and disease. Its presence had been previously published in *Nature* in 1988 under the title, "Body light points to health". Subsequently, it was largely forgotten until more pronounced ultra-weak photon emission was scientifically documented both when tumors begin to grow and during the beginning stage of rheumatoid arthritis. It was also increased under the influence of stress. Although the awareness of enhanced ultra-weak photon did not explain its functional role, it did stimulate the possibility that such data might be useful for early diagnostic purposes.

Ultra-weak photon emission had not yet been a subject taught in general biology nor in the field of biomedicine. That made it difficult to discuss and understand not only how it could be measured but also how it is related to the state of the organism. Its potential use in diagnosis could not be based on the presence (increase or decrease) of specific molecular substances. Rather, it had to be related to another development in the field of biological system diagnostics that included substance/field dualism and its interpretation. Substance/field dualism had often been illustrated when iron filings are scattered on a card held over a magnet. The iron filings arrange themselves vis-à-vis the pattern of lines of force of the magnetic field. If the filings are thrown away and fresh ones are scattered, the new filings will assume the same pattern as the old. Something analogous may happen in the cell or the body. Molecules and cells are constantly being torn apart and rebuilt with fresh materials. The combined pattern of molecular structure and a magnetic field is a very simple example of the substance/field dualism.

The basics of systems biology include how a system develops, how it is maintained, how its maintenance can fail, and how repair is possible in that system. They are the core questions utilized in the disciplines of embryology, pathology (tumor biology), and regeneration. It is the "program" that determines the outer and the inner forms of biological organisms. The term "program" will be defined later in the book. The most intriguing aspect of the "program" determines the exact "positioning" of the many differentiated cell types of tissues and organs as well as the thousands of different proteins and other large molecules that restore the overall pattern of molecular aggregates inside an injured cell (Figure 1.1). Cancer is directly related to this mysterious "program" reflecting disturbances of the most fundamental rules of behavior of "normal" cells.

The substance/field dualism in a biological system is based on its associated radiant (electromagnetic) structure, a stationary field obtained by superimposition of all fields associated with its sub-systems. Under such

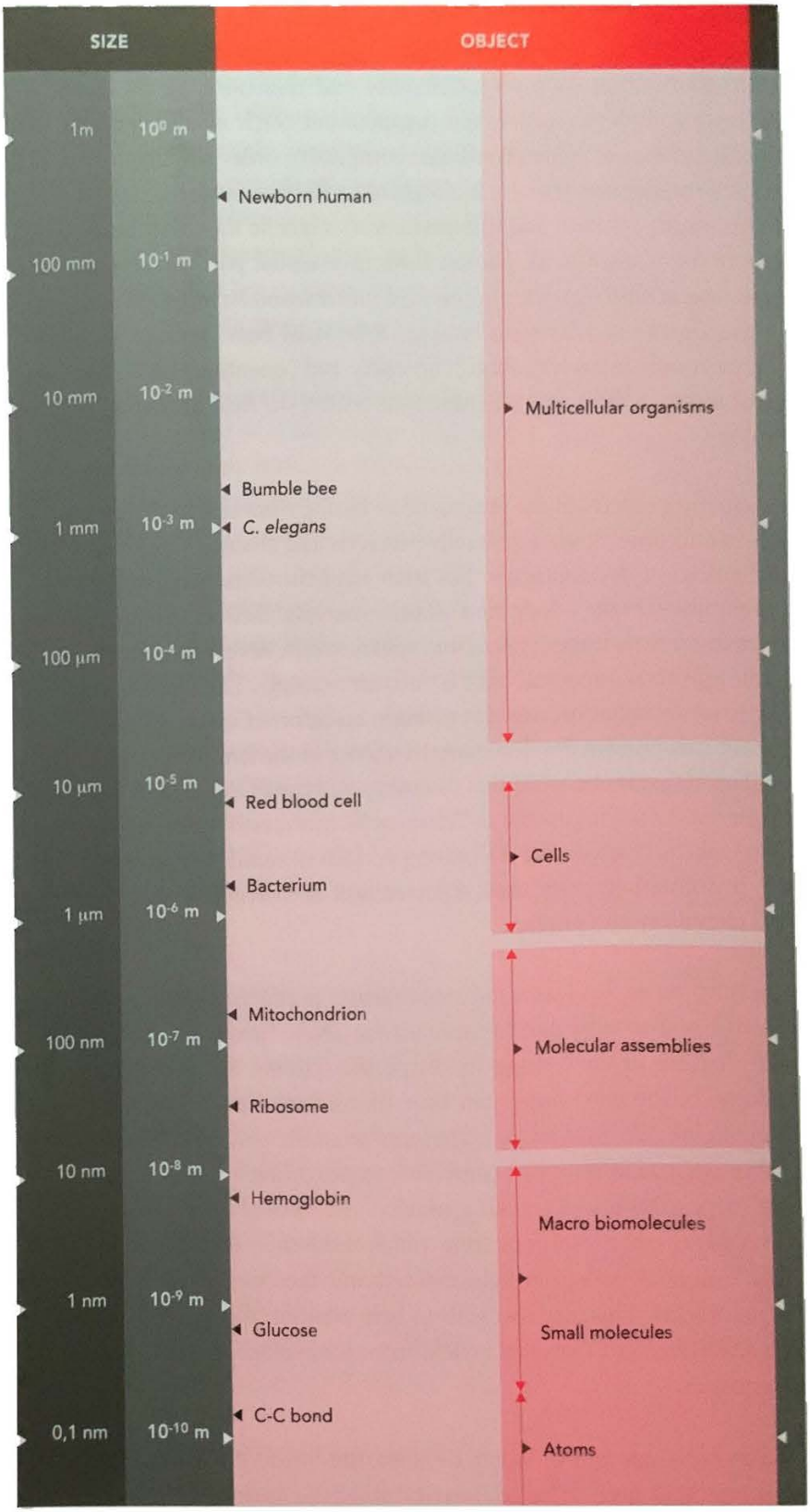
circumstances, the interferences of oscillations are one process of conserving information (an optical code). The generation of codes associated with information vis-à-vis interference and resonance are the pivots for creating complexity, order, and organization. Such interactions can lead to adjustments as systems optimize complexity, order and organization. It is such interactions that are highlighted in the two fundamental processes of becoming (growth and differentiation). Here lie the most fundamental questions. Is ultra-weak photon field an essential part of the substance/field interaction? Can the photon field information be included in the field of diagnostics in a "systems biology" approach? New studies must, therefore, not only focus on (photo)chemistry and (quantum) physics but also must aim to collect new scientific facts within the field of (living) systems biology.

Fascinating aspects in the process of accepting what is scientific are expectation and time. Science generally proceeds and changes rapidly with new technological developments. But what happens when such technological developments take a long time? Then, scientific facts are easily forgotten since they are no longer part of the rapid scientific developments. The ultra-weak universal biological light is such an example. The discovery of light informative radiation was not a single unexpected event. Instead, it was part of mainstream developments in science in the late 1900's. However, in the last 100 years between that discovery and the present, the scientific attitude vis-à-vis the concept of "ultra-weak (universally present) biological light" has fluctuated. The alternating periods of rapid progress and standstill in technology, over time, reflected lack of interactivity between biology, chemistry and physics.

The purpose of this text is to bring together pertinent aspects of previous research and to raise questions about the above "program" of substance/field dualism in the complexity of specific systems. Such may contribute to some of the most important (but almost forgotten) issues in the field of medicine. We must try to understand what the science of biophotons is and in what manner it can contribute to providing a more "truthful picture". Along this line of thinking, much of this book, *Light in Shaping Life* is about history, the traditions from which modern knowledge about ultra-weak universal biological light evolved and the controversies that arose in the process. This text also aims to help position the above information in a scientific field that may benefit from the science of biomedicine and diagnostics.

Two main issues follow: where to begin the history and the sequence of questions that need to be addressed during the journey? In the first part of the book, the reader will embark at the beginning of the journey 300

Figure 1.1
Biologists are interested in both the shape and shaping of living organisms from atoms and electrons to the entire human. The ordering of biological objects is aligned on a logarithmic scale.



years ago and subsequently travel through the first 200 years of research on "Formation of the Form". This ultimately led to the discovery of mitogenetic radiation. Subsequently, the latest century will be presented in four major parts representing progress in the understanding of "universal biological light" as it unfolded during alternating periods of active and inactive research within the inter-connected disciplines of biology, chemistry and physics (Figure 1.2). The building of such integrated understanding regarding various molecular components that underlie cellular functions in a living cell were explored within the realms of cell biology, biochemistry, developmental biology, bioelectromagnetism and biophotonics facilitating the presence of many additional pertinent questions clamoring to be explored.

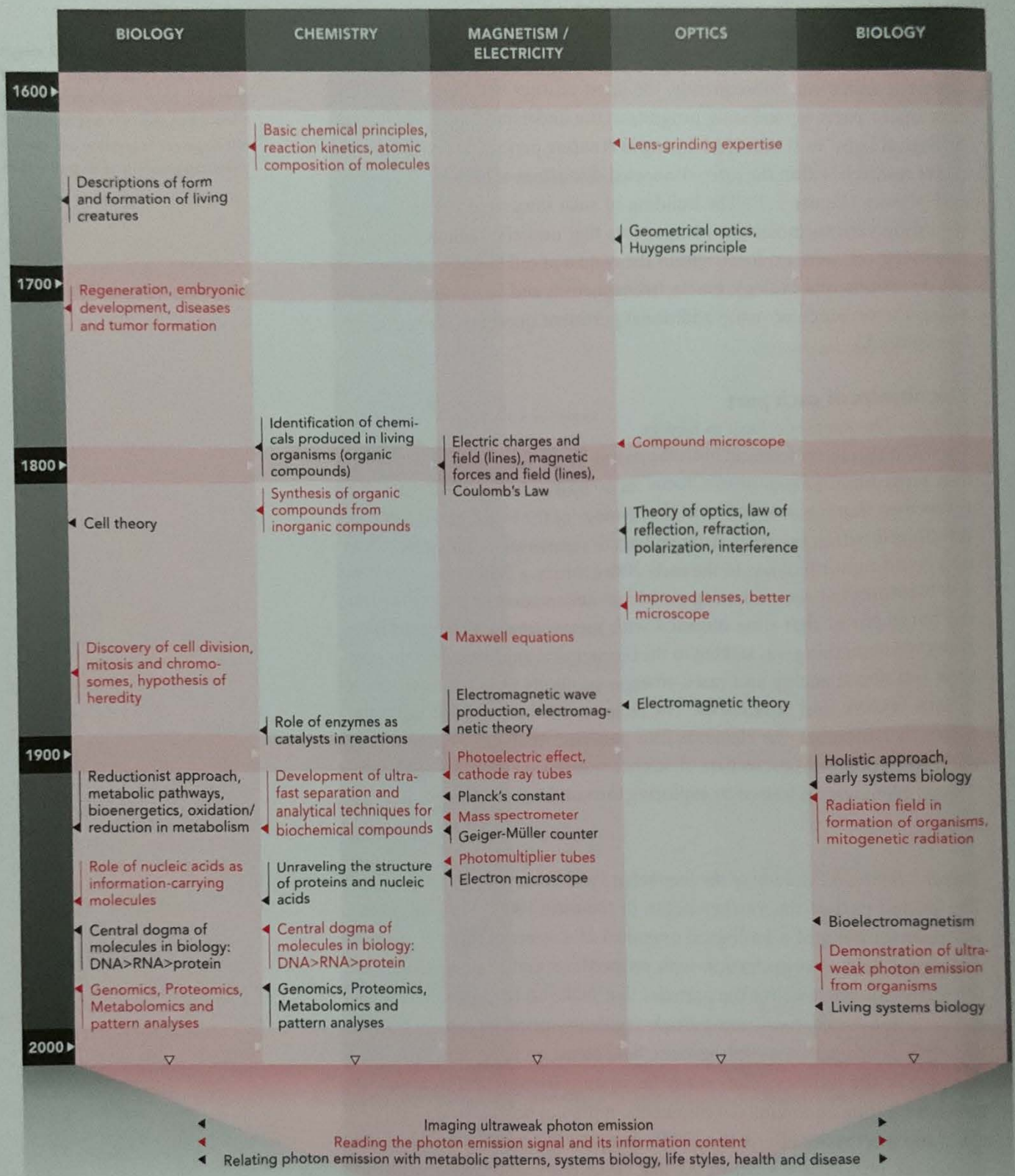
The objects of each part

Part A – The Roots of a Field in Biology

We will begin to understand both the nature of the blue print and instruction program of an organism's "form" as we look at biology 300 years ago. It was then that the description of the "biology of the form" became visibly intermixed within the three strong pillars of regeneration biology, embryology, and tumor biology. In the early 20th century, a field of organization was hypothesized encompassing positional information. It evolved within the paradigm of that time obsessed with metabolism and biochemistry. However, something was lacking in the biochemical approach. Metabolism then was about energy and rates, whereas positions were addressed with scalars, vectors, and boundaries. The recipe needed an accounting of the forces that mobilize the elements into patterns. At that time, the aspect of force in organization as part of science was not included in textbooks. The problem was addressed by exploring the radiation of a morphogenetic field.

Part B – Birth and Growth of the Biophoton Field Concept

The second part of the journey began in the early 1900's vis-à-vis a better understanding of a biological organism as a system. This perspective accepted a field of organization with properties specific to the totality of the form, not solely about the particles that make up the form. In searching for a non-mechanical/non-particle regulatory principle, an alternative principle developed: a morphogenetic field based on radiation. Although this part of the journey offers many fascinating scientific explorations, it took a long time and many developments in physics before biology definitively and internationally, in the 1970's, accepted the existence of universal ultra-weak biological radiation. At that time, one could finally begin to document the richness of information from measurements of biological photon emission. The new photovoltaic and multiplier technologies connected photon emission with the biochemistry of oxygen and oxygen reac-


BIOLOGY
CHEMISTRY
MAGNETISM / ELECTRICITY
OPTICS
BIOLOGY
1600
1700
1800
1900
2000
Basic chemical principles, reaction kinetics, atomic composition of molecules
Lens-grinding expertise
Descriptions of form and formation of living creatures
Geometrical optics, Huygens principle
Regeneration, embryonic development, diseases and tumor formation
Identification of chemicals produced in living organisms (organic compounds)
Electric charges and field (lines), magnetic forces and field (lines), Coulomb's Law
Compound microscope
Synthesis of organic compounds from inorganic compounds
Cell theory
Theory of optics, law of reflection, refraction, polarization, interference
Improved lenses, better microscope
Maxwell equations
Discovery of cell division, mitosis and chromosomes, hypothesis of heredity
Electromagnetic wave production, electromagnetic theory
Electromagnetic theory
Role of enzymes as catalysts in reactions
Photoelectric effect, cathode ray tubes
Holistic approach, early systems biology
Reductionist approach, metabolic pathways, bioenergetics, oxidation/reduction in metabolism
Development of ultrafast separation and analytical techniques for biochemical compounds
Planck's constant
Mass spectrometer
Geiger-Müller counter
Radiation field in formation of organisms, mitogenetic radiation
Role of nucleic acids as information-carrying molecules
Unraveling the structure of proteins and nucleic acids
Photomultiplier tubes
Electron microscope
Bioelectromagnetism
Central dogma of molecules in biology: DNA>RNA>protein
Central dogma of molecules in biology: DNA>RNA>protein
Demonstration of ultraweak photon emission from organisms
Genomics, Proteomics, Metabolomics and pattern analyses
Genomics, Proteomics, Metabolomics and pattern analyses
Living systems biology
Imaging ultraweak photon emission
Reading the photon emission signal and its information content
Relating photon emission with metabolic patterns, systems biology, life styles, health and disease

◄ **Figure 1.2**
Main ingredients of the synthetic theory on the role of information-carrying photon field in shaping organisms. The summary flow diagram shows, in roughly chronological order, how many disparate ideas from the disciplines of biology, chemistry and physics were gradually combined to create the synthetic theory of an informative biophoton field. The diagram is extended to show the co-development of technology and knowledge that typifies the progress of science. This diagram may also be considered as presenting the parts and subdivisions of the book.

tive species. However, during this part of the journey, the original interest in the informational content of radiation related to "form" was perhaps forgotten, or at least it was no longer obvious, or when present, only an issue of minor importance.

Part C – Cancer: Metabolism, Biochemistry and Photon Emission

The third part of the journey focuses on a separate line of research which progressed during the 1920's-1960's. It dealt with oxygen and metabolic energy and their role in tumor formation and differentiation. During this period, the glycolytic and respiratory pathways were discovered and the connection with differentiation and tumor established. The data in this period suggested that there are two kinds of energy available in life processes, the energy of fermentation (glycolytic pathway only) and a "superior" energy of respiration which includes functional mitochondria in the presence of oxygen. Respiratory energy creates and maintains a high differentiation of body cells. Fermentation energy can only maintain a low differentiation corresponding with increased growth. A large variety of tumors of the same tissue gave evidence for a systematic change in the biochemistry of metabolic pathways (the Molecular Correlation Concept). The replacement of respiration by fermentation corresponds with the disappearance of the high differentiation state resulting in the appearance of cancer cells. The question, "How may tumor cells arise and progress *in vivo*?" became evident as a result of a severe cell stress response resulting in fluctuations of oxygen limitation and re-oxygenation (reperfusion). The role of reactive oxygen species (ROS) became known during this part of the journey. With the advent of modern technology and increasing knowledge of the relationship between mitochondrial functioning and glycolysis, cancer might be interpreted as a mitochondrial dysfunction. Although the link between photon emission as part of the reactive oxygen dependent processes and tumor development was more firmly established, the question whether photon emission is able to regulate the mitotic process required an additional concept: the specific properties of an electromagnetic field. At that time, it was not part of molecular cell biology. We must now include another interface between biology and physics.

Part D – From Bioelectromagnetism to the Photon Field Concept

The fourth part of the journey deals with a contribution evolving from the concept of the bioelectromagnetic field and its organizational role within the living organism. From the 1930's on, it became evident that biological systems can emit electromagnetic waves and can also have sensitive physiologic responses to the entire spectral range. The organism embodies diverse interconnections of electromagnetic fields (in addition to the known diverse biochemical connections). Research data suggested a link with the mitogenetic radiation fields as well as a link that accounts for the *forces* that *mobilize* molecules of different kinds into a pattern. The "optical photon emission window" may, then, offer a view of the electromagnetic interaction field with the way a body develops and keeps its "shape" within the ceaseless shifting metabolism of material. It may even be leading the pattern development of chemical "morphogens" (which are then interpreted into body pattern by something else, such as, special genes). It was this journey at the end of the 1970's that suggested the possibility of integrating all previous scientific hypotheses resulting in the revival of the photon field concept.

Part E – Improving Understanding and Application of the Photon Field Concept in Health and Disease.

The last part of the journey began in 1977. The role of the photon field in metabolic and cell biological regulation was considered, bringing expertise simultaneously from biology, chemistry and physics. The idea emerged that a biological system not only has the property of emission but also the capacity to store (or trap) photons. The trapping, as a photochemical process, may influence metabolic events. It resulted in a cell resonance cancer theory. Data supported the fact that tumor cells with a lower degree of differentiation have a high-short storage and a low-long-term resonance system capacity. In biophysics, field/matter interaction studies focused on protein and DNA levels as well as on the process of differentiation.

The "optical window" provides a key to understanding the organization of living systems. It creates a challenge to collaborate on the theme of photon emission from animal and human systems linking it to metabolism in health and disease. Since the late 1990's, such collaboration has resulted in novel techniques that can both estimate the human body emission pattern as well as novel procedures to analyze the language of human light. Present developments focus on the relationship to life style, health and disease.

Conclusion

A fundamental message of this text is that different disciplines collaborating (over a long time) have been co-creating an improved understanding of the underlying "program" (blue print, instructions and forces) that result in the formation of the "form" of a living system. The journey in "universal

ultra-weak biological light" is not finished. However, the golden age may be just around the corner. To embrace it, we must set aside the old patterns and reference frames of the past, let go of rigid habits of thought that serve us no longer, and be willing to accept a permanent shake-up in our outlook regarding the photon field.

PART A

The Roots of a Field in Biology

Introduction Part A

The introduction into biology of the "field" concept in 1912 is a fundamental step in understanding one of the most enigmatic problems in biology, the process of morphogenesis. It was one of the pillars of the early story of the biology of the "Formation of the Form". This was the major focus of three disciplines in biology: regeneration biology, embryology, and tumor biology. Historically, the thinking about both the formation and deformation was strongly influenced by the cell division concept and its relationship with differentiation. An introduction to the three disciplines will present the scientific scene in which the notion of a regulative (light) radiation field developed.

Historical developments within the understanding of regeneration

We begin the history of regeneration research in 1712, when René-Antoine Ferchault de Réaumur presented his study to the French Academy of Sciences regarding regeneration of crayfish limbs and claws. It stimulated many scientists in the 1700's to follow in his footsteps, initiating a shift in thinking from "preformationism" to "epigenesis". Cells were unknown at that time. Cell biology was born in the late 1820's and 1830's. It took until the 1870's and 1880's for the stages of cell division to be identified. Subsequently, it was definitively established that all living things were composed of cells as the basic units of life. This marked the renewal of interest in the development of the form which was not limited to regeneration but also to the regulation of cell division in both eggs and early embryological development.

Further discoveries acknowledge a field that guides generation

In the 1880's, embryology matured as an experimental science. A debate unfolded addressing the question: Are future parts and functions preformed in the fertilized egg and then unfold in a deterministic manner, or is the formation epigenetic (the fate of a cell is a function of its position in the system)? The identification of both polarity and gradients led, in 1921, to the introduction of the "field of organization". Subsequently, the biochemical approach of a "patterning" phenomenon prevailed and still does.

Does a field also guide the development of cancer?

As the 20st century began, embryology and regeneration biology were not the only pillars of science addressing the formation of the form. The third pillar addressed the development of cancer theorizing that it might be analogous to both embryological development and the phenomenon of

regeneration. Cancer does not observe the "boundary laws" of "normal" differentiated tissue. Instead, it encroaches imperialistically upon its neighbours. Some fragments break off initiating colonies (metastases) throughout the body. Such events were observed in the 1910's and 1920's during "homoplastic implantation" research wherein structures in various stages of development encountered other differentiated structures. Even more interesting was the fact that an environment of regeneration could control the primitive cells of cancer. That was the revival of the embryonal hypothesis regarding the origin of carcinomata.

2

Historical Developments within the Understanding of Regeneration

Experimental biology was born in the efforts of eighteenth-century naturalists to document regeneration (Figure 2.1). Regeneration is the reactivation of development in postembryonic life to restore missing tissues. The regeneration experiments of Trembley (*Hydra*), Réaumur (*Crustacea*), and Spallanzani (salamanders) set the standard for experimental research and for the academic discussion of one's data (Dinsmore 1991).

More than two centuries later, we are beginning to find answers to the great problem of regeneration. We may soon be able to understand why we cannot grow back our arms and legs. What gives salamanders this ability we sorely lack?

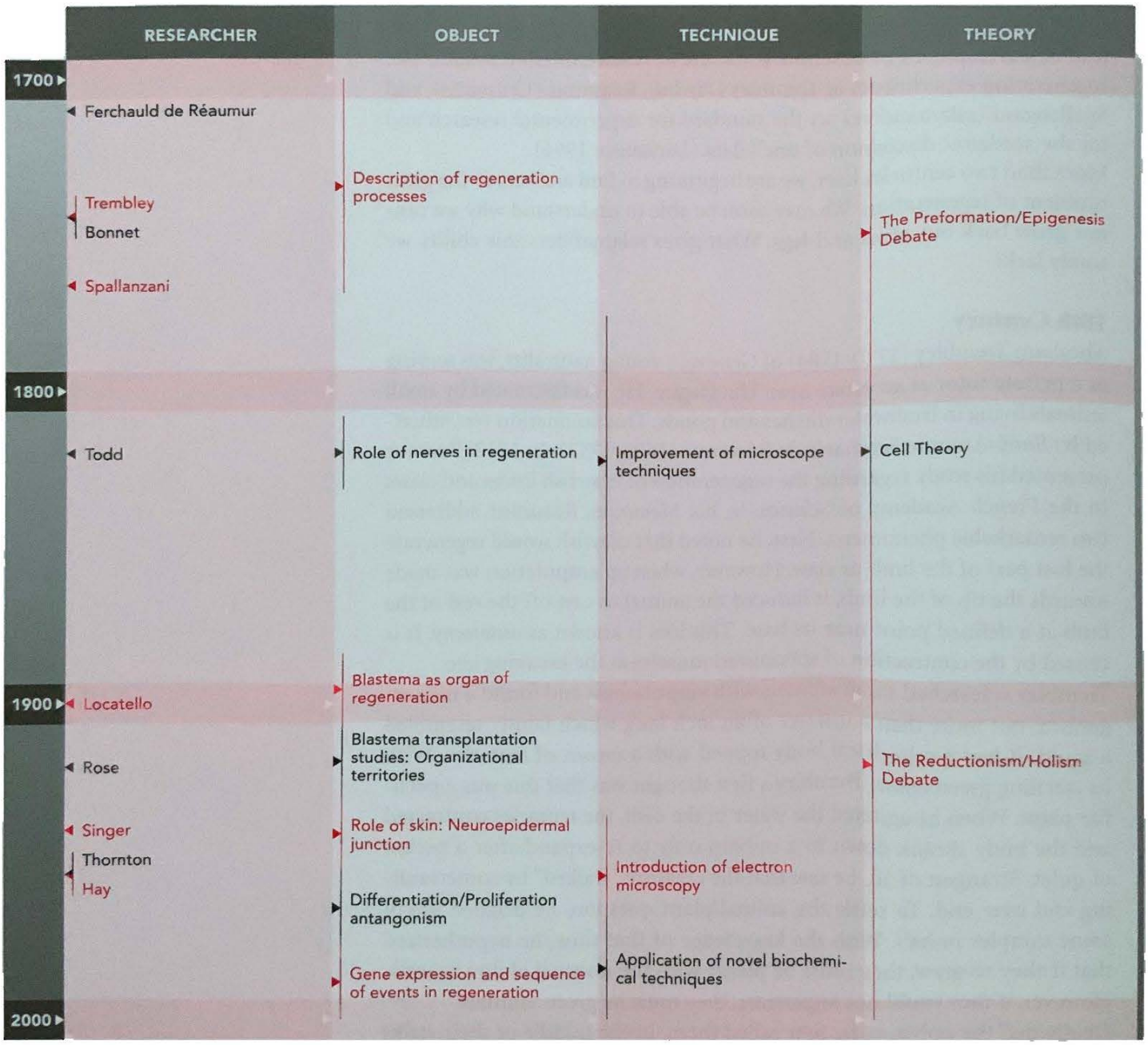
18th Century

Abraham Trembley (1710-1784) of Geneva, a young naturalist, was serving as a private tutor at an estate near The Hague. He was fascinated by small animals living in freshwater ditches and ponds. This fascination was initiated by René-Antoine Ferchault de Réaumur (1683-1757). In 1712, Réamur presented his study regarding the regeneration of cray fish limbs and claws to the French Academy of Science. In his *Memoires*, Réaumur addressed two remarkable phenomena. First, he noted that crayfish would regenerate the lost part of the limb or claw. However, when an amputation was made towards the tip of the limb, it induced the animal to cast off the rest of the limb at a defined point near its base. This loss is known as *autotomy*. It is caused by the contraction of specialized muscles at the breaking site.

Trembley researched small animals with amputations and found a new organism, not more than a quarter of an inch long which faintly resembled a squid. It had a cylindrical body topped with a crown of tentacles. With its startling green color, Trembley's first thought was that this was a peculiar plant. When he agitated the water in the dish, the tentacles contracted and the body shrank down to a nubbin only to re-expand after a period of quiet. Strangest of all, he saw that the creature "walked" by somersaulting end over end. To settle the animal/plant question, he decided to cut some samples in half. With the knowledge of that time, he hypothesized that if they re-grew, they must be plants with the unusual ability to walk. However, if they could not regenerate, they must be green animals.

He divided the polyp, as he first called them, in the middle of their stalks

Figure 2.1
Time-course of some salient contributions to previous thinking related to the early development of regeneration biology. This diagram may be seen as a detailed fragment of Figure 1.2 and may also be considered in conjunction with Figure 3.1 and 4.1.



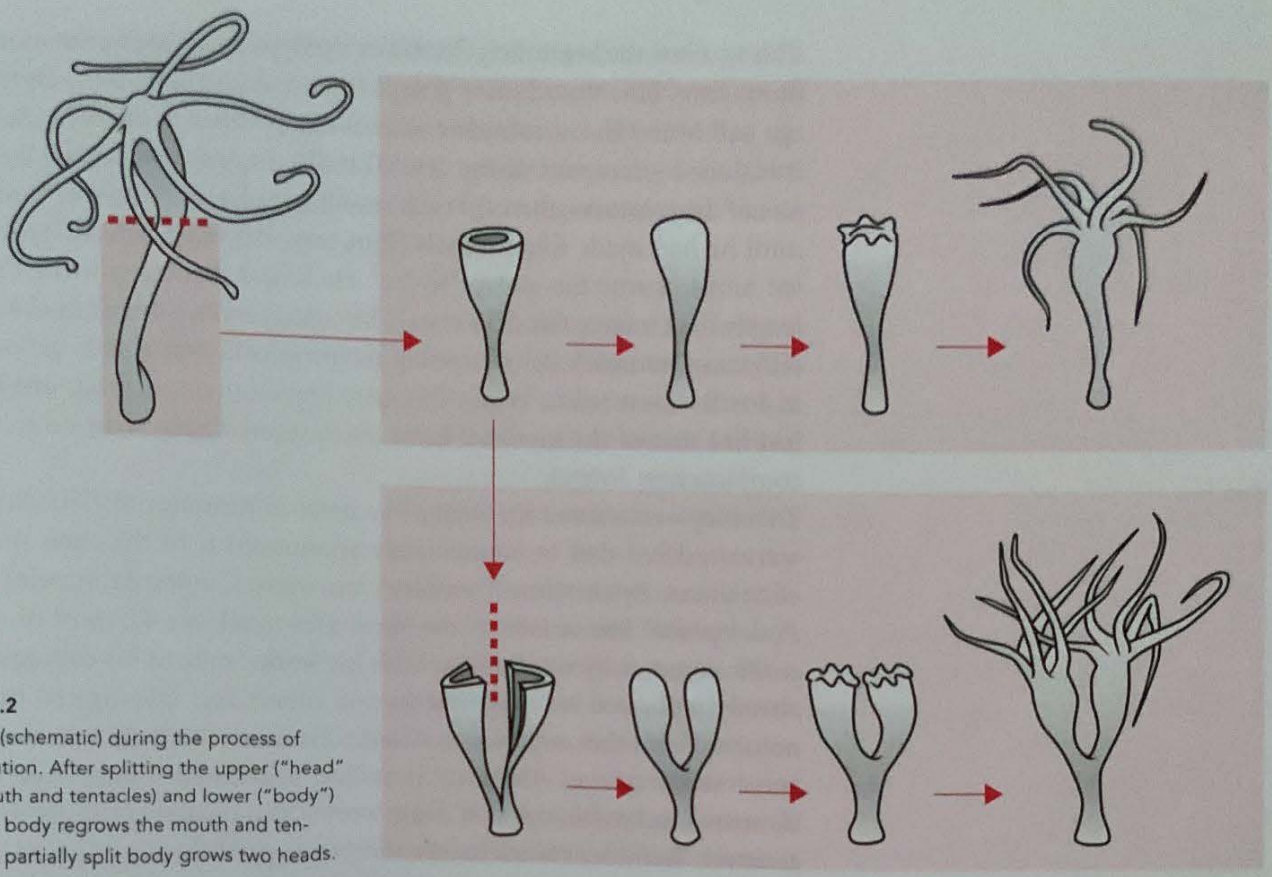


Figure 2.2
A *Hydra* (schematic) during the process of regeneration. After splitting the upper ("head" with mouth and tentacles) and lower ("body") part, the body regrows the mouth and tentacles. A partially split body grows two heads.

(Figure 2.2). He then had two short pieces of stalk, one with attached tentacles, each of which contracted down to a tiny dot. Watching patiently, Trembley eventually witnessed the two pieces expand. The tentacle portion began to move normally as though it was a complete organism. The other portion lay inert and apparently dead. After Trembley continued watching the motionless object for nine days, he noted that the cut had sprouted three little "horns". Within a few more days, the complete crown of tentacles was restored. Trembley now had two complete polyps as a result of cutting one in half. Even though they regenerated, more observations convinced Trembley that the creatures were small animals. Not only did they move and walk, but their arms captured tiny water fleas and moved them to the "mouth" (located in the center of the ring of tentacles) which promptly swallowed the prey.

This was just the beginning. Trembley's polyps performed even more wondrous feats. He minced some polyps into as many pieces as he could manage and found that a complete animal regrew from each piece (as long as it included a remnant of the central stalk). In one instance, he quartered one of the creatures, then cut each resulting polyp into three or four pieces until he had made fifty animals from one. His most famous experiment led him to name this polyp "hydra". He found that by splitting the head lengthwise (leaving the stalk intact) he could produce one animal with two crowns of tentacles. By continuing the process he was able to get one animal with seven heads. When Trembley lopped them off, each one regrew, just like that of the mythical beast. Each severed head went on to form a complete new animal.

Trembley wrote about his results in a letter to Réaumur in 1741. Réaumur was so excited that he immediately announced it to the Paris Academy of Sciences. By the time Trembley's discoveries appeared in print in the *Philosophical Transactions of the Royal Society* volume 42, most of the scientific community was familiar with his work. Some of his colleagues had already replicated his experiments and others had investigated the phenomenon in other organisms. Charles Bonnet (1720-1793), a Swiss scientist and cousin of Abraham Trembley, performed similar experiments in worms and published that some worms also displayed the ability to regenerate. Trembley's work in its entirety was published in 1744: *Mémoires, pour Server à l'Histoire d'un Genre de Polyps d'Eau Douce, à Bras en Forme de Cornes.* It was translated into English by Sylvia and Howard Lenhoff under the title *Hydra and the Birth of Experimental Biology – 1744.*

A furious debate raged at the time of Trembley's announcement. It concerned the origin of the individual. It was a debate on preformation and epigenesis. This debate often seemed for many people to represent distinguishable positions regarding how organisms evolve to a differentiated form. Both preformationists and epigenesists addressed the question: When does the embryo arrive at its expected final differentiated form? Or, at what point does the individual assume the characteristics of its type? Preformationists traditionally held that the form is already predetermined at the earliest stages of an individual's existence. Epigenesists had traditionally maintained that form emerges only gradually during the developmental processes.

It seemed a debate without end since knowledge was so limited compared to our present understanding. Thus, in this period, it was even difficult to distinguish, on firm grounds, between an animal and a plant. We also have to be aware that cells and cell division (or mitosis) were unknown at that time. In fact, in 1740, knowing nothing about cells, yet beginning to insist on logical, scientific explanations, epigenesis seemed rather absurd. Instead, the idea of preformation (meaning that a frog or person already exists in

miniature form inside, which then could possibly transform the gelatin of eggs and sperm into a frog or a human) was a more widely accepted hypothesis. This was also the opinion of Charles Bonnet. He defended the preformationist philosophy while attacking epigenesis. Trembley's sliced *Hydras* and their regeneration suddenly raised new arguments in favour of epigenesis. It was part of the ongoing furious debate.

In the middle of the debate, another series of discoveries by Lazzaro Spallanzani (1729-1799) became known. Born in Northern Italy in 1729, Spallanzani studied at a Jesuit seminary in Reggio Emilia, matriculated in 1749 at the University of Bologna, and earned his doctorate in 1754 in natural sciences and mathematics. He became professor of philosophy at Modena in the University and College of Nobles and later at the University of Pavia. He published extensively and his work has been celebrated for its creative approach and rigorous use of scientific methodologies. His attitude was scientific: he read voraciously and preferentially believed anything that he could prove himself. He had a strong interest in regeneration as we have seen with others. In twenty years of meticulous observation, he studied regeneration in worms, slugs, snails, salamanders, and tadpoles. Spallanzani's most important contribution to science was his discovery of the regenerative abilities of the salamander. The salamander is a marvelously complex animal, almost as complicated as a human. Its forelimb is basically the same as that of humans. Yet, after amputation, all its interrelated parts grow back in the proper order. Interested in the origin of regenerating tissue, he closely examined the interface between the stump and the regenerated tail. He had trouble believing that an organized tail could result from a simple outgrowth and he continued to look for evidence that could support his inclination toward the existence of preformed germs. He was a great admirer of Charles Bonnet and was deeply influenced by the Swiss scientist's philosophy. He initially tried to interpret his regeneration data in a way that supported Bonnet's preformationist philosophy. However, he openly reported his observations questioning preformationism. In contrast, he suggested that the tail regenerates in tadpoles as the result of an elongation vis-à-vis an epigenetic process. In 1768, he published *An Essay on Animal Reproductions* in Italian, which was translated a year later into English.

20th Century

Later studies have confirmed the crucial conclusions of Spallanzani and have stepwise produced more insight into the details of the miraculous process of regeneration. The later studies, particularly on a cell level were pertinent. Thomas Hunt Morgan (1866-1945), a biologist, did, in fact focus on that area. He is probably better known for his fundamental work on *Drosophila* genetics. However, his initial research addressed the regenera-

MORE ABOUT...

BOX 2.1

■ EPIGENESIS AND PREFORMATIONISM

Epigenesis and Preformation are two ways of attempting to explain the development of an individual organic form. The core question underlying the existence of these two competing philosophical traditions is the extent to which something is considered either as being formed or organized from the "beginning" or whether organization and form arise only over time. The question is in part metaphysical. What is it that exists: form or also the unformed that becomes the formed? The debate has persisted since ancient times. Even today, the debate continues in terms of nature or nurture, genetic determinism or developmental free will. Included in the debate is when life begins (with profound bioethical and policy implications).

Epigenesis

The concept of epigenesis, was supported by Aristotle (384 BC-322 BC), a Greek philosopher, a student of Plato and a teacher of Alexander the Great. Together with Plato and Socrates (Plato's teacher), Aristotle was one of the most important founding figures in Western philosophy. Among many things, Aristotle was a keen observer of embryos. Looking at chicks, for example, he observed "material, final, formal, and efficient causes" at work in developing individual organisms. The early egg was not already a little chick. Instead, it gradually acquired form. The essence of the material may be there from the beginning, but its destiny is gradually directed internally.

In sexually reproducing species, the fluids from the mother and the father combine. From that point on, the process is internal. The process leads to development of the form of an individual's type ("Once a thing has been formed, it must of necessity grow"). Aristotle believed that an individual organic life requires a "soul" which must be present from the outset residing within the material body. The soul guides the gradual epigenetic process of development. This is the Aristotelian and not the Christian soul. Such a soul consists of the vegetative (for all organisms), locomotory (for those organisms that move, primarily animals), and rational (as Aristotle considered for those organisms that think, namely man). This soul is the teleological drive of the potential that actualizes the individual and its form and function (epigenetically, gradually, and internally).

Aristotelian epigenesis provided the background assumptions about individual development as the 18th century began. Researchers sought to observe the gradual emergence of form from non-form through the process of "ensoulment". In effect, this interpretation of epigenesis depended on a life force within the organism driving its emergence of form. Those who accepted epigenesis also accepted a form of vitalism. Matter in motion, by itself, would not have the capacity to produce these results. How could matter become formed when it was not? How could the emerging form acquire the capacity to function without some vital force or factor that was not strictly material? This was the problem for materialists.

Preformationism

In the history of biology, "preformationism" was the specific contention that all organisms were created at the same time and that succeeding generations grow from homunculi, animalcules, or other fully formed but miniature versions of themselves that have existed since the beginning of creation. Later, the notion that the form of living things (in some sense) preexisted their development. In 1651, William Harvey (1578-1657) published *On the Generation of Animals* (*Exercitationes de Generatione Animalium*) that contradicted many of Aristotle's fundamental ideas. Harvey's ideas regarding the process of development were fundamentally epigenetic. However, this epigenesis was more mechanistic and less vitalistic than the Aristotelian version. There was no available mechanical explanation for epigenesis because of technological limitations. It was, thus, more compatible with the natural philosophy of the time.

The Dutch microscopist Antonie van Leeuwenhoek (1632-1723) was one of the first to observe spermatozoa. He thought he saw in semen, "all manner of great and small vessels, so various and so numerous that I do not doubt that they be nerves, arteries and veins... And when I saw them, I felt convinced that, in no full grown body, are there any vessels which may not be found likewise in semen." After this discovery in 1677, the epigenist theory proved more difficult to defend. How could complex organisms such as human beings develop from such simple organisms? Henceforth, Marcello Malpighi (1628-1694) made observations using microscopes in the late 17th century.

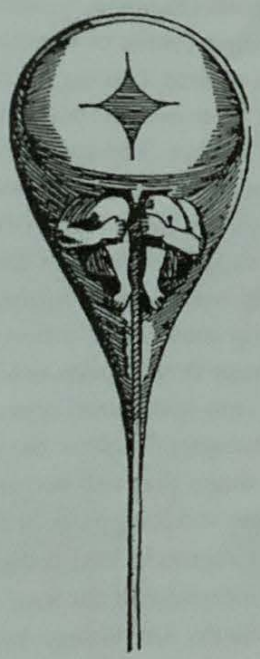


Figure B.2.1.1
A "homunculus" inside a spermatozoon. The imaging drawing by Nicolaas Hartsoeker in his *Essai de Dioptrique* (1694).

Malpighi demonstrated that the unincubated chick egg already had a great deal of structure. This observation provided him with reasons to question epigenesis. He interpreted these findings while developing the preformationist theory which further invigorated the controversy. Organisms were not seen to be "constructed", but rather "unrolled". No extra mysterious force was needed for embryonic development. A preformation perspective was a popular representation of the alternative. In 1694, Nicolaas Hartsoeker (1656-1725), in his *Essai de Dioptrique* (concerning things that could be seen with optical lenses) produced an image of a tiny human form curled up inside the sperm. This image has become iconic of the theory of preformationism (Figure B.2.1.1).

For two centuries (until the invention of cell theory) preformationists opposed epigenicists. Preformationism was the dominant theory of generation during the 18th century. Only with preformation could one be both a materialistic mechanist and a good Christian in the 18th century. Epigenesis must be rejected on the grounds that inert matter could not produce life without God's intervention.

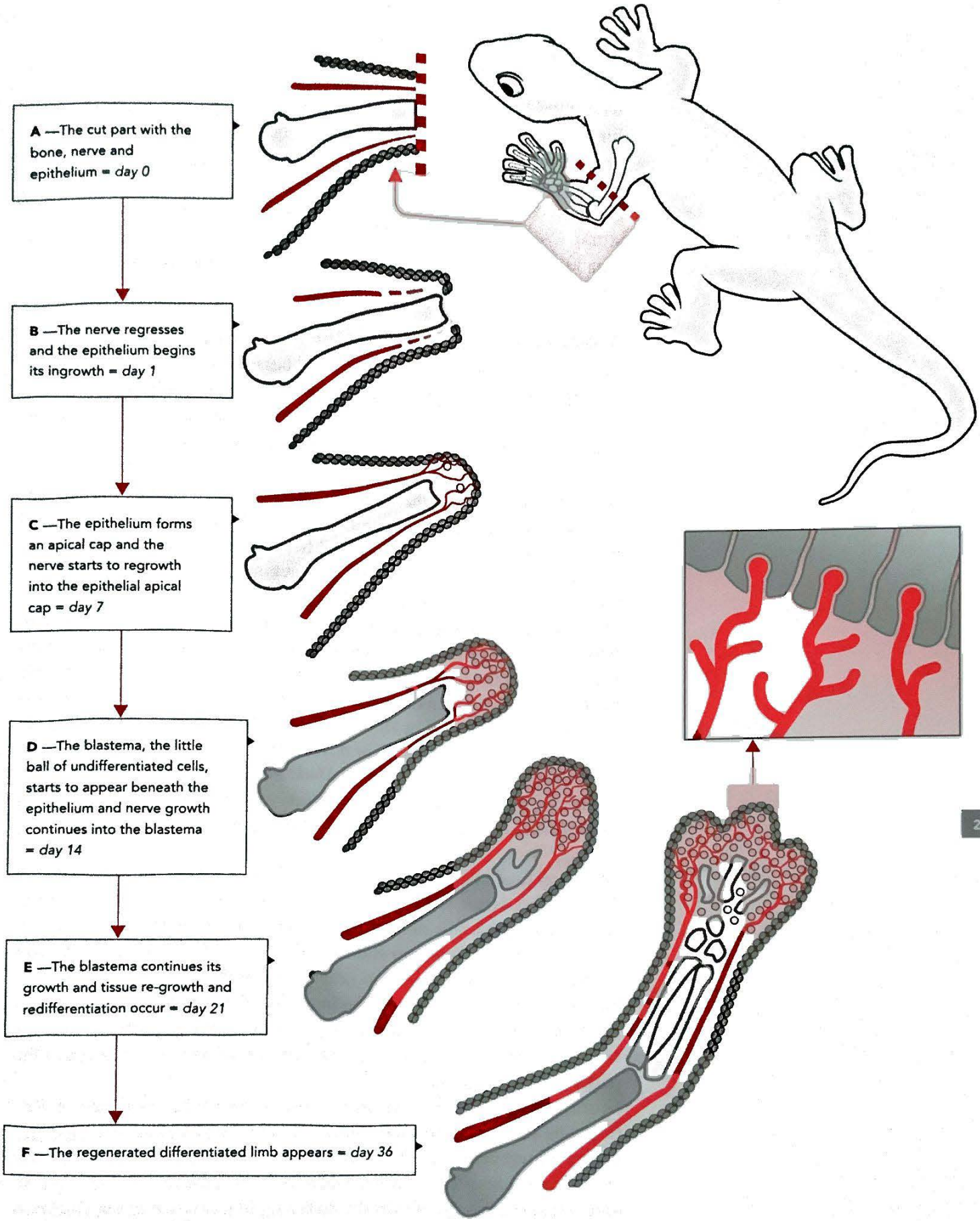
A reconciliation between preformation and epigenesis was attempted by the biologist Johann Friedrich Blumenbach (1752-1840). Blumenbach postulated a mechanical, goal directed force called the Bildungstrieb ("developmental force"). Such a force, he said, was not theoretical, but could be shown to exist by experimentation. A *Hydra*, when cut, regenerates its amputated parts by rearranging existing elements. Some purposive organizing force could be observed in operation and this force was a property of the organism itself.

Figure 2.3 ►
Regeneration of a salamander's limb. After a limb has been cut off, it regenerates through different steps that are schematically presented as A-F. The neuroepidermal junction is presented in an enlarged detail on the right

tive capacity of various organisms. He demonstrated that the same day that a limb is cut off, debris from dead cells is carried away in the bloodstream. His major works in this field appeared in the period 1898-1904; one of these was *Regeneration* (1901). Morgan chose the terms "morphollaxis" to describe the process of *Planaria* in which lost body parts are replaced by the remodeling of the remaining part and "epimorphosis" to describe the type of regeneration that, in contrast to morphollaxis, requires active cell proliferation prior to the replacement of the lost body part.

Limb regeneration of several urodele amphibians and fin regeneration of teleost fish are examples of epimorphosis (Figure 2.3). Early in epimorphic regeneration, immediately after the injury, some of the intact tissue begins to die back a short distance from the wound. During the first two or three days, cells of the epidermis (the outer layer of skin) begin to proliferate and migrate inward covering the wound surface. The epidermis then thickens over the apex of the stump into a transparent tissue called the apical cap. This stage is finished in about a week. By then, the blastema, a little ball of undifferentiated cells, has started to appear beneath the apical cap. The blastema appeared to be the "organ" of regeneration formed on the wound. Its cells are totipotent, able to develop into all the different kinds of cells needed to reshape the limb. After about three weeks, the blastema cells at the inner edge begin to differentiate into specialized types with a cartilage collar around the old bone shaft. Subsequently, other tissues form and the new limb, beginning with a paddle shape that will become the hand, appears as if "out of the mist". The elbow and long parts of the limb coalesce behind the hand and the process of regrowth and reshape is completed after about eight weeks with the reappearance of the four digits.

This beautiful process is full of problems for biology. What is the origin of the undifferentiated cells of the blastema? What organizes the growth of all these different types of cells? What is the factor in control of the cell interactions? And, last but not least, how does the blastema "know" that it must make a foreleg instead of a hind leg? The answers to those questions were sought by transplanting the blastema to other positions on the animal (Figure 2.4). If the blastema from an amputated foreleg was removed


A —The cut part with the bone, nerve and epithelium = day 0
B —The nerve regresses and the epithelium begins its ingrowth = day 1
C —The epithelium forms an apical cap and the nerve starts to regrowth into the epithelial apical cap = day 7
D —The blastema, the little ball of undifferentiated cells, starts to appear beneath the epithelium and nerve growth continues into the blastema = day 14
E —The blastema continues its growth and tissue re-growth and redifferentiation occur = day 21
F —The regenerated differentiated limb appears = day 36

within five to seven days (after it first appeared) and then grafted near the hind leg, it grew into a second hind leg, This may fit into the idea that there are "organizational territories" which provide information about the local anatomy. However, other experiments introduced some confusion. It was discovered that transplantation of a slightly older blastema from a foreleg stump to a hind-limb area produced a *foreleg*. Apparently, the young blastema knew where it was transplanted to and the older one knew where it had been!

A Role for Nerves and Skin in the Regenerative Process

To understand what comes out of the blastema, it would be easier if we understood what stimulates the blastema to form and where its cells came from? The assumption that differentiated cells would become dedifferentiated cells by loosing their characteristic tissue properties and structure seemed, at that time, not likely. Instead, it led to the belief that all regeneration had to be the work of "reserve cells" left over from the embryo and warehoused throughout the body in a primitive, unspecialized state. Perhaps, there was a biological bell that called them to migrate to the stump and form the blastema. In searching for what triggers the blastema's origin, the best candidate are the nerves. In complex multicellular animals, there is no regeneration without nerve tissue. In 1823, the English naturalist, Tweedy John Todd found that if the nerves in a salamander's leg were cut at the time of the amputation, the limb would not regrow. He made many observations about such nerve issue. His findings and the possible consequences were in 1831 published in a book with the remarkably long title: *The Book of Analysis or a New Method of Experience; whereby the Induction of a Novum Organon is Made Easy of Application to Medicine, Physiology, Meteorology, and Natural History; to Statistics, Political Economy, Metaphysics, and the more Complex Departments of Knowledge.*

Over a century later, the Italian biologist, Piera Locatelli demonstrated the reverse effect. Namely, that an extra leg would grow if a nerve was rerouted. She cut the large sciatic nerve down the salamander's hind leg leaving it attached to the spinal column and carefully threaded it up beneath the skin so that its end touched the skin near to one of the forelegs. Subsequently, an extra foreleg sprouted near the intact leg. Placing the nerve end near a hind leg resulted in an extra hind leg. This indicated that some sort of energy from the nerves was adapted by local conditions that determined the pattern of what grew back.

Shortly thereafter, other researchers found that when they sewed full-thickness skin grafts over the stumps of amputated salamander legs, they prevented regeneration. This discovery led Sylvan Meryl Rose, in the early 1940's, to surmise that in frogs, where it is *not* possible to regenerate an amputated leg, it is specifically due to the rapid formation of full-thickness

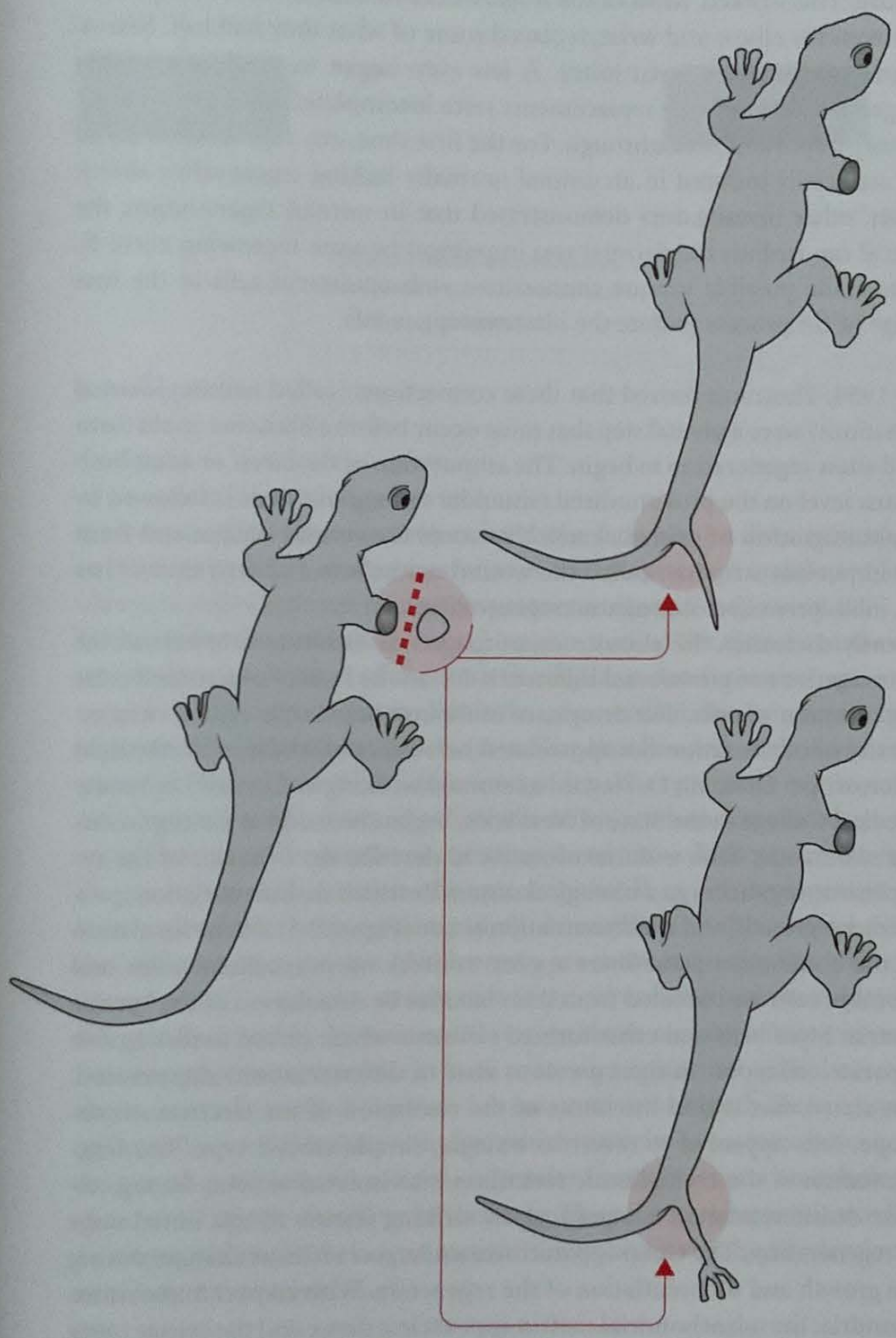


Figure 2.4
Transplantation of a foreleg stump blastema to other positions on the animal demonstrates "spheres of influence" or "organizational territories". If the blastema is removed early (within 5-7 days) after it appeared and grafted near the hind leg, it grows into an extra hind leg. If the blastema was slightly older it would produce a foreleg in the hind-limb area.

skin over the stumps. To study this question, Rose tried to prevent (or at least inhibit) skin formation. Rose dipped the wounds in saturated salt solution several times a day to prevent the dermis from growing over the stump. This worked. Most of the frogs, whose forelimbs he had amputated between the elbow and wrist, replaced some of what they had lost. Several regrew well-formed wrist joints. A few even began to produce one new finger. Even though the replacements were incomplete, this was a tremendously important breakthrough. For the first time, any regeneration could be artificially induced in an animal normally lacking regenerative ability. Later, other investigators demonstrated that in normal regeneration, the apical cap (minus the dermis) was important because regrowing nerve fibers made possible unique connections with epidermal cells in the first stage of the process (before the blastema appeared).

By 1954, Thornton proved that these connections (called neuroepidermal junctions) were a pivotal step that must occur before a blastema could form and allow regeneration to begin. The amputation of the larval or adult limb at any level on the proximodistal (shoulder to fingertip) axis is followed by rapid migration of epithelial cells that cover the wound surface and form an important structure called the wound epithelium (whose removal has an inhibitory effect throughout regeneration).

Shortly thereafter, the electron microscope was introduced to extend the investigations of growth and differentiation to the level of macromolecular organization of cells. The cytoplasm of differentiated cells exhibits a complexity of organization not appreciated heretofore in studies with the light microscope. Elisabeth D. Hay, an anatomist working at Cornell University Medical College in the State of New York, began the study of the regenerating salamander limb with the objective to describe the behavior of the cytoplasmic organelles in a biological system in which dedifferentiation, proliferative growth, and redifferentiation occur (Figure 2.5). In the formation of the blastema, muscle fibers appear to break up into cellular units and cartilage cells are liberated from their lacunae by dissolution of the hyaline matrix. Myofibrils and other formed elements which served to distinguish separate cell types (in their previous state of differentiation) disappeared. She stated that within the limits of the resolution of the electron microscope, cells appeared to revert to a single, simplified cell type. The fragmentation of the endoplasmic reticulum into isolated vesicles during cellular dedifferentiation is a particularly striking feature of this initial stage of regeneration. The Golgi apparati also undergo a series of changes during the growth and differentiation of the regenerate. With respect to the mitochondria, the mitochondrial matrix appears less dense and the cristae more irregular in early blastema cells (a feature which has been noted in certain immature cells). One of the most interesting features of the blastema cell is its acquisition of large numbers of free ribonucleoprotein granules in its

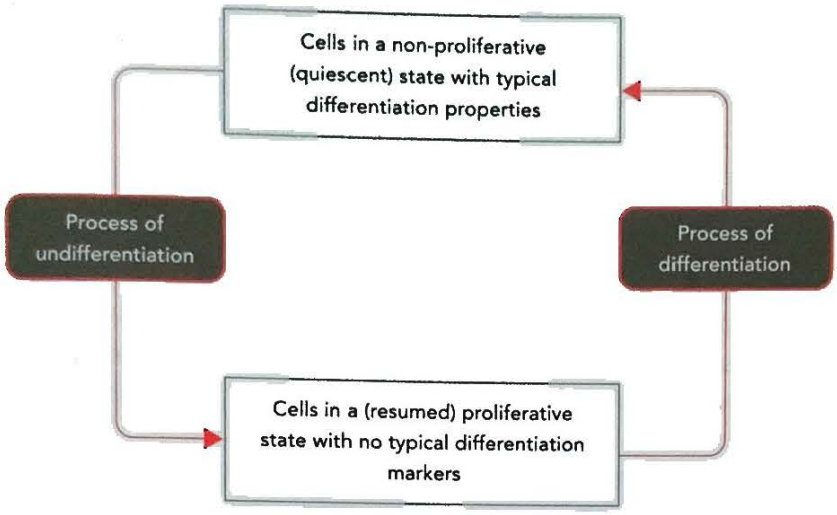


Figure 2.5
The reversible "differentiation/proliferation switch".

cytoplasma. Since these granulaes had been shown to be directly involved in protein synthesis, their appearance in the cytoplasm of dedifferentiated blastema cells suggested that active protein synthesis is beginning.
Utilizing the electron microscope, Hay also studied the changes in the extracellular components of the limb during blastema formation and subsequent redifferentiation. In particular, she studied the neuroepidermal junction. She found that as each nerve fiber bundle reached the end of the stump, it broke up and each fiber snaked into the epidermis in a separate way. Each nerve fiber formed a tiny bulb at its tip, which was placed against an epidermal cell's membrane, nestling there into a little pocket. In fact, the intraepidermal nerves in the apical epithelial cap of the regenerating larval salamander limb remain entirely extracellular in their course. Only the bulbous enlargements of the nerves are intimately related to the epidermal cells. The plasma membranes of the nerve bulbs and the epidermal cell are separated by a space of 20 nm. In these regions of close apposition, the plasma membranes of both nerve and epidermal cell appear very dense and an osmophilic material is concentrated beneath the epidermal cell membrane. These nerve bulbs, which contain numerous mitochondria, small granules, and vesicles are similar (in their general morphology) to nerve terminals elsewhere in the body. The arrangement was much like a synapse, although, the microscopic structure was not as highly developed as in long-term connections.

The role of the nerve tissue was further studied by Marcus Singer from the mid-1940's to the mid-1950's. He performed, at Harvard Medical School, a series of experiments with newts proving that nerves were needed only

during the first week until the blastema was fully formed. It is important to know that blastema cells, the progenitor cells of the regenerate, divide under the control of the brachial nerves of the limb forming a conical mound of undifferentiated cells known as the blastema. If the limb is denervated by transecting the brachial axons at the shoulder, blastema cells do form after amputation but do not divide. If denervation is performed during the first 2 weeks after amputation in an adult newt, proliferation stops and regeneration is arrested. If the sciatic nerve is transplanted from hind leg to foreleg, regeneration is restored. At later stages, denervation has a less radical effect in that regeneration proceeds but the resulting regenerate is smaller than normal. After that, regeneration proceeded even if the nerves were cut.

By the early 1950's, Singer was ready to look at biochemistry. He hypothesized that a growth-inducing chemical was emitted from the nerves. The most promising possibility was the neurotransmitter acetylcholine, one of several compounds known to relay nerve impulses across synapses. Indeed, the nerves secreted acetylcholine more abundantly than normal during blastema formation (just when nerve supply was crucial). Its production fell back to normal when regrowth was well under way. In order to find more evidence, it was necessary to add acetylcholine artificially and then see if this facilitates blastema formation. In this regard, Singer was interested in previous failures where experimenters had injected acetylcholine into the blastema. He thought these methods were too artificial and henceforth invented a micro-infusion apparatus to continuously release tiny amounts of acetylcholine duplicating what the nerves did. However, even then, he did not observe any regeneration. He concluded that the growth factor was almost certainly not acetylcholine. The molecular basis of a nerve's capacity to initiate limb regeneration remained unclear. It was then that he also speculated that the nerve exerts a direct positive influence by releasing what would now be referred to as a mitogenic growth factor required for division in the blastema (Singer 1974).

A fibroblast growth factor was another substance found in the axons innervating the amphibian limb (Figure 2.6). It also seemed critical for the process. In 1979, Mescher and Gospodarowicz were able to restore mitosis in denervated blastemas by infusing them with fibroblast growth factor. Mullen and co-workers in 1996 were able to induce regeneration in denervated blastemas by implanting beads containing fibroblast growth factor into the blastema. This particular factor may play more than one role in regeneration. It may also serve as an angiogenesis factor since the regenerating tissues need to develop a blood supply very shortly after amputation.

The knowledge of nerve-dependent processes in early regeneration was further elucidated using the aneurogenic limb model. It was possible to allow the limb bud of a urodele embryo to develop in the complete absence of a nerve supply. Such aneurogenic limbs are of normal morphology. If

amputated they will regenerate despite the absence of nerves. In a series of experiments by Thornton and Thornton (1970), aneurogenic limbs were transplanted to a normal embryo in place of the forelimbs. The host brachial nerves grew into this aneurogenic limb and during days 10-13 of ingrowth, the limb established a state of nerve dependence necessary for regeneration. This means that if the limb is then denervated, regeneration does not occur. The implication from the results of these experiments was that the nervous system superimposes nerve dependence for regeneration over any other mechanism operating during development and regeneration of the aneurogenic limb.

Another approach used the ectopic limb formation. Usually, if a limb was wounded, the skin healed. However, if a nerve was rerouted (along with skin fibroblasts from the other side of the limb) to the wound site, an ectopic limb would form. Deviating the nerve to the wound site caused a bump-like blastema to form. Those blastemas were composed largely of dermal fibroblasts. The neural signal induced such cells to proliferate and dedifferentiate. However, without fibroblasts from the contralateral region, those blastemas regressed. Although these bumplike blastema are not "natural" regeneration blastema, they do indicate that there is a temporal sequence to the formation of the regeneration blastema.

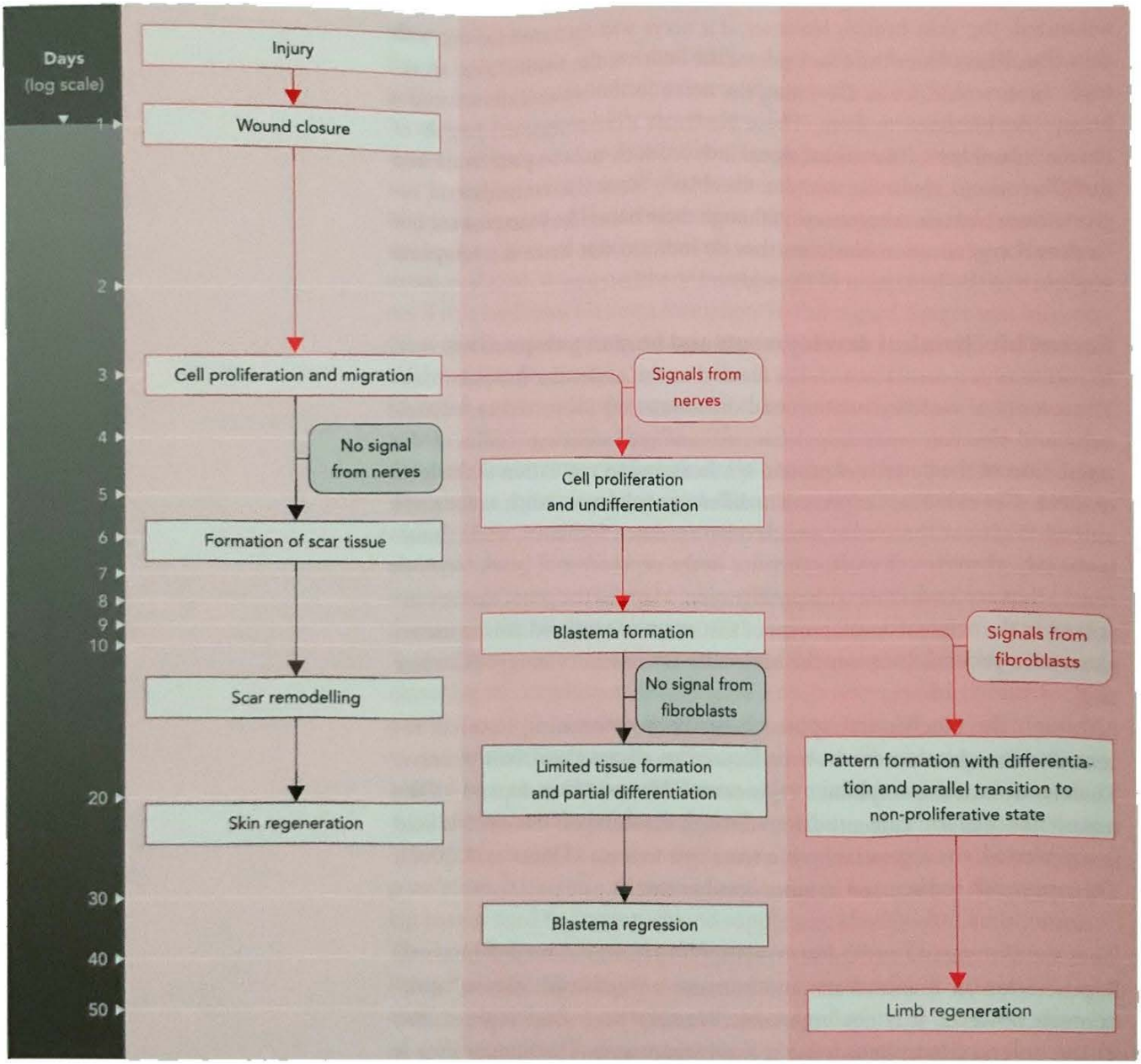
Recent biochemical developments and human perspectives

In recent times, most research has focused on the molecular fundamentals. The events of dedifferentiation and differentiation (observed in microscopy and electron microscopy) has been analyzed utilizing studies of the regulation of the pattern of protein synthesis and its regulation at the level of DNA. Genes that are expressed in differentiated tissues (such as the mrf4 and myf5 genes expressed in muscle cells) are down regulated, while simultaneously, there is a dramatic increase in the expression of genes (such as msx1) that are associated with proliferation. Many of the genes that are involved in the original development of tissues are reinitialized during the regenerative process. [Also see the Molecular Correlation Concept (Chapter 10)].

Although the biochemical approach has been dominating research, recent attention has sometimes been focused on electrical currents in nerve. The creation of the amphibian regeneration blastema may depend on the maintenance of ion currents driven through the stump. If this electric field is suppressed, the regeneration blastema fails to form (Altizer *et al.* 2002). This approach is discussed in more detail in Part D.

Does the aforementioned information apply to mammalians and humans? Regeneration in mammalians and humans is considered "almost" nonexistent: however, it is not impossible. Humans have some regeneration within several tissue; knowledge is slowly increasing. The human liver is

Figure 2.6
A model of amphibian limb regeneration depicting typical steps in skin and limb regeneration and their relationship. After injury, epithelial cells proliferate and migrate. Without signals from the nerves a scar is formed cacilitating skin regeneration. In the presence of nerve signals, cells at the wound site proliferate and undifferentiate to form a blastema. If signals from mesenchymal fibroblast are present, the blastema is patterned and then grows into a regenerated differentiated limb. If signals are not present, only limited renewal takes place and the blastema regresses.



one of a few glands in the body that has the ability to regenerate from as little as 25% of its tissue. It regenerates by the proliferation of existing tissue. The intensity of the liver's response is directly proportional to the mass resected. The regenerating liver cells only partially dedifferentiate when they re-enter the cell cycle. A regeneration blastema is not formed. Rather, the five types of liver cells (hepatocytes, duct cells, fat-storing cells, endothelial cells, and Kupffer macrophages) all begin dividing in order to produce more of the same. Each cell type retains to some extent, its cellular identity. The liver retains its ability to synthesize the liver-specific enzymes necessary for glucose regulation, toxin degradation, bile synthesis, albumin production, and other hepatic functions. [Also see Molecular Correlation Concept (Chapter 10)].

Regeneration of the kidney is also possible based on mammalian studies. The basic functional and structural unit of the kidney is the nephron which is composed of four components: the glomerulus, tubules, the collecting duct and peritubular capillaries. Regeneration of the tubular component following an acute injury is well known. In the damaged proximal tubule, surviving epithelial cells undergo migration, dedifferentiation, proliferation, and redifferentiation to replenish the epithelial lining of the proximal tubule.

In rats, heart damage can be regenerated under some conditions. Normally, in mammals cardiomyocytes (heart muscle cells) cannot proliferate (multiply) and subsequently, heart damage initiates scarring and fibrosis. However, under some conditions (inhibition of p38 MAPkinase and treatment with a fibroblast growth factor) it has been found that mitosis might be induced in adult mammalian cardiomyocytes. Such treatment might regenerate the heart, reduce scarring, and improve cardiac function in rats with cardiac injury.

Conclusion

Historical applicability of regeneration data was the beginning. In the 20th century, as described in modern textbooks, 4 major categories are detailed. The first involves the use of stem cells to regrow organs or tissues that have been lost. Examples of stem cell-mediated regeneration include the continual replacement of blood cells from the hematopoietic stem cells in the bone marrow. The second mechanism involves the dedifferentiation of adult structures to form an undifferentiated mass of cells that then become respecified. This type of regeneration, epimorphosis, has been observed in the planarian flatworm and regenerating of amphibian limbs. The third mechanism, morphallaxis, displays regeneration vis-à-vis the repatterning of existing tissues with little new growth. Such regeneration has been seen in *Hydra*. A fourth type of regeneration can be thought of as compensatory

regeneration. Here, the differentiated cells divide but (only partially) maintain their differentiated functions. This type of regeneration is characteristic of the mammalian liver. The trend to further distinguish categories of regeneration illustrates lack in fundamental knowledge regarding the common denominators in the program of formation of the form. However, Chapter 10 details the omission in modern textbooks, the common denominator of the four types of regeneration, i.e., the shift in the different underlying fields regulating the differentiation/proliferation cycle.

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3

Further Discoveries Acknowledge a Field that Guides Generation

After 1850, biology began to break up into various specialties. In the 1880's, embryology matured as an experimental science providing a better understanding of the physiologic phenomenon of mitosis (Figure 3.1). The concept that cells were the basic structural units of all living things as well as the process of mitosis greatly enhanced the already existing knowledge about how embryos grew and how hereditary traits were transferred. Up to that point in time, scientists believed that the fertilized egg "seemed" to be an unstructured single cell. Embryonic growth occurred when the fertilized egg divided into two other cells, which promptly divided again. As cells proliferated, they also differentiated. That is, they began to show specific characteristics of muscle, cartilage, nerve, and so forth.

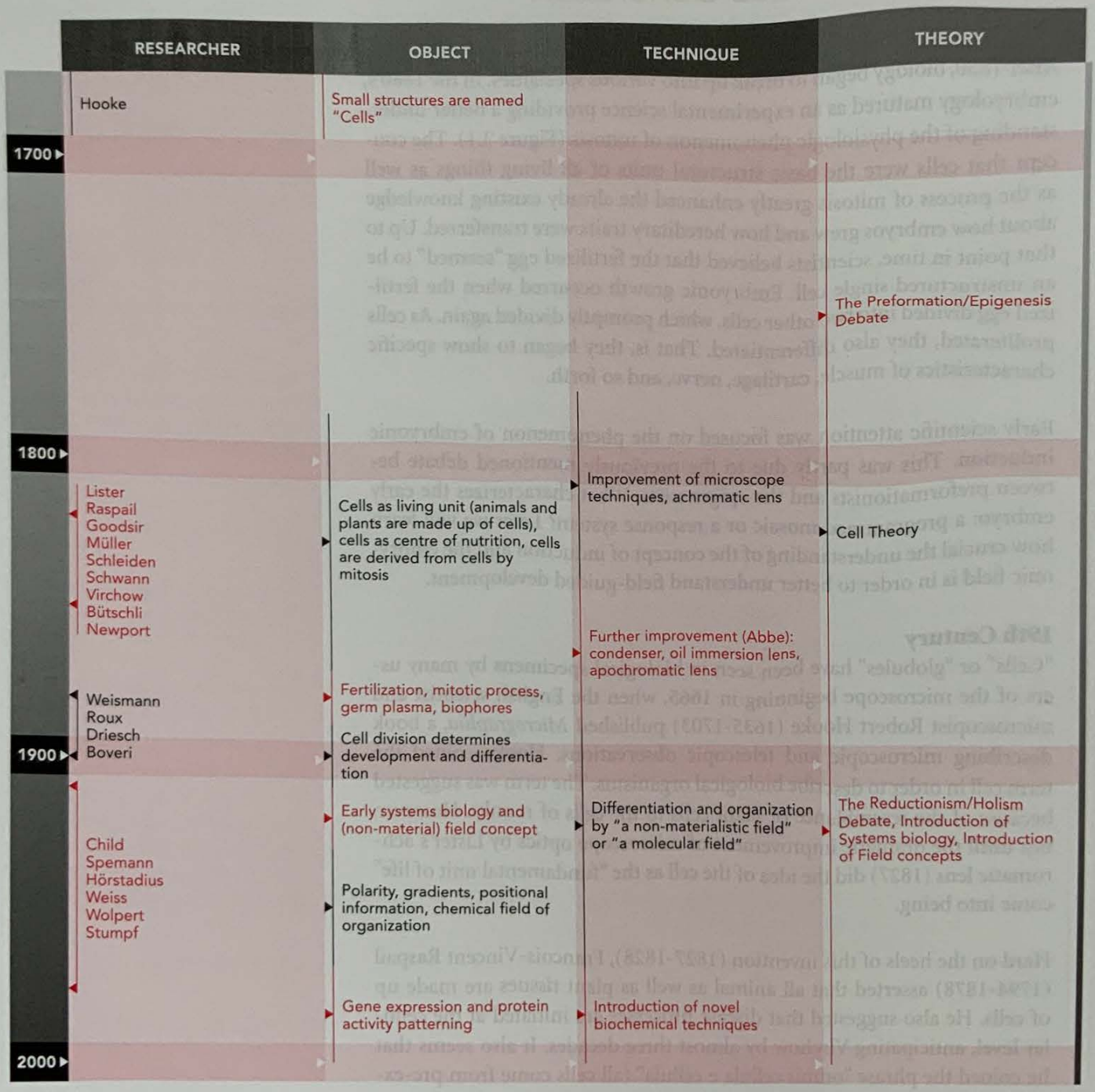
Early scientific attention was focused on the phenomenon of embryonic induction. This was partly due to the previously mentioned debate between preformationists and the epigenesists. What characterizes the early embryo: a programmed mosaic or a response system? Later we will learn how crucial the understanding of the concept of induction and the embryonic field is in order to better understand field-guided development.

19th Century

"Cells" or "globules" have been seen in biological specimens by many users of the microscope beginning in 1665, when the English scientist and microscopist Robert Hooke (1635-1703) published *Micrographia*, a book describing microscopic and telescopic observations. Hooke coined the term cell in order to describe biological organisms. The term was suggested because of the resemblance of plant cells to the cells of monks. However, not until the dramatic improvement of microscope optics by Lister's achromatic lens (1827) did the idea of the cell as the "fundamental unit of life" come into being.

Hard on the heels of this invention (1827-1828), Francois-Vincent Raspail (1794-1878) asserted that all animal as well as plant tissues are made up of cells. He also suggested that disease processes are initiated at the cellular level, anticipating Virchow by almost three decades. It also seems that he coined the phrase "omnis cellula e cellula" (all cells come from pre-ex-

Figure 3.1
Time-course of some salient contributions to thought related to the early embryology and developmental biology. This diagram may be seen as a detailed fragment of Figure 1.2 and may also be considered in conjunction with Figure 2.1 and 4.1.



isting cells) as early as 1825. The anatomist and surgeon, John Goodsir (1814-1867), accepted Raspail's work and identified the cell as the "centre of nutrition". The period 1835-1840 saw the emergence of "cell theory" in other European countries, particularly in Germany. Johannes Peter Müller (1801-1858), a German physiologist and anatomist recognized that cells in plants and animals are essentially similar. By the late 1830's, the botanist Matthias Schleiden (1804-1881) asserted that all cells have a common principle of origin. Theodor Schwann (1810-1882), a student of Müller agreed. The traditional German founders of cell theory, Schleider and Schwann, who proposed the cell theory in 1838, were aware of Raspail's pioneering discoveries but held them in contempt: an illustration of the antipathy between French and German scientists that persisted throughout much of the 19th century.

Another of Müller's students, Rudolf Virchow, became the founding father of cellular pathology. It was Virchow's authority that was instrumental in making the cell the centre of attention among biologists. He regarded the cell as the physiological as well as the morphological unit of life and emphasized its continuity in development. By the 1850's, cell theory was established.

This was the context in which George Newport (1802-1854) made the first explicit observation of fertilization. Newport's work can be considered the beginning of experimental embryology. The next major advances in experimental embryology took place immediately after Ernst Abbe joined the firm of Carl Zeiss and initiated a series of important innovations in microscope technology: the introduction of the substage condenser (1873), the oil immersion lens (1878), and the apochromatic lens (1882) which eliminated spherical as well as chromatic aberration. The effect on cell biology was almost immediate. A clear idea of the origin of cells arose from the descriptions of mitosis by Otto Bütschli (1873). In addition, many intracellular structures other than the nucleus were identified.

August Weismann (1834-1914), German biologist and one of the founders of genetics, is best known for his "germ plasm" theory, the forerunner of DNA theory. He was highly interested in the sex cells of *Hydrozoa.* However, experiencing trouble with his eyesight, he had to abandon microscope work in 1884. Henceforth, he concentrated on theory. Weismann hypothesized that the germ cells of animals contain "something essential for the species, something which must be carefully preserved and passed on from one generation to another." It was the birth of the theory of the germ plasm which he embodied in his book *Die Kontinuität des Keimplasmas als Grundlage einer Theorie der Vererbung* (1885). Its essence was the notion that all living things contain a special hereditary substance. He proposed

MORE ABOUT...

BOX 3.1

EGG CLEAVAGES AND CELL MOVEMENTS IN CREATING MULTICELLULARITY

The production of multicellularity in all known animal species, begins by a process called cleavage, a series of mitotic divisions wherein the enormous volume of egg cytoplasm is divided into numerous smaller, nucleated cells (Figure B.3.1.1). These cleavage-stage cells are called blastomers.

At first, the egg is divided in half, then quarters, then eights, and so forth. This division of egg cytoplasm occurs much faster than other types of cell division (even in tumor cells). A frog egg, for example, can divide into 37,000 cells in just 43 hours. One consequence of this rapid division is that the ratio of cytoplasmic to nuclear volume gets increasingly smaller as cleavage progresses. In many types of embryos, this manifold decrease in the cytoplasmic-to-nuclear volume ratio is crucial in timing the activation of nuclear genes. At that time, the rate of cleavage decreases and the blastomers become motile.

Pattern of embryonic cleavage

The pattern of embryonic cleavage is determined by the yolk protein within the cytoplasm. The amount and distribution of yolk determines where the cleavage can occur and the relative size of the blastomers. When one pole of the egg is relatively yolk free, the cellular divisions occur at a faster rate than at the opposite pole. The yolk rich pole is referred to as the *vegetal pole*. The yolk concentration in the animal pole is relatively low. In zygotes with relatively little yolk, cleavage is *holoblastic* (meaning that the cleavage furrow extends through the entire egg). Zygotes containing large accumulations of yolk protein undergo *meroblastic* cleavage wherein only a portion of the cytoplasm is cleaved. Meroblastic cleavage can be *discoidal* (as in birds' eggs) or *superficial* (as in insect zygotes) depending on whether the yolk deposit is located to one side or in the center of the cytoplasm, respectively.

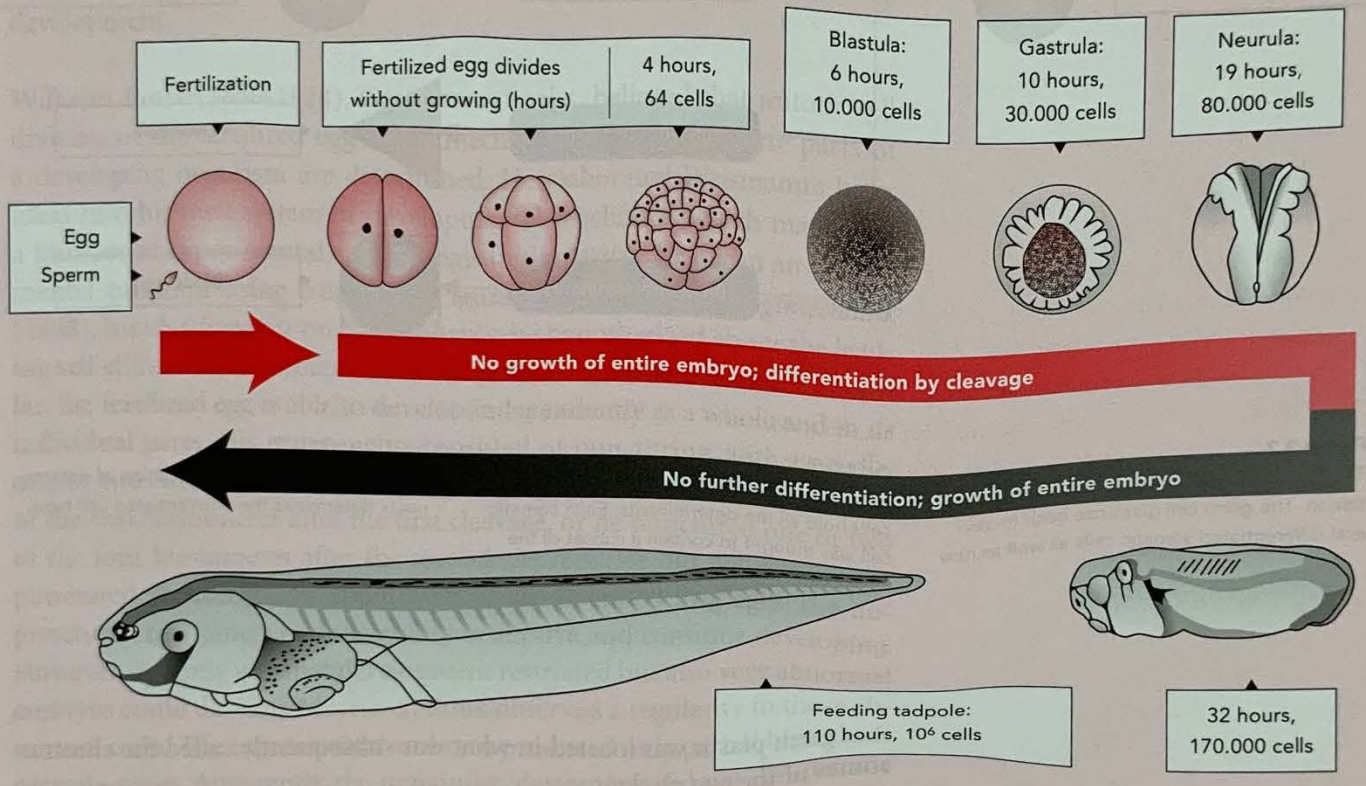
Cell movements shape the body

In the developing embryo, the movement of most cells are often extensive and dramatic. The movements themselves rearrange certain parts of the cells and place them into their final appropriate positions. During the period of cleavage, the frog embryo becomes transformed from a solid sphere of cells into a hollow ball, a fluid filled cavity surrounded by cells. The embryo is then labelled as a *blastula*. Soon after this, the coordinated movements of gastrulation begin. This process transforms the hollow ball of cells into a multilayered structure with a central gut tube and bilateral symmetry. Many of the cells on the outside of the embryo are moved inside. Subsequent development depends on the interactions of the inner, outer, and middle layers of cells thus formed. *Ectoderm* is the precursor of the epidermis and of the nervous system. A part of this sheet becomes tucked into the interior to form the *endodermis*, the precursor of the gut and its appendages such as the lung and liver. Another group of cells move into the space between ectoderm and endoderm, forming the *mesoderm*: the precursors of muscles, connective tissues, and various other components. From these three germ layers, the tissues of the adult vertebrate body will be generated preserving the basic body plan established through gastrulation. In the process, the three principle axes of the body are established: antero-posterior from head to tail; dorso-ventral from back to belly; and medio-lateral from the midline outwards to the left or to the right.

Cell dying in later development

As a tadpole changes into a frog, the cells in the tadpole tail are induced to undergo apoptosis. As a consequence, the tail is lost. A cell that undergoes apoptosis dies neatly without damaging its neighbors. The cell shrinks and condenses. The cytoskeleton collapses, the nuclear envelope disassemblies, and the nuclear DNA breaks up into fragments. Most important, the cell sur-

Figure B.3.1.1
Creating multicellularity in the development of *Xenopus laevis* from fertilized egg to feeding tadpole. The developmental stages are viewed from the side, except for the 10-hour and 19-hour embryos, which are viewed from below and from above, respectively. Upon fertilization, the egg cleaves very rapidly – initially at a rate of one division cycle every 30 minutes – forming a multicellular tadpole within a day or two. The cells get progressively smaller with each division, and the embryo remains the same size. During this non-growth stage cell differentiation and primary organ formation is (almost) completed. Then, growth of the "whole" begins. This parallels the begin of the tadpole's feeding.



face is altered, displaying properties that cause the dying cell to be rapidly phagocytosed, either by a neighboring cell or by a macrophage (a specialized phagocytic cell) before any leakage of its content occurs. This allows the organic components of the dead cell to be recycled by the cell that had just ingested it. All of the changes that occur during apoptosis in the tail are stimulated by an increase in thyroid hormone in the blood. The intracellular machinery responsible for apoptosis depends on a family of proteases that are called *caspases*.

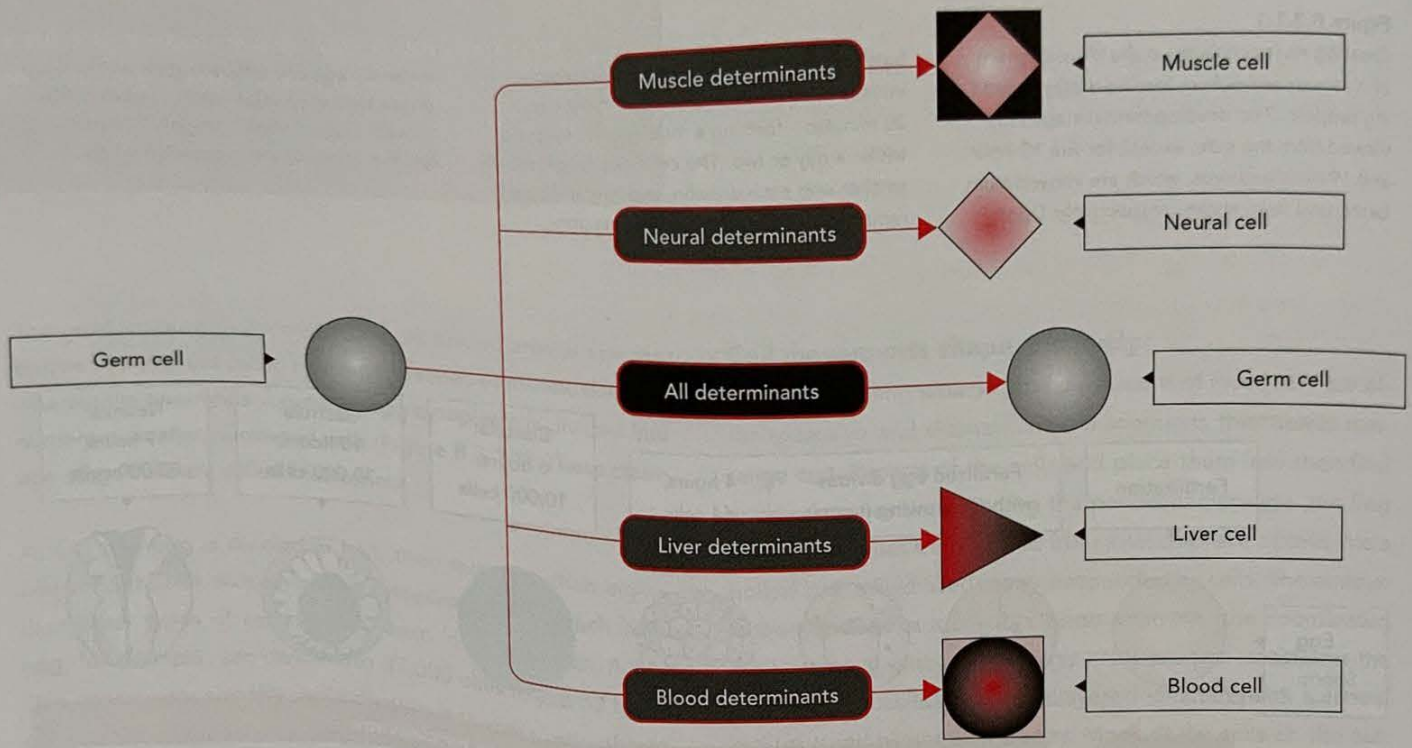


Figure 3.2
The Weismann germ plasm theory of differentiation. The germ cell gives rise both to several differentiated somatic cells as well as new germ cells. According to Weismann, only germ cells hold all the determinants. Each somatic cell was thought to contain a subset of the determinants and the combination of determinants determines the differentiated cell type.

that germ plasm was located in what was subsequently called the chromosomes of the egg nucleus.

It is interesting that Weismann served as a primary spokesman for a revised perspective of the preformationists movement (see also Box 2.1). He reformulated the traditional question about preformation and epigenesis questioning if the embryo constitutes a self-differentiating mosaic rather than a regulative body (which is responsive to changing internal and external conditions). In his classic *Das Keimplasm* (1892, translated into English in 1893), he voiced his opinion that body parts become diversely differentiated. According to Weismann, this differentiation must result from different kinds of material particles (or biophores). The various parts of the body somehow indicate the exact number of particles of germ plasm required for a particular composition (Figure 3.2).

For a multicellular system that is complexly differentiated, one would require many different types of biophores. Even if those biophores were quite

small, there would possibly not be sufficient space in the germ plasm for all of them. Weismann was forced into accepting the preformationist position, turning to particles which indirectly determine what will become manifest. He explained that the development of the individual depends on a series of gradual qualitative changes in the nuclear substance of the egg cell. Nuclear changes, presumably by divisions, act to distribute the determinants for each body feature to different cells. Each cell becomes a particular cell type (i.e., nerve, muscle, epithelial, etc.) simply because of the action of the determinants distributed to it. Thus, each cell has its predestined role because of the (inherited) determinants distributed to different cells during development.

Wilhelm Roux (1850-1924), German zoologist, believed that mitotic cell division of the fertilized egg is the mechanism by which future parts of a developing organism are determined. He elaborated Weismann's basic ideas into his own system of developmental mechanics which made him a founder of experimental embryology. In the 1880's, he began an experimental program using frog eggs (Figure 3.3). In *Entwicklungsmechanik* (1888), his classic paper on half embryos, he hypothesized about the leading self-differentiation theory of development as to whether, and if so how far, the fertilized egg is able to develop independently as a whole and in its individual parts. His experiments consisted of puncturing, with a needle, one or two blastomeres of a dividing frog egg. He chose to puncture one of the two blastomeres after the first cleavage, or he punctured one or two of the four blastomeres after the second cleavage. He did not remove the punctured blastomers. In about 20% of the operated frog eggs, the unpunctured blastomers had the ability to survive and continue developing. However, not only was that development restricted but also very abnormal embryos could develop. Moreover, Roux observed a regularity in those abnormal cases. The experimental embryos produced only partial blastula or gastrula stages. Apparently, the remaining blastomeres did not compensate for the injured blastomeres. Roux concluded that the early developing embryo acts as a mosaic of independent parts. His thinking clearly supported mosaic development wherein no regulative action could occur. He did, however, open the possibility that such action might occur in later stages when the embryo becomes more complex.

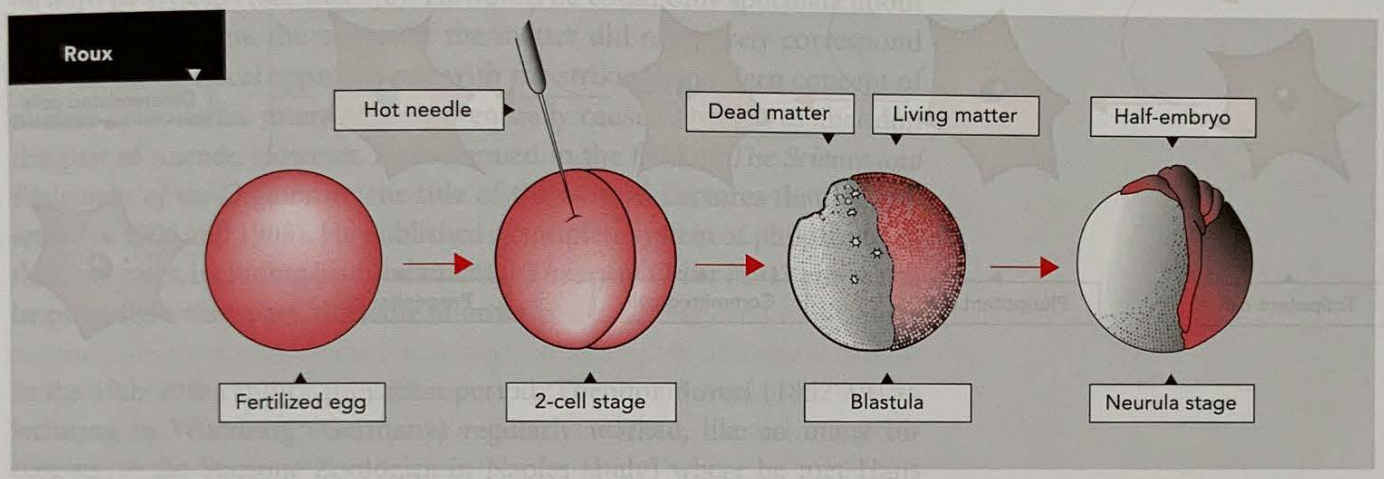
Hans Adolf Eduard Driesch (1867-1941) was another German experimental worker in embryology. He is also noted for his neo-vitalist philosophy. Driesch began to study under August Weismann, and continued testing the mechanistic embryological theories of Wilhelm Roux. He used *Echinoderm* eggs instead of frog's eggs. In 1891, Driesch set out to repeat the experiments by Roux on sea urchin embryos. Instead of puncturing one of two blastomeres with a needle, Driesch vigorously shook the two cells apart (or placed

them into calcium-free seawater). His experimental results pushed him to different conclusions (Figure 3.3). In his first of a series of *Experimental Studies*, Driesch mentioned: "But things turned out as they are bound to do and not as I had expected; there was a typically whole gastrula on my dish the next morning, differing only by its small size from a normal one" (1908). In the dividing cells of the embryo after the first celldivision, he also expected each cell to develop into the corresponding half of the animal to which it has been destined or preprogrammed. But instead, he found that each developed into a complete sea urchin. This also happened at the four-cell stage: complete larvae ensued from each of the four cells, albeit smaller than usual. Driesch (1893) also changed the direction of the third cleavage by gently compressing early embryos between two glass plates thus causing the third division to be meridional like the preceding two. After he released the pressure, the fourth division was equatorial. If segregation of nuclear determinants had occurred (as had been proposed by Weismann and Roux), the resulting embryo should have been strangely disordered. However, Driesch obtained normal larvae from these embryos. By 1895, Driesch's experiments on the sea urchin embryo suggested that it was possible to remove large pieces from eggs, shuffle the blastomeres and interfere in many ways without affecting the resulting embryo. It appeared that any single monad in the original egg cell was capable of forming any part of the completed embryo. This important refutation of both preformation and the mosaic theory of Wilhelm Roux was to be subject to much discussion during the ensuing years.

20th Century

In fact, the experiments demonstrated the development of the "whole" from a "part". If one considers the naked facts, the situation became remarkably lucid: the main issue was that the experiments clearly illustrated that morphogenesis. "Formation of the Form" was not realized by the unequivocal connections between the cells participating in the morphogenesis. However, these results were crucial and eventually led Driesch (1899) to suggest two principal concepts: equipotentiality and equifinality. The former means all the parts (cells) of a whole (embryo) are equal in their potential morphogenetic capability. The latter means that the final result of the development (formation of a highly specific mature organism) may actually be reached by quite different pathways. Driesch's findings also brought about the adoption of the terms "totipotent" and "pluripotent" cells, referring respectively to cells that can generate every cell in an organism and one that can generate almost every cell (Figure 3.4). Driesch's concept that the fate of a blastula cell is a function of its position in the system was an early step in thinking about positional information or some form of "polarity". The concept of polarity already existed as we have seen from discussing regeneration.

Figure 3.3
The contradictory outcome of the Roux's and Driesch's studies on early development. Upper panel: Roux's demonstration that destroying (but not removing) one cell of a 2-cell embryo results in the development of only one half of the embryo. Lower panel: Driesch's demonstration that when one isolates each of the four cells of a 4-cell embryo, each cell can form a smaller, but normal, pluteus larva.



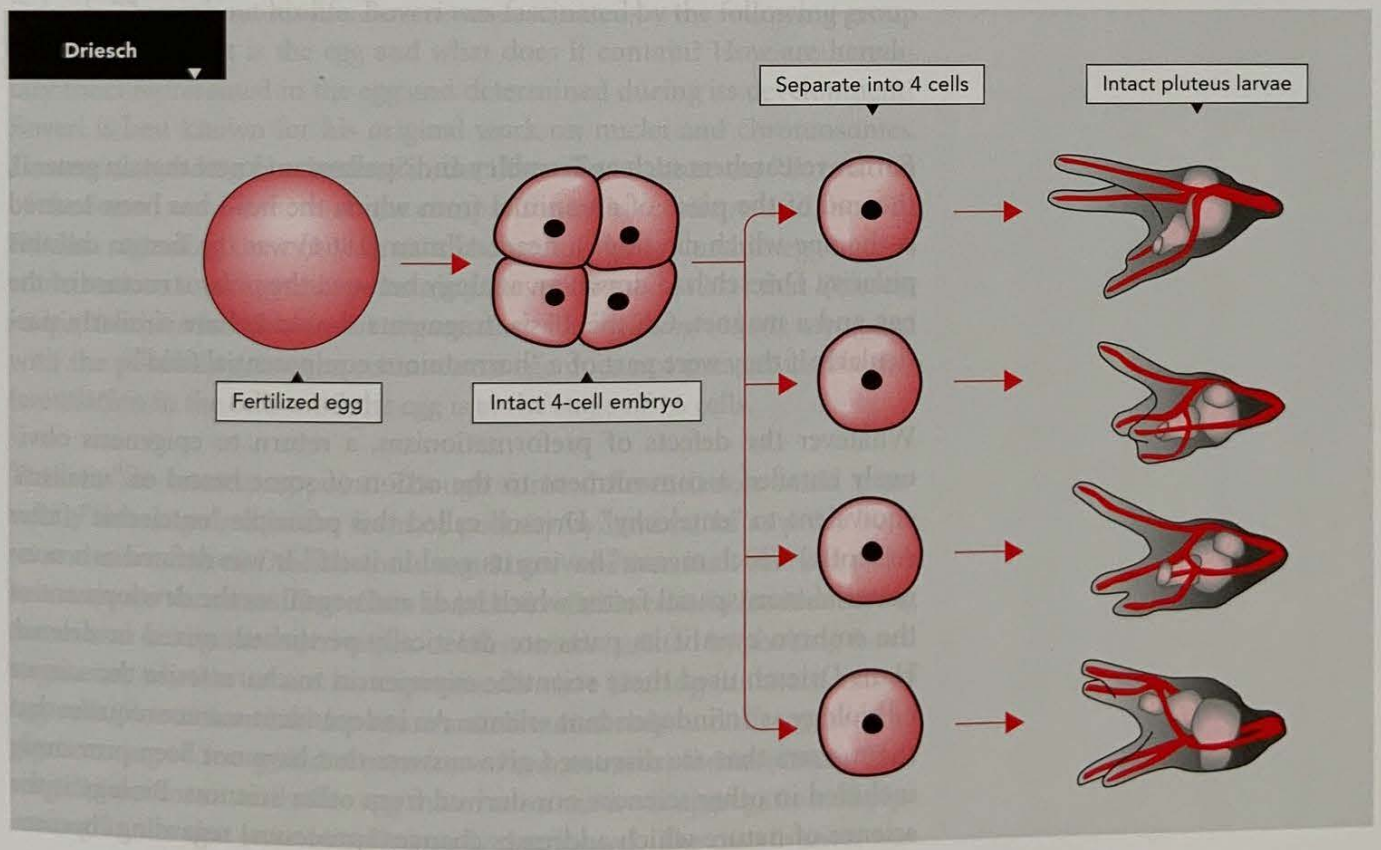
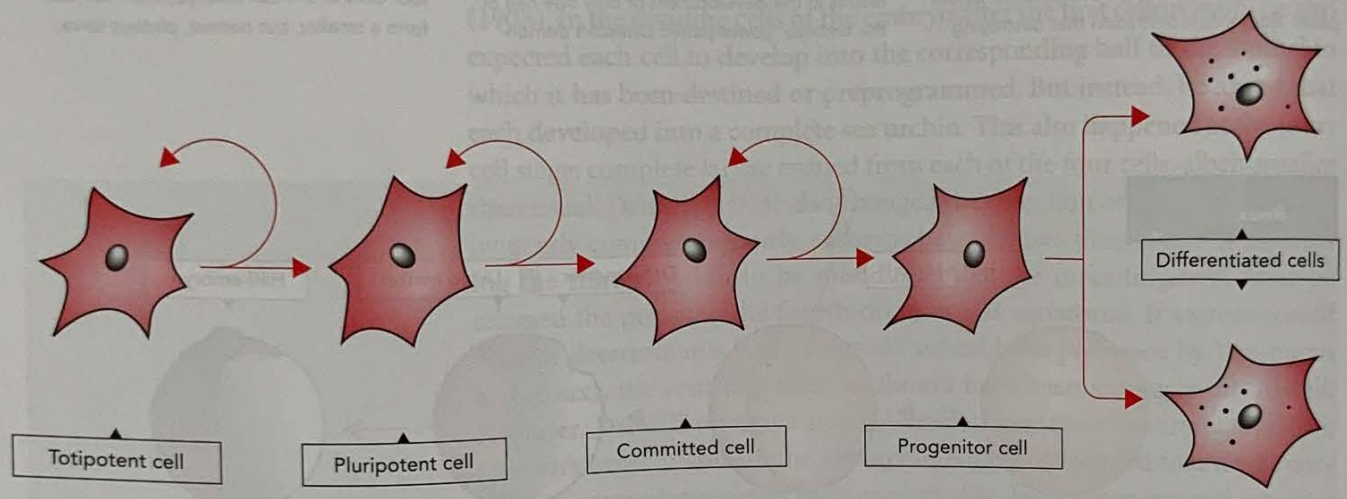


Figure 3.4
A generalized view of the sequence from totipotent (stem) cell through pluripotent (stem) cell through committed cell, through progenitor cell, to differentiated cell. Stem cells divide asymmetrically forming a more committed cell and another stem cell.



Earlier researchers such as Trembley and Spallanzani knew that, in general, the end of the piece of an animal from which the head has been severed is the one which develops a head. Allman (1864) was the first to call this polarity. Driesch had drawn an analogy between the polar structure of the egg and a magnet. On this basis, fragments should behave similarly, particularly if they were part of a "harmonious equipotential field".

Whatever the defects of preformationism, a return to epigenesis obviously entailed a commitment to the action of some brand of "vitalism" equivalent to "entelechy". Driesch called this principle "entelechia" (after Aristotle) which means "having its goal in itself". It was defined as a non-material, non-spatial factor which leads and organizes the development of the embryo even if its parts are drastically perturbed, mixed or deleted. Hans Driesch used these scientific experiences to characterize the science of biology as an independent science. An independent science requires that the matters that are discussed give answers that have not been previously included in other sciences nor derived from other sciences. Biology is the science of nature which addresses changes (processes) regarding "becom-

ing" and it includes existence in nature as well as how this and that evolves. It also includes how such changes unite and become the essence. He expressed his vision in *Die Biologie als selbständige Grundwissenschaft und das System der Biologie* (1911). This search for the essence has often led to misunderstanding about "where to look": at the matter in nature or at the "carrier" of the matter. His view stimulated a demonstration regarding how entelechy can influence matter. How does entelechy influence matter so that it is arranged only around this spatially natural deterministic principle? This whole series of fascinating questions was beautifully put forward by Driesch (see Box 5.2). However, he could only speculate about answers. In his view, the carrier of the matter did not purely correspond with the biochemical approach nor with the strikingly modern concept of nuclear-cytoplasmic interaction. It eventually caused Driesch to abandon this part of science. However, he continued in the field of *The Science and Philosophy of the Organisms* (the title of the Gilford Lectures that he presented in 1906 and 1908). He published a complete system of philosophy in three volumes, including his fundamental *Theory of Order* (1912) in which he proposed a threepart "doctrine of order".

In the 19th-20th century transition period, Theodor Boveri (1862-1915), lecturing in Würzburg (Germany) regularly worked, like so many biologists, at the Stazione Zoologica in Naples (Italy) where he met Hans Driesch. Throughout his life, Boveri was fascinated by the following group of problems: What is the egg and what does it contain? How are hereditary traits represented in the egg and determined during its development? Boveri is best known for his original work on nuclei and chromosomes. However, regarding our interest in the discussions about the "Formation of the Form", we will focus on the comparison of his concepts and those of Driesch regarding the cytoplasmic structure of the sea urchin egg and its contributions to embryonic patterning. Boveri argued (1901) that Driesch, in his *Analytische Theorie der organischen Entwicklung* (1894) struggled with the possibility that the echinoderm egg does not present visible differentiation in the cells until the egg is at the stage of 16 cells.

This was an interesting point. Though some differentiation could transiently be recognized in the form of polarity by landmarks as polar bodies and micromeres, Driesch could not know whether such marked polar axis is always the same. Therefore, he lacked reliable criteria for identifying the animal or vegetable derivation of blastomers isolated by shaking 8-cell stages, nor whether or not his experiments were altering the main axis of egg cells. Boveri searched for more suitable materials and discovered the pigment ring of *Strongylocentrotus* (now *Paracentrotus*) *lividus* (1901). This ring did not form until after egg maturation vis-à-vis aggregation of pigment granules that were initially evenly distributed. The distribution was

invariably oriented at right angles to the axis marked by the polar bodies. Using the polarity markers and especially the pigment ring, Boveri confidently stated that axial polarity was maintained from oogenesis through fertilization and cleavage up to gastrulation and beyond. He further localized specific functions in the polar cytoplasm. For example, the animal pole of the embryo could and the vegetable pole could not gastrulate.

The crucial type of experiment involved eggs strongly deformed by the experiment. The events that Boveri (1901) observed could only be explained by a gradient in the egg. For this reason, Boveri is usually credited with being the first to have identified polarity with gradients. He explained that Driesch's concept could work only if a certain (polar) location were to act as a "centre of the first differentiation" from where all other parts and their roles have to be determined. Referring to the uniform plutei that Driesch (1896) had obtained after severe scrambling of the blastomeres of an 8-cell stage, Boveri suggested "that each region of the blastula is ready to form mesenchyme or to gastrulate, and that the localization to a single point is effected because these processes begin easier in this [most vegetal] region than in others." In his view Driesch's famous sentence: "Each part, if need to be, can do everything" should be modified to: "Each part can do that, provided the one which could do better is no longer present". The best is the (relatively) most vegetative. It postulates a graded vegetal property which at the site of its relative maximum serves as the first centre of induction.

The ideas of polarity and gradients were taken up by Charles Manning Child (1869-1954), an American zoologist who also studied in Leipzig (Germany). He later worked in experimental zoology at the Naples Zoological Station. Returning to the United States, Child began a long series of experiments on the regeneration of coelenterates and flatworms. During the decade 1900 to 1910, he constantly sought for a governing factor in regeneration by experimenting with *Planaria*. For some years, he believed the transformation of a piece into a normal whole resulted from tensions and activities, or more generally speaking, that the organism is constructed by functioning. He began to hypothesize that the unity of the organism is a matter of correlation which led to the gradient theory (as the mechanism of correlation). The concept emerged about 1911 and numerous researches from 1910 to 1915 confirmed the concept of the existence of a gradation in rate of physiological processes along the axis. Child believed that he had found the chief factor regarding correlation: the antero-posterior dominance, i.e., each level dominates the region behind and is dominated by that in front. In the work titled: *Die physiologische Isolation von Teilen des Organismus als Auslösungsfaktor der Bildung neuer Lebewesen und der Restitution* (1911), Child developed his concept of antero-posterior dominance with special reference to distance diminution of the dominance. According to this con-

cept, parts sufficiently distant from the dominant anterior end are permitted to escape from that dominance and establish themselves as new individualities, thus forming buds, zoids, and the like. He termed such escape from dominance physiological isolation and supported it with a wealth of facts. It was covered in his book, *Individuality in Organisms* published in 1915. The characteristics of the individual: definite size, form, structural pattern, and correlation of activities are maintained by transmission of dynamic change along the axis. Such a gradient in transmission of excitation represents the simplest expression of unity and order in the living material. It is the simplest axis of polarity and symmetry. Along such a gradient a relationship of dominance and subordination exists because the most active region dominates the others. He made a further, important statement: "A polarity with a stable gradation of substances requires a continuous metabolic process in one part of the gradient and, thus involves migration". He wrote that such was lacking and therefore the most unsatisfactory feature of this hypothesis.

He argued further that regeneration is fundamentally the same as embryonic development. Both consist in the formation of the apical dominant region first and then the appearance of other parts in correlation with this. These concepts appeared in *Senescence and Rejuvenescence* (1915). Focused on the role of metabolic processes in gradient formation, Child devoted his later studies to the demonstration of the existence of physiological gradients in a great variety of materials, plant and animal, adult and embryonic. His method was to place the organism in a lethal solution and observe the progress of death and disintegration. In many of these studies, he employed agents which were known to influence oxidative processes. It was found to definitely correlate with the axes of the organism.

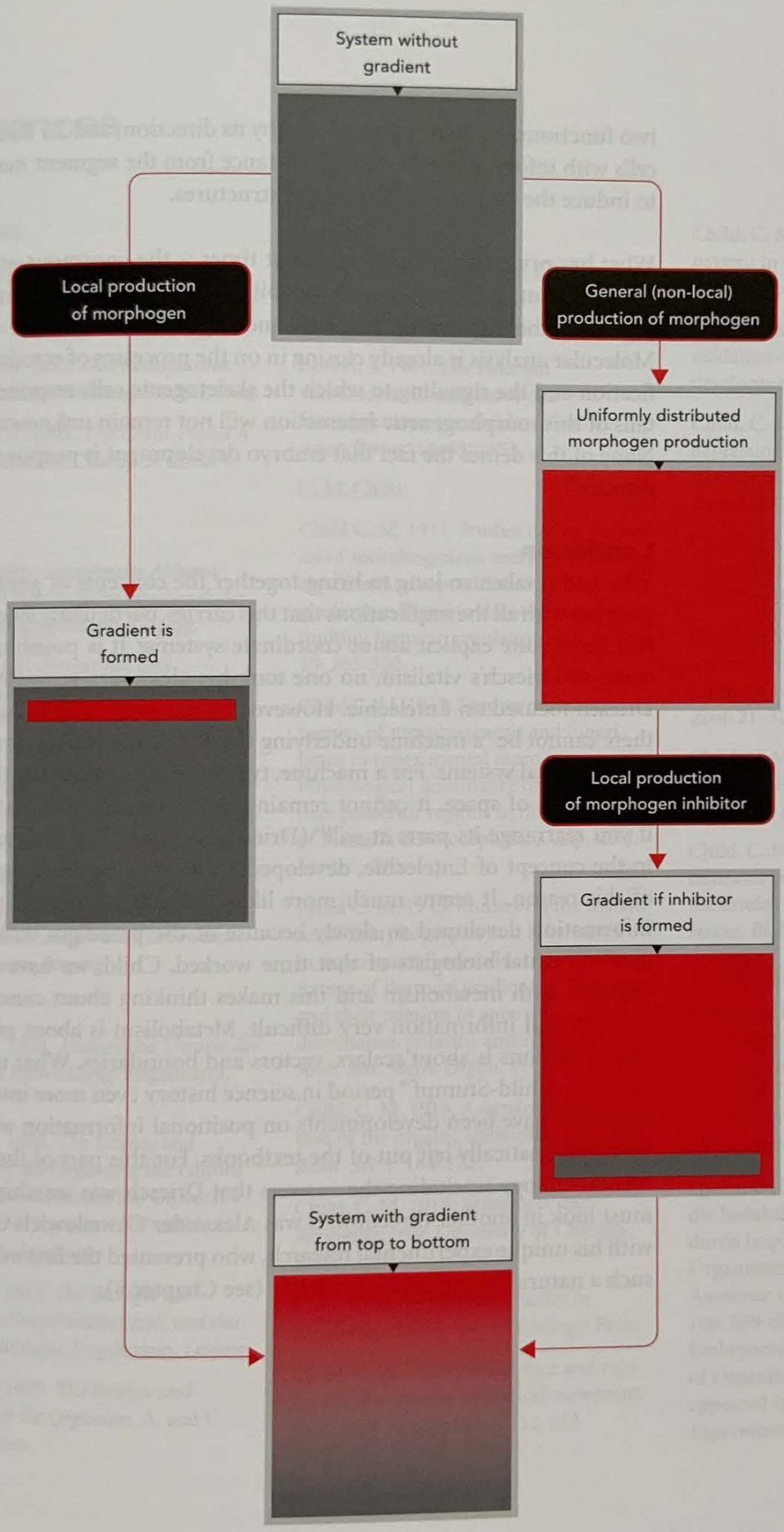
It was Spemann's work in the 1920's and 1930's on induction in amphibian development that created a revolution in developmental biology. His work demonstrated, for example, that the nervous system of a frog would not develop unless induced to do so by the underlying mesoderm. More dramatically, he demonstrated that one part of the embryo, the dorsal lip of the blastophore, could, when appropriately grafted, induce an almost complete second axis. He was not the first. Earlier demonstrations of induction had already been presented by Lewis in 1904 focusing on the lens. Lewis grafted the developing optic cup under the epidermis and found that a lens formed above the transplanted optic vesicle. This type of data led Spemann to introduce (in 1921), the field concept into embryology: a "field of organization". When the reacting tissue has the appropriate competence and is in contact with the right organiser at the right time, it begins to develop in a programmed way to form more elaborate structures. Correctly timed, contact with the organiser determines the fate of an embryonal tissue depending on its competence.

Figure 3.5 ►
Two principle manners of formation of a morphogen gradient. Left: A gradient is formed by polar (the local) production of an inducer (a morphogen) that diffuses away from its source. Right: A non-polar (general) development resulting in a uniform distributed morphogen production which is followed by a polar (local) production of a morphogen inhibitor that diffuses away from its source and blocks the action of the uniformly distributed inducer.

During the 1930's, Sven Hörstadius (1898-1996) established that organisers produce chemical substances that affect the development of embryonic structures. These substances (morphogens) form concentration gradients between the poles of the blastula. Reacting tissues respond to particular concentrations of morphogens and these responses determine the fates of their component cells. From the 1930's through the 1950's, gradient models were utilized to explain conditional cell specification in sea urchin and amphibian embryos (Hörstadius and Wolsky 1936; Hörstadius 1939; plus Toivonen and Saxen 1955). In the 1960's, these gradient models were further extended to explain how cells might be told what their position is along an embryonic axis (Figure 3.5). According to such models, the morphogen diffuses from its site of synthesis (source) to its site of degradation (sink).

The morphogenetic field has been referred to as a "field of organization" (Spemann 1921) and as a "cellular ecosystem" (Weiss 1923, 1939). The ecosystem metaphor is quite appropriate in that recent studies have shown that there are webs of interactions among the cells in different regions of a morphogenetic field. A morphogenetic field can be described as a group of cells whose position and fate are specified with respect to the same set of boundaries (Weiss 1939; Wolpert 1968,1969,1977). Thus, a particular field of cells will give rise to its particular organ (forelimb, eye, heart, etc) even when transplanted to a different part of the embryo.

Any clear demonstration of the concept of positional information was not until Hildegard Stumpf's paper entitled *Mechanism by which Cells Estimate their Location in the Body* (1966). She hypothesized: "It may be as a concentration gradient in substances which were made at one margin of the segment and destroyed at the other." Using the abdominal segments in the pupal cuticle of *Galleria mellonella*, she did experiments to determine whether the postulated concentration gradient is the means by which the partition is determined. The experiment demonstrated that this was the case. She thus concluded that the concentration gradient, obviously has


System without gradient
Local production of morphogen
General (non-local) production of morphogen
Uniformly distributed morphogen production
Gradient is formed
Local production of morphogen inhibitor
Gradient if inhibitor is formed
System with gradient from top to bottom

two functions: 1.) To orient the scales by its direction; and 2.) To supply the cells with information about their distance from the segment margins and to induce the corresponding circular structures.

What has primarily changed in recent times is the enormous, unforeseen enhancement in technological capabilities for largescale measurements regarding the sequencing of DNA and regulation of protein synthesis. Molecular analysis is already closing in on the processes of ectoderm specification and the signaling to which the skeletogenic cells respond. The details of this morphogenetic interaction will not remain unknown for long. None of this denies the fact that embryo development is purposeful (goal-directed).

Conclusion

Why had it taken so long to bring together the concepts of gradients and position with all the implications that this carries, particularly since Driesch had been quite explicit about coordinate systems? It is possible that, because of Driesch's vitalism, no one took his ideas very seriously. Indeed, Driesch focused on Entelechie. However, he did so because in his opinion there cannot be "a machine underlying the differentiation of harmonious-equipotential systems. For a machine, typical with regard to the three chief dimensions of space, it cannot remain itself if you remove parts of it or if you rearrange its parts at will" (Driesch 1908). In fact, Driesch, driven to the concept of Entelechie, developed a theory of vitalism just because of this reason. It seems much more likely that the concept of positional information developed so slowly because of the paradigm within which developmental biologists of that time worked. Child, we have seen, was obsessed with metabolism and this makes thinking about concepts such as positional information very difficult. Metabolism is about energy and rates; positions is about scalars, vectors and boundaries. What makes this "Driesch-Child-Stumpf" period in science history even more interesting is that there have been developments on positional information which have been systematically left out of the textbooks. For this part of the developmental biology (including the answers that Driesch was searching for) we must look in another direction. It was Alexander Gawrilowich Gurwitsch, with his unique experimental research, who presented the first evidence for such a natural deterministic principle (see Chapter 5).

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4

Does a Field also Guide the Development of Cancer?

The body of an animal or human operates as a society whose individual members are cells, reproducing by cell division, and organized into collaborative assemblies or tissues. The anatomical organization of the adult body is stable over time. This is best illustrated by the regulation of tissue volume. The volumes of the various tissues are proportional to the size of the organism. At the same time, the stability of anatomical form is a dynamic situation. Constancy of tissue volume means that the rates at which cells are added by mitosis are equal to the rates at which cells are lost by cell death. In many organs, the rates of cell division can increase when injury occurs or when an appropriate physiological demand is made on the system. In rodents, for example, when a portion of the liver is surgically removed, the rate of cell division in the remaining liver tissue increases until the original volume is restored. An example of a physiological demand is the increase in number of muscle cells and tissue upon exercise. The body as society is peculiar because, in a healthy body, selfsacrifice is the rule. Ultimately, all somatic cell lineages are committed to die. They leave no progeny. Instead, somatic cells dedicate their existence to the support of the germ cells. In this society, the cells of a multicellular organism are committed to collaboration. Each cell behaves in socially responsible manner by resting, dividing, differentiating, or dying as needed for the good of the organism.

Cancer doesn't observe the "boundary laws" of normal tissue (see Box 4.1). Instead, it encroaches imperialistically upon its neighbours, not adhering to the appropriate structures. Some of them, breaking off, flow through the blood and lymph setting up colonies (metastases) throughout the body. Just at this stage, the cancer clearly begins to show its metabolic priority. The tumor tissue greedily takes first choice of all nutrients circulating in the blood. The healthy part of the body obtains what is left over until, finally, the host dies. The major lesson to be learned is that less differentiation corresponds with extended growth.

18th Century

From the earliest times, physicians have puzzled over the causes of cancer (Figure 4.1). However, for the foundation of a scientific oncology, the study

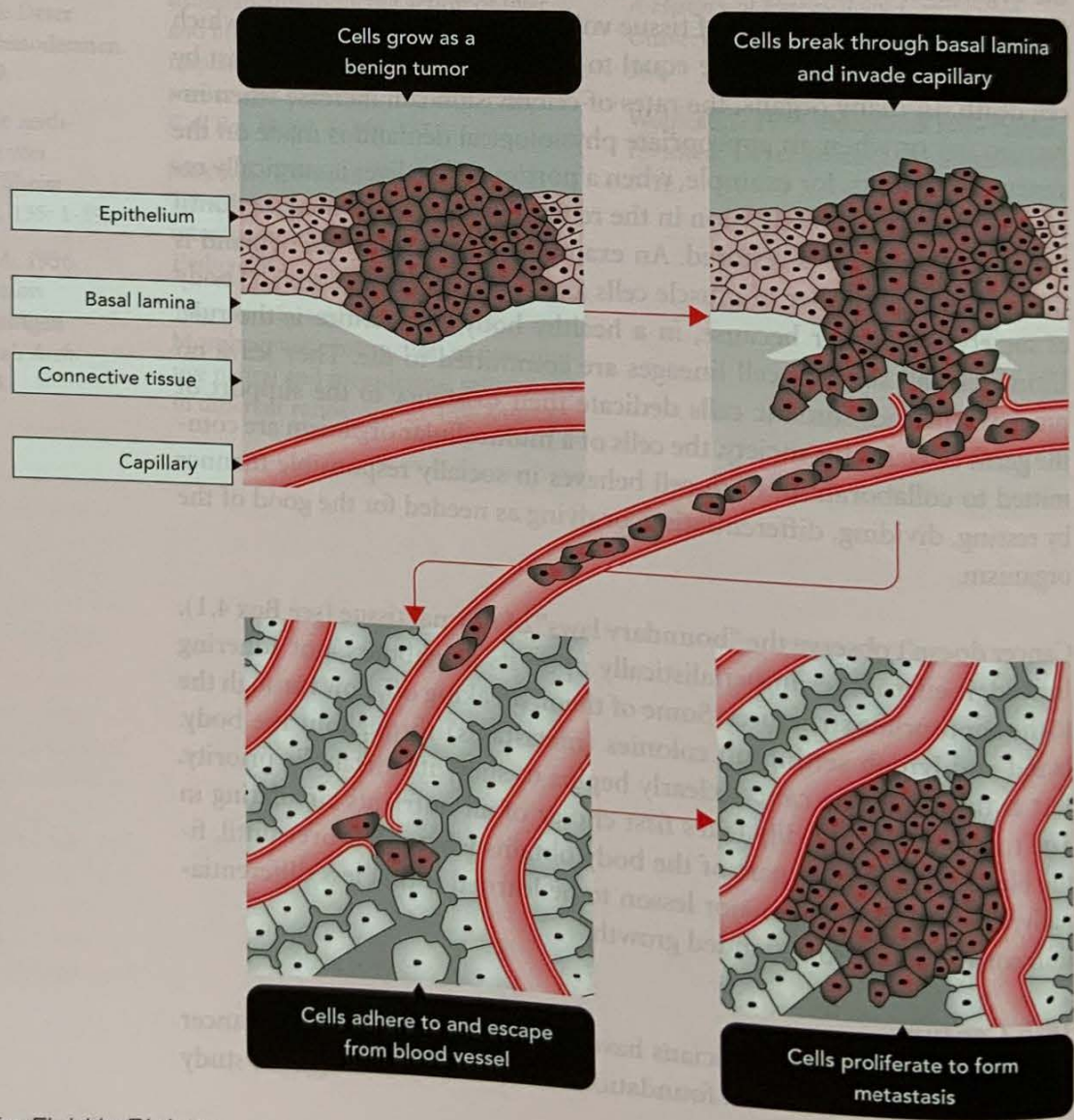
MORE ABOUT...

BOX 4.1

■ CANCER AS A MICROEVOLUTIONARY PROCESS AND ITS STAGING

If a cell's proliferation is out of control, it will give rise to a tumor (a growing mass of abnormal cells). As long as the neoplastic cells remain clustered, however, the tumor is said to be benign. A tumor is considered a cancer only if it is malignant (that is, only if its cells have acquired the ability to invade surrounding

Figure B.4.1.1
The process of metastasis. Tumor cells grow as a benign tumor (upper left panel). They may break through the basal lamina and enter the bloodstream by crossing the wall of a blood vessel (upper right panel). The tumor cells in the vessel may adhere to the vessel wall and escape from the vessel (lower left panel). They then may proliferate to form a metastasis at a new site (lower right panel).



tissue). Invasiveness usually implies an ability to break loose, enter the bloodstream or lymphatic vessels, and infiltrate other sites in the body where they form secondary tumors called *metastases*. (Figure B.4.1.1)

Cancers are classified according to the tissue and cell type from which they arise. Cancers arising from epithelial cells are termed *carcinomas*. Those arising from connective tissue or muscle cells are termed *sarcomas*. Cancers that do not fit in either of these two broad categories include the various leukemias, derived from hemopoietic cells as well as cancers derived from cells of the nervous system. In parallel with the set of names for the malignant tumors, there is a related set of names for benign tumors. An adenoma, for example, is a benign epithelial tumor with a glandular organization. The corresponding type of malignant tumor is an adeno-macarcinoma. Similarly, a chondroma and a chondro-sarcoma are, respectively, benign and malignant tumors of cartilage. About 90% of human cancers are carcinomas. Perhaps because most of the cell proliferation in the body occurs in epithelia or because epithelial tissues are most frequently exposed to the various forms of physical and chemical damage that favor the development of cancer.

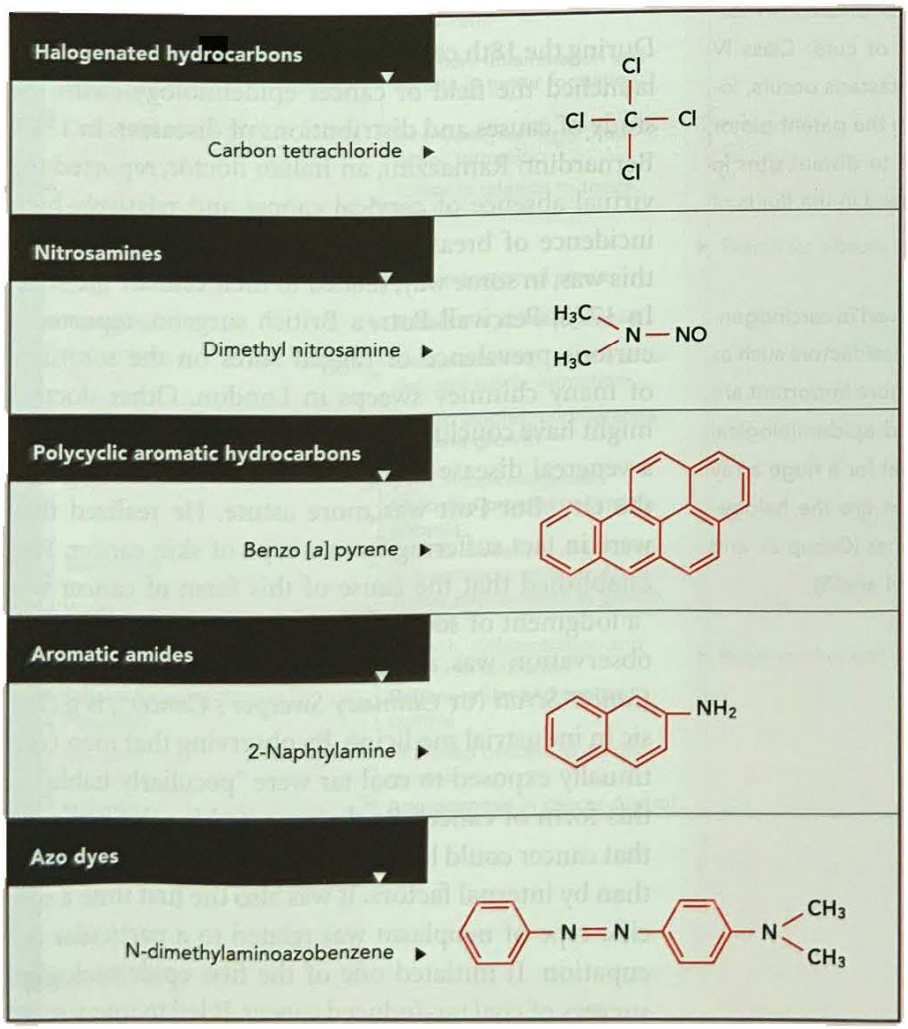


Figure B.4.1.2
Main classes of chemicals in carcinogenesis. From each main class an example (and chemical formula) is presented.

Cancers, in general, seem to arise by a process wherein an initial population of slightly abnormal cells evolves from bad to worse through successive stages. At each stage, one cell acquires an additional adaptation making it better able to thrive in its environment inside a tumor with low levels of oxygen, scarce nutrients, and the natural barriers to growth presented by the surrounding normal tissues. In general, the rate of evolution in any population would be expected to depend on a collection of subversive new skills. These include disregarding external and internal signals that regulate cell proliferation, avoiding suicide by apoptosis, circumventing programmed limitations (such as replicative senescence) and avoiding differentiation, escaping from their home tissue (becoming invasive) as well as surviving and proliferating in foreign sites (metastasize).

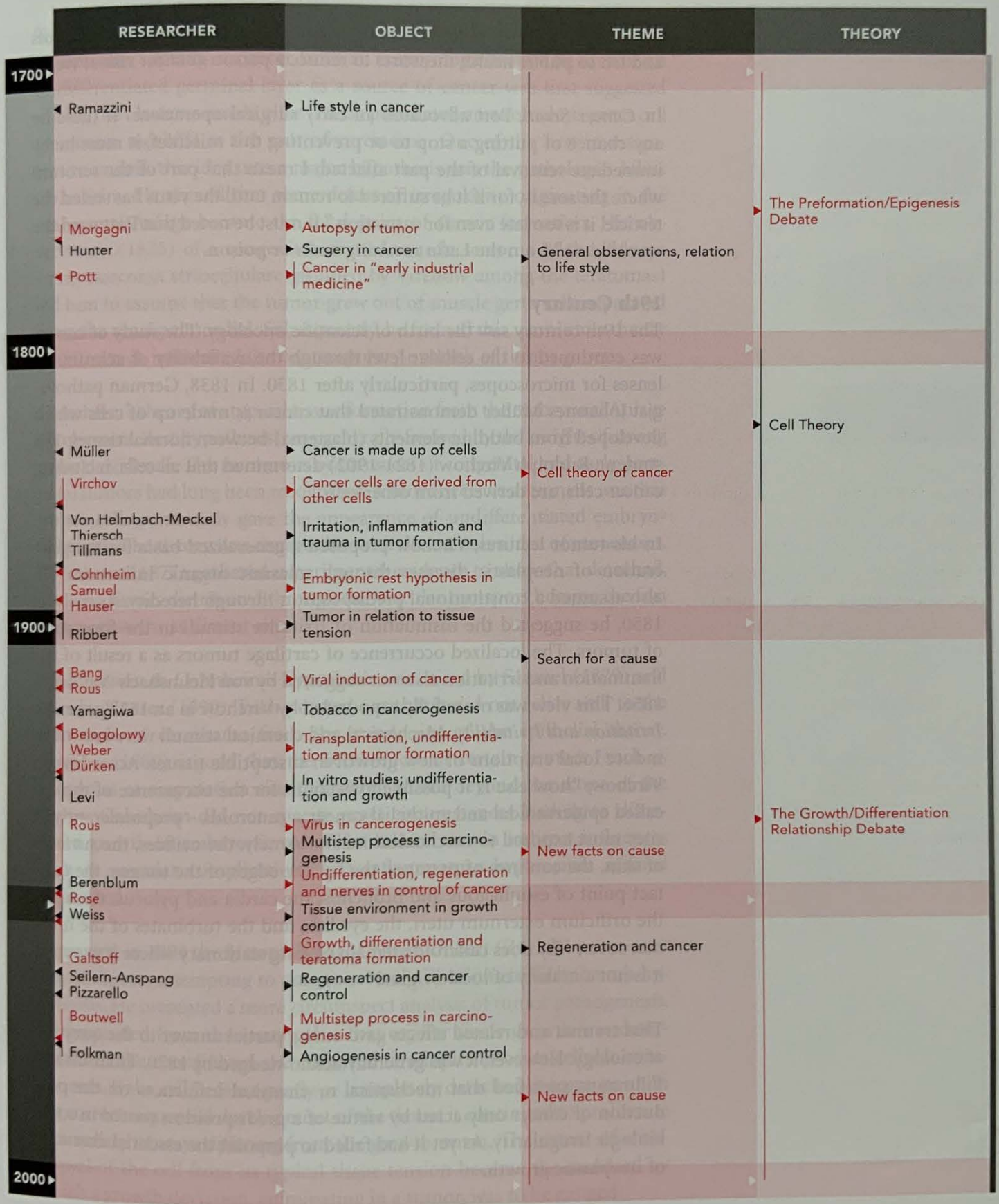
The tumor progression (the incremental development of increasingly malignant states) has allowed science to stage the tumor. Clinically, tumors are categorized into Classes I-IV according to their progression and likelihood of cure. Class IV (malignant) is the most dangerous. When metastasis occurs, local dissemination of the malignant tissue from the parent tumor mass evolves spreading the malignant tissue to distant sites in the body. Clumps of tumor cells are transported in the fluids of the body cavities and the vascular system.

Environmental conditions are frequently involved in carcinogenesis. Environmental carcinogens include physical factors such as ionizing radiation and ultraviolet light. Even more important are chemical carcinogens. Both experimental and epidemiological studies have indicated an oncogenic potential for a huge array of chemicals (Figure B.4.1.2). Most important are the halogenated hydrocarbons (Group 1), the nitrosamines (Group 2), and the aromatic organic compounds (Groups 3, 4 and 5).

Figure 4.1 ►
Time-course of some salient contributions related to tumor biology. This diagram may be seen as a detailed fragment of Figure 1.2 and may also be considered in conjunction with Figure 2.1 and 3.1.

of cancer, we have to go back to 1761 when Giovanni Morgagni of Padua did what has become routine today: utilizing autopsies to relate pathologic findings after death to the patient's illness. It was about the same time that John Hunter (1728-1793), a Scottish surgeon, suggested that some cancers might be cured by utilizing surgery. He described how the surgeon might decide which cancers to operate on. If the tumor had not invaded nearby tissue and was "moveable", he said, "There is no impropriety in removing it".

During the 18th century, a few important observations launched the field of cancer epidemiology (with the study of causes and distributions of diseases). In 1713, Bernardino Ramazzini, an Italian doctor, reported the virtual absence of cervical cancer and relatively high incidence of breast cancer in nuns and wondered if this was, in some way, related to their celibate life style. In 1775, Percivall Pott, a British surgeon, reported a curious prevalence of ragged sores on the scrotums of many chimney sweeps in London. Other doctors might have concluded that the men were afflicted with a venereal disease that was then rampant throughout the city. But Pott was more astute. He realized they were in fact suffering from a type of skin cancer. Pott established that the cause of this form of cancer was "a lodgment of soot in the rugae of the scrotum". His observation was a medical milestone, and his work *Cancer Scroti* (or *Chimney Sweeper's Cancer*), is a classic in industrial medicine. By observing that men continually exposed to coal tar were "peculiarly liable" to this form of cancer, he documented for the first time that cancer could be caused by an external agent rather than by internal factors. It was also the first time a specific type of neoplasm was related to a particular occupation. It initiated one of the first epidemiological surveys of coal tar-induced cancer. It led to many more


RESEARCHER
OBJECT
THEME
THEORY
1700
Ramazzini
Life style in cancer
Morgagni
Autopsy of tumor
Hunter
Surgery in cancer
Pott
Cancer in "early industrial medicine"
General observations, relation to life style
The Preformation/Epigenesis Debate
1800
Müller
Cancer is made up of cells
Cell theory of cancer
Cell Theory
Virchov
Cancer cells are derived from other cells
Von Helmbach-Meckel
Thiersch
Tillmans
Irritation, inflammation and trauma in tumor formation
Cohnheim
Samuel
Hauser
Embryonic rest hypothesis in tumor formation
1900
Ribbert
Tumor in relation to tissue tension
Search for a cause
Bang
Rous
Viral induction of cancer
Yamagiwa
Tobacco in cancerogenesis
Belogolowy
Weber
Dürken
Transplantation, undifferentiation and tumor formation
Levi
In vitro studies; undifferentiation and growth
Rous
Virus in cancerogenesis
Multistep process in carcinogenesis
New facts on cause
The Growth/Differentiation Relationship Debate
Berenblum
Undifferentiation, regeneration and nerves in control of cancer
Rose
Weiss
Tissue environment in growth control
Galtsoff
Growth, differentiation and teratoma formation
Regeneration and cancer
Seilern-Anspang
Pizzarello
Regeneration and cancer control
Boutwell
Multistep process in carcinogenesis
Folkman
Angiogenesis in cancer control
New facts on cause
2000

studies that identified a number of occupational carcinogenic exposures and led to public health measures to reduce a person's cancer risk at work.

In *Cancer Scroti*, Pott advocated an early surgical operation: "If there be any chance of putting a stop to or preventing this mischief, it must be by immediate removal of the part affected; I mean that part of the scrotum where the sore is, for if it be suffered to remain until the virus has seized the testicle, it is too late even for castration." It must be noted that Pott used the word "virus" from the Latin meaning toxin or poison.

19th Century

The 19th century saw the birth of scientific oncology. The study of cancer was continued to the cellular level through the availability of achromatic lenses for microscopes, particularly after 1830. In 1838, German pathologist Johannes Müller demonstrated that cancer is made up of cells which developed from budding elements (blastema) between normal tissues. His student, Rudolph Virchow (1821-1902) determined that all cells, including cancer cells, are derived from other cells.

In his tumor lectures, Virchow proposed a generalized basis for the generation of neoplastic diseases through miasmic organic influences. He also assumed a constitutional predisposition through heredity. As early as 1850, he suggested the insinuation of irritative stimuli in the formation of tumors. The localized occurrence of cartilage tumors as a result of inflammation and irritation also was suggested by von Helmsbach-Meckel in 1856. This view was more fully expounded by Virchow in an 1858 paper on *Irritation and Irritability*. Mechanical and chemical stimuli were known to induce local eruptions of new growth in susceptible tissues. According to Virchow: "how else is it possible to account for the occurrence of the so-called epidermoidal and epithelial cancer – cancroids – preponderantly at sites most exposed to mechanical insult; namely, the orifices, the surfaces of skin, the confines of passages, the lip, the edges of the tongue, the contact point of esophagous and bronchus, the cardia and pylorus, the anus, the orificium externum uteri, the eye lids, and the turbinates of the nose; moreover, why does cancroids remain so long stationary where it occurs if it is not a malady of local origins?"

That trauma and related effects gave only a partial answer to the question of etiology. However, it was generally acknowledged by 1870. Thiersch and Tillmanns specified that mechanical or chemical influences on the production of cancer only acted by virtue of a predisposition rooted in some biologic irregularity. As yet it had failed to pinpoint the essential character of neoplastic growth.

To fill this gap in the 1870's under Virchow's influence, several scientific groups were looking at cancer from an embryologic view. The idea of an undifferentiated germinal layer as a source of cancer was first suggested during the blastema era. Müller (1838) postulated an anomalous germ cell (seminum morbid) as the root of carcinoma. One of the first investigators to rework this idea into a major hypothesis was the pathologist Julius Cohnheim. He argued that some defect or irregularity originating in the embryonal Anlage became the focal point of tumor formation. His observation (1875) of a congenital striated muscle sarcoma of the kidneys (a myosarcoma striocellulare grouped by Virchow among the teratomas) led him to assume that the tumor grew out of muscle germ cells separated from the renal primordial. Subsequently, he framed this view into a theory (1880) which influenced the contemporary outlook of tumor genesis.

Cohnheim's theory of atypical tissue formation from the embryonal Anlage (Embryonic Rest hypothesis) was generally favored by the weight of strong clinical evidence. The numerous occurrences of congenital and early postnatal tumors had long been recognized. A more decisive argument was that tumor cells commonly gave the appearance of undifferentiated embryonal types. The theory was compatible with the embolus idea of metastases. Cohnheim (1877) gathered experimental evidence to show that detached, growing tissues distributed through the vascular transport became the foci of new growths at distant sites.

An objection to Cohnheim's hypothesis was raised by Samuel (1879) and later by Hauser (1890). Each pointed out that it did not relate well to the formation of carcinoma from the standpoint of the epithelial theory. Virchow (1880) argued that the occurrences of certain tumors (such as neuromas at amputation sites) appeared to fall beyond the scope of the theory. Cohnheim did not specify exact circumstances leading to disordered growth in embryonic rests. The role of certain indirect influences such as vascular complications and inflammatory hyperaemia seemed implicated.

Beginning in 1894 the histopathologist Hugo Ribbert (Zürich) wrote a series of reports attempting to answer several objections to Cohnheim's hypothesis. He presented a more circumspect analysis of tumor pathogenesis. He proceeded from the assumption that regular tissue growth was determined by the coordinated and balanced development of its cellular components. If the cell became partly or completely detached at some point in the correct sequential development of the tissue, it continued to proliferate in a variety of ways differing from the growth norm. The extent of withdrawal of the cell from its typical tissue tension became the standard by which a growth deviation, culminating in a tumor, was to be gauged.

Just at the beginning of the 20th century, pathologists recognized Cohnheim's concepts how malignant neoplasms resembled their embryonic bases (utilizing terms such as dedifferentiation, blastoma, etc. in the description of both benign and malignant neoplasms). Also of interest (for the history of the association of cell growth and division with cell differentiation) is the work *Trattato di Istologia* by Giuseppo Levi (1927). Levi was one of the pioneers of *in vitro* cell culture. As part of a culture, cell proliferation is stimulated by the addition of embryonic (cell-free) extracts. He observed that by stimulating cell proliferation, he could inhibit cell differentiation. The loss of differentiation, named anaplasia, also occurs during enhanced growth in regeneration. Most prominent, however, it is seen in the establishment of cell lines in culture. This antagonism between cell proliferation and cell differentiation may be an important avenue when approaching carcinogenesis (Figure 4.2).

We can add to this another amazing line of research. The embryonal hypothesis of the origin of carcinomas, popular at the turn of the Nineteenth Century, also stimulated considerable interest in experiments involving the transplantation of eggs and embryos onto the tissue and organs of various vertebrates. Of particular theoretical interest were the results of experiments with amphibia (toads and frogs) in which eggs at early stages of development and embryos were transplanted into the abdominal cavities of adults. It is a type of homoplastic implantation (Figure 4.3). These

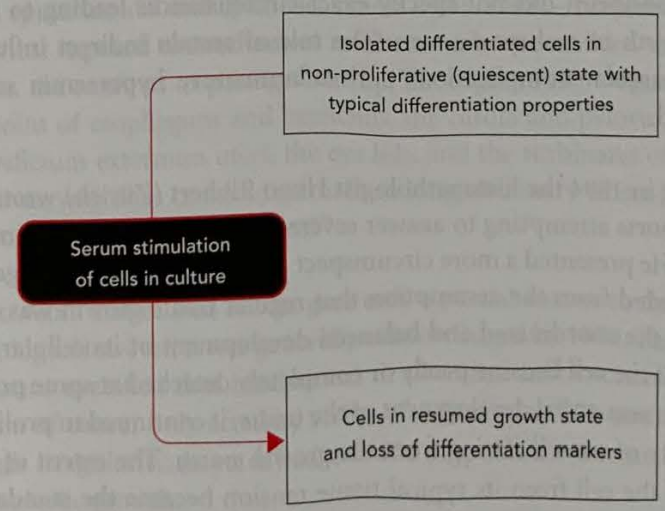


Figure 4.2
The "differentiation/proliferation switch" in anaplasia. Loss of differentiation in growth stimulation according to Levi (1927).

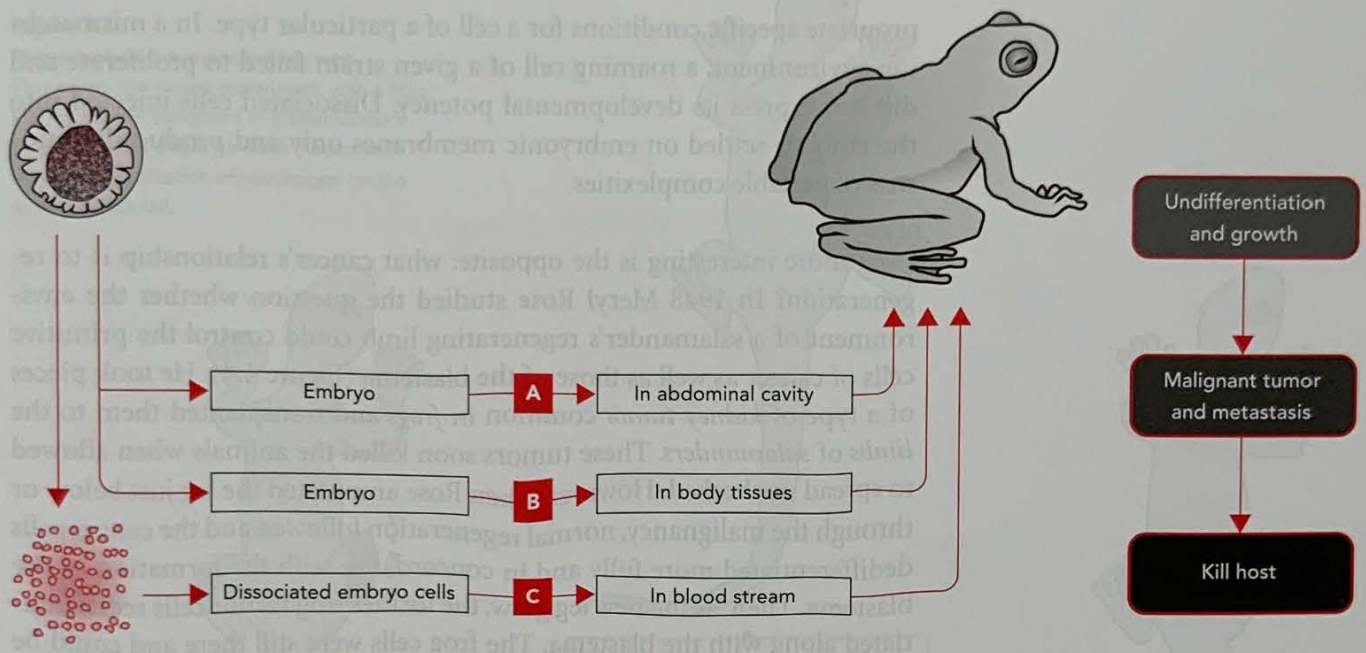


Figure 4.3
Homoplastic implantation and cancer development. Embryos or embryonic cells were transplanted into the adult animal in three types of experiments: A. in the abdominal cavity, B. in body tissues, and C. as dissociated cells in the blood stream. The right panel presents the outcome if cells are not rejected: cells dedifferentiate, a malignant metastasizing tumor develops which kill the host.

embryos then followed another development. In many cases, the embryo was rejected. However, it was claimed by Belogolowy (1918), Weber (1920), and Dürken (1926) that in case the host did not simply reject the graft, the embryo underwent gradual dedifferentiation, formed large masses of simplified cells and degenerated into a highly malignant metastasizing tumor. In 5 to 6 months it killed the host. Similar results were obtained by Galtsoff and Galtsoff (1953, 1959). They studied the relationship between growth and differentiation by implanting frog embryos in various body tissues of adult frogs.

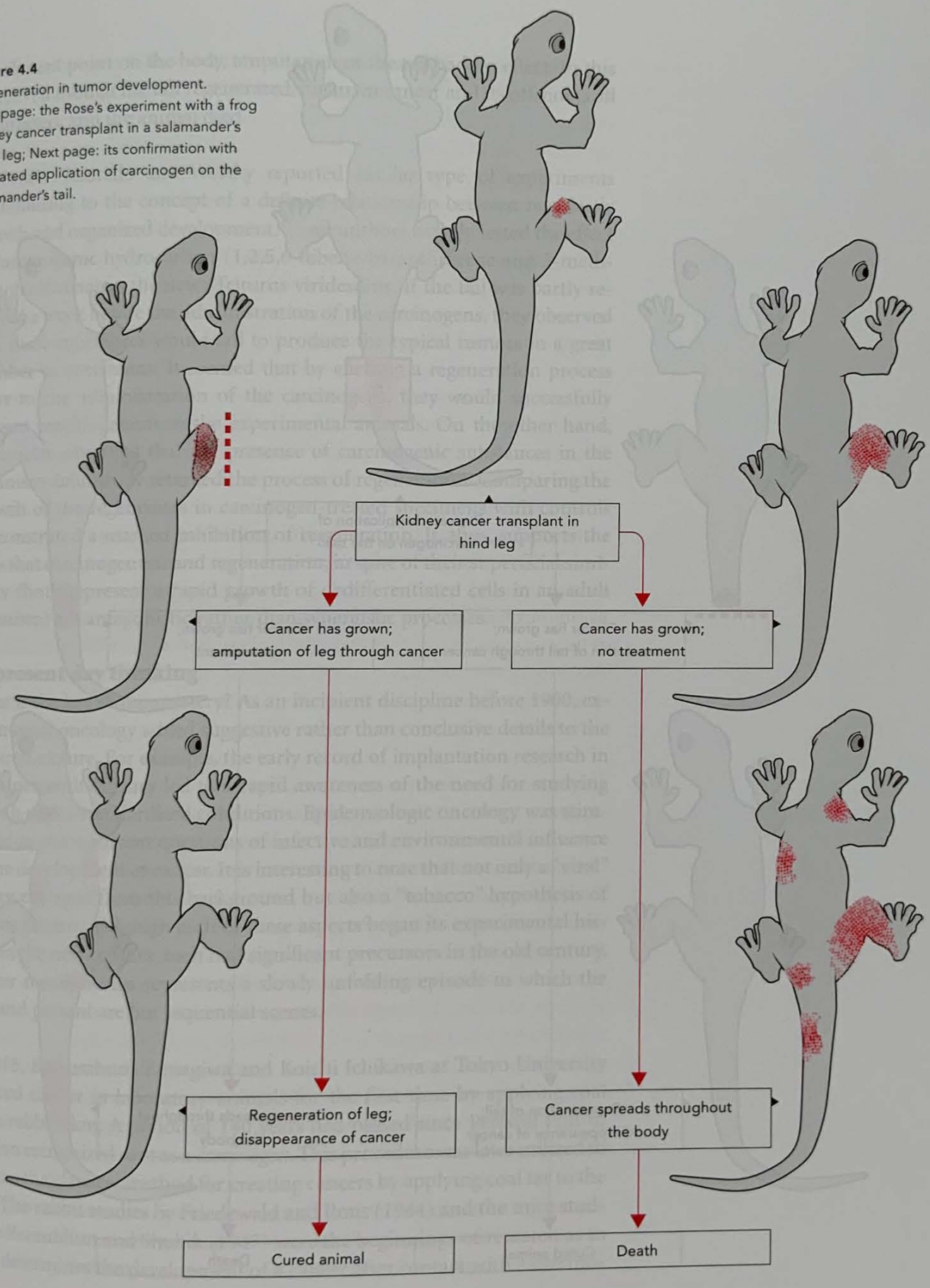
In other experiments, using dissociated chick embryos, Weiss and Andres injected dissociated cells intravenously into adult birds and into their embryos. In their studies (1952-1953), they documented that various embryonic cells (dispersed by the blood stream throughout the adult organism) may eventually leave the blood vessels and take up their proper positions in the body, proliferate profusely, and colonize the locations that offered ap-

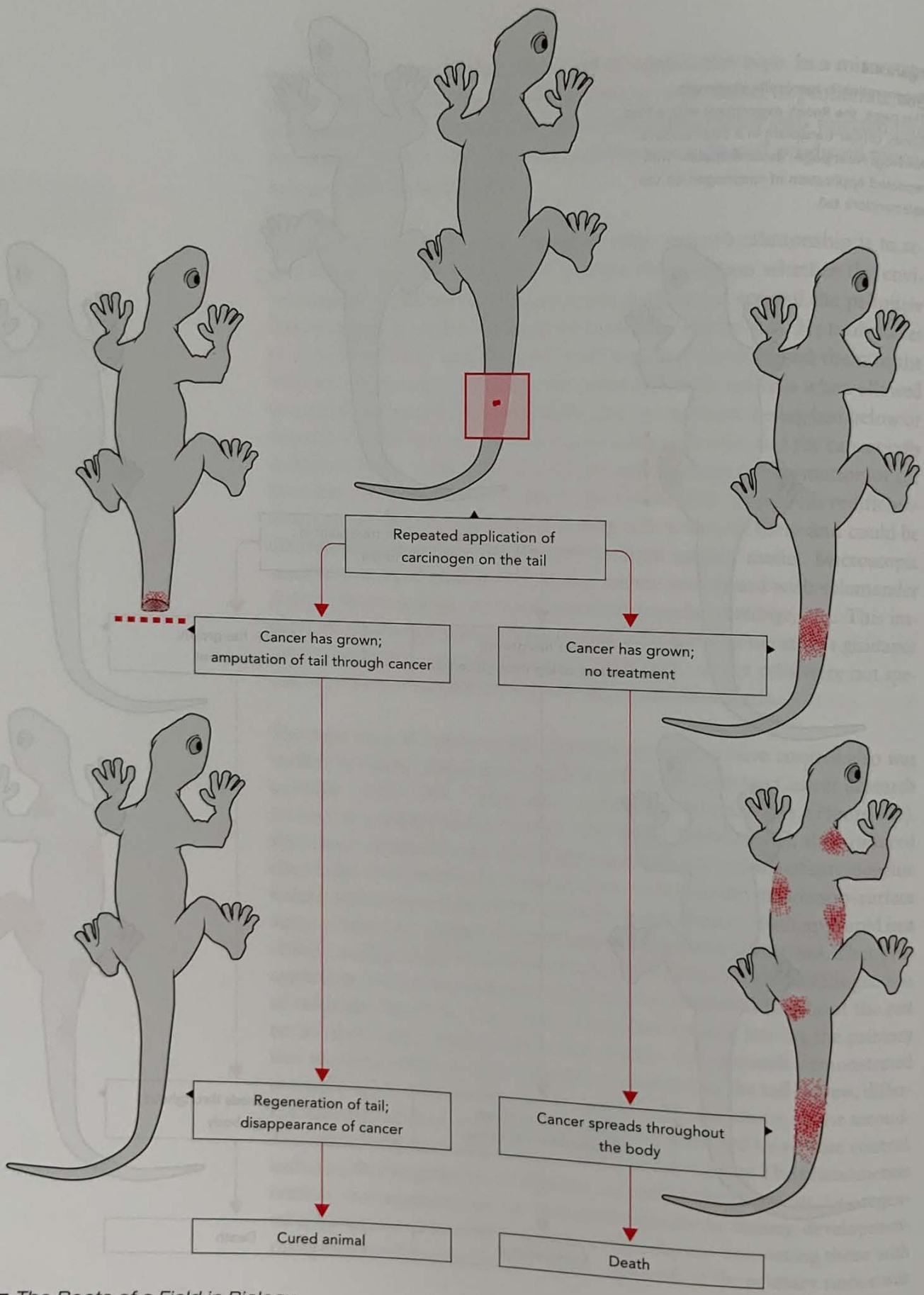
propriate specific conditions for a cell of a particular type. In a mismatching environment, a roaming cell of a given strain failed to proliferate and did not express its developmental potency. Dissociated cells injected into the embryo settled on embryonic membranes only and produced teratomas of variable complexities.

Even more interesting is the opposite: what cancer's relationship is to regeneration? In 1948 Meryl Rose studied the question whether the environment of a salamander's regenerating limb could control the primitive cells of cancer as well as those of the blastema (Figure 4.4). He took pieces of a type of *kidney tumor* common in *frogs* and transplanted them to the *limbs* of *salamanders.* These tumors soon killed the animals when allowed to spread unchecked. However, when Rose amputated the leg just below or through the malignancy, normal regeneration followed and the cancer cells dedifferentiated more fully and in concordance with the formation of the blastema. Then, as the new leg grew, the former frog tumor cells redifferentiated along with the blastema. The frog cells were still there and could be distinguished from salamander cells by their smaller nuclei. Microscopic examinations demonstrated that frog muscle was mixed with salamander muscle. Frog cartilage cells were amid salamander cartilage, etc.. This important experiment proved Rose's hypothesis that regeneration's guidance system could also control cancer. It implied that cancer cells were not special but merely embryonic cells in a post-embryonic body.

The regression of tumor under appropriate regenerative control also was studied by Seilern-Aspang and Kratochwill at the Austrian Cancer Research Institute (1962-1963). They also worked on salamanders (Figure 4.5). Instead of implanting frog tumor cells in the limbs or tail, they induced skin cancer (epitheliomas) with large, repeated applications of carcinogenic chemicals. They eventually initiated tumors that would invade sub-surface tissues, metastasize, and kill the animals. A spectacular result appeared in a series where they applied the carcinogens to the base of the tail. That procedure resulted in the formation of a primary tumor where carcinogen was applied as well as in metastases appearing at random throughout the rest of the body. However, if they then amputated the tail, leaving the primary tumor intact, this tumor would often regress. Cell research demonstrated that the tumor did not die or degenerate. Instead, as the tail regrew, differentiation apparently reverted to normal skin. Furthermore, all the secondary tumors vanished as if they were surgically removed by remote control. The salamander ended up with a new tail and no cancer. The phenomenon indicates that in periods of intensive secondary development, i.e., regeneration, the organism has an increased capacity to convey developmental information to its undifferentiated cells, thereby converting those with malignant tendency to normal tissues. However, if the primary tumor was

Figure 4.4
Regeneration in tumor development.
This page: the Rose's experiment with a frog kidney cancer transplant in a salamander's hind leg; Next page: its confirmation with repeated application of carcinogen on the salamander's tail.




Repeated application of carcinogen on the tail
Cancer has grown; amputation of tail through cancer
Cancer has grown; no treatment
Regeneration of tail; disappearance of cancer
Cancer spreads throughout the body
Cured animal
Death

at a distant point on the body, amputation of the tail had no effect. In this case, even though the tail regenerated, the main cancer and its offshoots all progressed, and the animal died.

In 1966, Pizzarello and Wolsky reported similar type of experiments contributing to the concept of a definite relationship between neoplastic growth and organized development. These authors usually tested the effect of carcinogenic hydrocarbon (1,2,5,6-dibenz-[α]anthracene and 3-methylcholanthrene) in the newt Triturus viridescens. If the tail was partly removed a week before the administration of the carcinogens, they observed that these substances would fail to produce the typical tumors in a great number of specimens. It seemed that by eliciting a regeneration process prior to the administration of the carcinogens, they would successfully prevent carcinogenesis in the experimental animals. On the other hand, it was also observed that the presence of carcinogenic substances in the organism definitively retarded the process of regeneration. Comparing the growth of the regenerates in carcinogen-treated specimens with controls demonstrated a marked inhibition of regeneration. It, thus, supports the view that carcinogenesis and regeneration, in spite of their superficial similarity (both represent a rapid growth of dedifferentiated cells in an adult organism) are antagonistic rather than synergistic processes.

To present day thinking

What is the key of the mystery? As an incipient discipline before 1900, experimental oncology added suggestive rather than conclusive details to the research picture. For example, the early record of implantation research in the nineteenth century led to a rapid awareness of the need for studying growth under standardized conditions. Epidemiologic oncology was stimulated by the recurrent questions of infective and environmental influence on the development of cancer. It is interesting to note that not only a "viral" theory emerged from this background but also a "tobacco" hypothesis of carcinogenesis. Although each of these aspects began its experimental history in the new century, each had significant precursors in the old century. Cancer research thus represents a slowly unfolding episode in which the past and present are but sequential scenes.

In 1915, Katsusaburo Yamagiwa and Koichi Ichikawa at Tokyo University induced cancer in laboratory animals for the first time by applying coal tar to rabbit skin. A period of 140 years had passed since Percival Pott of London recognized soot as a *carcinogen*. This procedure was later utilized to develop the classical method for creating cancers by applying coal tar to the skin. The rabbit studies by Friedewald and Rous (1944) and the mice studies by Berenblum and Shubik (1947) were the beginnings of research as to what determines the development of a cancer after contact with a carcino-

gen (Figure 4.5). Skin cancers can be elicited, for example, by repeatedly painting the skin with a mutagenic chemical carcinogen such as benzo[α] pyrene or related compounds. In skin, the stages of tumor progression can easily be observed. These studies suggested that carcinogenesis was not a single step event, but instead a multistage process. The characteristic stages in skin carcinogenesis were described by Boutwell (1974). A single application of the carcinogen does not, by itself, give rise to a tumor or any other lasting abnormality. Yet it does cause latent genetic damage (mutations) that set the stage for a greatly increased incidence of cancer when the cells are exposed either to further treatments with the same substance or to certain other, quite different, insults.

A carcinogen that sews the seeds of cancer in this way is said to act as a tumor initiator. Even though the carcinogen may be the seed of cancer, the cells require further exposure to other insults to grow and develop. Simply wounding skin that has been exposed once to such an initiator can cause cancers to develop from some of the cells at the edge of the wound. Alternatively, repeated exposure over a period of months to certain substances known as tumor promoters, which are not themselves mutagenetic, can cause cancer selectively in skin previously exposed to a tumor initiator. Based on these findings, Boutwell discriminated at least two major stages in the sequential cancer development process that he called "initiation and promotion". Subsequently, stages analogous to initiation and promotion demonstrated carcinogenesis in other tissues such as hepatocarcinogenesis in rodents.

The tumor initiation/promotion studies initiated considerable discussion about environmental risks. A typical example was with burned coal resulting in small amounts of coal tar. We now know that this brown/black material is a mixture of several hundred compounds: phenols, polycyclic aromatic hydrocarbons (PAHs), and heterocyclic compounds (see Box 15.2). Coal tar is commonly used in parking lot sealing which is marketed to protect and beautify the underlying pavement. The International Agency for Research on Cancer has raised substantial concern in its report *Coal Tars and Coal Tar Pitches* (1980). However, the defenders of coal tar pointed out that humans are exposed to PAHs through many pathways. According to the International Agency for Research on Cancer, preparations that include more than five percent of crude coal tar are in the top tier of carcinogens. Coal tar concentrations between 0.5% and 5% are classified as safe and thereby may be used for psoriasis.

Another potential induction source of cancer are viri. Certain families of viri are able to trigger neoplastic transformation of the cells they infect. Members of both the DNA and the RNA classes of viri have such capacity.

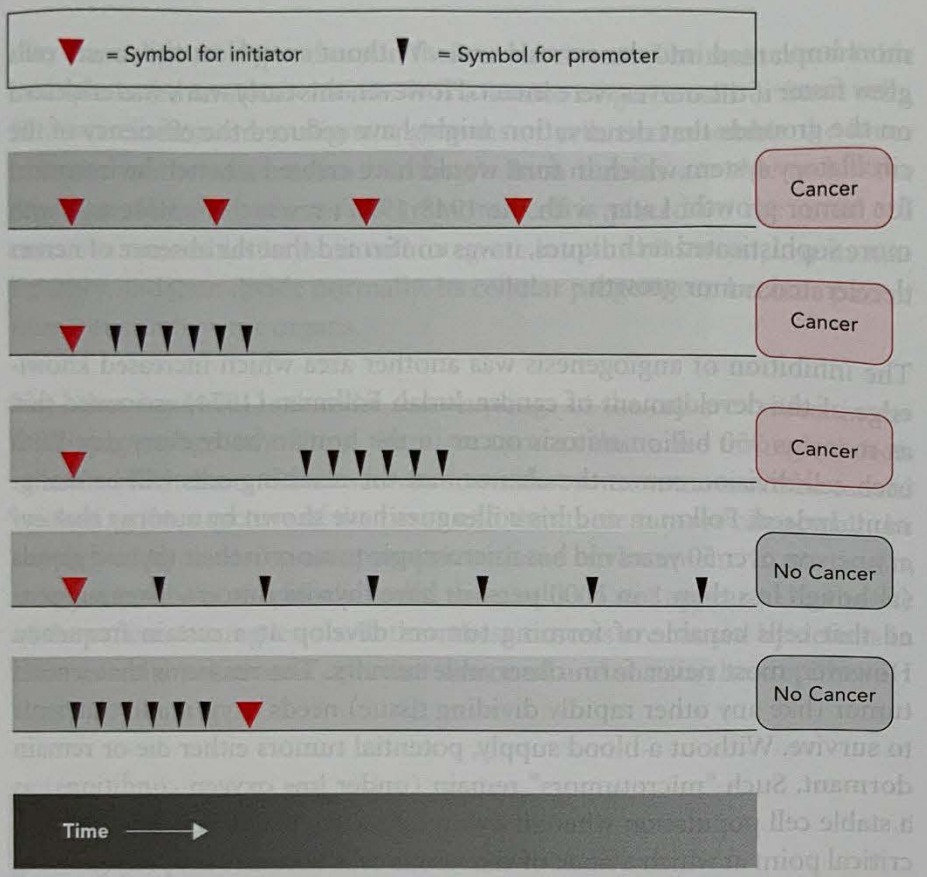


Figure 4.5
Schedules of exposure to a tumor initiator and a tumor promoter and the outcomes. Cancer can occur as a result of repeated exposure to the initiator alone. In case of only one exposure to a tumor initiator, cancer ensues if the exposure to the promoter follows exposure to the initiator and only if the intensity and frequency of this exposure exceeds a certain threshold.

The theory that cancer could be caused by a virus began with the experiments of Oluf Bang and Vilhelm Ellerman (1908) who initially demonstrated that avian erythroblastosis (a form of chicken leukemia) could be transmitted by cell-free extracts. This was subsequently confirmed for solid tumors in chickens (1910-1911) by Peyton Rous. By the early 1950's it was known that viri could remove and incorporate genes and genetic material into cells. It was suggested that these new genes inserted into cells could make the cell cancerous. Many of such viral oncogenes have been identified to cause cancer. Generally, tumor viri cause little or no disease after infection in their hosts nor do they cause non-neoplastic diseases such as acute hepatitis B or mononucleosis by Epstein-Barr. A minority of persons (or animals) will develop cancers after infection. So far, it has been difficult to determine whether or not a given virus causes cancer.

Since regeneration cannot occur without the stimulus and control of nerves, one expects that nerves would exert some control on cancer. This apparently can happen. As far back as the 1920's, many researchers studied tu-

mors implanted into denervated areas. Without exception, the cancer cells grew faster if the nerves were intact. However, this early work was criticized on the grounds that denervation might have reduced the efficiency of the circulatory system which in turn would have created a better environment for tumor growth. Later, with the 1948-1960's research by Rose and with more sophisticated techniques, it was confirmed that the absence of nerves decelerated tumor growth.

The inhibition of angiogenesis was another area which increased knowledge of the development of cancer. Judah Folkman (1974) estimated that as many as 350 billion mitosis occur in the human body every day. With each cell division comes the chance that the resulting cells will be malignant. Indeed, Folkman and his colleagues have shown by autopsy that every person over 50 years old has microscopic tumors in their thyroid glands (although less than 1 in 1000 persons have thyroid cancer). They suggested that cells capable of forming tumors develop at a certain frequency. However, most never form observable tumors. The reason is that a solid tumor (like any other rapidly dividing tissue) needs oxygen and nutrients to survive. Without a blood supply, potential tumors either die or remain dormant. Such "microtumors" remain (under low oxygen conditions) as a stable cell population wherein dying cells are replaced by new cells. The critical point at which a node of cancerous cells becomes a rapidly growing tumor occurs when it becomes vascularised. A microtumor can expand to 16000 times its original volume in the 2 weeks after vascularization.

To accomplish this vascularization, the microtumor secretes substances called tumor angiogenesis factors. These often include the same factors that produce blood vessel growth in the embryo. Tumor angiogenesis factors stimulate mitosis in endothelial cells and direct the cell differentiation into blood vessels in the direction of the tumor (see Figure 11.6). Folkman and his colleagues have pioneered attempts to find and use artificial angiogenesis inhibitors. These compounds act to prevent the endothelial cells from responding to the angiogenetic signal of the tumor. Interestingly, thalidomide, the teratogen responsible for birth defects in the 1960s, was one of them. The reduction of growth of cancers in rats and mice by thalidomide was suggested by D'Amato and Dredge.

Conclusion

It is evident that the genetic approach to explain the formation of numerous tumor types is not the whole story. It must be complemented by a field approach that facilitates epigenetic, cell to cell, and extracellular interactions that are pivotal both in tumor induction and in tumor progression. In finishing this chapter, we mention a few more arguments to substantiate this view. Many tumor cells have normal genomes. Whether or not

they are malignant depends upon their environment. The most remarkable example is the teratocarcinoma. It is a tumor of germ cells or stem cells. Teratocarcinomas are malignant growths of cells that resemble the inner cell mass of the mammalian blastocyst. Such tumors are able to kill the organism. However, if a teratocarcinoma cell is placed on the inner cell mass of a mouse blastocyst, it will integrate into the blastocyst, lose its malignancy, and then divide normally. Its cellular progeny can become part of numerous embryonic organs.

The other argument is that the surroundings of the cell are critical in determining malignancy. Thus, there are tumors that arise through defects in tissue architecture. Studies have shown that some tumors can be caused by altering the structure of the tissue and that these tumors can actually be suppressed by restoring an appropriate tissue environment. In particular, in human tumors of epithelial cells, these cells do not appear to be the site of the cancer causing lesion. Rather, these cancers are caused by defects in the mesenchymal stromal cells that surround and sustain these epithelia.

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Conclusion Part A

At the end of our first journey through the history of embryonal development, regeneration and tumor formation, we have become acquainted with the universality of the "program for the formation of the form", a teleonomic process characterized by properties of unfolding, goal-directedness and adaptedness. This "program" is more than a "blueprint" for the ultimate goal (structure, functioning). It includes "instructions for using the information of the blueprint". The program is universal: it directs development. However, it also provides instructions how to react in case of intrinsic disturbances or injury.

Increasing knowledge about embryonal development included "cell theory" and the concept of "positional information". The cell theory (established in the 1850's) documented the fact that cells are the basic structural units of all living things and that their mitotic division is the mechanism by which future parts of a developing organism are determined. Development and differentiation were discussed in terms of a.) "preformation" and "epigenesis" (the Roux-Driesch discussion), b.) "equipotentiality" (i.e., all cells of an embryo are equal in their potential morphogenetic capability) and "equifinality" (final result of development may be reached by different pathways), and c.) "totipotent" and "pluripotent" (referring respectively to a cell that can generate every cell in an organism and one that can generate nearly every cell).

The concept of positional information documented the fate of a blastula cell as a function of its position in the system. That concept was discussed in terms of "polarity", "gradients" (Driesch, Boveri, Child, and Spemann) leading, in 1921, to the "field of organization". Biochemically, these organisers were considered producers of chemical substances (morphogens) and concentration gradients which react with tissues determining the fates of their component cells [i.e., induce differentiation by changing the subset of genes that are expressed in that cell or tissue (Hörstadius, Strumpf, Wolpert)].

The structural outcome of the "program" is a body operating as stable and, at the same time, as a dynamic society whose individual members are cells. Such a body has a miraculous capacity to withstand disturbances specifically in the process of *regeneration* after amputation in some species. This process was described for centuries in all its magnificence and discussed (like in the research on the early development) in terms of "preformation" and "epigenesis". After the establishment of the cell theory, the discussion

focused at the local cell level addressing changes in differentiation and cell divisions.

The positional information evidently changed. Intact tissue begins to die back a short distance from the wound, epidermal cells begin to proliferate and migrate inward forming the apical cap, and beneath it all is a ball of undifferentiated cells (blastema). The blastema is the start of new, precisely directed cell divisions and growth followed by differentiation. This entire process illustrates that the "program" constantly reads the entire functioning structure (developed according to the *blueprint*) and opens the *instructions for adaptations* within itself, emphasizing locally a partial reversal of the teleonomic process. It then starts again with the *instructions to renew the body structure.*

The nerve has a special role in this process. Regeneration is less. If the limb is denervated by transecting the axons, blastema cells form after amputation but do not divide. It is time dependent. Denervation, early after amputation, stops proliferation and arrests regeneration. If denervation occurs at later stages regeneration proceeds but, the resulting regenerate is smaller than normal. The opposite effect was also discussed. Namely, that an extra leg would grow if a nerve was re-routed. It indicated a temporal sequence in the formation of the regeneration blastema and a time-specific influence by some energy emitted from the nerve which determines what might grow back. The knowledge of the complex interactions between regenerating tissue and nerves is further stimulated by experimentation with the "aneurogenic limb" (the limb bud develops in the absence of a nerve) and the "ectopic limb" (resulting from rerouting the nerve to a wound site along with skin fibroblasts from the other side). Biochemically, the developments have focussed on growth factors emitted from the nerves which may play a role in regeneration.

The instructions to adapt to intrinsic threatening conditions by a partial reversal of the teleonomic process (i.e., dedifferentiation and increased division of cells) also apply in tumor formation. At the birth of scientific oncology (Virchow), it was established that cancer cells are derived from other cells and metastasize through spreading of malignant cells. The belief that trauma could cause cancer, although a common belief until the 1920's, was *not* supported by experiments that were aimed to demonstrate just that. Much later it was finally demonstrated that when a carcinogen has seeded a cancer cell, the cells still require further exposure to other insults in order to grow and develop. Then, simply wounding could cause the cancer to develop.

Thus, we are back to the "embryonal theory of cancer" which compares cancer with the dedifferentiation and increased growth properties within blastema formation. The embryonal theory stimulated interesting experiments involving "homoplastic implantation" (transplantation of eggs and embryos in tissue and organs of adults). In the event that the host did not reject the graft, the embryo underwent dedifferentiation forming large masses of cells ending up with malignant metastasizing tumor that eventually kills the host (Belogolowy, Weber, Dürken, Galtsoff and Galtsoff, Weiss and Andres). Even more interesting were the observations that the regeneration's guidance system could control cancer. The regression of a tumor under appropriate regenerative control was not only observed in a primary tumor but was also pertinent for metastases which had appeared in the rest of the body (Rose; Seilern-Aspang and Kratochwill; Pizzarello and Wolsky).

Summary and Perspective

This journey has described that a similar "program" (containing the blueprint and the instructions for the use of the blueprint) is set in motion in early development as well as by disturbances, exterior and interior. The unfolding goal-directed proces laid down in the development of the form, is the most recognizable part of the "program". At both cell and biochemical levels, it is recognized that the field of organization encompassing positional information is etiologic. It has been studied by biochemistry and cell biology in line with the paradigm of the 20th century which was obsessed with metabolism and biochemistry resulting in the considerable knowledge of today.

However, that knowledge also has definitive limitations. Something was lacking in the biochemical approach. Metabolism was about energy and rates. Positions were addressed with scalars, vectors and boundaries. The recipe required an accounting of the forces that mobilize the elements into patterns. Are these forces properties of the molecules themselves? This mystery is increasingly recognized within the framework of Living Systems Theory. A living creature has an organic form, levels of organization and a composition containing particles. Where to find these forces?

We will therefore, during the next leg of our journey learn how the early 1900's helped to better understand a biological organism as a system. That system perspective accepted the fact that a "field of organization" with properties specific to the totality of the form was not dependent only on the system's particles. In searching for other, non-mechanical (non-particle) aspects of the "program" (and blueprint), an alternative regulatory principle became evident: a morphogenetic (biological light) radiation field, the predecessor of the biophoton field.

PART B

Birth and Growth of Biophoton Field Concept

Introduction Part B

The understanding of a biological organism as a system arose in the early 1900's. Hans Driesch (1911) asked the question if the program that determines "the formation of the form" can be explained solely on molecular grounds. Alexander Gawrilowich Gurwitsch (1912) considered a radiation process as a non-mechanical deterministic principle in mitosis. The physics for detecting radiation was only beginning at that time. In the early 1960's researchers were able to detect (without any doubt) ultra-weak photon emission from biological organisms.

Early systems biology and the morphogenetic (ultraviolet) radiation field

A biochemical field of organization faces a fundamental problem. The program for the formation of the form should include a non-mechanical principle. Gurwitsch hypothesized that a *radiation process* is the *exogenous* division stimulating factor which energizes the mitosis. In 1923, Gurwitsch performed "the" crucial experiment documenting that the living organism emits photons (in the ultraviolet range) that stimulate the process of cell divisions in biological (detector) systems. By the mid-1930's, other studies documented that living cells required oxygen to emit such radiation.

Biology looks at physics

Subsequent demonstration of radiation vis-à-vis biological detectors resulted in a cry to research such inherent physiologic radiation with physical mechanical detectors. The attention of biologists then focused on progress in the field of photoelectric detectors beginning with Albert Einstein (1905). However, no scientist utilizing these detectors was able to document ultraviolet radiation emanating from organisms. However, a more sensitive radiation detection device was the Geiger counter. Although some researchers proved the existence of radiation, others were unable to find such an effect. The overall interest in Gurwitsch's radiation declined.

The entrance of the photomultiplier

The interest in photon emission of biological organisms revived in the 1950's and 1960's with the arrival of the photomultiplier tube with its enormous increase in sensitivity. In the 1960's, Russian groups headed by Tarusov, Vladimirov, Zhuravlev and Konev estimated ultra-weak photon emission from all types of biological organisms. They continued to produce emission data in relationship to physiology and biochemistry. They also demonstrated that photon emission is dependent on the presence of oxygen, documenting their observations vis-à-vis chain oxidation reactions and reactive oxygen species.

World-wide expansion of photon emission research

From the late 1960's on, six major centers took birth in Japan, Poland, Australia, Germany, USA and China. They began to form the beginning of world-wide research addressing ultra-weak photon emission of living systems and the use of this emission as a sensitive assay for oxidative radical reactions. Despite such international developments, interest regarding informational aspects of inherent biological radiation did not really catch on at a continuing pragmatic research level.

5

Early Systems Biology and the Morphogenetic (Ultraviolet) Radiation Field

At the transition of the 19th to the 20th century, biological issues informally discussed could not be "formally" admitted and published into other sciences nor be derived from those other sciences (including the laws and instructions for "the formation of the form"). Such "transgressions" eventually evolved in biology to the status of an independent science in the same manner that chemistry, geology, history and economics became "independent" sciences.

Hans Driesch played a major role in helping to bring this transition about. He expressed his vision in *Die Biologie als selbständige Grundwissenschaft und das System der Biologie* (1911). Biology is the science of nature's "program" that delineates how this and that comes about. It also includes how these changes emerge and become central. This search for the "program" (essence) often led to misunderstanding regarding where to look: for *matter* in nature or the *"carrier" of the matter.* How can a "form" develop and exist in time with all its richness that certainly could not be explained solely on molecular-mechanical grounds? Instead, it really should require, most likely, a nonmechanical, entelechy principle.

Systems biology as the basics of biology

Until far into the twentieth century, philosophers were almost consistently confounded about analysis and reduction. Owing to this unbroken history of successes, no one would question the heuristic importance of analysis. The mechanists, in their opposition to vitalism, demanded that all living phenomena be analyzed down to the lowest component elements to show that no residue was left after everything had been explained in terms of physics and chemistry. This culminated in the famous Berlin declaration. Mechanistic materialism was articulated by the Berlin Physical Society [founded in 1845 and originally dedicated to explaining all aspects of life (including mental processes) in terms of Newtonian mechanics]. This position was outlined as early as 1841. When du Bois-Reymond approvingly quoted the French materialist and physiologist Henri Dutrochet (1776-1846) he said: "The more one advances in the knowledge of physiology, the more reasons one will have for ceasing to believe that the phenomena of

MORE ABOUT...

BOX 5.1

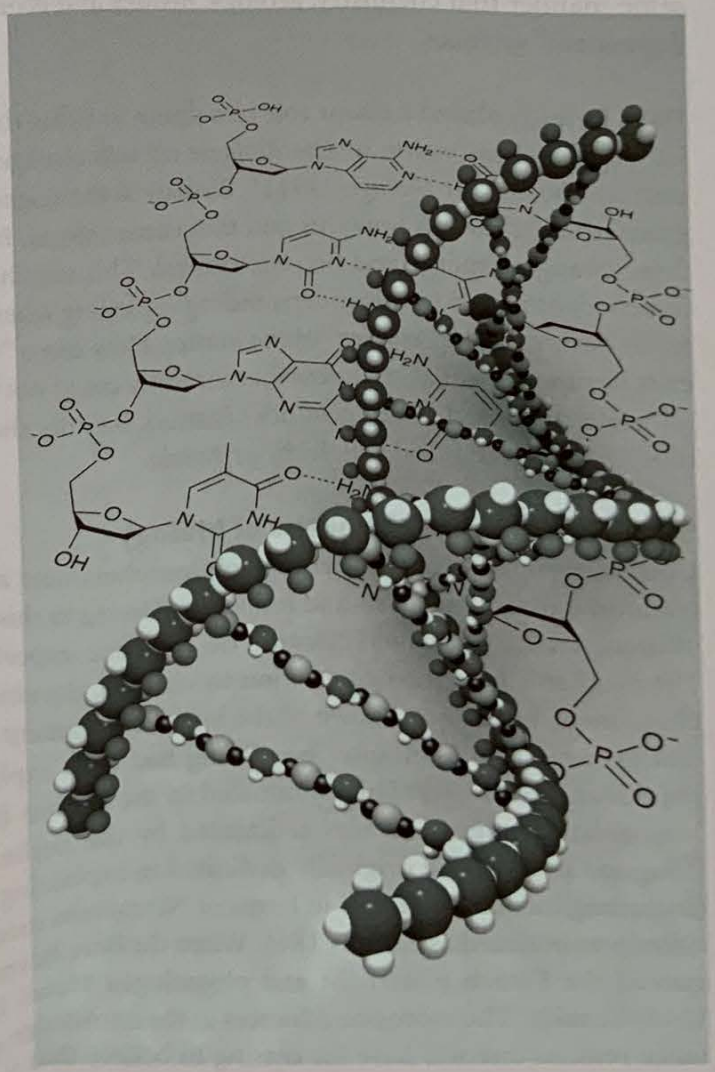
■ ANALYSIS AND EXPLANATORY REDUCTION

Much of the history of biology is a tale of the triumphs of the analytical approach. The anatomist attempted to understand the workings of the body by actually dissecting it into the component parts. The objective, then, of microscopy was the study of smaller and smaller components of both tissues and cells. The proponents of strict reduction claimed that no higher level of biological phenomenon could be understood until it had been analyzed into the components of its next lower level and then continued downward to the level of the purely physicochemical components and processes. They also claimed that knowledge of the components at the lowest level permitted the reconstruction and understanding of the higher levels. Such claims were based on the conviction that wholes were not more than the additive sums of their parts. Thus, newly emergent properties would not appear.

Such an endeavor would take the analysis to even lower levels which would carry severe limitations when attempting to explain. Experience has shown that claims by the reductionists were only rarely validated. Biological organization was realistically impossible to explain utilizing physicochemical reductionism. Reduction no longer assists the analytical approach when smaller components of a system loose applicability in providing complete information about all of the properties of a system.

What counts in a complex system is its organization. No one would be able to infer the structure and function of a kidney even if given a complete catalogue of all the molecules of which it is composed. In fact, it is now quite evident that even an exhaustive knowledge of elementary particles would not help us to explain differentiation during ontogeny. This does not deny that analysis occasionally produces "upward illumination". For instance, the discovery of the structure of DNA by Watson and Crick made it possible to explain two major properties of DNA: its mode of replication and that of information transfer. Analysis is continued downward only as long as it yields practical new information and it does not (according to reductionists) claim that the "smallest parts" give all of the answers.

Figure B.5.1.1
DNA: a prime example of explanatory reduction.



life are essentially different from physical phenomena". One year later, du Bois-Reymond and Ernst Brücke (1819-1892) had sworn to validate the basic truth that in an organism, no other forces have any effect other than the common physicochemical ones. Helmholtz had been recruited to this cause by the end of 1845, and Ludwig by the early part of 1847.

The experimental physiologist must proceed with physical and mathematical exactness, always seeking to explain biological phenomena in terms of mechanics. Du Bois-Reymond defined "mechanics" as the motion of particles of matter. The explanatory power of this approach seemed so persuasive that even the naturalist Weismann spoke of certain biological processes as being due to the "movement of molecules". There is no doubt that du Bois-Reymond, Brücke, Helmholz and Ludwig made invaluable contributions to physiology. However, they could offer no satisfactory answer to the problem of purpose. Rather than offer any alternative, they dismissed and even ridiculed the issues of "purpose" and "entelechy". Since du Bois-Reymonds opinions the mechanistic doctrine had to prevail. Organisation, development and purposiveness were simply disregarded as matters unworthy of serious scientific discussion.
However, before the end of the 19th century, mechanistic materialism in its original form had faltered. Ludwig and his student Adolf Fick agreed that physiology was not wholly reducible to mechanics after all. Nevertheless the spirit of mechanistic materialism survived inspiring the early genetic work of Thomas Hunt Morgan (1866-1945) and the pioneers of biochemistry. The mechanistic materialists had spread their influence throughout Europe and North America thanks to the numerous students they (especially Ludwig) had trained. Many of those students became professors of physiology.

However, to have isolated all the parts, even the smallest ones, was not enough for a complete explanation of most systems (as claimed by the reductionists). For a complete explanation one also needs to understand the interaction among these parts. In experimental physiology, we trace this tradition to 19th century France. Claude Bernard (1813-1878) accomplished remarkable research on the pancreas, the liver, smooth muscle tissue and brain lesions leading to crucial insights into metabolism and its control. He became increasingly impressed by what we now call homeostasis, the set of mechanisms by which body water content, body temperature, blood pressure, blood glucose concentration and a host of other variables are maintained within narrow limits. This focus was quite different from any that derived from mechanistic materialism. There was a clear gulf between the tradition of French experimental physiology and the mechanistic materialism of the post 1847 Berlin school.

MORE ABOUT...

BOX 5.2

■ DRIESCH'S LIVING SYSTEMS THEORY

Biology as science (of "Living" and "Becoming") needs a definition of "Life". In the search for a definition, one could look to scientific laws previously established. For example, any living creature has an expected composition containing certain particles. In the most extreme case, those particles possess a purely chemical and physical property which can be studied in the non-living sciences (such as physics and chemistry). A "form", then, implies that it is composed of particles. Simultaneously, a living creature does not always have the same form, but instead, possesses a "Form" that is able to "form". In biology, looking at the totality of organic forms, one can observe at the lowest level a large uniformity throughout all living creatures. In addition, the intracellular ingredients of all living creatures demonstrate a large degree of similarity both in animals and plants. The "mitochondria", "membranes" and "nuclei" are found everywhere. That is why scientists are allowed to speak about cytology. The same has been documented for groups of cells (i.e., tissues and organs). Nerves and muscles have been found in medusa, worms, man, as well as, to some degree, in certain plants. Simultaneously, biology became aware that in the same tissue, different forms had been observed. That has been beautifully illustrated by the many different forms that bone can have. The highest degree of multiplicity in form has been observed at the level of individuals. It led to the recognition that the variety of forms between individuals includes the lower layer, the organs, and even the ordering of the cells.

The early 20th century description of this principle regarding the totality of organic forms (its levels of organization) was the first attempt to conceptualize "a Living Systems Theory". Significant questions of that era were: Who has certain properties? Does this organized form of units have the sum of the properties of the units or, does this organized form have its own specific property? For example, a piece of iron and its properties can easily clarify what is meant by who owns the properties. One can distinguish the temporal as well as constant properties of iron. Temporal properties are temperature, form, weight, and color. Constant properties are specific density, specific heat coefficient, heat conductance or electrical resistance. All are properties. The question is: who or what has those properties? The answer is: the "mass". In biology, the matter has been very complicated. From the beginning of biology, two dual categories are recognized. The first was the simultaneous "matter/property" category. The second dual category was "whole/parts". Both dual categories are involved in a complex, composite body. Who has eyes or a liver, for instance, in the case of the lion? But, who is this lion? Is the lion just a "composition of its parts"? The "parts" are certainly not sufficient to describe the lion. In fact, one has to conclude that "property" as a single term cannot be used when discussing a living system. The word "property" must be used in combination with the duality of the "whole/parts". The matter is evidently such that a natural body has spatially distinct parts and those parts have temporal and constant properties. Thus, an organism has organs as a first layer and the organs have tissues as parts of the second layer. The parts of tissues are the cells (the third layer) whereas the parts of cells are the intracellular forms (the fourth layer). Where are the "properties" to be found? One needs not much imagination to understand that relative parts, such as organs or cells have their own specific properties. They can, for example, have a quite distinct "hardness" or "heat conductance" compared to the whole.

This discussion about the subdivisions in the whole has a deeper meaning: The organism has "properties" (i.e., capacities and potencies) that are not the result of the properties of its parts. With this idea in mind, it was possible to further the argument. The rain worm, for example, has the following properties: it has a multitude of organs which, in itself, are composite. Every organ and every organ part has its own form. Every organ has the capacity to physiologically and spatially "tune" with the environment. Furthermore, in its embryological form, the rain

worm must be considered to be filled with (not yet developed) form-giving potencies. Finally, the rain worm has the capacity to form germs that are able to carry forth its "proper rain worm" potencies. All of that is possessed by the rain worm. But what is and where is that which has this all? How such a form exists in time with all its richness of forms is not easily explained solely on molecular-mechanical grounds.

At this juncture, we move towards another challenge: an information problem. The multiplicity of a system cannot be increased intrinsically within itself. We cannot simply step from an information that is intrinsically poor into one that is richer. If it appears to have increased, it is an increase that seems to have evolved from itself. This basic and novel principle is limited to living nature. It qualifies the science of "biology" as an independent basic science.

The so-called "holistic materialism" (or "organicism") of early 20th century physiology can be seen as a resolution of this conflict. The position taken by John Scott Haldane (1860-1936) was close to that of Virchow, while contemporaneous physiologists such as Charles Sherrington (1857-1952) and Walter Cannon had subtly different views. In opposition to the mechanistic materialists, they held that the whole was greater than the sum of its parts (i.e. that emergent properties in biology are real and essential for understanding). However, they still believed that the study of the parts rather than of the whole would ensure the advancement of knowledge whereas Haldane favoured the study of the parts as well as the whole.

An approach that includes a study of the interaction of higher levels in a complex system is called a holistic approach. It is in conflict with the attempts of some biologists "to reduce biology to physics and chemistry". However, within the holistic approach, it fits the search for the program (essence). Is it in the *matter* or in a "*carrier*" of the matter? In the latter case, it should require a nonmechanical, entelechy principle. How does entelechy influence matter so that it is arranged just around this spatial natural deterministic principle? The whole series of fascinating questions was beautifully put forward by Driesch. However, he could only speculate about answers. For these answers, we must look elsewhere. It was Alexander Gawrilowich Gurwitsch (with his unique experimental approach) who presented the first evidence for such a natural determinative principle. It was the introduction of the (light) radiation field concept in biology.

Alexander G. Gurwitsch and the field concept

Alexander Gawrilowich Gurwitsch was born in 1874 in the Ukrainian town of Poltava. He graduated from the medical faculty of Munich University and worked for nearly a decade in German and Swiss leading biology centers. Like many others at that time, he was deeply interested in one of the most enigmatic problems in biology, morphogenesis. He wished to understand the mechanism of emerging complex organs as well as entire organisms having unique architecture which

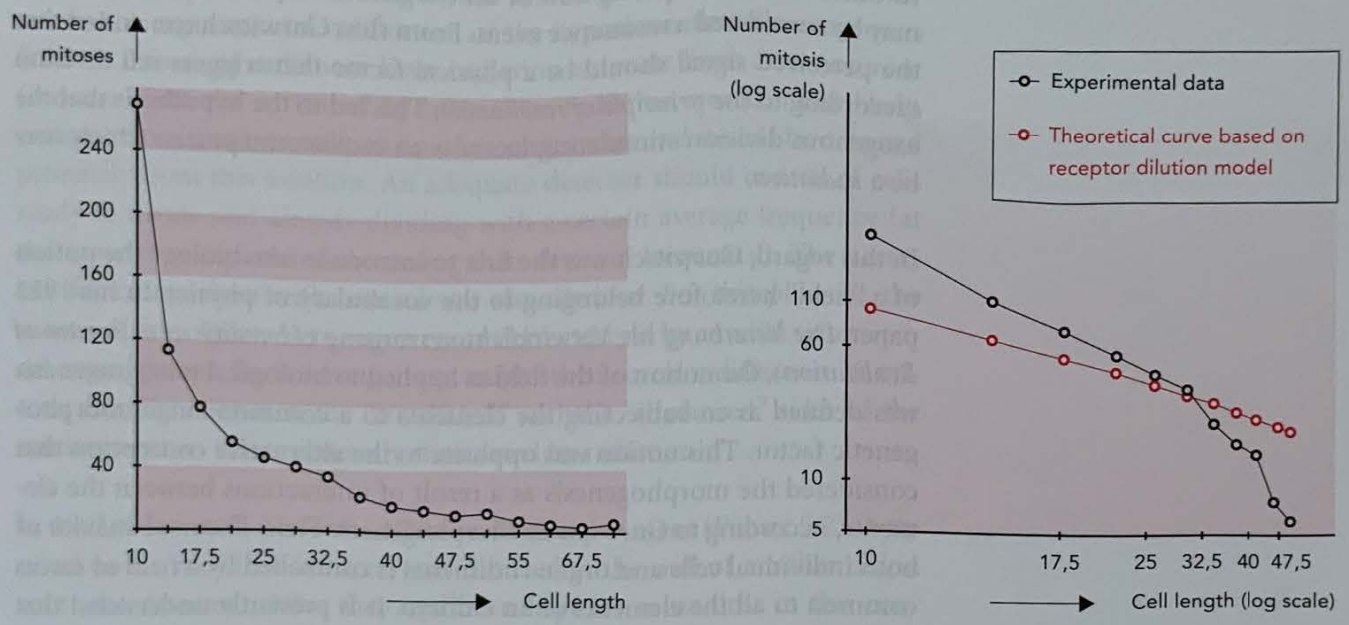
evolved from rather primitively structured embryo cells. He thus supported the significance of mitosis writing "A never-weakened interest in the miracle of caryokinesis was the basis of all my further attemps. The most mysterious of all was for me the sporadic arising of mitoses in meristems". In 1906, Gurwitsch moved to St Petersburg where he became Professor of Histology at Bestoughev Womens College.

Gurwitsch made numerous observations related to the morphological development of a variety of organisms including animals and plants. He was particularly interested in situations that developed into a strict geometrically regular form. His question was whether, generally speaking, such precise form observed at a macroscopic (morphologic) level was due to a similar preciseness such as the spatial distribution of cells constituting a particular morphological contour. In fact, what the field of biology understood was that the spatial distribution must be related to the growth of the cells up to different sizes plus their specific directed mitotic activity and movements (relative velocities, directions and spatial orientation of the moving cells). On this basis, Gurwitsch formulated a distinction between "The Enveloping and the Enveloped" wherein "The Enveloping" is the morphological contour ("the level of the Whole") while "the Enveloped" includes the cells constituting this contour ("the level of the Whole's parts"). Gurwitsch found that the microscopic (histological) picture of the geometrically precise morphological contour demonstrated a rather irregular spatial distribution of the cells at the microscopic level. With these facts in hand, Gurwitsch inferred the rule that in biological reality, "The Enveloping" is much more precise than "The Enveloped".

In the process of embryonic development, the number of cells increase as they divide. Gurwitsch questioned which factor or factors causes cell reproduction. He studied the statistical distribution of mitosis and came to the conclusion that mitosis occurs when two independent events coincide. The first event is the accomplishment of cell maturation. A cell must synthesize all the components necessary for two viable daughter cells before it proceeds into the process of mitosis. This condition depends on the given cell's metabolic activity. Gurwitsch defined it as the "possibility factor" wherein the cell's possibilities must have already matured. But even a mature cell does not enter mitosis unless it is triggered by some external impulse. This external factor is called the "realization factor". The latter must originate from the whole organism to which the cell belongs.

Reflecting upon the nature of the external signal, Gurwitsch noticed that in symplasts or syncytia (in tissue regions containing a mass of cytoplasm having many nuclei but no internal cell boundaries), the nuclei divide rather synchronously in common waves of mitotic divisions. Meanwhile,

Figure 5.1
The type of experimental data suggesting that cell division is stimulated by a resonance event. The number of mitosis (N_m) in an onion root is related to cell length (units arbitrary). In the left panel cell length (X-axes) and N_m (Y-axes) are plotted on linear scales. In the right panel cell length and N_m are plotted on logarithmic scales. In addition, the right panel demonstrates a theoretical curve constructed on the assumption of a model assuming the dilution of receptors with cell elongation. See text.



in tissues consisting of uni-nucleated cells (cells with a single nucleus), the cells always divide asynchronously. In his opinion, that should indicate that a cell membrane (or cell environmental interface) was the structure that would perceive the external signal(s) initiating cell division. In order to define the specific molecular structures of cell surfaces responsible for such a perception, Gurwitsch used the term "receptor" long before it was established in modern molecular biology.

Such study and thinking eventually resulted in the suggestion that a theory of mitosis should be based upon a dual principle. That is, one of the factors which enables a cell capable of division is endogeneous (a "possibility factor"). The other was labelled the "realization factor". This exogenous factor may also evolve within the same organism. In order to obtain more precise information about these receptors properties, Gurwitsch studied the length of onion root cells in relationship to the frequency of cell division (Figure

5.1). He assumed that: 1.) the cell elongation is associated with random insertion of new non-receptive surface particles between the receptors, and 2.) the perception of mitotic signals is proportional to the density of receptors. Such dependence should be inversely proportional and fit a first order hyperbole. However, he found that it was much steeper than a first order hyperbole indicating that "receptors" did not act as independent units. Rather, the data suggested that there existed a kind of holistic (cooperative) "receptor" system, which may have the properties of a resonating contour. In other words, the perception of an exogenous impulse by a cell surface may be considered a resonance event. From this, Gurwitsch concluded that the perceived signal should be a physical factor that triggers cell division (according to the *principle of resonance*). This led to the hypothesis that the exogenous division stimulating factor is an *oscillatorial process* which may be a *radiation.*

In this regard, Gurwitsch was the first to introduce into biology the notion of a "field" heretofore belonging to the vocabulary of physics. In his 1912 paper *Die Vererbung als Verwirklichungsvorgang* (*Heredity as a Process of Realization*), the notion of the field as applied to biological morphogenesis was defined as co-subjecting the elements to a common single morphogenetic factor. This notion was opposite to the alternative conception that considered the morphogenesis as a result of interactions between the elements. According to Gurwitsch's Morphogenetic Field Theory, behavior of both individual cells and organ rudiments is controlled by a field of forces common to all the elements of an embryo. It is presently understood that the field regulates the behavior of individual cells in a developing embryo, routes their movements, controls their divisions and differentiation, and evolves itself with embryo growth.

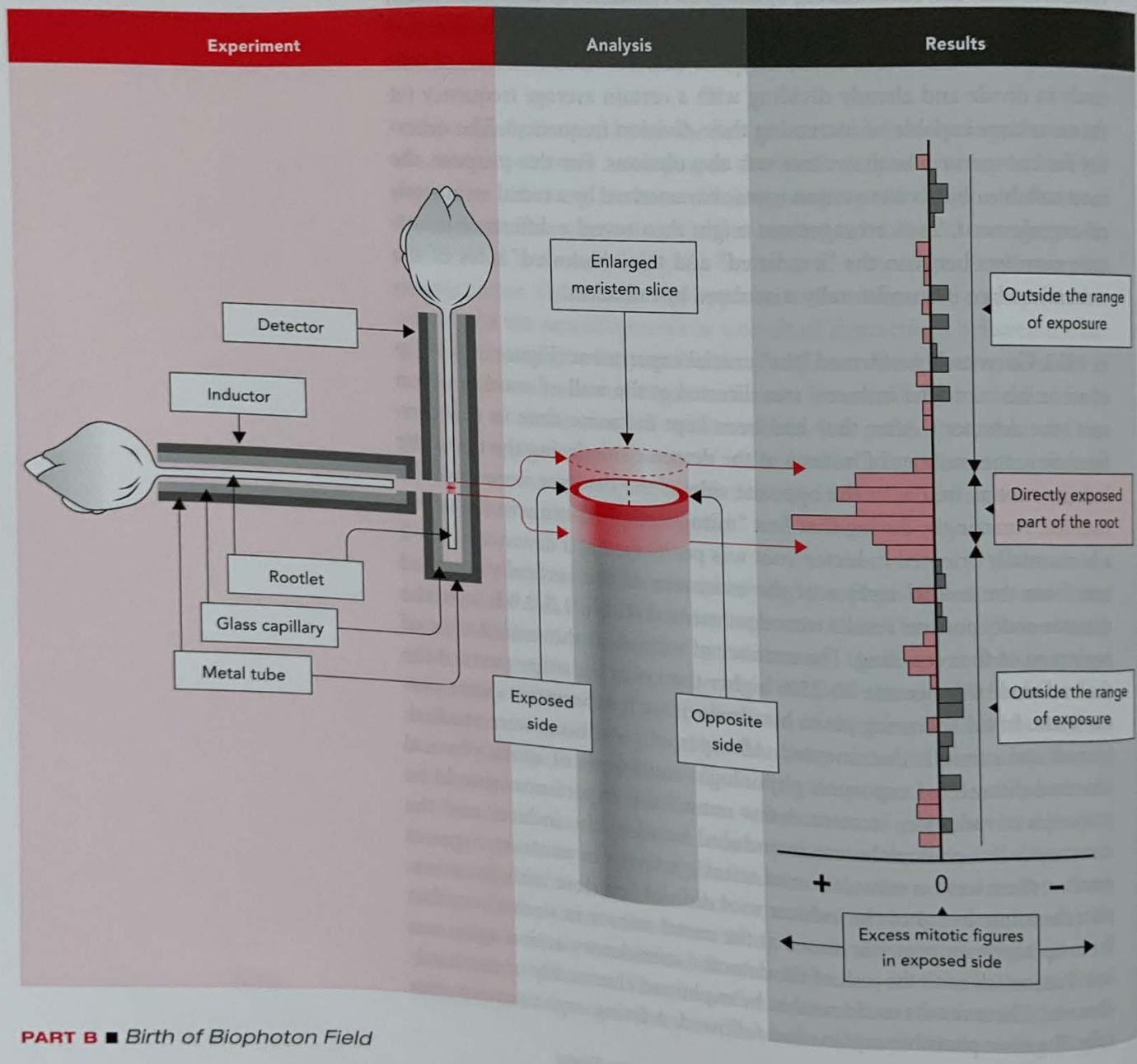
Gurwitsch was cautious not specifying the physical nature and initial source(s) of this field. The Morphogenetic Field Theory explained many facts and, therefore, attracted great interest. However, at the same time, there were a number of opponents of the field theory (primarily because the theory did not keep within the lines of thinking that had become the cornerstone of physiology and biochemistry of the 20th century). Namely, that all biological phenomena (including the process of development of a living organism) result from mere summations of usual chemical processes. Gurwitsch considered that only experimental evidence could persuade his opponents. However, at the institution where he worked, in Petersburg, it was impossible to think of such experimental work. It was just after the Bolshevik revolution when terror, hunger and ruin were reigning. Gurwitsch moved to a newly established Taurida University in Crimea. In 1918, that university admitted its first class of students and was (at that time) the only self-governed free university in the whole of Russia. Outstanding Russian

scientists who did not want to leave their country gathered there. Such prominent figures as the physicists Alexander Loffe and Igor Tamm, the geologist Vladimir Obruchev plus the biologists Michail Zavadovsky and Alexander Lubischev were gathered there. The Chair of Histology of the Medical and Natural Sciences was Alexander G. Gurwitsch.

Gurwitsch emphasized that a conclusion about the radiation field controversy may evolve from the data of an "onion experiment". The choice for such experiment was in no way casual. It was specifically designed to focus on the experimental verification of the hypothesis that the exogenous factor is a form of radiation. Does radiation emanate from an onion root (inducer) into the surrounding space. The radiation would, most likely, eminate from the cone-shaped tip of an onion root and should be detected primarily from this location. An adequate detector should consist of cells ready to divide and already dividing with a certain average frequency (at the same time capable of increasing their division frequency). The necessity for comparison with control was also obvious. For this purpose, the most suitable objects were onion roots characterized by a radial symmetrical arrangement. Such arrangement might then reveal a difference in mitotic numbers between the "irradiated" and the "shadowed" sides of the meristem when it is unilaterally stimulated by radiation.

In 1923, Gurwitsch performed "the" crucial experiment (Figure 5.2). A tip of an onion root (the inducer) was directed at the wall of another onion root (the detector). After they had been kept for some time in this configuration, the number of mitoses at the detector side facing the tip of the inducer root as well as at the opposite side of the detector were carefully counted. Amazingly. during that first "mitogenetic" experiment (in which a horizontally oriented inductor-root was positioned at a distance of 1.5-2 mm from the medial surface of the meristem of the vertically oriented detector-root) positive results were documented within 1.5-2.0 h from the beginning of the recording. The number of mitoses at the medial zone of the "radiated" side became 20-25% higher than over the other parts of the meristem. In the following years, hundreds of such experiments were performed and carefully documented. All types of conditions were studied: time and distance of exposure, physiologic conditions of roots, physical properties of radiation, etcetera. A few remarkable experiences should be mentioned. If a glass plate was introduced between the inducer and the detector, there was no stimulation of mitotic activity. In contrast, a quartz plate shielding the tip of the inducer root did not interfere with its action. If the tip of the inducer was aimed at the metal mirror in such a way that its reflection fell onto the wall of the detector, stimulatory action again was observed. These results could neither be explained chemically or mechanically. The most plausible explanation followed. A living organism can emit

Figure 5.2
The Gurwitsch experiment with onion roots (schematic representation). The left panel ("Experiment") shows an inducer root and a detector root. Each root is fixed in a capillary glass tube that is enclosed by a metal tube. The inducer and the detector roots are separated by a glass or quartz window. The middle panel ("Analysis") shows enlarged the slice of a detector root with its "exposed" and "opposite" side. The number of mitotic figures in successive 10 µm thick cross sections is counted for the exposed side and opposite sides. The right panel ("Results") shows the difference in mitotic numbers between the exposed side and the corresponding opposite side of the root. The symbol "+" indicates that the exposed side has more mitotic figures compared to the opposite side. The symbol "–" indicates that the exposed side has less mitotic figures compared to the opposite side. The directly exposed part of the root shows highest difference.



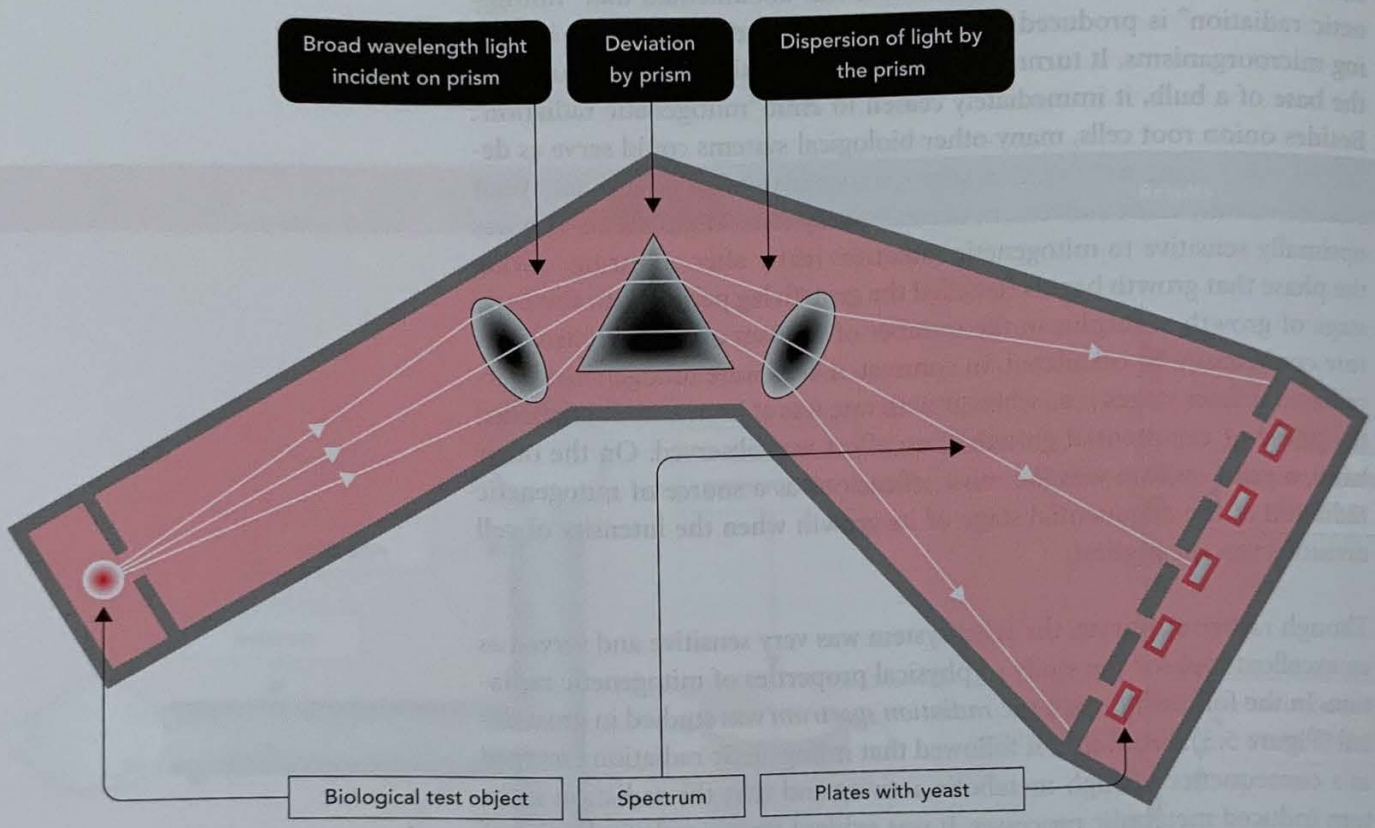
photons that stimulate cell divisions. That is why photon emission from a tip of the root stimulating mitosis in another root was named "mitogenetic radiation". However, these photons belong to the ultraviolet region of the electromagnetic spectrum wherein quartz (but not glass) will transmit.

In 1924, Gurwitsch became the Chair of the Histology Department at the Medical Faculty of Moscow State University. There, the investigations of this new phenomenon were continued. It was documented that "mitogenetic radiation" is produced by various animal and plant tissues including microorganisms. It turned out that when an onion root was cut off at the base of a bulb, it immediately ceased to emit "mitogenetic radiation". Besides onion root cells, many other biological systems could serve as detectors. After comparing many systems, it turned out that an ordinary yeast culture was the most convenient *detector* testsystem. The yeast culture was optimally sensitive to mitogenetic radiation [early after culturing, during the phase that growth begins (labelled the growth lag period)]. In this early stage of growth, a surplus in the number of mitoses over the control culture could easily be calculated. In contrast, if cells were mitogenetically irradiated in later stages [i.e., when growth rate was at its maximum (labelled the phase of exponential growth)], no effect was observed. On the other hand, a yeast culture was the most efficacious as a source of mitogenetic radiation at the exponential stage of its growth when the intensity of cell divisions was the highest.

Though rather elaborate, the yeast system was very sensitive and served as an excellent tool for the study of physical properties of mitogenetic radiation. In the following years, the *radiation spectrum* was studied in great detail (Figure 5.3). From this, it followed that mitogenetic radiation emerged as a consequence of high metabolic activity and that the radiation in its turn induced metabolic processes. It was evident from a technical point of view, that investigation of mitogenetic radiation required an understanding of the physiology of both biological detectors and emitters of such radiation. Unfortunately, many of the studies which refuted the existence of mitogenetic radiation were methodologically ignorant.

Particularly important observations were made by Gurwitsch's student, Gleb Frank. Frank made the analysis of the color spectrum of mitogenetic radiation from different biological sources using plates with yeast cultures as detectors. He later became a Member of the USSR Academy of Sciences and directed the Institute of Biophysics of the USSR Academy of Sciences. The analysis of the spectrum appeared to be a crucial step in the studies of chemical and metabolic reactions related to the different aspects of mitogenetic radiation.

Figure 5.3
Spectral analysis of mitogenetic radiation in the Gurwitsch' type of experiment. The principle set up includes 1.) a biological object for estimating the spectrum of its radiation, 2.) a quartz prima for deviating the radiation and producing a spectrum, and 3.) the biological detector yeast.



Mitogenetic radiation is a general phenomenon

In previous chapters, we have encountered the role of the nerves in controlling the differentiated state in case of injury, playing a role in the "program" which instructs regeneration (Chapter 2), and in the process of tumor formation (Chapter 4). In this regard the studies by the Gurwitsch team regarding mitogenetic analysis of nerves are of interest. This work started around 1931 and was carried out for 6 years. Eleven collaborators as well as laboratory guests took part in the work. The analysis of the data was published in the monograph entitled *Mitogenetic Analysis of the Excitation*

of the Nervous System (1937). However, at that time, the results did not fit into the limits of the doctrines of the classical physiology of the nervous system and, therefore, were not accepted. The monograph was, according to Gurwitsch, the result of more than 5000 experiments. The research methodology utilized yeast cultures.

The yeast method is based on the principle of a "mitogenetic effect", [i.e. regarding the stimulating action of the ultraviolet rays (within limits approximately between 190-250 nm) on cell multiplication]. The procedure also includes spectral analysis which was one of the most important methods used for the study of mitogenetic phenomena. For this purpose the above group of scientists had at their disposal two quartz spectrographs. The source of radiation (in this case it was the nerve preparation) was placed as near to the slit of the collimator as possible, generally using a slit of nearly 1 mm. The detector (i.e., the yeast culture) was placed exactly in front of the monochromatic slit.

The experiments to estimate mitogenetic radiation focused first on the nerve that was at rest and subsequently on the nerve that was excited. The excitation was electrical or utilizing a mechanical approach. Of the first category, 41 of 45 experiments were positive. Within the latter category, 27 of 30 were successful.

The researchers expected that the mitogenetic radiation would be concomitant with metabolic processes. They further expected that it would appear during the excitation as well as during the state of rest of the nerve (but in a diminished intensity). They observed that the sciatic nerve behaves in just such a manner (that is in a quasi-ideal state of rest a mitogenetic radiation was detectable). In a state of excitation, the mitogenetic radiation was increased by the artificial stimulation (which was described as "adequate"). An "adequate" stimulation focused on the result (which in the case of the sciatic nerve, was the contraction of the muscle). Other "adequate" (strictly physiological), sciatic excitations arose via a spinal reflex and for the optic nerve by illumination of the eye.

To briefly summarize the main conclusions, it can be stated that mitogenetic radiation in nerve was first discovered by Vassiliev, Goldenberg and Frank. Outside of Gurwitsch's laboratory, a radiation from the brain cortex and the sciatic nerve of a rabbit was discovered by Marinesco and his collaborators. They also utilized the yeast method as a detector, publishing their data at the *First Congress of Electroradiobiology* in Venice (1934). In 1935 the radiation in nerve was confirmed by two French scientists, Audubert and Levy, who used purely physical photon counting methods for their work (Figure 5.4). More details in Chapter 6.

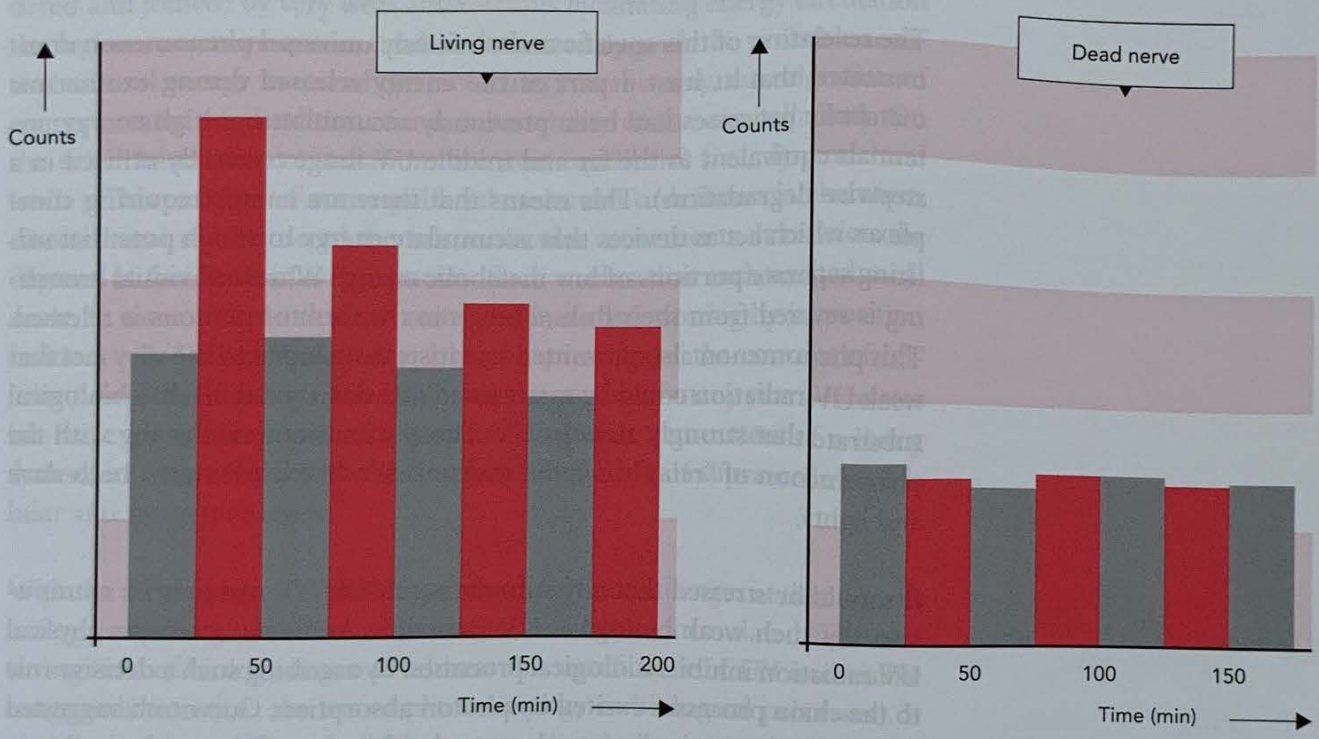
The origin of the radiation was frequently discussed. The researchers expected that only emission emanating from the surface of the nerve would have an induction effect on a detector. They considered it unreasonable to expect that the "feeble" ultraviolet radiation, emitted by deep nerve fibers, would almost not be entirely absorbed by the superficial fibers. The researchers advanced the hypothesis that the neurofibrils were both the site of origin as well as the conduction of the mitogenetic emission. However, they also proposed that the radiation that affects the (biological) detectors may be a partial secondary radiation. The most interesting situation was that of traumatic stimulation. Kalendaroff demonstrated that each scissor cut of the nerve resulted in the production of a different radiation from the nerve (judged by the character of the spectrum) which remained for at least two minutes.

The other problem that was approached by the Gurwitsch team focused on the question whether the nervous elements that produce the emissions were at the same time influenced by them. In their studies of the mitogenetic radiation of the central nervous system (CNS) of the frog and rabbit they observed that in the state of quasi-rest, the radiation was extremely low and that the slightest general excitement of an animal was accompanied by emission. In comparing models during this part of the research, the system most easily adaptable to experimentation was the visual system. This served as a basis for the research of Anna Gurwitsch. Generally she did the work on isolated heads of frogs. However, many control experiments were also carried out on other living specimens.

Numerous experiments on all parts of the optic system documented that the radiation was absent when the eyes were not exposed to light. However, after illumination of the eye, the mitogenetic radiation was present not only at the optic nerve but also at the optic lobes, the hemispheres and the medulla oblongata. The intensity of the radiation, as judged solely by the time of exposure, was of the same magnitude for all parts of the physiologic system(s) involved. The reaction of the cerebral visual tract to the illumination of the eye was also studied within Gurwitsch's own laboratory by Marinesco and his collaborators (Bucharest), as well as that of Braines.

These observations implicate many new phenomena. Such might eventually change the biological view, but not without initial considerable skepticism from the scientific world. To illustrate the skepticism after the appearance of the first series of papers dealing with the nerve radiation, it is of interest to know the somewhat ironical note of the great scientist Hill in *Nature* (April 8, 1933, pp. 507-508) wherein he wrote: "If it be true that excited nerve gives out a characteristic radiation... then indeed a new day has dawned in the very difficult problem of the physical nature of nerve ac-

Figure 5.4
Early result of registration of radiation from the excited nerve of a frog leg using a modified Geiger-Müller counter. The photocathode is sensitive in the UV range of 215-240 nm. The left panel shows the counts of the nerve both at rest and an excited condition. The right panel exhibits the counts of a dead nerve destroyed with alcohol. The dead nerve has lower counts than both the living nerve at rest and during excitation. Excitation of living nerve reveals stimulation by counts. In contrast dead nerve does not react on excitation. The "Rest" and "Excitation" of the nerve is indicated by colors grey and red respectively.



tivity". "What is to be done now, after a confirmation by physical methods of the French authors?"

More about biological radiation: the role of reactions underlying metabolic chain processes

Around the mid-1930's, the formation and recombination of short-lived free radicals within enzymatic reactions became known. It was established that such reactions correspond with high energy. Those reactions rarely occur. Hence, they do not influence the overall balance of cells. On the other hand, the high energy is sufficient to generate UV radiation of a very low

intensity. Studies discovered that atmospheric oxygen is necessary in order for the mitogenetic radiation to have its effect during the enzymatic processes. During the initial years of investigation, it also became evident that all of the events connected with mitogenetic radiation were based upon *chain reactions* in which light and dark high-energy reactions are linked and combined. Such high-energy could be transferred and stored in other compounds or, instead, may be transformed into radiation in subsequent reactions. It was these types of processes that led to the widespread understanding of "secondary radiation".

The revelation of this specific and obviously universal phenomenon demonstrated that at least a part of the energy released during exothermic metabolic processes had been previously accumulated at high energy potentials equivalent to the far and middle UV range (typically utilized in a stepwise degradation). This means that there are energy requiring complexes which act as devices that accumulate energy to a high potential utilizing separate portions of low metabolic energy. When individual branching is severed from their links, energy, in the form of photons is released. This phenomenon also permitted scientists to understand the very fact that weak UV radiation could be maintained and distributed inside a biological substrate that strongly absorbs UV. Those scientists were dealing with the phenomenon of "relay" (i.e., the transmission of excited states, both dark and light).

It should be stressed that any stimulating role of UV can only be manipulated by such weak biological UV radiation. As a rule, intensive physical UV radiation inhibits biological processes. By ascribing such a decisive role to the chain processes excited by photon absorption, Gurwitsch suggested that the "organization" of a substrate should create the special conditions that regulate the use of the absorbed energy. This led to the idea that photon absorption and emission is part and parcel of organization. Slowly, it became clear how important this knowledge was to better understand the biological phenomenon.

From relay to organization

The primary action of the absorbed photons was obviously considered. The photons are a very important trigger activating (as described above) as well as participating in chain processes. Such processes simultaneously maintain specific conditions throughout a biological system. Therefore, the main biological problem was (and still is) to elucidate the conditions that promote the specific "organization" of the cellular processes (arising as secondary or tertiary links in response to the absorption of single photons). Obviously, a necessary condition for the development of chain processes is a certain degree of molecular "orderliness". The concept of orderliness

added a completely novel aspect to the understanding of cell organization. Within classical biological concepts, the term "orderliness" was no more than an unnecessary synonym for "structure". However, mitogenetic radiation led to a novel concept of cell organization.

Another major development in biology was the eventual understanding that single molecules are important in that they form molecular complexes or, even better, molecular "constellations" with a very specific purpose. Associations of molecules are brought together (and to some extent ordered and joined) by very weak interactions facilitating energy circulation throughout an entire system (and, simultaneously, with the probable accumulation of energy in one of the molecules). In this way, the migration of energy creates the conditions for a metastability of the constellations. On the other hand, the constellations are non-equilibrial (i.e., dynamic and labile). This creation of non-equilibrial molecular orderliness is, therefore, directed against disordered thermic molecular movements. It restricts the degrees of freedom in living systems. Such means that each excited molecule, to some extent at least, is restricted in its functioning by its position inside a cell. This formulation is a brief expression of the concept of cellular *fields* elaborated by Gurwitsch over the course of many years. It relates his basic ideas that constant regulation of spatial parameters of a living system are intertwined with the coupling interaction of supra-cellular, cellular and molecular levels.

With the accumulation of the above new data, the challenges at that time in biology requiring further elucidation became better formulated. The maturing field of biology began to address the significance of UV and visible range radiation (i.e., the use of photon energy in biological processes). It also studied how UV and visible range radiation functioned as a subtle indicator of those molecular processes related to the different functional states of living matter. Such a division is arbitrary to some extent since there is now more and more evidence that photons of different energetic levels participate in practically all biological processes.

Mitogenetic biology reached its zenith by the middle of 1930's. From 1923 on and into the 1930's, hundreds of papers and a dozen comprehensive reviews on mitogenetic radiation appeared. However, by the beginning of the 1940's, the generally positive world-wide attitude to mitogenesis was ousted with indifference and even hostility. In 1943, Gurwitsch noted in an article devoted to the 20th anniversary of mitogenetic radiation discovery: "Many observers from different countries who did not dare even to try a single experiment and who are hardly acquainted with the current literature regarding the mitogenetic radiation phenomenon, claimed that the number of published research papers with negative results steadily grew

while the number of confirming papers declined. The discrepancy of such statements with the real state of things is so striking, that if similar statements were made on ordinary, rather than scientific questions, those who make them had to answer according to the law. One should just turn to the recent review by Maxia (Italy) to realize that hundreds of papers with data confirming mitogenetic radiation effects are opposed by less than a couple of dozens of reports with negative results."

WW II destroyed all the European research centers where mitogenetic radiation was studied. Independent research regarding this issue came to a halt. In 1945, Gurwitsch founded and chaired the Moscow Institute of Experimental Biology of the Academy of Medical Sciences of the USSR. In a short period of time, he and his associates managed to document much new data. But this activity was soon interrupted. In 1948, when Lysenko regained full power in Soviet biology, Gurwitsch was dismissed. After Alexander Gawriliwich Gurwitsch passed away in 1954, the unexplained answers to questions about mitogenetic radiation were forgotten for decades and all of his discoveries fell into question.

Conclusion

Nature's "program" that delineates the biological organization could not be explained solely on molecular-mechanical grounds. Instead, it required an additional non-mechanical, spatial natural deterministic principle. Such principle was first presented by Gurwitsch, who introduced, at the same time, the field concept in biology.

The idea that the field directed biological morphogenesis was defined as co-subjecting the elements to a common single morphogenetic factor. However, according to Gurwitsch's Morphogenetic Field Theory, behavior of both individual cells and organ rudiments is controlled by a field of forces common to all the elements of an organism from the embryo to the adult. This notion was opposed to the alternative conception that considered the morphogenesis as a result of interactions between the elements. Instead it was understood that the field controls the divisions and differentiation of cells and evolves with embryo growth.

The field was revealed as mitogenetic radiation. It was a general phenomenon in living nature. The events connected with this radiation were based upon *chain reactions* in which light and dark high-energy reactions are linked and combined suggesting that photon absorption and emission is part and parcel of organization. This view automatically led to many interesting questions. Does this organizational conception have a direct link to the balance and switch between differentiated and undifferentiated states (and their corresponding field states)? Does Nature's "program" have a link

to a photon field? Initially, it was necessary to demonstrate light radiation objectively. The search for physical detectors began.

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6

Biology Looks at Physics

After years of effort with biological detectors, Gurwitsch and co-workers found many effects. The news about the apparent documentation of biological radiation spread very quickly both throughout the scientific and public circles. A large number of investigators, among them physiologists, microbiologists, medical scientists, and physicists in Russia, Germany, France, Italy, and other countries tested the effects discovered by Gurwitsch and further studied his hypothesis. This fascinating period is sometimes called the Golden Age of Mitogenetic Rays which brought many hundreds of papers to scientific journals and books. This early mitogenetic work was described in detail in the mid and late 1930's in reviews by Bateman (1935) as well as by Hollaender & Claus (1937). Gurwitsch's work was supported by many Russian and several Western scientists. To name a few of the Western researchers, one can refer to the 1930 study of Borodin, the 1932 study of Wolff & Ras, and the 1936 study of Rahn. But many others in the same period [Hollaender & Schoeffel (1931), Richards & Taylor (1932) and Bateman (1935)] were unable to detect any mitogenetic effect. One problem at that time was that most of the publications that contained much valuable data were written in Russian and were hence almost inaccessible to the Western scientific community. This led to a statement by Gurwitsch that in several cases the appropriate biological conditions for testing were not utilized. The contradictory outcomes resulted in louder and louder cries for the greater use of physical detectors. Finally, the scientific world focused on investigations only utilizing such detectors.

At that time, what approaches were possible? Progress in research regarding mitogenetic radiation was clearly hampered by the lack of objective read-out instruments that would provide sufficient sensitivity. Both sensitivity and objectivity of instruments were crucial. The problem then was that one did not know what sensitivity for any particular biological issue was required. This chapter will follow the search for instruments that could objectively measure radiation in the UV and visible parts of the spectrum and how they were eventually applied to test biological radiation.

The photoelectric effect

In the late 19th and early 20th centuries, the photoelectric effect journey continued. That effect was first observed by Heinrich Hertz in 1887. Since then, the phenomenon has also been known as the "Hertz effect". Hertz

MORE ABOUT...

BOX 6.1

■ PHOTONS AND THEIR INTERACTION WITH MATTER

The photoelectric effect is one interaction mechanism between photons and atoms. Depending on the energies carried by photons, the effects of photons on matter vary wildly.

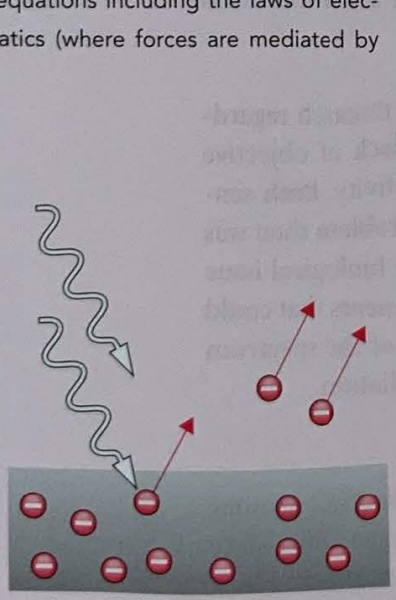
A summary of these effects is given in the following:

- Low-energy phenomena (of eV range: visible light from approximately 400 to 800nm): photoelectric effect.
- Mid-energy phenomena (keV range): the Compton effect dominates wherein electrons are simply kicked with an energy so great that the atomic binding of them is negligible.
- High-energy phenomena (MeV and higher): photons carry enough energy to make electron-positron (e^+ e^-) pairs (provided that there is an atomic nucleus against which to recoil).

Figure B.6.1.1 illustrates the Feynman diagram of interaction of a photon with a charged particle.

Photons actually hit electrons as the incoming and outgoing moments of the electrons indicate. The photons actually do not necessarily always need to be photons. By allowing them to travel slower than the speed of light, such diagrams actually contain the full Maxwell equations including the laws of electrostatics and magnetostatics (where forces are mediated by such "virtual" photons).

Figure B.6.1.1
Interaction of a photon with a charged particle.



made use of a spark gap. This consisted of an arrangement of two conducting electrodes separated by a gap. The gap is usually filled with a gas such as air designed to allow an electric spark to pass between the conductors. Such a spark forms when the voltage difference between the conductors exceeds the gap's breakdown voltage. When the spark forms and the gas is ionized, these coupled phenomena drastically reduce the electrical resistance. Then, an electric current (sparks) flows until either the path of ionized gas is broken or the current decreases below a minimum value (the "holding current"). This usually happens when the voltage drops. The electrons are emitted from metals and non-metallic solids, liquids or gases.

To observe such sparks, different conditions had to be met. Typically, placing the apparatus in a darkened box made it easier to observe the spark. Hertz noticed, however, that although the spark itself became more visible, the length of the spark was reduced. In fact, the formation of the spark was enhanced as a consequence of the absorption of very short electromagnetic energy radiation such as visible or ultraviolet light. A glass panel placed between the source of electromagnetic wave radiation and the receiver absorbed ultraviolet radiation that assisted the electrons in jumping across the gap. When the glass panel was removed, the spark length increased. He observed no decrease in spark length when he substituted quartz for glass. Quartz does not absorb UV radiation. Hertz concluded that the electrodes (illuminated with ultraviolet light) created the electric sparks more easily. Thus, electrons emitted in this manner may be referred to as "photoelectrons". Hertz concluded his months of investigation and published these observations in the journal, *Annalen der Physik* 1887. Subsequently, he no longer pursued this investigation.

Shortly thereafter, the scholar Aleksander Stoletov made a detailed analysis of the photo-effect. He utilized a new experimental setup and discovered that the light-induced photoelectric current was directly proportional to the intensity of light. This became the first law of photo-effect (Stoletov's Law). In the period

from 1888 to 1891, Stoletov made several more discoveries. One was the dependence of the intensity of the electric photo current on the gas pressure. He found that an optimal gas pressure corresponded to a maximum photocurrent. His results were published in 6 works. Four of them were inluded in the *Comptes Rendus* of 1888 and 1889 and the others in 1890 in the *Journal de Physique* and in 1892 in *Physikalische Revue.*

The light emitted by a spark does not come from the current of electrons itself, but rather, from the material of the medium. Without the intervening matter capable of electromagnetic transitions, the spark would be invisible. It is impossible for a visible spark to form in a vacuum. The medium fluoresces in response to collisions from the electrons. When electrons in the gap collide with molecules of air, they excite their orbital electrons to higher energy levels. When they fall back to their original energy levels, they emit the energy as light.

The discovery of the ionization of gases by ultraviolet light was made by Philipp Lenard in 1900. He observed that the energy of individual emitted electrons increased with the frequency of the light (which is related to the color). In 1902, he published these important findings under the title, *Über die lichtelektrische Wirkung.*

Maxwell's classical theory on electromagnetism

The above data were, at that time, not only interesting, but also appeared to be at odds with the previous wave theory of light that was developed by James Clerk Maxwell. Maxwell's theory was thought to predict that the electron energy would be proportional to the intensity (and not the wavelength) of the radiation. James Clerk Maxwell (1831-1879) was a Scottish physicist and mathematician who developed the so called "four" equations. These equations formed the basis of the classical electromagnetic theory. As a student he spent much time experimenting with light and electricity (working on Faraday's ideas about electricity and electromagnetism). As a professor at London's King's College (1860-1865), he continued his research and then published his work on electromagnetism. His work was extremely important and considered equal to that of Isaac Newton and Albert Einstein. In 1871 he became the first Cavendish professor in experimental physics at the University of Cambridge. The completely new laboratory, the Cavendish, was officially started in 1874.

The primary "Maxwell equation" articles regarding electricity and magnetism began as early as 1855-1856 when his paper *On Faraday's Lines of Force* was presented to the Cambridge Philosophical Society. The paper presented a simplified model how electricity and magnetism were related. He also reduced all of the current knowledge into a linked set of differ-

ential equations, a work that was published as *On Physical Lines of Force* in 1861. Close to 1862, Maxwell calculated that the speed of propagation of an electromagnetic field is approximately that of the speed of light. He considered this to be more than just a coincidence. As he continued his work on this challenge, he demonstrated that his equations actually predicted the existence of waves of oscillating electric and magnetic fields that travel through empty space at a speed that could be predicted from simple electrical experiments using the data available at the time. He calculated a velocity of 310,740,000 m/s. In his 1864-1865 paper, *A Dynamical Theory of the Electromagnetic Field*, he wrote, "The agreement of the results seems to show that light and magnetism are affections of the same substance, and that light is an electromagnetic disturbance propagated through the field according to electromagnetic laws".

In another major research endeavor, Maxwell investigated the kinetics of gases. In 1866, he statistically formulated (independent of Ludwig Boltzmann) the Maxwell-Boltzmann kinetic theory of gases. His formula (The Maxwell Distribution) gave the fraction of gas molecules moving at a specified velocity at any given temperature. In 1871, he established the Maxwell's Thermodynamic Relations. Finally, his famous equations appeared in fully developed form in his textbook, *A Treatise on Electricity and Magnetism*. Maxwell also introduced the concept of the electromagnetic field in comparison to force lines that Faraday had discovered. At that time, Maxwell believed that the propagation of light required a medium for the waves (dubbed the "luminiferous aether"). However, over time, the existence of such a medium, permeating all space and yet apparently undetectable by mechanical means, proved more and more difficult to reconcile with experimental data.

Albert Einstein's photoelectric effect

These difficulties inspired Albert Einstein to formulate a theory eliminating the requirement for a luminiferous aether. In 1905, Einstein solved this apparent paradox regarding Lenard's frequency dependency on light-induced electrons plus the Maxwell theory predicting proportionality to the intensity. In one of his 1905 papers, Einstein described, in mathematical terms, how the photoelectric effect was caused by absorption of a quanta of light (presently called photons). This paper, entitled, *On a Heuristic Viewpoint Concerning the Production and Transformation of Light*, explained the features of the phenomenon and the characteristic frequency.

Einstein's idea was based upon Max Planck's theory of black-body radiation. Max Planck published his theory in the paper, *Über das Gesetz der Energieverteilung im Normalspectrum* (*On the Law of Distribution of Energy in the Normal Spectrum*) published in the 1901 *Annalen der Physik*. It as-

sumed that Hertzian oscillators could only exist at energies E proportional to the frequency of the oscillator by $E = h\upsilon$ (where h is Planck's constant). By assuming that light actually consisted of discrete energy packets, Einstein wrote an equation for the photoelectric effect that agreed with experimental results. He theorized that the energy in each quantum of light was equal to the frequency multiplied by a constant (later called the Planck's constant). A photon above a threshold frequency has the required energy to eject a single electron creating the observed photoelectric effect.

Einstein's theory explained why the energy of photoelectrons was dependent only on the frequency of the incident light and not on its intensity. A low intensity, high frequency source could supply a few high-energy photons, whereas a high intensity, low frequency source would supply no photons of sufficient individual energy to dislodge any electrons. This discovery led to the quantum revolution in physics and earned Einstein the Nobel Prize in Physics in 1921.

The use of a photoelectric detector in biological UV emission. It became more and more clear (even to other disciplines such as biology) that the photoelectric principle may offer a perspective to solve the discrepancy about biological radiation. As mentioned previously, biological radiation was estimated in the same way that Hertz concluded about photoelectrons. Both concluded, by substituting quartz for glass, that it was ultraviolet light that created the "photoelectrons". In biological organisms, in similar fashion, ultraviolet radiation may be detectable. In both Germany and France, groups began to investigate the radiation of biological samples using this photoelectric method to detect the radiation of biological materials. In the1930's, five studies on the use of photoelectric effects were executed (Figure 6.1). Due to the properties of the device, the detection of the radiation took place in the region of 230-240nm.

In Germany, F. Seyfert focused on the detection of radiation from the onion, *Allium cepa*, the most widely cultivated species of the genus *Allium*. It is of interest that this model was also used by Gurwitrsch in his first biological model. The data were published in 1932 in the *Jahrbuch für Wissenschaftliche Botanik*. The study did not give data that presented statistical evidence. The same technique was used in two French studies researched by R. Audubert & R. Levy. In one study, the eggs of the Corsican painted frog (Discoglossus) were used. In the other study, these authors studied nerves under excitation. Both studies were published in 1935. However, they missed the calculations that would have made those studies statistically convincing. Two more studies were carried out in 1937 in Japan and America. The Japanese study, led by Odani was focused on tumors. The American study by Hollaender and Claus utilized bacteria. Neither

Figure 6.1
Photoelectric detectors in biological UV radiation.
The studies were performed without proper statistical analysis.

Year	Author(s)	Object	Statistics
1932	Seyfert	Onion roots	No
1935	Audubert & Levi	Frog eggs	No
1935	Levi & Audubert	Nerves	No
1937	Odani	Tumors	No
1937	Hollaender & Claus	Bacteria	No

study could present any quantitative proof for the existence of biological radiation.

Overall, no scientist guided by the photoelectric principle utilizing photoelectric detectors was able to statistically document ultraviolet radiation from the rapidly dividing cells. Such outcomes drastically reduced enthusiasm to continue studies on Gurwitsch radiation. However, the apparent discrepancy between the outcomes of the two scientific approaches with biological and physical detectors could still be due to the sensitivity of the two types of detectors.

The entrance of the Geiger-Müller counter

A few people still continued to look for more sensitive radiation detection procedures. In fact, another device with improved capacity to detect radiation was being developed. Let us first look at this development and what actual improvements were made. The Geiger counter is a type of particle detector. It is commonly associated with the detection of nuclear radiation emission: alpha particles, beta particles or gamma rays. It is named after Johannes Wilhelm Geiger (1882-1945), a German physicist. In 1902, Geiger began to study physics and mathematics at the University of Erlangen receiving his doctorate in 1906. In 1907, Geiger began work with Ernest Rutherford at the University of Manchester. In 1908, they together developed the Geiger counter and published under the title, *An Electrical Method of Counting the Number of Alpha (α) Particles from Radioactive Substances.* In the beginning, this counter was only capable of detecting alpha particles but, in time, was continuously improved.

One such improvement was published by H. Geiger in 1913 under the title, *Über eine einfache Methode zur Zählung von α- und β-Strahlen* (*On a Simple Method for Counting α- and β-Rays*). The instrument was utilized in experiments that led to Rutherford's atomic model. A further improvement was created by Geiger and his Ph.D student, Walther Müller. At that time Geiger was a professor in Kiel. They published in 1928 on this improved version, the Geiger-Müller counter, in the article *Elektronenzählrohr zur Messung schwächster Aktivitäten* (*Electron Counting Tube for the Measurement of the Weakest Radioactivities*). With the improved counter more types of ionizing radiation could be detected.

The principle of operation of a Geiger-Müller counter (G-M counter) is based on a wire which is stretched along the axis of a hollow metal cylinder that is enclosed in a glass tube (Figure 6.2). Both are enclosed in a tube which is filled with a suitable gas, usually helium, neon or argon (with halogens added) at low atmospheric pressure. A negative voltage is applied to the cylinder-electrode (cathode). The wire (anode) is connected through a small condenser to a vacuum tube amplifier and then through a high resistance to ground. The value of the voltage is adjusted so that it is nearly enough to cause a brush discharge between the electrodes. When in this sensitive state, any radiation passing through the tube and liberating

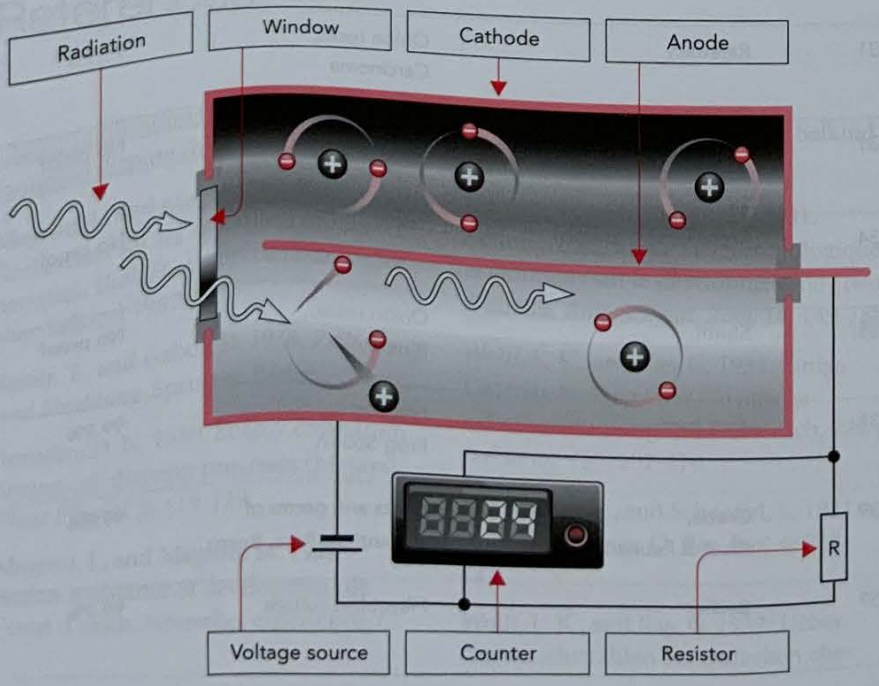


Figure 6.2
The principle of a Geiger-Müller counter. It is based on a wire (anode) along the axis of a hollow metal cylinder (cathode). The voltage is adapted to nearly cause a brush discharge between the electrodes. Any radiation liberating ions initiates a small discharge and outputs a current pulse. The pulse is displayed by a counter.

one or more ions initiates a small gas discharge between the electrodes. The tube amplifies this conduction by a cascade effect and outputs a current pulse. This pulse is then displayed by a needle or lamp and/or audible clicks. The counters used to detect ionizing radiation were of three general types: 1.) gamma-ray counters, suitable for measuring gamma rays, x-rays, and cosmic rays; 2.) photoelectric quantum counters which combine the characteristics of photocells and counters and were used for measuring weak ultraviolet light; and 3.) particle counters for measurements of α and β. The Geiger-Müller counters have been, since their invention, adapted to a large variety of uses in physical laboratories and have become one of the physicist's most powerful tools for measuring weak radiations.

It is evident that the photoelectric quantum counters offered better opportunities to objectively detect the radiation that was introduced by Gurwitsch and co-workers. It required technical adaptations and led to counters consisting of a counting tube, an amplifier (usually with radio

Figure 6.3
Geiger-Müller counter in biological UV radiation. Some studies suggested the emanation of UV radiation from biological studies based upon statistical analysis. The sensitivity of these counters is rather low. The counts/photon ratio ranged between 10^{-2} and 10^{-5}.

1930	Schreiber & Friedrich	Yeast	No proof
1931	Rajewsky	Onion roots, Carcinoma	99.9%
1931	Frank & Rodinow	Frog heart, Frog muscle	No proof
1934	Kreuchen & Bateman	Yeast	No proof
1935	Sturm	Onion roots, Blood	No proof
1938	Audubert	Frog nerve, Frog Spawn	99.9%
1939	Grebe, Krost & Peukert	Roots and germs of *Helianthus*, Peas, Beans	99.9%
1957	Becher	Fibroblast culture	99.9%

tubes), a recording device for registering the number of discharges of the counter, and a suitable source of (negative) high voltage for the counting tube.

Its application in biology research was initiated in the late 1930's. At least eight studies were carried out during those years plus another study published in 1957. As an illustration, Figure 6.3 presents an overview how it was applied in biology research to investigate the Gurwitsch radiation. This overview also summarizes the outcome of the different studies.

Some authors did eventually prove the existence of ultraviolet radiation emanating from biological tissues. Others were unable to find such an effect even though they worked with equipment of similar sensitivity.

Conclusion

The search for instruments that could objectively measure light radiation in the UV and visible parts of the spectrum resulted in the late 1920's in the Geiger-Müller counter. Its use in biology resulted (for the first time) in evidence for the Gurwitsch radiation. These types of counters stood at the beginning of technical development that eventually led to the recent presence of photomultiplier equipment. However, simultaneously, biological interests continued to decrease as World War II destroyed essential parts of the biological research data.

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7

The Entrance of the Photomultiplier

The primary critical response in the mid 1930's came from Gray and Quellet (1933), Lorenz (1934), as well as Hollaender and Claus (1937), all playing a fatal role by smearing the possible existence of biological radiation. This seems amazing since the Lorenz article was critiqued scientifically as unimportant. A number of other scientists, also working in this field (not only Gurwitsch and others on his team) publically and frequently addressed obvious experimental errors uncamouflaged in the team's work. Among them was Rahn (USA). The issue was that yeast cells, at the stage being utilized, were not sensitive to external photons. However, such criticism was ignored by the scientific community.

Even though there were some positive research outcomes using physical detectors to actually document radiation from biological organisms, the data, in general, were variable, thus strengthening the scientific rejection of the data.

However, we will discover that, notwithstanding all of these scientific and political developments, interest in photon emission research of biological organisms finally returned after the World War in the 1950's and 1960's. This revival can be traced back to the invention of the photomultiplier tube which provided an enormous increase in sensitivity. That invention was associated with continuing developments in physics and concomitant technical developments that can be traced back to a combination of the science of the photoelectric effect as well as that of secondary emission.

As mentioned in a previous chapter, the photoelectric effect has perhaps been most associated with Albert Einstein. The phenomenon of secondary emission was first limited to purely electronic inventions (i.e., those lacking photosensitivity). In 1902, Austin and Starke reported that a metal surface impacted by an electron beam emits a larger number of electrons than were previously present. However, the use of secondary emission as a means for signal amplification was not proposed until after World War I. Joseph Slepian entered a 1919 patent with Westinghouse. The combination of the photoelectric effect and secondary emission was named a photomultiplier. Nowadays, the omnipresence of photomultiplier tubes is striking (Figure 7.1). They are used practically in every kind of experimental study including space research, archaeology, medicine, geology, biology, art, as-

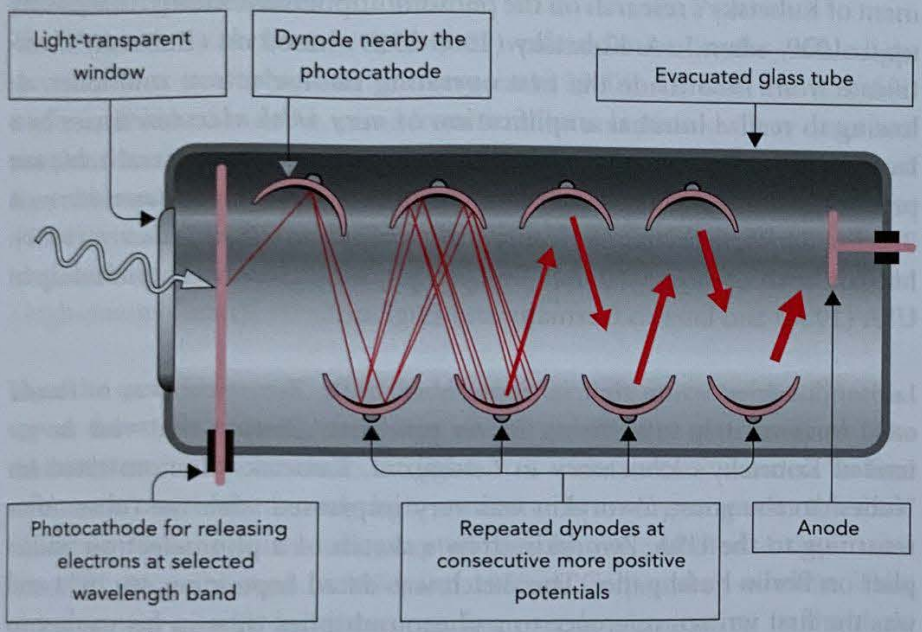
tronomy, metallurgy, chemistry, agriculture, and many others. The photomultiplier tubes have, most likely, become the most utilized, widespread vacuum electronic device.

Invention of the photomultiplier: Just in time

This brings us to several questions. When and where was the photomultiplier invented? Amazingly, the answer is different for the East and West scientific worlds. How was the highly increased sensitivity developed? What exactly was the technical improvement? In the Western scientific arena, most people acknowledge that the photomultiplier was invented in 1936. In contrast, in the USSR, tribute was given to the memory of Soviet-Russian physicist and engineer, Leonid Aleksandrovitch Kubetsky. He was born in 1906 in Tsarskoye Selo (now Pushkin, Leningrad region). His childhood coincided with very hard times in Russia. In 1923, he started his studies in physics at the Petrograd University (now Saint Peterburg State University). Not being fully satisfied with the style of teaching at the University, he moved in 1925 to the famous electromechanical faculty at Leningrad Polytechnical Institute and started to work in the field of low current electrical engineering. In 1929 (still a student) he developed a gas discharge device with a heated cathode and a controlled electrode. The actual invention of the photomultiplier took place when he proposed a new method and device to amplify weak photocurrents without using traditional radio-lamps. This patent was registered as certificate #24040, giving priority and dated 4 August 1930. In accordance with Kubetsky's proposal, the device was furnished with a source of primary photoelectrons (photocathode) and consecutive secondary electron emitters (dynodes). Each subsequent emitter was supplied with higher electrical potential in comparison with the preceding emitter. The electrical potential came from a high voltage power supply. Photoelectrons produced by the photocathode illumination were accelerated to the first dynode knocking out from its surface secondary electrons. The latter were accelerated to the next one knocking out secondary electrons as well, etc.. The device can have arbitrary numbers of emitters. The electron flux from the last emitter is captured by a collector (anode).

In 1933-34, Kubetsky developed a number of different photomultiplier tubes with silver-oxide-cesium (Ag-O-Cs) photocathodes and circular secondary electron emitters also made from Ag-O-Cs. The multi-stage electron multiplier system also included constant magnets for the purpose of electron focusing (electrostatic electron optics were not sufficiently developed at that time). The total gain of the tubes reached at least 10^3-10^4. Throughout the 1930's, such tubes were known in Russia as "Kubetsky's tubes".

Figure 7.1
The principle of a photomultiplier. It is based on an evacuated glass tube with a transparent window allowing light to hit the cathode. Nearby, another electrode, called a "dynode" with a slightly more positive voltage than the cathode, draws the electrons liberated from the photocathode by the electric field between the two. These electrons knock loose "secondary" electrons. Repeated dynodes at consecutive more positive potentials produce multiplications so that after, say, 8 stages with multiplications of about 10, one find that one single electron liberated from the cathode will produce 10^8 electrons at the end. This signal is then large enough to be detected with standard electronics and amplified into a measurable pulse. The pulse is displayed by a counter.



The majority of the physics community in the west had a deep conviction that the first photomultiplier tube was developed by Vladimir K. Zworykin and colleagues at RCA in 1936. Just prior to this, in 1935, H. E. Iams and B. Salzberg (also at RCA) published about a single-stage photomultiplier. That device consisted of a semi-cylindrical photocathode, a secondary emitter mounted on the axis, and a collector grid surrounding the secondary emitter. The tube had an approximate gain of eight. In the 1936 publication, Vladimir Zworykin reported success with a tube that amplified the current of photo-emitted electrons in multiple stages. This model used, as in the Kubetsky's tube, a Ag-O-Cs photocathode having a typical peak quantum efficiency of 0.4% at 800 nm. Also in 1936, a much improved photocathode, Cs_3Sb (cesium-antimony) was reported by P. Gorlich. This was particularly dramatic regarding the quantum efficiency improvement to 12% at 400 nm. It was the first commercially successful photomultiplier manufactured by RCA.

More historical detail about the invention of the photomultiplier were collected by Bayarto K. Lubsandorzhiev at the Institute for Nuclear Research of RAS (Moscow, USSR). He highlighted the controversial issue of who actually invented the first photomultiplier tube. In 2005, he presented, *On the History of the Photomultipleir Tube Invention* at the occasion of the 4th Beaune Conference on New Development in Photodetection (Beaune, France, 19-24 June, 2005). Lubsandorzhiev was amazed that in the West there was almost no mention of Kubetsky's name in the English scientific literature. However, he did find in English one unequivocal acknowlegement of Kubetsky's research on the photomultiplier invention: "...However up to 1930, when L. A. Kubetsky (1906-1959) based on (Kubetsky's) certificate from 1930 made the first operating photoelectron multiplier, allowing to realize internal amplification of very weak electron fluxes by a factor of 10^3-10^6, this idea has not been considered as neither realizable nor practically useful." This was cited in W. Summer's book, *Photosensitors – A Treatise on Photoelectric Devices and their Application to Industry* (1957). Lubsandorzhiev suggested that "Kubetsky's results have been picked up in USA (1934) and later in Germany and England".

Lubsandorzhiev wrote that in September 1934, Zworykin was in Russia on a business trip advertising RCA's products. During that visit he attended Kubetsky's laboratory in Leningrad. Kubetsky demonstrated his "tubes" to the guest. Zworykin was very impressed with the tubes. After returning to the USA, Zworykin drew a sketch of a photoelectron multiplier on Berlin hotel paper. The sketch was dated September 18, 1934 and was the first written reference to a photomultiplier tube in his papers. In Zworykin's laboratory journal, the first note concerning a photomultiplier tube was dated November 22, 1934. At last, in 1936, V. K. Zworykin and his colleagues published their paper on their photomultiplier invention. Comparison between Zworykin's and Kubetsky's tubes demonstrates great similarities. Thus ended the period of confusion in the East and West scientific communities as to who was finally credited with the invention and introduction of the photomultiplier.

As previously mentioned, the combination of high gain, low noise, high frequency response, and large area of collection earned photomultipliers an essential place in many scientific fields. What continued was the improvement of the spectral response of photocathodes. This is the wavelength range that the photomultiplier is sensitive to. The spectral response of the photomultiplier was sometimes confusing which led, in the early 1940's, to the development of a standard system of names and numbers. The JEDEC (Joint Electron Devices Engineering Council), an industry committee on photosensitive devices, developed this system to designate a variety of spectral responses still in use today. The various types of photocathode

devices were assigned "S-numbers" (spectral numbers) ranging from S-1 through S-40. For example, S-11 uses the cesium-antimony photocathode and S-25 uses a so-called "multialkali" photocathode (Na-K-Sb-Cs-or sodium-potassium-antimony-cesium) that provides extended response in the red portion of the visible light spectrum. No suitable photoemissive surfaces had yet been reported that could detect wavelengths longer than approximately one micrometer.

Studies focusing on biological photon emission with photomultiplier tubes

The detection (without any doubt) of weak radiation in the visible light spectrum was performed in the very early 1950's. One of the very first studies, published in 1951, came from Strehler and Arnold. They described photon emission from green plant organisms including three species of algae. However, such light could only be detected following initial irradiation with visible light. Such was the scientific recognition of the delayed luminescence phenomenon (light energy in plants stored for some time in a high-energy form).

Then the question arose, after the delayed luminescence "seemed" to disappear from the material, whether or not it still kept a capacity to continuously emit tiny amounts of light in a spontaneous manner. To find an answer, one had to study light emission from organisms in the dark. Subsequently, long-term dark adapted research was initiated. A step in this direction was published in 1954 and 1955 by Colli and co-workers. They had observed a weak luminescence from seeds germinating in the dark. The light intensity emitted by seedlings of wheat, beans, lentils and corn varied between 700 cps (counts per second) and 250 cps. For comparison, the background of the tube was 130 cps. This fifferential indicated that the seedlings in the dark were able to emit photons. The intensity was found to vary with the age of the seedlings. Commonly, it reached a maximum between the fourth and seventh day. The researchers also reported a close connection between the radiation of light and the capacity of the seed to germinate. Another interesting observation was that the seedlings that had been cut into pieces radiated 2 to 3 times more than intact seedlings. The researchers were also interested in the color of the emitted light. To determine this color, they placed specific color filters between the seedlings and the photomultiplier. That protocol facilitated the transmittance of a specific part of the color spectrum and absorbed other parts. In that manner, Colli and co-workers were able to estimate the so called spectral distribution of the emitted light. It extended from 400 nm to 600 nm culminating at approximately 550 nm.

With this type of data, some critics could hypothesize that the light was not produced by the seedlings but instead came from luminescent bacteria growing on the seedling. To test this hypothesis, the research scientists included sterilized seedlings in their studies. In that manner, they demonstrated that the photon emission came from the seedlings themselves and not from some distinct bioluminescent organisms like bacteria. This work on light emission of germinating plants was published in 1954 in *Nuovo Cimento*, as well as one year later in the more widespread journal *Experientia*. The authors did not refer to the Gurwitsch mitogenetic radiation. It certainly was possible that, in those days, they knew nothing about Gurwich's general concept of a regulatory photon radiation from organisms. Nevertheless, it was a clear step forward.

Much progress was also made in the USSR. In the 1960's several Russian groups headed by Tarusov, Konev, Vladimirov, and Zhuravlev began to take an interest in the ultra-weak photon emission from biological tissues. The Tarusov group was focused on seedlings of plants. Their data confirmed the work of Colli and co-workers. The emission of the plants was located in the blue-green range. Further investigations were carried out with the roots of plants to demonstrated the extent to which the intensity of photon emission was dependent on the concentration of oxygen in the atmosphere. They observed a nearly linear relationship. The radiation of the plant tissue could be stopped at any time by withholding oxygen. The researchers also observed that photon emission consisted of two components. The first controlled its decline with time that may originate from the light already absorbed before the beginning of the experiment. The second, nearly constant in time, depended on the absence or presence of oxygen.

In the Tarusov studies, temperature dependence of photon emission was evaluated. Most of the results were obtained using grain seedlings. The seedlings were allowed to germinate at temperatures roughly between -8°C and 50°C. Between approximately 0°C and 40°C, the seedlings remained in a steady state and emitted photons at a constant level. This level rose with temperature and did not depend on the rate of the temperature change. However, there was a temperature point ("upper critical temperature" or UCT) beyond which no steady state could exist. The intensity then began to fall with a rising temperature and increased again between 45°C and 50°C. Thermal death of the seedlings occured in the region of maximum radiation.

A similar behavior was detected in the lower temperature ranges. The emission became minimal at the "lower temperature glow limit" (LGL) point. If the seedlings were cooled below that point, a further emission was observed reaching its maximum in the range of 0°C and -5°C. Such a sudden

burst indicated the death of the seedlings. Now, the surprising fact follows. The UCT was related to the heat resistance of the given grain species. Those which were more resistant to heat had a higher "upper critical point". There also was an analogical relationship between frost resistance and the LGL. A reduced LGL points to a higher frost resistance.

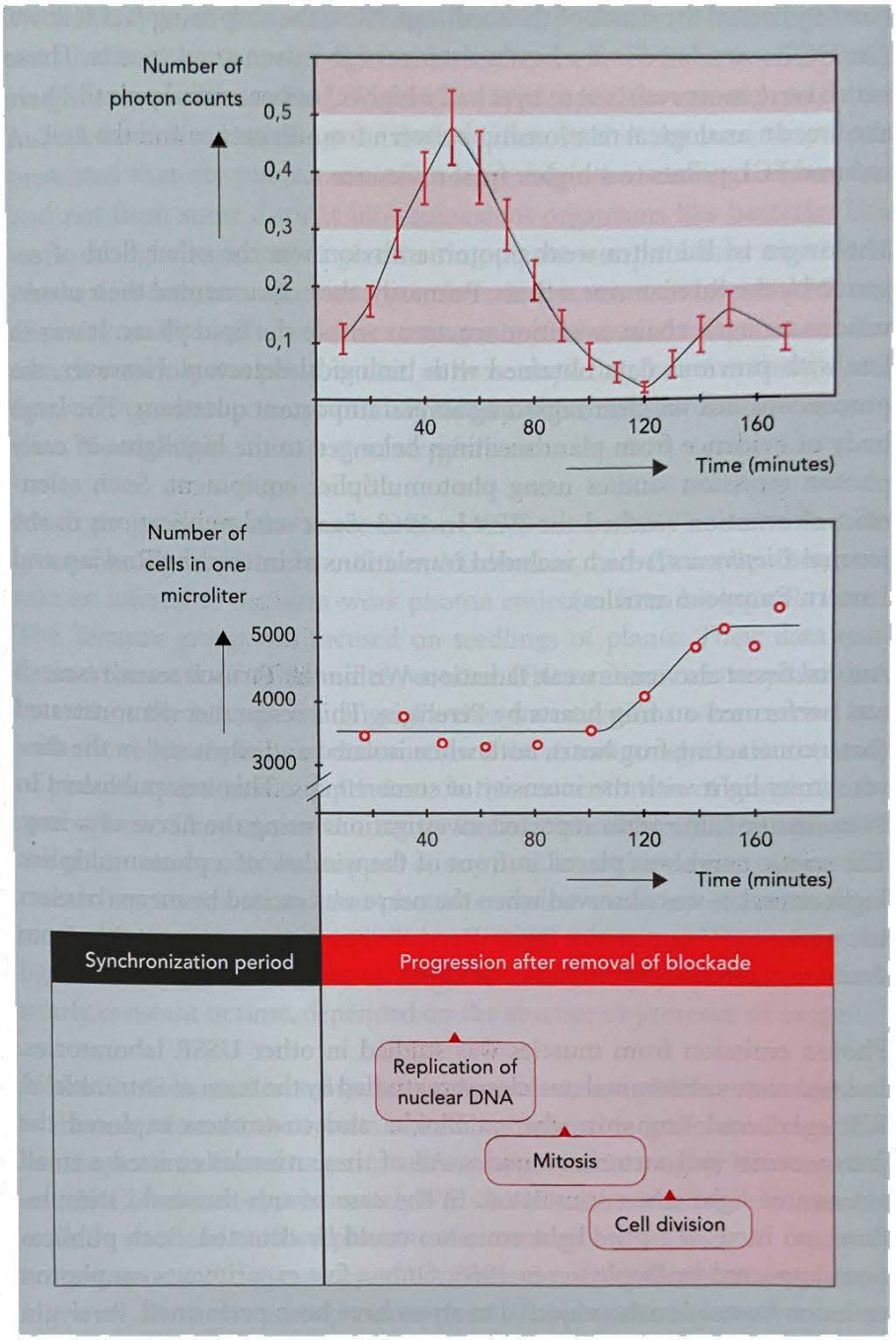
The origin of the ultra-weak photon emission was the other field of research by the Russian researchers. Primarily, they documented their observations vis-à-vis chain oxidation reactions within the lipid phase. It was in line with previous data obtained with biological detectors. However, the process was not yet clear regarding several important questions. The large body of evidence from plant seedlings belonged to the highlights of early photon emission studies using photomultiplier equipment. Such scientific information reached the West in 1963 via several publications in the journal *Biophysics* (which included translations of interesting Russian and Eastern European articles).

Animal tissue also emits weak radiation. Within the Tarusov team, research was performed on frog hearts by Perelygin. This researcher demonstrated that a contracting frog heart, both when isolated and when still in the thorax, emits light with the intensity of some 10 cps. This was published in 1966. In 1967, the team reported investigations using the nerve of a frog. The sciatic nerve was placed in front of the window of a photomultiplier. Light emission was observed when the nerve was excited by means of electric pulses (15V, 6 ms pulse time). Dead nerves or preparations taken from dead frogs did not produce any reaction.

Photon emission from muscles was studied in other USSR laboratories. Isolated rectus abdominal muscles were studied by the team of Shtrankfel'd, Klimenko, and Komarov whereas Blokha and co-workers explored the gastrocnemis and sartorius muscles. All of these muscles emitted a small amount of light when stimulated. In the case of sub-threshold stimulations, no increase in the light emission could be detected. Both publications appeared in *Biophysics* in 1968. Only a few experiments on photon emission from animals subjected to stress have been performed. Perelygin and Tarusov found that emission from a frog's heart, earthworm and spawn increased 2-15 times when treated with toxic chemicals.

In the USSR, Konev and his team employed the ultraviolet sensitive photomultiplier tube to detect such photon emission from yeasts in the early 1960's. They also repeated some of the classical mitogenetic work. Their observations regarding emission by yeast cells appeared in the West in 1966. That particular study was not unique for Konev's group. It was also explored by researchers Stauf and Reske. They described radiation

Figure 7.2
Photon emission from the yeast *Torula utilis* during its cell cycle. The yeast cells were synchronized by specific blocking of the cells at an early phase in their cell cycle. (Read more on the cell cycle in Box 16.3). After the blockage of cells in that cell cycle phase, the blockage was removed and the cells moved synchronously through their cycle, starting to growth, duplicating their DNA, dividing their nucleus (mitosis), and forming buds in a synchronized manner. The upper panel illustrates photon emission. The middle panel demonstrates the increase in the number of cells. That stepwise increase illustrates the cell's synchronization. The lower schematic panel illustrates the temporal sequence of a few major cell cycle events.



from yeast cultures in a publication that appeared in 1964 in the journal *Naturwissenschaften*. The special question addressed by the Konev team was whether photon emission was related to a special part of the cell cycle of yeast cells. They used *Torula utilis* yeast cells that were synchronized (Figure 7.2). When yeast was grown in a culture medium, the yeast cells commonly divide asynchronously just when they reached the critical point

of size that allowed them to divide. It meant that those cells continued cycling through all the phases of their growth (doubling all their components) and then, they divided. However, a culture of yeast cells could be synchronized by depriving the cells of a certain required component of their nutrition essential for a specific step in the yeast cell life cycle. In the absence of such a component, the yeast cells can grow up to this stage, but, are no longer able to follow their journey to the completion of cell division. If one waits long enough, all cells in the culture will have reached this stage of cell cycling (waiting for the essential component to arrive). After the addition of the missing substance, they all continue their growth simultaneously. This is the experimental condition that Konev used to study the emission of photons in relationship to the cell cycle. In fact, Konev and co-workers detected a maximum of emission roughly one hour before morphological cell division begins. The phenomenon was also present during the second wave of cell divisions.

Another research topic by Konev and co-workers was spectral distribution, the color of the light. Yeast cells emitted between 250 and 380 nm with a maximum near 330 nm (the region of Gurwitsch's mitogenetic radiation). Konev and co-workers demonstrated that the emission spectrum was not uniform because the emission of the plants was located in the blue-green range. Their early data became available by translation and publication in *Biophysics* in 1966.

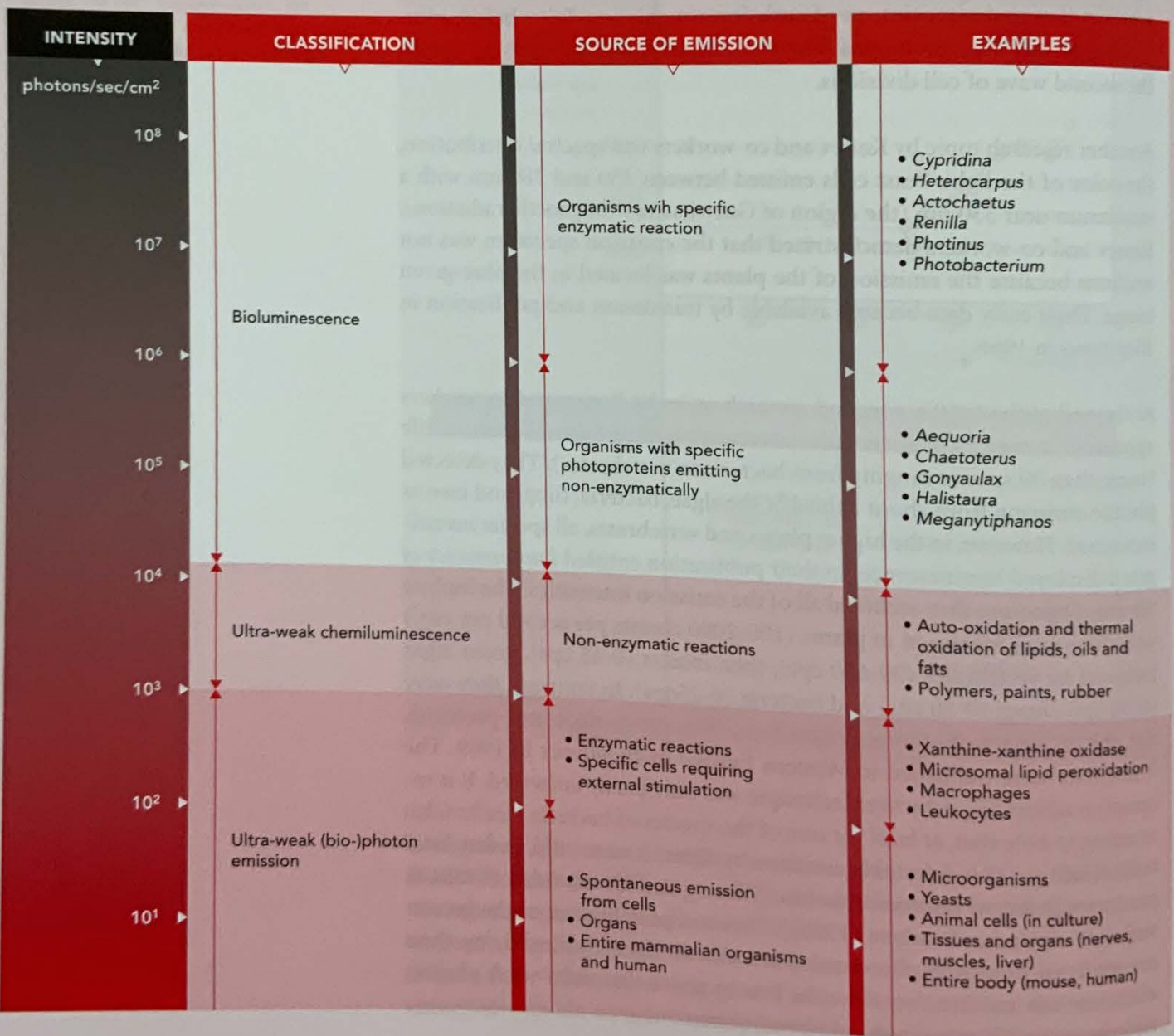
At the end of the 1960's, a second research series by Konev and co-workers appeared. It was their systematic investigation of different forms of life (more than 90 species ranging from bacteria to vertebrates). They detected photon emission from about a third of the algae, bacteria, fungi and insects examined. However, in the higher plants and vertebrates, all species investigated displayed luminescence. In their publication entitled *Luminescence of Various Organisms* they included all of the emission intensities. The highest emissions were measured in plants (100-2000 counts per second per cm^2) followed by vertebrates (30-600 cps), then insects (0-45 cps), green algae (0-50 cps), fungi (0-50 cps) and bacteria (0-25cps). In contrast, they were not able to detect photon emission from blue green algae and protozoa. These data were published in Western Europe in *Biophysics* in 1969. The question addressing detection technique was only partly answered. It is interesting to note that, at least for one of the species of bacteria (*Escherichia coli*) (which gave no detectable emission by Konev's team) did, in fact, later produce significant photon emission [Tilbury and Quickenden (1988) as well as Wang and co-workers (1990)]. This was possibly due to the greater sensitivity of improved photomultiplier tube designs. Summarizing their work, one can say that they were the first to prove that ultra-weak photon emission is a widespread natural phenomenon.

MORE ABOUT...

BOX 7.1

■ THE VARIOUS LIGHT EMISSION PHENOMENA IN LIVING ORGANISMS

Figure B.7.1.1
Classification of light emission phenomena in relation to the emission intensity and their association with life phenomena.



Some general scientific understanding of what causes the emission also slowly increased. The many biological systems that display true ultra-weak photon emission were now available for intracellular research. This meant that research could start on the homogenization of cells and tissues to study which cellular parts are responsible for photon emission. Mitochondria seemed to be a possible source of photon emission from cells. When isolated from rat liver, they emit weak radiation. The first such publications were by Vladimirov and L'vova (1964), Stauff and Ostrowski (1967), plus Zhuravlev and colleagues (1973). It was the beginning of the biochemistry of the ultra-weak radiation.

In the Western European scientific world hardly any progress was found during the 1960's. The only research that documented photon emission measurements (that referred to the Gurwitsch mitogenetic radiation) originated in the other hemisphere by Metcalf (New Zealand) and Quickenden (Australia). Their work began because they wanted to check the radiation data from rapidly dividing cells and tissues as reported by the Soviet Union research teams. They constructed a photon counter attached to a photomultiplier with a large photocathode to view large areas of cell culture. The yeasts (*Schizosaccharomyces pombe, Candida albicans and Saccharomyces cerevisiae*) and bacteria (*Escherichia coli*) were suspended in a nutrient medium and then tested. They reported that no light could be detected from any of the yeast or bacterial suspensions. This paper was published in *Nature* in 1967. Although it lacked technical details on the experimental procedure, that publication in this journal (which is considered a major impact journal in Western scientific world) may have been one of the reasons that, for a long time, such research almost continued exclusively in the Soviet Union.

Conclusion

The invention of the photomultiplier shortly before World War II led, in the early 1950's, to the detection of photons in the visible light range of the spectrum. Russian research in the 1960's demonstrated the presence of photon emission in almost any tested living

Classification and characteristics of various light emission phenomena associated with life and biological activities are presented in Figure B.7.1.1. The scale provides an approximate estimation of the magnitude of the emission intensity. They are based on the available reports. The natural light sources of biological origin referred to as bioluminescence (or biological luminescence) are the most familiar types such as luminescent bacteria, fireflies, jelly fishes, and some fish. Light emission from such sources have a recognized purpose in nature (e.g., mating signals or to attract prey). It is the light that we are able to observe with the naked eye. Its intensity is in the order of magnitude of 10^{-12}-10^{-9} W.cm^{-2}. That intensity is orders of magnitude higher than the spontaneous ultra-weak light emission that are only detected with the highly sensitive photomultiplier system. The latter (ultra-weak biophoton emission) can be observed generally from all organisms both at the microscopic and macroscopic scale without any external inducement. The emission is down to the order of about 10^{-15} W.cm^{-2} or less. Emission occurs over a wide spectral range spanning from the UV to the near-IR wavelength regions.

MORE ABOUT...

BOX 7.2

■ THE DIVERSITY AND FUNCTIONALITY OF BIOLUMINESCENCE

Searching for scientific evidence that light exists in living organisms has led to a fascinating conclusion. There are numerous animal species in the world capable of producing light.

There are many examples of bioluminescence on land. However, the vast majority of luminous organisms make home in the ocean. One idea to consider regarding the diversity of bioluminescent creatures may evolve if one could have a look at the systematically listing of all bioluminescent organisms. However, such list would be too extensive. We prefer to present a partial list. This list begins with small bacteria of low complexity through the full range of animal types up to highly complex chordate species:

- Luminescent *bacteria* are present primarily in the intestine of organisms. In the sea, they are cohabitating with larger organisms that play a role, for example, in the lure of Anglerfish and in the ventral counter-illumination of the bobtail squid (*Euprymna scolopes*).
- Luminescent *fungi* can be found on wood, where they may cause "foxfire".
- *Dinoflagellates* are well known luminescent organisms. They are part of the phytoplankton protists which may become very abundant during red tides. They are thought to use their light to alarm animals grazing close to predators.
- *Radiolarians* is another type of marine protists. These single celled organisms are like amoebe living in shallow oceanic gyres as well as in deep oceans.
- Bioluminescent *Cnidarian* groups include *Scyphozoa* (true jellyfish), *Hydrozoa* (*Hydroids*, *Siphonophores*, and *Hydromedusae*), and *Anthozoa* (sea pens and sea pansies). Different species employ photoproteins (or a variety of luciferases) to trigger the production of luminescence.
- In the group of *Ctenophores*, the comb jellies are almost all bioluminescent. Like *Hydrozoa*, they also use photoproteins to produce light. Some species, when stressed, secrete luminous material into the water.
- A variety of *Mollusks* are able to make light. The *Cephalopods* have the most advanced luminescent systems. Some squids may have three or more types of luminous organs.
- Within the segmented *Annelid* worms, many types of marine *Polychaetes* produce light. Some interesting examples include the "fireworms" which use luminescence as part of their mating display.
- Many *Crustacea* have luminous members. These include *Amphipods*, *Copepods*, *Decapods*, *Euphauslids* and *Ostracods*.
- Within the *Echinoderms*, luminescence is most prominent among brittle stars (*Ophiuroids*) and sea cucumbers (*Holothuroids*).
- *Hemichordate* worms are also luminous. The most common are the *Enteropneust tornaria* (larva).
- In the *Chordates*, many species of fish use photophores as counterillumination, defense, as well as fishing food. Some have even evolved as a combination of intrinsic and bacterial light organs.

It is quite evident that bioluminescence in nature is found in a wide spectrum of living species. It must serve important ecological roles, given its widespread taxonomic distribution in the ocean and on land. Many questions regarding its ecological communication functions have already been answered. However, many others remain unanswered.

To discuss the functionality of this light, we begin with a visit to the Waitomo Caves at the Northern Island of New Zealand. Nowhere else on earth can one view a more spectacular sight of this radiation. The cavern roof is dotted with thousands of tiny, blue-green lights, each being produced by a single glow worm. The first record of the New Zealand glow worms was made by Rev. A. G. Purchas who, in 1871, collected the female fly and larvae of this luminous insect. The scientific analysis by G. V. Hudson clarified that it was, in fact, a small gnat or fly. With regard to its food, Wheeler and Williams, in 1915, documented that the glow worms are carnivorous, attracting prey by light. The generic name changed a few times vis-à-vis *Keroplatus*, *Bolitophilia* and *Arachnocampa* until, finally, Edwards (1924) suggestion of *Arachnocampa luminosa* became the established scientific name of the New Zealand glow worm. Within the name *Arachnocampa luminosa*, "Arachno" refers to "spider" and the ability of the glow worm to spin silk threads. "Campa"

refers to its grub-like qualities and "luminosa" to the light it emits.

Let us now turn to the glow worm's life cycle in connection with light radiation. Like that of most insects, the glow worm life cycle has four stages: egg, larva, pupa and adult (imago). The entire cycle takes approximately ten to eleven months. This spectacular voyage through life begins with a spherical egg (less than 1 mm in diameter). The eggs are sticky and adhere to the cave conto walls where they are deposited. In this situation, they "incubate" for a period of 20-24 days until the time of hatching. In this period, luminescence in the (glow worm) egg has not yet been observed. At hatching, the larva splits the skin of the egg and crawls out. The new hatched larva (or "worm") immediately commences building a nest. By letting down sticky threads and switching on its light, it is able to attract its first meal. The threads are the "fishing lines" composed of long and very thin (10 micron) twin-thread silk with a series of mucus droplets arranged at regular intervals. The New Zealand glow worm thus makes use of an inherent and instinctive behavior of all species of insects.

Flying insects, even nocturnal ones, tend to be attracted to light. The tiny light spots of the glow worm larvae are, to them, indicative of the night "sky". They fly upward towards the fake stars ending in the sticky threads of the glow worm. Within a few seconds to a few minutes, the larva reacts to the vibration of its prey getting stuck to a fishing line. With considerable accuracy, it chooses the correct line to haul in.

The larva is the only stage of the life cycle that feeds. The larva must store enough food reserve to "feed" the pupa and (if the adult is a female) also for the eggs of the next generation. Before pupation, the larva shrinks and becomes opaque. Both the male and female pupae are luminescent even though the light is intermittent. Luminosity in the male pupa is suppressed during the last two or three days before emergence of the adult. In contrast, luminescence in the female becomes more noticeable in the latter stages of its development and also possibly changes its spectral characteristics (a device to attract male glow worm flies). When reaching his adult stage, the fly "escapes" from its pupal case by muscular contraction, expansion of the body, and wriggling of the legs.

The adult glow worm fly lives only a few days and the environment around it is almost totally dark. Mating within that short time frame must be assured, otherwise, there would be no future generations. Female pupae when gently rocked or touched, as well as when a male fly alights on her, she "lights up" even more brilliantly. It is not uncommon to see one or more male flies sitting on one female pupa waiting for the female fly to emerge and "light up one male life". Immediately, or shortly after fertilization, the female lays her eggs and then, usually and quickly dies.

How these glow worms produce light radiation is an interesting question. In fact, to perform that task, the genus, *Arachnocampa* is the only group of insects where the light organ is formed from the swollen tips of the thin excretory tubes termed Malpighian tubes. This light producing organ lies within a layer of respiratory tissue which acts as a reflector directing the light downwards. The respiratory tissue is composed of a network of fine blind ending tubes called trachea which supply oxygen to the insect's internal organs. The oxygen is essential for the chemistry underlying the light production. The chemical reaction producing this light involves several other components in addition to ATP. One component is luciferin, a waste product formed in the glow worm's digestive system. Then, there is luciferase, an enzyme that acts on luciferin by catalyzing a chemical reaction which, in the presence of oxygen, leads to the emission of "cold light". The amount of heat produced in the reaction is negligible. The light has a blue-green colour with a maximum emission peak of 487 nm. When disturbed by noise, chemicals, surface vibration, or bright light, the glow worm douses its own light. The quenching of the light is thought to be brought about by a restriction of oxygen flow and/or other chemicals to the light producing organ.

An interesting question to elucidate is: can glow worms see? There are numerous indications that glow worms do see light. By inserting electrodes into the eyes of male adult glow worm flies, it is possible to electrically record responses of the eyes to brief equal quanta flashes of spectral light. Maximum response was found to be at 540 nm wavelength accompanied by a significant secondary peak near to 460 nm. This peak at 460 nm, more specifically, may be aimed at detecting the glow of the female fly.

organism from bacteria and yeasts to plants and vertebrates. The photon emission was dependent on physiological conditions. It also varied within the cell cycle. Progress in the understanding of the biochemistry of ultra-weak photon emission demonstrated the role of oxygen and reactive oxygen species.

The novel observations regarding ultra-weak photon emission facilitated the comparison of this general category of photon emission with that of another, more rare, category of photon emission, biological luminescence (Box 7.1). The intensities of two different categories of natural light sources of biologic origin differ approximately 3-4 orders of magnitude.

Although bioluminescence is a rare phenomenon, it is not limited to only a few species. Instead it is found in a wide spectrum of species from bacteria to vertebrates. Many questions regarding its ecological communication functions have already been answered (Box 7.2).
It resulted in numerous questions regarding the functionality of ultra-weak photon emission. Does it have a role in the communication between organisms or within the metabolic network? Or is it just a loss of energy without any physiological significance?
This was the search that began world-wide in the 1970's.

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8

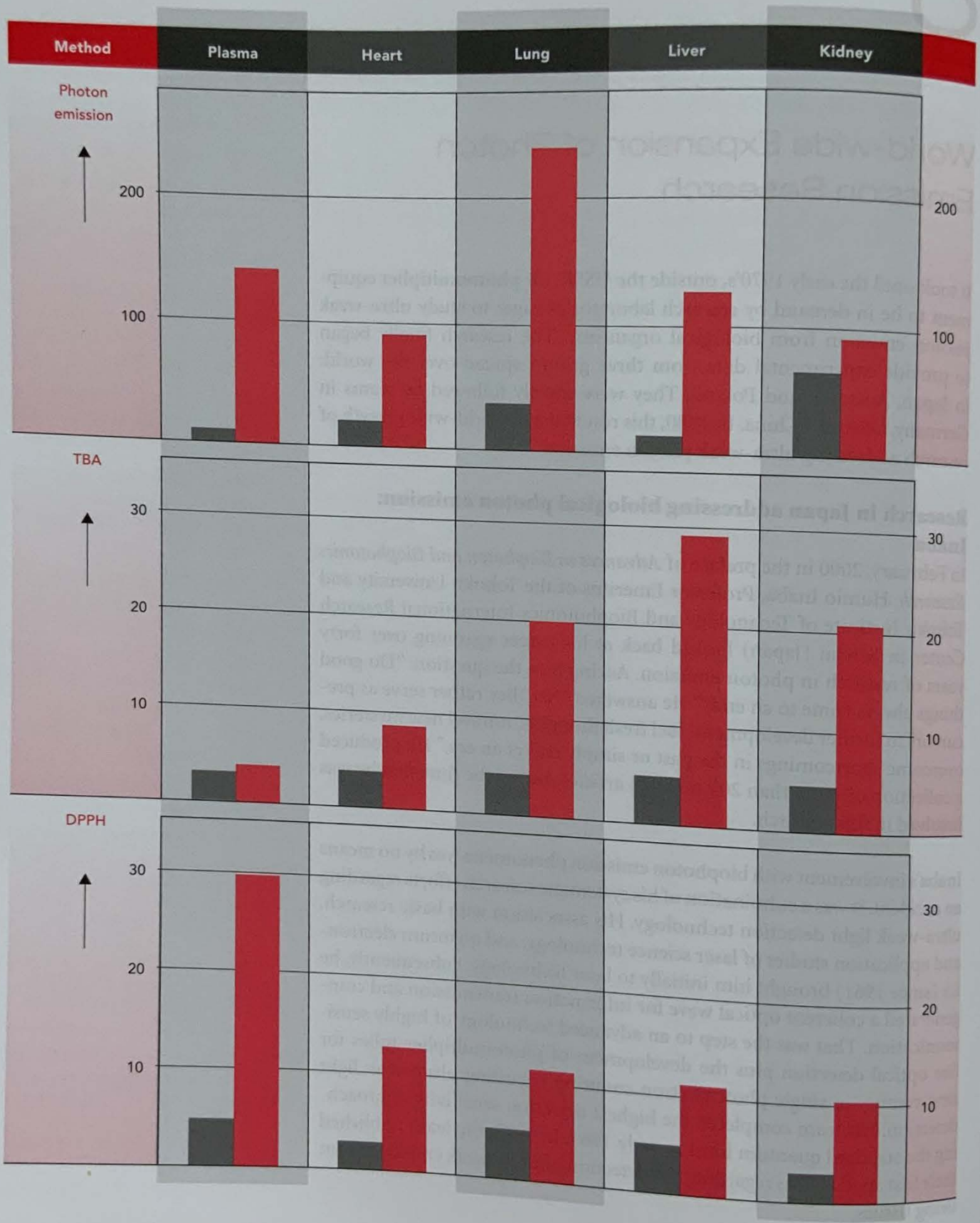
World-wide Expansion of Photon Emission Research

It took until the early 1970's, outside the USSR, for photomultiplier equipment to be in demand by research laboratories eager to study ultra-weak photon emission from biological organisms. The research finally began to provide experimental data from three groups spread over the world: in Japan, Australia and Poland. They were shortly followed by teams in Germany, USA and China. In 1980, this resulted in a world-wide growth of research addressing ultra-weak photon emission.

Research in Japan addressing biological photon emission: Inaba

In February, 2000 in the preface of *Advances in Biophoton and Biophotonics Research*, Humio Inaba, Professor Emeritus of the Tohoku University and Tohoku Institute of Technology and Biophotonics International Research Center in Sendai (Japan) looked back at his career spanning over forty years of research in photon emission. Asking him the question: "Do good things always come to an end?" He answered "No, they rather serve as precursors to further development, fuel fresh thoughts, unravel new mysteries, overcome shortcomings in the past or simply reflect an era." He produced a collection of more than 200 research articles during the time that he was involved in this research.

Inaba's involvement with biophoton emission phenomena was by no means an accident. It was a culmination of his systematic research efforts regarding ultra-weak light detection technology. His association with basic research, and application studies of laser science technology, and quantum electronics (since 1961) brought him initially to laser technology. Subsequently, he generated a coherent optical wave for information transmission and communication. That was the step to an advanced technology of highly sensitive optical detection plus the development of photomultiplier tubes for new avenues in single photoelectron counting regarding ultra-weak light detection. His team completed the highest detection sensitivity approaching the standard quantum limit in early 1969. In 1973, the team published their first applications regarding the detection of ultra-weak emission from living tissues.


Method
Plasma
Heart
Lung
Liver
Kidney
Photon emission
200
100
TBA
30
20
10
DPPH
30
20
10

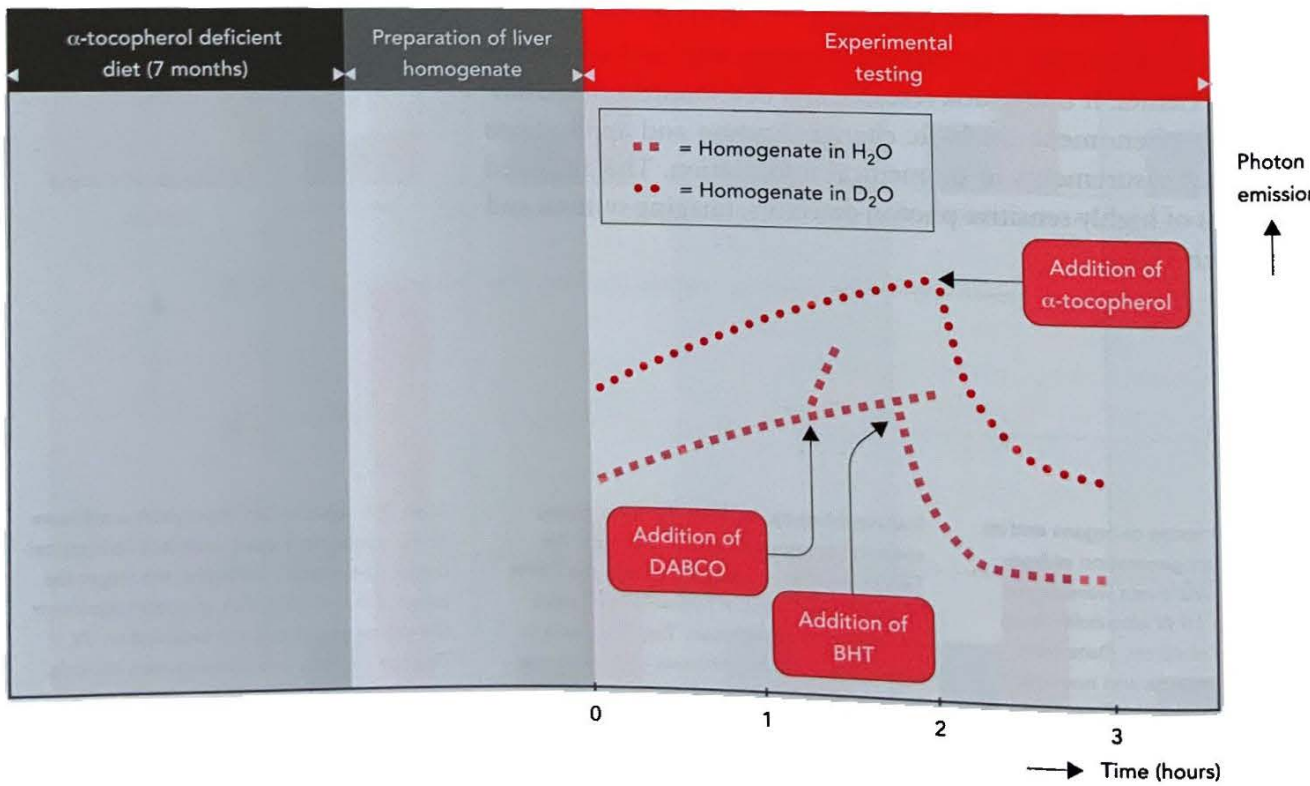
Another important development from the Inaba school was the spectral analysis system for ultra-weak light intensities. They developed a filter spectral analyzer system that was superior by approximately 2 orders of magnitude compared to the conventional spectrometers. This new system provided excellent spectral measurements of ultra-weak photon emission. In 1975, the team published regarding the application of this system to ultra-weak photon emission from various biological and chemical systems. They studied living tissues, malignant tumors, organs, blood, urine as well as enzymatic reactions and biochemical processes involving free radicals, lipid peroxidation and oxidative deterioration of oils, fats and foods. The progress and performance of this technique received encouraging attention from scientists and engineers working in various life science fields such as biochemistry, pharmacology, food technology and medicine.

The exciting outcome of such research facilitated in 1986 the launch of the "Inaba Biophoton Project", one of the national Japanese research projects of Exploratory Research for Advanced Technology (ERATO) organized by Research Development Corporation of Japan under the auspices of the Japanese Science and Technology Agency. It was funded from 1986 to 1992. The Inaba Biophoton Project included dozens of researchers from different disciplines (partly Japanese and the rest from all over the world). At the conclusion of that Project, a new school of thought emerged. It was decided to codify the research data in order to develop future technological demands in the area of biomedicine, agriculture and industry. "Biophotonics Information Laboratories, Inc." was established in Yamagata for a period of six years beginning in 1993. The research company was funded by Japan Key Technology Center. It undertook research and development of the biophoton emission phenomena for basic characterization and applications to non-invasive measurements of biomedical information. This included the development of highly sensitive photon detectors, imaging systems and spectrum analyzers.

◄ Figure 8.1
Ultra-weak photon emission of organs and its response: UV-enhanced generation of free radicals (Inaba 1983). Male rats were irradiated with light from a 15 W ultraviolet lamp for 90 h at a distance of 40 cm. Data were obtained from blood plasma and homogenates of internal organs such as heart, lung, liver, and kidney. At the same time, classic chemical parameters were measured. The TBA (thiobarbituric acid) value and the DPPH (α, α-diphenyl-β-picryl hydrazil) value were measured for comparison. The TBA assay is one of the common methods utilized for the measurement of lipid peroxides in living tissues. The value of DPPH provides a measure of the antioxidant used to detect lipid peroxidation (the smaller the value, the larger the antioxidant activity). The grey bars represent the values of animals not exposed to UV. The red bars are from UV-exposed animals.

A few highlights from the early period must be mentioned. Using rat tissues, a study addressed ultra-weak photon emission associated with the process of lipid peroxidation. The Inaba team studied the response to stress. It was generally known that the exposure of living systems and tissues to ultraviolet light resulted in the enhanced generation of free radicals. It was hypothesized that this generation lowers the antioxidative activity vis-à-vis acceleration of lipid hydroperoxidation. This was verified by comparing the ultra-weak photon emission intensities of various tissues from animals before and after ultraviolet light radiation (Figure 8.1). The data were obtained from blood plasma and homogenates of internal organs such as heart, lung, liver and kidney. At the same time, classic chemical

Figure 8.2
Ultra-weak photon emission from liver homogenates prepared from rats that were fed a α-tocopherol-deficient diet for 7 months. The liver homogenate was prepared in physiological saline or in 0.9% NaCl – D_2O rats. D_2O possesses an elongation effect on the lifetime of singlet oxygen ($^1O_2$) molecules. Tocopherol is supposed to quench $^1O_2$ and free radicals. In the same studies the stimulating effect of DABCO (1,4-diazabicyclo[2,2,2] octane) was tested. The compound is known as a stimulant for the generation of singlet oxygen molecules. Also, the effect of BHT (butyl hydroxytoluene) was investigated. It acts as a free radical scavenger. Hence the results verify the involvement of ($^1O_2$) and free radicals in ultra-weak photon emission.



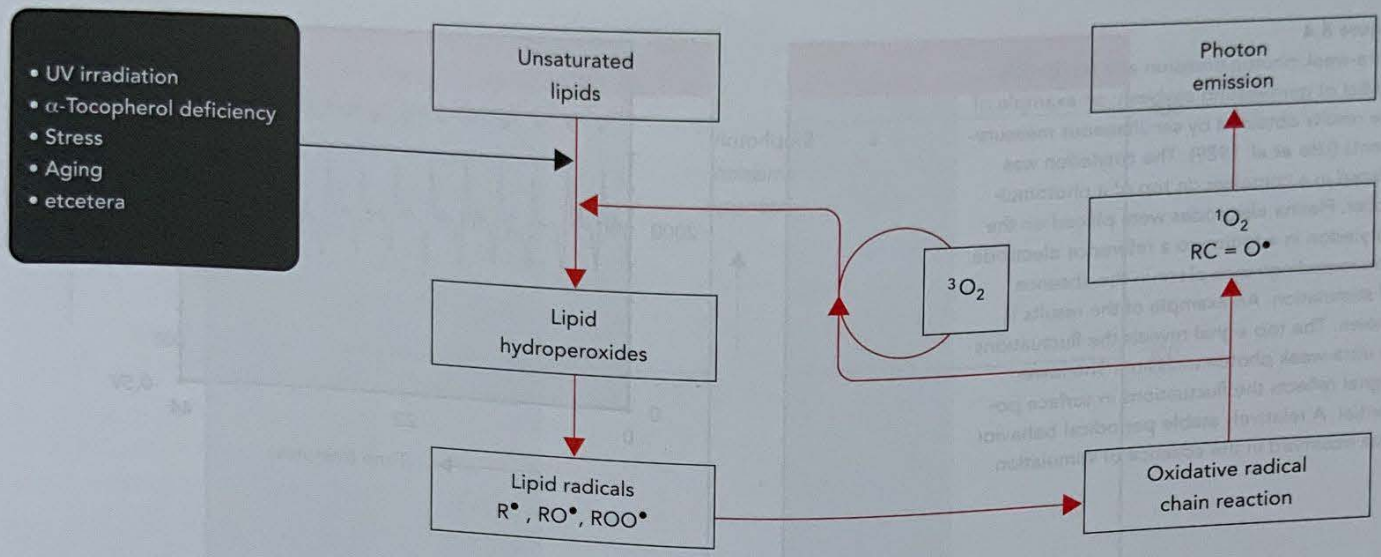


Figure 8.3
Relationship of ultra-weak photon emission and lipid peroxidation in biological tissues. $^1O_2$, singlet molecular oxygen, RC = O•, excited carbonyl compounds.

parameters were estimated. Such data again supported the usefulness of ultra-weak photon emission detection as a monitoring method for tissue lipid peroxidation.

Another interesting contribution addressed the role of α-tocopherol deficiency in rat tissue lipid peroxidation (Figure 8.2). The rats were divided into two groups. They were fed either an α-tocopherol containing diet (normal diet) or an α-tocopherol-deficient diet (which consisted of components similar to those of the normal diet). After 7 months of feeding, the α-tocopherol-deficient liver had a markedly enhanced photon emission compared to that of a normal diet. This confirmed that α-tocopherol quenches free oxygen radicals. To further elucidate the chemistry involved in such emission, the effect of α-tocopherol was combined with substances known as stimulants in order to generate singlet oxygen ($^1O_2$). Hence, the data verified the involvement of excited ($^1O_2$) molecules and free radicals in the production of ultra-weak photon emission (Figure 8.3).

A last example of the early innovative research by Inaba's team was the synchronous estimation of ultra-weak biophoton emission and bioelectrical activity in plant tissues. Measurements of ultra-weak photon emission

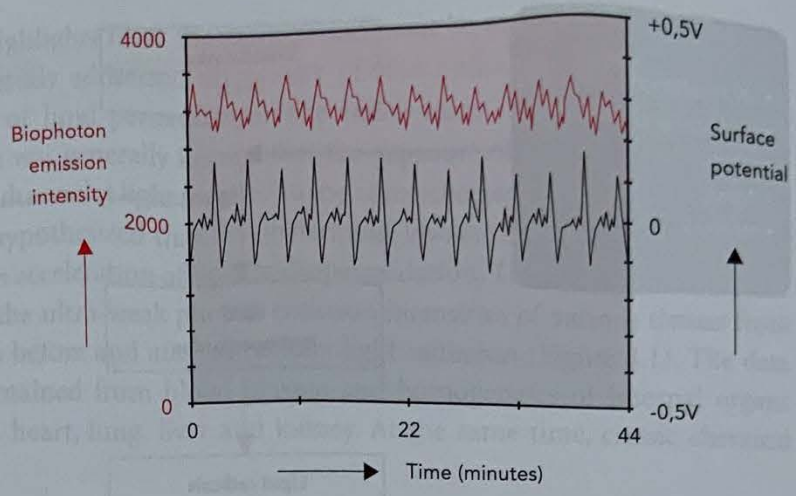


Figure 8.4
Ultra-weak photon emission and surface potential of germinating soybean: an example of the results obtained by simultaneous measurements (Usa *et al.* 1989). The cotyledon was placed in a container on top of a photomultiplier. Platina electrodes were placed on the cotyledon in addition to a reference electrode. The recordings took place in the absence of stimulation. An example of the results is shown. The top signal reveals the fluctuations in ultra-weak photon emission. The lower signal reflects the fluctuations in surface potential. A relatively stable periodical behavior was observed in the absence of stimulation.

intensity and biosurface electric potential were initiated on germinating soybean. The results obtained by simultaneous measurements demonstrated that the two different physiological phenomena occurred almost synchronously (Figure 8.4).

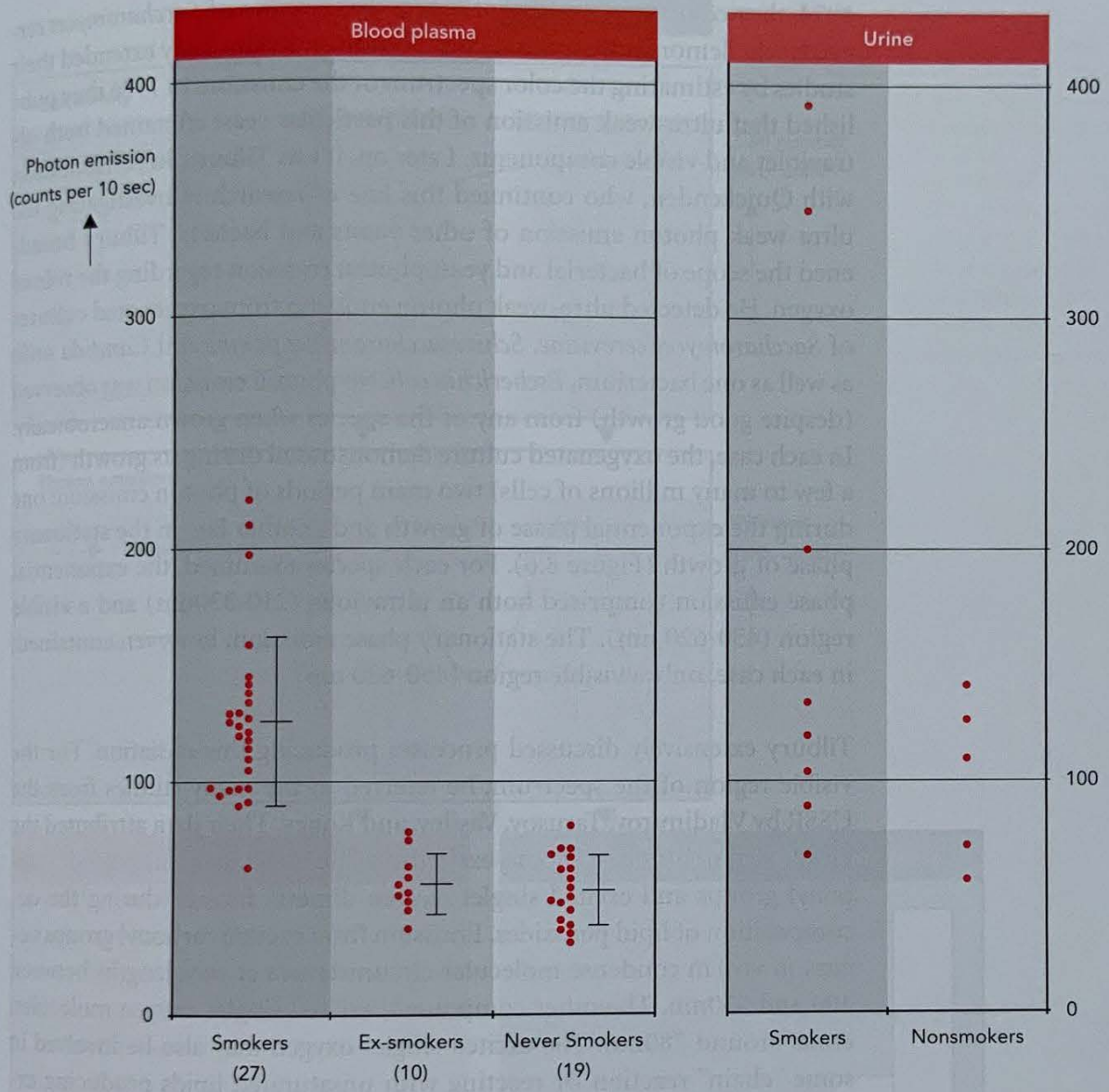
The group continued their research in the health and life style arena. They studied the ultra-weak photon emission of plasma from smokers and nonsmokers. They were able to discriminate between these two groups. Additionally, the level of plasma ultra-weak photon emission was estimated in a man that quit smoking. This rapidly decreased the level of photon emission in plasma.

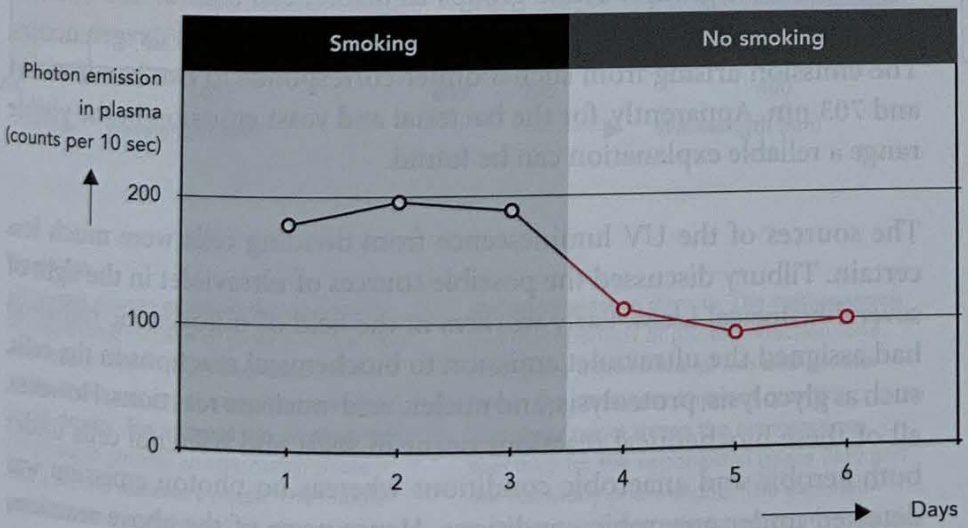
The Inaba team also developed novel two-dimensional photon recording equipment. Later, they were the first to use highly sensitive CCD equipment. This is discussed more in Chapter 19.

Research in Australia addressing biological photon emission: Quickenden and Tilbury.

Many years later, after his 1967 denial of biological radiation (see page 123), Quickenden and Que Hee studied ultra-weak photon emission of yeast. In

Figure 8.5 ►
Ultra-weak photon emission in studies regarding smoking (Yoda *et al.* 1985; 1987; Inaba 1987). Ultra-weak photon emission was estimated in blood plasma (upper left panel) or in the urine (upper right panel) of smoking and non-smoking subjects. In some cases a distinction within the non-smokers was made between "never smokers" and "ex-smokers". Additionally, the level of plasma ultra-weak photon emission was estimated in a man that withheld from smoking at the end of the third day. It was concluded that smokers demonstrate significantly higher ultra-weak photon emission from blood plasma. Data from urine samples are less distinct. The effect of discontinuing cigarette smoking was rapidly detected in the decrease in plasma photon emission.


Blood plasma
Urine
Photon emission
(counts per 10 sec)
400
300
200
100
0
Smokers
(27)
Ex-smokers
(10)
Never Smokers
(19)
Smokers
Nonsmokers


Smoking
No smoking
Photon emission
in plasma
(counts per 10 sec)
200
100
0
1
2
3
4
5
6
Days

1974, they confirmed the data of others that cultures of *Saccharomyces cerevisiae* do demonstrate spontaneous photon emission. They extended their studies by estimating the color spectrum of the emission. In 1976 they published that ultra-weak emission of this particular yeast contained both ultraviolet and visible components. Later on, it was Tilbury, in collaboration with Quickenden, who continued this line of research reinvestigating the ultra-weak photon emission of other yeasts and bacteria. Tilbury broadened the scope of bacterial and yeast photon emission regarding the role of oxygen. He detected ultra-weak photon emission from oxygenated cultures of *Saccharomyces cerevisiae, Schizosaccharomyces pombe* and *Candida utilis* as well as one bacterium, *Escherichia coli.* No photon emission was observed (despite good growth) from any of the species when grown anaerobically. In each case, the oxygenated culture demonstrated during its growth (from a few to many millions of cells) two main periods of photon emission: one during the exponential phase of growth and another late in the stationary phase of growth (Figure 8.6). For each species examined, the exponential phase emission comprised both an ultraviolet (210-330nm) and a visible region (450-620 nm). The stationary phase emission, however, contained, in each case, only a visible region (450-620 nm).

Tilbury extensively discussed processes producing this radiation. For the visible region of the spectrum, he referred to the many studies from the USSR by Vladimirov, Tarusov, Vasilev, and Konev. Their data attributed the visible region luminescence to excited specific molecular compounds (carbonyl groups and excited singlet oxygen dimers) formed during the decomposition of lipid peroxides. Emission from excited carbonyl groups occurs *in vivo* in condense molecular circumstances at wavelengths between 400 and 550nm. The other component, excited singlet oxygen molecules, emits around 780nm. The excited singlet oxygen may also be involved in some "chain" reaction by reacting with unsaturated lipids producing excited carbonyls groups. These groups as before, can emit at 400-550 nm. The other possibility is that dimerization of excited singlet oxygen occurs. The emission arising from such a dimer corresponds to two bands at 634 and 703 nm. Apparently, for the bacterial and yeast emission in the visible range a reliable explanation can be found.

The sources of the UV luminescence from dividing cells were much less certain. Tilbury discussed the possible sources of ultraviolet in the light of several historical ideas. Early workers in the field of mitogenetic radiation had assigned the ultraviolet emission to biochemical reactions in the cells, such as glycolysis, proteolysis, and nucleic acid-nuclease reactions. However, all of these biochemical reactions occur in yeast and bacterial cells under both aerobic and anaerobic conditions whereas no photon emission was detected under anaerobic conditions. Hence none of the above reactions

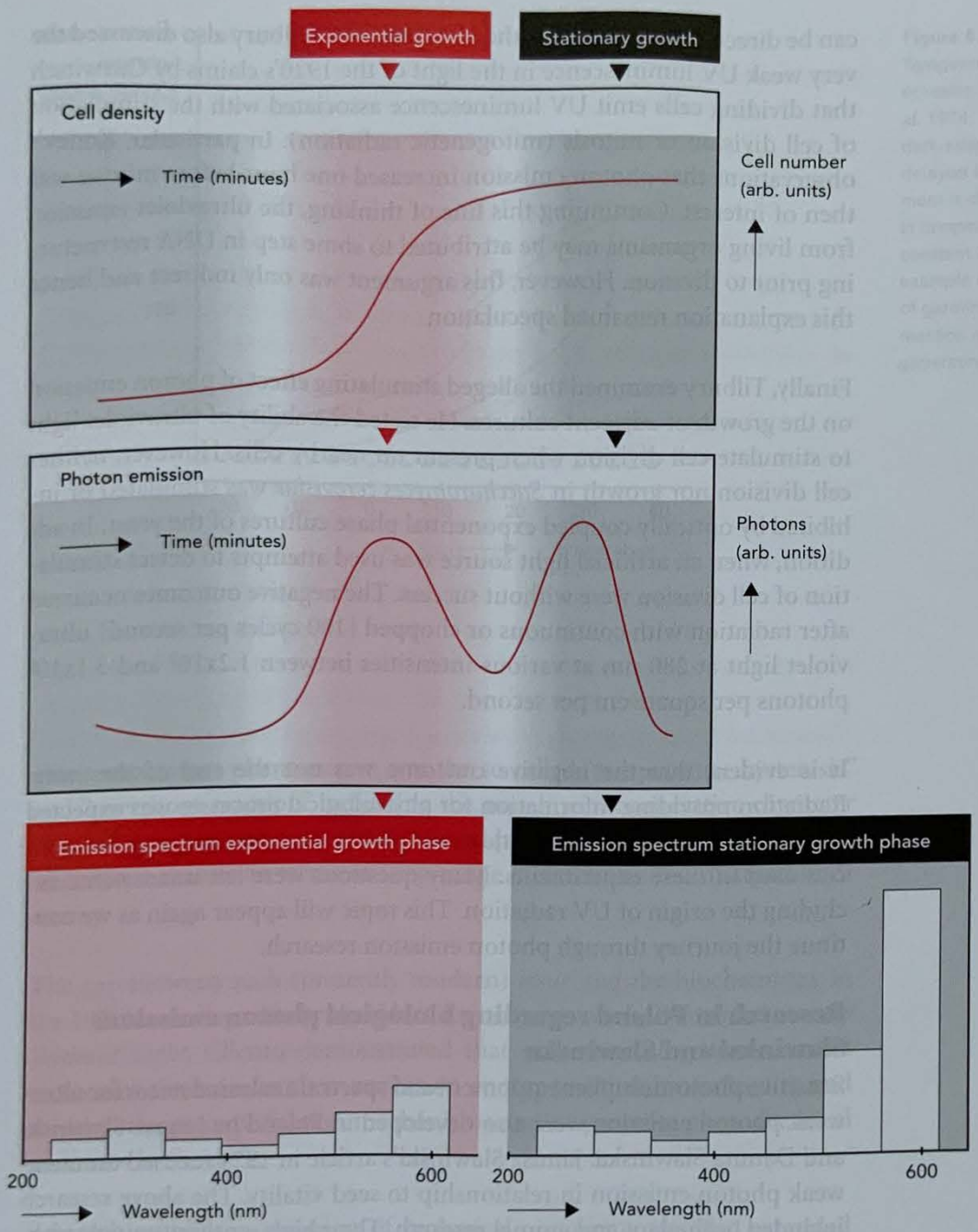


Figure 8.6
Ultra-weak photon emission during growth of oxygenated cultures of yeast (Quickenden and Tilbury 1983; Tilbury and Quickenden 1988). The upper panel shows the increase in cell density. The increase has an exponential phase in growth, an adaptation phase and a second stationary phase. The middle panel shows the ultra-weak photon emission during increase in density. The culture shows two main periods of photon emission: one during the exponential phase and another late in the stationary phase of growth. The lower panel shows the luminescence spectrum for the exponential phase (left) and the stationary phase (right). The exponential phase emission comprised both an UV (200-330 nm) and visible (450-620 nm) region (with the latter always being several times more intense than the former). The stationary phase emission is more bright than the exponential phase emission. The spectrum reveals that the brighter emission in the stationary phase mainly occurs above 450 nm.

can be directly responsible for the UV emissions. Tilbury also discussed the very weak UV luminescence in the light of the 1920's claims by Gurwitsch that dividing cells emit UV luminescence associated with the stimulation of cell division or mitosis (mitogenetic radiation). In particular, Konev's observations that photon emission increased one hour before mitosis was then of interest. Continuing this line of thinking, the ultraviolet emission from living organisms may be attributed to some step in DNA restructuring prior to division. However, this argument was only indirect and hence this explanation remained speculation.

Finally, Tilbury examined the alleged stimulating effect of photon emission on the growth of adjacent cultures. He tested the ability of ultraviolet light to stimulate cell division when present on nearby cells. However, neither cell division nor growth in *Saccharomyces cerevisiae* was stimulated or inhibited by optically coupled exponential phase cultures of the yeast. In addition, when an artificial light source was used attempts to detect stimulation of cell division were without success. The negative outcomes occurred after radiation with continuous or chopped (100 cycles per second) ultraviolet light at 280 nm at various intensities between 1.2×10^2 and 3.1×10^9 photons per square cm per second.

It is evident that the negative outcome was not the end of the story. Radiation providing information for physiological processes was expected to have more complex and subtle modulations than the chopping frequencies used in these experiments. Many questions were left unanswered including the origin of UV radiation. This topic will appear again as we continue the journey through photon emission research.

Research in Poland regarding biological photon emission: Slawinksi and Slawinska

Sensitive photomultiplier equipment and spectral analysis devices for ultra-weak photon emission were also developed in Poland by Janusz Slawinski and Danuta Slawinska. Janusz Slawinski's article in 1974 focused on ultra-weak photon emission in relationship to seed vitality. The above research included both plant and animal research. Their high sensitivity single photon counting systems combined with the cutoff filter method facilitated measuring count rates in the very low range of 10-1000 photoelectrons per second in order to achieve the spectral resolution of 15-20 nm. Their analysis of the spectral distribution of ultra-weak photon emission from lower and higher plants confirmed the participation of electronic excited carbonyls and singlet molecular oxygen and its dimoles.

A special focus of their research addressed temperature-dependency of photon emission from biological objects (Figure 8.7). The most remark-

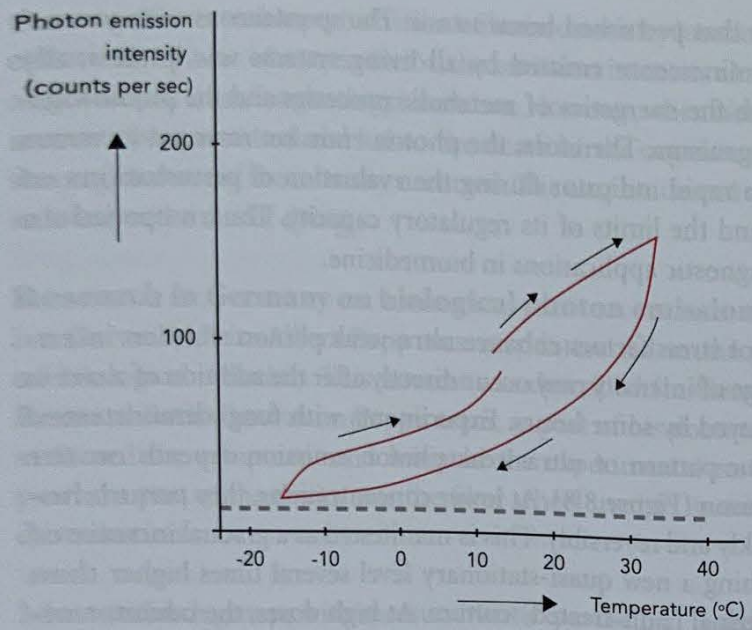


Figure 8.7
Temperature hysteresis of ultra-weak photon emission in germinating seeds (Slawinski *et al.* 1974; 1980). The germinating seeds were dark-adapted to eliminate photosynthetic delayed luminescence. The seed's environment is changing in temperature; the change in temperature (both up and down) was constant at 0.5 °C per min. The presented example illustrates the temperature hysteresis of germinating seeds. The "overshoot" reaction reveals the non-linear processes generating excited states and photons.

able temperature phenomenon was the so called "temperature hysteresis" and "overshoot reaction" indicating the non-linear, cooperative character of processes generating excited states and photons. They discussed this cooperative behavior in terms of long-range interactions and dissipation of energy.

The gap between such (presently modern) issue and the biochemistry in the 1970's was elegantly bridged by Cilento in his work, *Photobiochemistry Without Light.* Cilento demonstrated that in biological systems excited molecules can transfer their excitation energy to other biomolecules and thereby trigger amplification mechanisms and/or promote photochemical processes in the dark.

Research on photon emission of biological organisms was also extended in other directions. Newly formed Polish teams contributed to a better understanding of light producing processes during an immune response. The technique was also used for other applications. For example, photon emission of cereal grain, subjected to the effect of mechanical loads, was found to be related to microstructures with practical value regarding the evaluation of the mechanical quality of grain.

Another application of ultra-weak luminescence was its use as a noninvasive experimental tool for the description of biosystems under harm-

ful conditions that perturbed homeostasis. The spontaneous endogenous ultra-weak luminescence emitted by all living systems was intrinsically associated with the energetics of metabolic processes and the physiological state of organisms. Therefore, the photon emission response to stress may serve as a rapid indicator during the evaluation of perturbations of homeostasis and the limits of its regulatory capacity. Thus, it opened an avenue for diagnostic applications in biomedicine.

The majority of stress factors enhance ultra-weak photon emission intensity. The change of intensity may occur directly after the addition of a stress or may be delayed by some hours. Experiments with fungi demonstrated how the kinetic pattern of ultra-weak photon emission depends on the dose of the poison (Figure 8.8). At lower concentrations, they perturb homeostasis weakly and reversibly. This is manifested as a gradual increase of intensity reaching a new quasi-stationary level several times higher than that of the original (non-treated) culture. At high doses, the inhibitor results in a single peak of "degradative" ultra-weak photon emission reflecting an irreversible perturbation of homeostasis. This photon emission response corresponded with earlier observations regarding stress-induced ultra-weak luminescence from lower animals, described by Tarusov's team. They found that the intensity of a frog's heart, an earthworm and spawn increased 2-15 times when these objects were treated with toxic chemicals. In

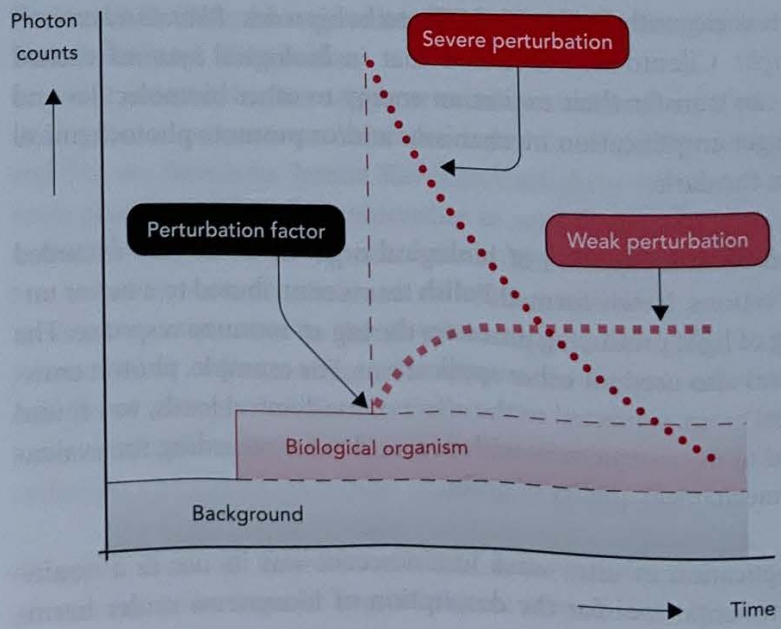


Figure 8.8
The ultra-weak emission response to stress: a generalized schematic representation. The response depends on the strength (dose). At low dose it perturbs homeostasis weakly which is manifested in a gradual increase reaching a new quasi-stationary level. At higher strength it results in a single sharp peak reflecting an irreversible perturbation of homeostasis.

the case of a reversible perturbation of homeostasis by low doses of chemicals or low power of a stress factor, the emission intensity slowly increased and reached a flat, longlasting maximum. However, when homeostasis was irreversibly perturbed and led to the death of the organism, a rapid and strong increase of the intensity occurred. The amplitude and duration were related to the rate of dying.

Research in Germany on biological photon emission: Popp

In Germany, Fritz-Albert Popp was very interested in the phenomenon of biological radiation. This interest was described (in German) in 1976. Bernard Ruth who was on Popp's team, as part of his doctoral thesis, constructed the equipment and initiated the experimental research An important part of the device was the filter wheel used to investigate spectral distribution.

Most research groups with measurement expertise had pointed to the fact that plant materials (in particular, seedlings) displayed high signals even after long-term dark adaptation. Ruth studied cucumber and potato seedlings plus *Tradescantia* roots. The 3 to 5 day old cucumber seedlings revealed a photon emission which in a few hours reached a uniform level. Even after more days in complete darkness, the light emission from those seedlings remained present (with fluctuations in intensity clearly exceeding the limit of error). The spectrum was characterized by a maximum near 550 nm and an increase in the long-wave range. No clear proof of emission was found at 400 nm, 369 nm or 331.5 nm. It confirmed data from several other research teams.

In case of the photon emission of cucumber seedlings was dependent on physiological processes, Ruth reasoned that the addition of chemical substances should influence photon emission. In fact, different chemical substances may influence the properties of photon emission in various ways. In his experiments, Ruth used acetone, solution of Cialit, saturated brine and ethanol. In the temporal course of the photon emission two different types could be seen. The addition of acetone or ethanol initially led to high activity of photon emission followed by a slow decrease over time. A completely different emission course occurred after the addition of a solution of Cialit or saturated brine. Initially, those seedlings behaved as if untreated. However, after a latency period, the intensity rose rapidly. Both Ruth and Popp confirmed the existence of so-called "degradation radiation". That is the relatively intensive emission of photons from dying cells, regardless of the cause of death, whether it be by refrigeration, centrifugation, heat or treatment with toxic agents. Ruth and Popp suggested that this phenomenon must be attributed to the irreversible destruction of well-ordered (high energetic) cellular states.

Popp was also very interested in the application of "degradation radiation" to evaluate the effect of cytostatica. Ruth selected Ehrlich ascites cells grown in the abdominal cavity of rats and mice to study the effects of cytostatics *in vitro*. The tumor cells were taken from the animals before their death and were put in culture. Because of the restricted experimental time, no clear proof was obtained, at that time, that the cells demonstrated a detectable radiation statistically different from background. However, the addition of the cytostatica of the phosphamid family to Ehrlich's ascites tumor cells actually did increase the emission. Popp speculated that such effect was a marker for the "good cytostaticum" and therefore should be used to select the specific cytostaticum after testing it on individual patients' tumor cells.

The role of Fritz Albert Popp in photon research specifically developed in the direction of light-induced photon emission measured as light-induced delayed luminescence. This is defined as the photon emission that is registered after the completion of the illumination of the object. It represents the light energy that could be stored in the biological system. This aspect has farreaching theoretical and experimental consequences and will be discussed in relationship to the revival of the information aspect of ultraweak photon emission (Chapter 16).

Research in USA addressing biological photon emission: Chance, Boveris, Cadenas and others

In the mid-70's, the interest in the occurrence of low level luminescence in leukocytes during phagocytosis began in the USA with the work of Allen. He and his colleagues were, in 1975, the first to describe this luminescence. However, such work made use predominantly of artifical light producing substances such as luminol. The leukocytes would interact with reactive oxygen species which produced the luminescence. At that time, leukocytes were known to be involved in the defense strategy of the organism. The ingestion of bacteria or other phagocytizable materials by leukocytes was also known to be associated with a respiration burst.

Allen's work was followed by the team of Britton Chance, Alberto Boveris and Enrique Cadenas at the University of Pennsylvania (Philadelphia, PA.). In 1977, Chance began to study leukocytes (he was interested in biochemical events when organisms are exposed to bacteria). Ultimately, his team was very productive with publications in high-impact journals. In 1979, they presented numerous data on the luminescence of leukocytes isolated from the peritoneal cavity of guinea pigs. Utilizing those leukocytes, they provided evidence that luminescence is related to the uptake of oxygen in the respiratory burst. The team also studied isolated alveolar macrophages from guinea pigs. Macrophages produced less luminescence intensity

than leukocytes. In most of those studies, the photon emission was different from the spontaneous ultra-weak photon emission by the Japanese, Polish, and Australian teams. Thus, luminol mediated luminescence lacked specificity because luminol can be oxidized by several O_2 intermediates (e.g., O_2^-, $HO^\bullet$, H_2O_2, and $^1O_2$). Unfortunately, spontaneous (non-luminol mediated) low level photon emission of (alveolar) macrophages had not been studied mostly due to technical limitations. In later studies, Chance and co-workers utilized a high sensitivity counter which permitted spectral analysis of spontaneous (non-enhanced) light emission. They described the kinetics of photon emission and its dependence on O_2 tension utilizing spectral evidence for singlet oxygen generation.

Later, in 1980, the Chance-Boveris-Cadenas team described the ultra-weak photon emission of intact and perfused organs. They directed their attention to mitochondria as an important contributor to cellular and organ emission. In that respect, microsomal fractions had already been recognized as an effective source of photon emission by Japanese researchers in 1975. The photon emission intensity of rat liver mitochondria was found to be dependent on the metabolic state and was markedly increased when the mitochondria were supplemented with hydroperoxides. The apparent requirements for the photon emission in liver mitochondria were hydroperoxide, molecular O_2 and a membrane redox system. O_2^- was believed to participate in the chain radical reactions that lead to lipid peroxidation. Superoxide anion and singlet oxygen were found to be involved but singlet molecular oxygen was found to be mainly responsible for the photon emission.

The chemiluminescence technique permitted continuous monitoring of lipid peroxidation reactions occurring in tissue homogenates (and therefore the detection of complex kinetic patterns that are difficult to follow with other techniques). This established an advantage over chemical methods (e.g., the detection of malondialdehyde by the thiobarbituric acid assay). Evaluation of oxidative stress in the intact organ approached by photon emission recording followed the 1960's Russian research. In an 1980 paper by the Chance team they reported spontaneous and hydroperoxide-induced photon emission of rat liver as well as a partial spectral analysis of the emission of the liver. Again, they confirmed that light emission indicates the generation of short-lived free radicals and excited states derived from the side reactions of the free radical process of lipid peroxidation. It was evident that the ultra-weak photon emission was a sensitive assay for oxidative radical reactions. It was concluded that spectral analysis is the most accurate method for identifying the photon emitting species. In 1981, they published under the title *Ultraweak Chemiluminescence: A Sensitive Assay for Oxidative Radical Reactions*, the state of the art in the USA's

ultra-weak photon emission research. Another article entitled, *Low-level Chemiluminescence as an Indicator of Singlet Molecular Oxygen in Biological Systems* appeared in 1981 in *Methods in Enzymology.*

Enrique Cadenas continued his research in Germany at the Institut für Physiologische Chemie, University of Düsseldorf in collaboration with Helmut Sies. They demonstrated that oxygenation of isolated hepatocytes led to a simultaneously increased emission of low level chemiluminescence and accumulation of malondialdehyde. Singlet molecular oxygen, formed during the free radical process accompanying lipid peroxidation, was identified as the main source of photon emission. It was concluded that low level chemiluminescence emitted from hepatocytes at wavelengths beyond 600 nm ("red band") provided a tool to examine oxygen dependent radical damage.

Alberto Boveris joined the Department of Biochemistry, Faculty of Pharmacy and Biochemistry, University of Buenos Aires. His team produced studies asking questions about the photon emission response of liver related to tumor growth in the animal as well as to thyroid induced oxidative stress.

Britton Chance continued with other collaborators. Together with Martin D. Williams, they studied the spontaneous photon emission of human breath. Breath is a voluminous and readily accessible waste product. They observed that lipid peroxide radicals (as well as other possible emitters including singlet oxygen and the hydroxyl radical) are sufficiently volatile to be found in breath. Such studies corresponded with Japanese research. The experiments were initiated in order to determine the aspects of a subject's daily life which produce physiological conditions detectable by photon emission measurements. The studies continued in the direction of mitochondrial functioning, oxidative stress, aging and chronic disease.

Research in China addressing biological photon emission: Yan Zhiquang and Zheng Rongrong

Reporting the ultra-weak photon emission of biological systems by sensitive photomultiplier equipment in China started by Yan Zhiqiang. The early studies published in 1979 and the early 1980's, focused on aspects of traditional Chinese medicine such as the search for meridians on the body surface and acupuncture points in humans (both healthy and diseased subjects). The studies belonged to a research program of the Chinese Academy of Science and were carried out at the Institute of Biophysics in Behjing. In the early 1980's, another research group at the Institute of Traditional Chinese Medicine in Shanghai led by Zheng Rongrong joined the research line on human body ultra-weak photon emission in relation-

ship to physiological and pathological states. The photomultiplier collected photons via a relatively small window. The sensitivity of the cathode was in the range of 285-450 nm with a peak value at 380 nm. Since the signal to noise ratio of such photomultiplier tubes is relatively low, the reliability of the experimental measurements was enhanced by using 30 s measurements and 10 times repeating the measurements per measured surface of the finger point.

In research on such intensity, the finger was placed in a small dark container (the tip directly pointed towards the photomultiplier window). Using the endpoint of index fingers this research studied the intensity of photon emission in different age groups. In experiments with almost 250 subjects, they observed a remarkable decline of photon emission with age. Comparative studies have also been made for men versus women (men, in general, reveal higher emission values than women). Other studies have focused on seasonal fluctuations. The research team also studied finger end point emission values in relationship to specific diseased states and in patho-physiological changes. The Chinese studies included important aspects of traditional Chinese medicine in which meridians and acupuncture points play an important role (see Chapter 20). The studies were based on Chinese common knowledge about the relationship between specific information points (mostly finger end points) and diseases. This approach is different from the Western medical approach. In Chinese traditional medicine, the common syndromes and diseased states are essentially not known. Instead, diseased states are linked to energies and subjects that could show different balance in energy distribution patterns. Such subdivisions in energy distribution and its reflections on finger endpoints have been used to correlate with corresponding photon emission intensities at these points. In this manner, treatment procedures like acupuncture needling, moxa, or the utilization of specific herb remedies have been correlated with the photon emission. Several dozens of publications have been produced. Since this highly interesting and valuable work has been almost exclusively published in the Chinese language, it is of utmost importance to have this literature more available in biophoton science.

Conclusion

It is evident that the 1970's and 1980's contributions to the field were enormous regarding the biochemistry and metabolic picture how to link ultraweak photon emission with basic metabolism. At least, at this point, one could begin to discern the richness of information which could be retrieved from measurements of photon emission.

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Conclusion Part B

At the end of the second journey, we learned that after the 1912 proposal of a morphogenetic radiation field for the regulation of the biological form, it took 50 years to definitively proof that living organisms emit radiation in the visible and ultraviolet range. It took almost another 20 years before this research was spread world-wide.

The assumptions of the morphogenetic field were based on systems thinking. The first attempt to conceptualize a Living Systems Theory was by Hans Driesch in 1911. He hypothesized that the organic form of a living creature has levels of organization with a "property of the whole" that should *not* be explained solely on the molecular/mechanical properties of "its parts", but, rather, should include a non-mechanical principle.

In 1912, Alexander G. Gurwitsch introduced the Morphogenetic Field Theory.
Shaping a form begins with directionally regulating the growth and mitosis of cells based upon the presence of an exogenous division stimulating factor which is a *radiation process.* The analysis of the radiation spectrum and identification of the active ultraviolet component was a crucial step in the eventual understanding that metabolic reactions underlie such radiation. By the mid-1930's (using biological detectors) other studies documented the role of short-lived oxygen radicals and their role in *chain reactions* (wherein light and dark high-energy reactions are linked). The radiation was emitted by all types of organisms and tissues and detailed data were collected from nerves.

The demonstration of radiation urgently needed the introduction of physical detectors. Such devices were not available. Our journey highlighted the developments in the field of the photoelectric effect. In 1905, Albert Einstein described how the photoelectric effect was caused by absorption of quanta of light (photons). However, investigating the radiation of biological samples using this photoelectric method did not document ultraviolet radiation from rapidly dividing cells. With more sensitive radiation detection devices (such as the Geiger counter) some researchers documented (in the late 1930's) the existence of ultraviolet radiation from biological tissues. Others were unable to find such an effect. However, in the 1950's and 1960's, the interest in photon emission of biological organisms was revived with the arrival of the photomultiplier tube and its enormous increase in sensitivity and definite detection capability of weak radiation in the visible light spectrum. It also resulted in the classification and characterization of two light emission phenomena associated with life (i.e., bioluminescence

and ultra-weak photon emission). The two phenomena were different both in intensity and spectral distribution.

Most of the progress in ultra-weak photon emission research was made in the 1960's by Russian research teams. They estimated that the emission from all types of biological organisms demonstrated its relationship with physiology and stress. They documented the role of oxygen and reactive oxygen molecule species. This information reached the Western scientific world via publications beginning in 1963. However, it took in the Western scientific world another decade before photomultiplier equipment reached the reliability, stability and sensitivity to do research with biological organisms.

Six major centers in Japan, Poland, Australia, Germany, USA and China formed the beginning of an international growth of research in ultra-weak photon emission of living systems. The different research teams contributed with their own specific expertise. The Japanese team, in 1973, published about the ultra-weak photon emission from living tissues, blood and biochemical processes involving free radicals and lipid peroxidations. They confirmed the Russian data that emission is related to physiology. In 1974, a Polish and an Australian research team published about photon emission in relationship to seed vitality and on yeast physiology respectively. Both teams progressed into various, but different directions. The Polish team utilized the method to assess perturbation of homeostasis in biosystems whereas, the Australian team focused on yeast spectral analysis. In Germany, the first experimental data regarding ultra-weak photon emission from biological systems were presented in 1976. Shortly thereafter, the interest in USA developed and in 1979-1980, they presented their data abou ultra-weak emission in phagocytosis plus intact and perfused organs. This confirmed that the ultra-weak photon emission was, indeed, a sensitive assay for oxidative radical reactions. The Chinese work pointed to a strong interest in human body ultra-weak photon emission, particularly in relationship to traditional Chinese medicine.

Summary and Perspective

In 1981, ultra-weak radiation (in the ultraviolet and visible light spectral emissions) by biological organisms was internationally accepted. This did not mean that the 1912 proposal of a morphogenetic radiation field that regulates the development of the biological form was also accepted. In fact, this "informative" aspect of the radiation field in the original idea was forgotten. This was probably due to the long duration of physical recording device development as well as the predominantly biochemical interest of the researchers. At the end of the Part B journey, the main question is whether this radiation plays a field organizational role set into motion in

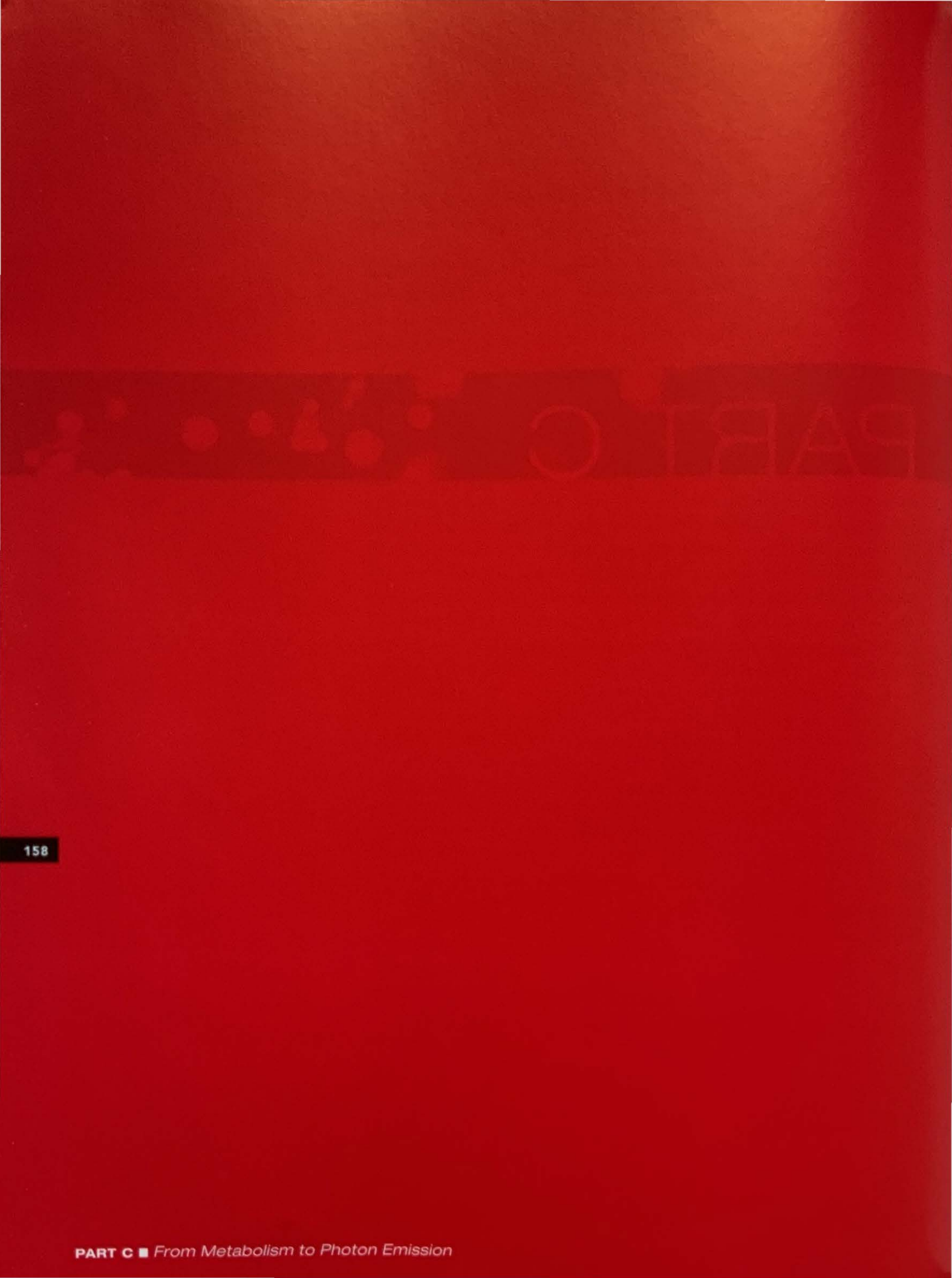
development both in transitions between the states of differentiation and undifferentiation as well as between the states of growth and non-growth (such as in tumor formation).

This complex question cannot be answered directly. In fact, it needs to be reformulated. Interestingly, during the time described in the Part B journey, another scientific direction was in full development which provided great help in reformulating the question. This development had as its main focus energy aspects of tumor development. At minimum, it could clarify the role of high-energy in the phenomenon of differentiation. Such information should be considered before we link photon emission from high-energy reactions to mitosis.

We will, therefore, during our third journey, focus on the question how cancer is viewed as the opposite of differentiation and how this is linked to a type of energy metabolism. The aspect of bioinformation in the ultra-weak light radiation (in division and differentiation) will not yet be touched. Instead, our journey will give some indirect evidence that the processes of high-energy formation and DNA replication/cell division are related in an opposite manner.

PART C

Cancer: Metabolism, Biochemistry and Photon Emission



Introduction Part C

An association of cancer with loss of differentiation has been recognized for more than a hundred years by both pathologists and oncologists. In the beginning of the 20th century, this opposite coordination between cell proliferation and cell differentiation became a new possible avenue how to approach cancer as a model in order to understand the biochemistry of metabolism in relationship to photon emission.

Glycolysis and respiration in cancer

The discovery of glucose fermentation (glycolysis) began in 1906 and finally ended in 1940. The biochemistry of respiration was lagging behind. Its first enzyme was discovered in 1924 followed (over two decades) by the other respiratory enzymes. At that time, the degree of oxygen dependent respiration available to cells was acknowledged as the pivotal transition from the existence of normal cells to cancer cells. In the 1920's to 1960's, research suggested that a greatly diminished oxygen pressure might be at the root of an increase of fermentation capability as well as an intrinsic stimulus for development of the cancer cell. It suggested two kinds of energy available in life. Respiration energy might create and maintain high differentiation. The energy of fermentation (an inferior energy), might only maintain low differentiation.

Transplantable hepatoma research opens new avenues in the 1950's

The dualities regarding 1.) differentiation/proliferation and 2.) respiration/fermentation were hotly disputed when this next step in the biochemical arena became active. Comparing many of the studies regarding hepatic carcinogenesis and then continued as transplanted hepatomas, correlations were, in fact, demonstrated between metabolic alterations. In both 1959 and 1961, the correlation studies resulted in the "Molecular Correlation Concept" of cancer. The "reprogramming" of gene expression was reflected in the biochemical balance between key glycolytic, respiratory and gluconeogenic enzymes. A similar situation was observed regarding key enzymes of synthesis and degradation in the pyrimidine and purine metabolic pathways. This systematic shift was not only specific for cancer but also for embryological development.

Oxygen supply and the role of free radicals

The suggestion that respiration corresponds both with a superior kind of energy and with differentiation led to the question regarding the nature of this type of energy. In the 1960's until the 1980's, the knowledge about the oxygen and respiration dependent formation of free radicals and the

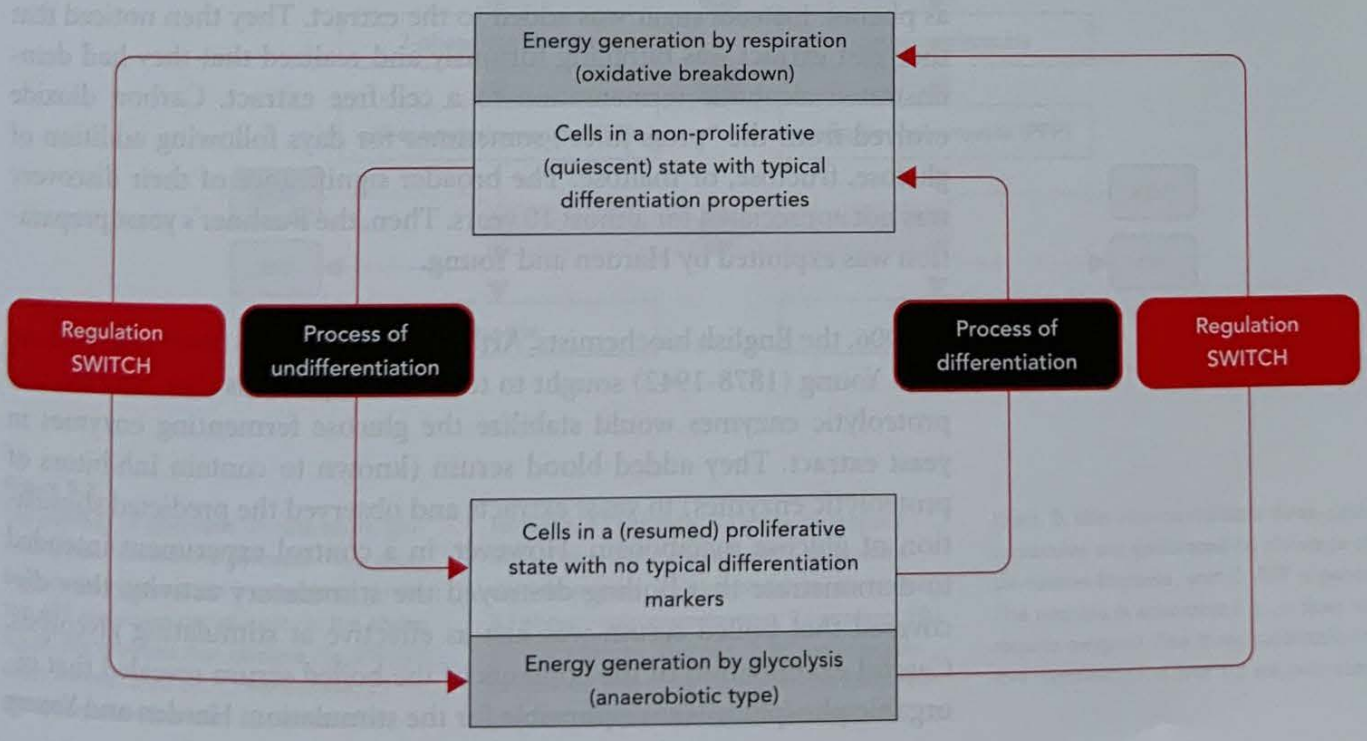
bioenergetics of their excited states drastically increased. The free radical part of cellular metabolism could result in molecular excited states with particularly high energy levels able to produce ultra-weak photon emissions. The association of photon emission with this part of metabolism was highly relevant for physiological conditions regulating the redox state such as anoxia, ischemia and ischemia/reperfusion fluctuations. The insights about the physiology of anoxia/reperfusion as well as the biochemistry of redox regulation provided a better understanding of tumor development and the link to ultra-weak photon emission.

9

Cancer as Model to Understand Energy Metabolism in Relationship to Differentiation vs. a Dedifferentiation Switch

The transition from the undifferentiated to a high differentiated state and subsequently to suppression of growth is most obvious during development of the early embryo. Adult organs reach their final differentiated states when cell proliferation is in a quiescent state. The reverse process, a loss of differentiation and increased growth rate is observed in early regeneration (blastema formation) as well as in tumor development. In the early 1920's, the opposite types of regulation became an interesting subject for the rising field of biochemistry (Figure 9.1).

Figure 9.1
The switch in energy generation in the "differentiation/proliferation switch".



It was suggested that the types of metabolism and generation of energy are part of the concomitant shift in differentiation and growth rate. This occurred at the time of a series of discoveries regarding the metabolism of sugars under aerobic and anaerobic conditions.

History of the understanding of glycolysis

Glycolysis is Greek for "the splitting of sugar". It is the process whereby glucose, the most abundant monosaccharide in living systems, is converted to simpler products. Glycolysis is the backbone of carbohydrate metabolism. Alcoholic fermentation in yeast was the very beginning of biochemistry. The name enzyme is a memorial to this historical fact, in that Old Greek, "εν ζιμος" means "in yeasts".

The mechanism was unclear till the work of Eduard Buchner (1860-1917), a German chemist, who, in 1907 was awarded the Nobel Prize in Chemistry for his research on fermentation. His older brother, Hans Ernst August Buchner (1850-1902), a German bacteriologist, was known for his extensive research involving anaerobic bacteria. In 1897, the Buchner brothers made a key discovery opening the door not only to the investigation of the mechanism of fermentation, but also to the concept "enzymes" and the whole of modern biochemistry. The brothers were attempting to make an extract of yeast, grinding yeast cells with sand with a pestle and mortar in order to disrupt the tough cell walls. The resultant juice was then filtered through cheesecloth to remove the debris (sand, unbroken cells and fragments of cell walls). Having obtained this liquid, they were faced with the problem of preservation. They did not use conventional antiseptics such as phenol. Instead, sugar was added to the extract. They then noticed that the yeast extract was bubbling furiously and realized that they had demonstrated alcoholic fermentation in a cell-free extract. Carbon dioxide evolved from the "press juice", sometimes for days following addition of glucose, fructose, or maltose. The broader significance of their discovery was not appreciated for almost 10 years. Then, the Buchner's yeast preparation was exploited by Harden and Young.

In 1906, the English biochemists, Arthur Harden (1865-1940) and William John Young (1878-1942) sought to test their hypothesis that inhibitors of proteolytic enzymes would stabilize the glucose fermenting enzymes in yeast extract. They added blood serum (known to contain inhibitors of proteolytic enzymes) to yeast extracts and observed the predicted stimulation of glucose metabolism. However, in a control experiment intended to demonstrate that boiling destroyed the stimulatory activity, they discovered that boiled serum was just as effective at stimulating glycolysis. Careful examination of the contents of the boiled serum revealed that inorganic phosphate was responsible for the stimulation. Harden and Young

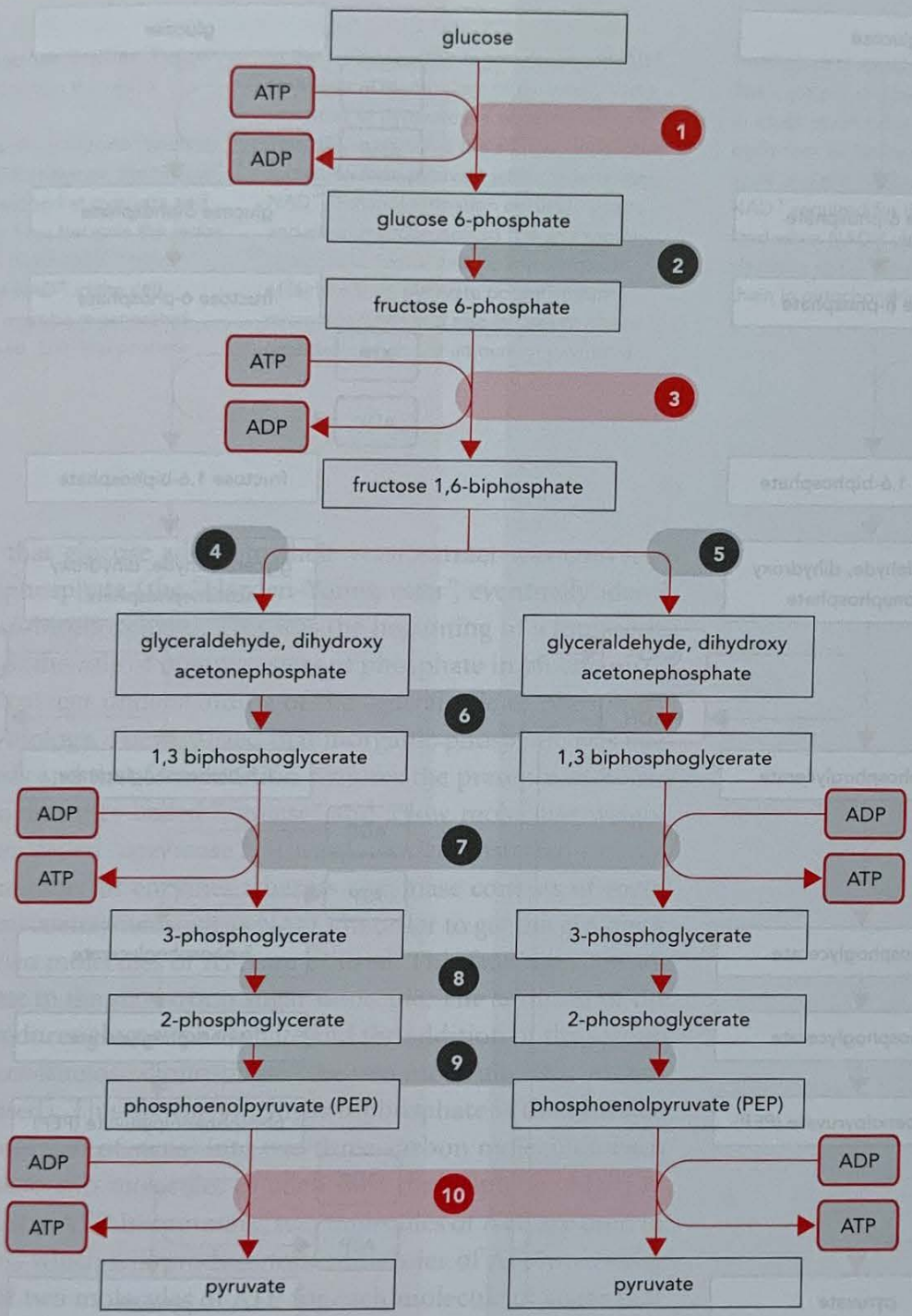
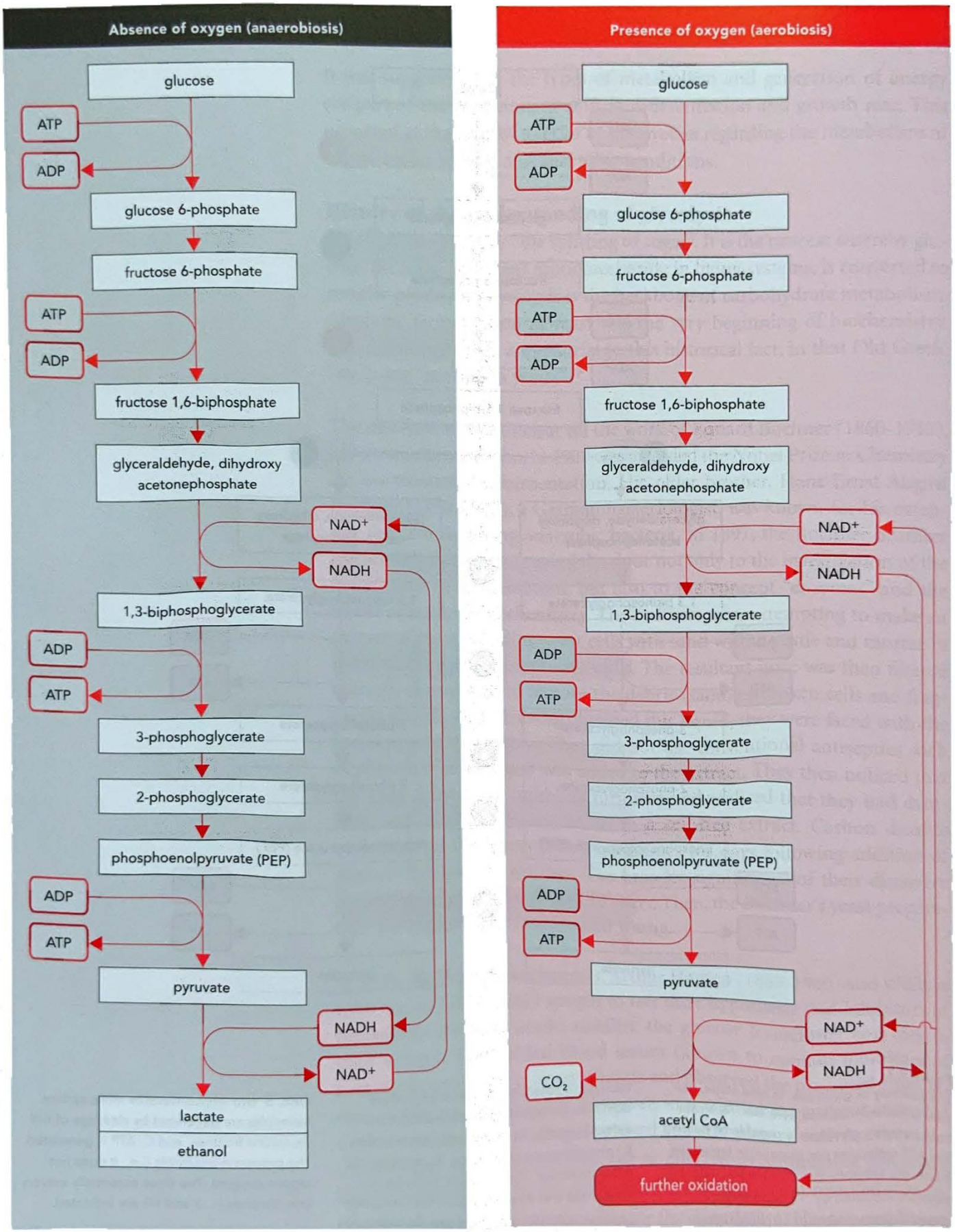


Figure 9.2
The pathway of glycolysis. In nine steps, glucose is transformed into pyruvate. As a catabolic pathway, glycolysis is capable of yielding two ATP molecules per glucose. In the above figure, the enzymes that catalyze the different steps are indicated by: 1.) hexokinase, 2.) phosphoglucoisomerase, 3.) phosphofructokinase, 4.) aldolase, 5.) triose phosphate isomerise, 6.) glyceraldehyde 3-phosphate dehydrogenase, 7.) phosphoglycerate kinase, 8.) phosphoglycerate mutase, 9.) enolase, 10.) pyruvate kinase. The glycolytic pathway can be divided into three stages: A. the six-carbon glucose molecule is trapped and destabilized, B. two interconvertible three-carbon molecules are generated by cleavage of the six-carbon fructose, and C. ATP is generated. The process is anaerobic (i.e., it does not require oxygen). The three essentially irreversible reactions (1, 3 and 10) are indicated.


Absence of oxygen (anaerobiosis)
glucose
ATP
ADP
glucose 6-phosphate
fructose 6-phosphate
ATP
ADP
fructose 1,6-biphosphate
glyceraldehyde, dihydroxy acetonephosphate
NAD+
NADH
1,3-biphosphoglycerate
ADP
ATP
3-phosphoglycerate
2-phosphoglycerate
phosphoenolpyruvate (PEP)
ADP
ATP
pyruvate
NADH
NAD+
lactate
ethanol
Presence of oxygen (aerobiosis)
glucose
ATP
ADP
glucose 6-phosphate
fructose 6-phosphate
ATP
ADP
fructose 1,6-biphosphate
glyceraldehyde, dihydroxy acetonephosphate
NAD+
NADH
1,3-biphosphoglycerate
ADP
ATP
3-phosphoglycerate
2-phosphoglycerate
phosphoenolpyruvate (PEP)
ADP
ATP
pyruvate
NAD+
NADH
CO2
acetyl CoA
further oxidation

◄ Figure 9.3
Anaerobiosis and aerobiosis requires diverse fates of pyruvate to maintain the redox balance.
Conversion of glucose into puryvate results in the net synthesis of ATP. However, the energy converting pathway stopped at pyruvate and would not proceed for long because the redox balance had not been maintained. There are limited amounts of NAD^+ in the cell. Consequently, NAD^+ must be regenerated for glycolysis to proceed. The final process in the pathway is the regeneration of NAD^+ through the metabolism of pyruvare. Three reactions of pyruvate are of prime importance. The left panel shows the ethanol and lactate formation from pyruvate which regenerates NAD^+. Ethanol formation occurs in yeasts and other microorganisms It is an example of alcoholic fermentation. The formation of lactate from pyruvate occurs in some microorganisms and also in cells of higher organisms when the amount of oxygen is limiting, as in muscle during intense activity. The right panel shows the aerobic condition in which much more energy can be extracted by further oxidation through the citric acid cycle and the electron transport chain. The NAD^+ required for the glycolysis is regenerated when NADH ultimately transfers its electrons to O_2 through the electron transport chain in mitochondria (see Chapter 10).

soon discovered that glucose added to their yeast extract was converted into a hexose bisphosphate (the "Harden-Young ester", eventually identified as fructose 1,6-bisphosphate). This was the beginning of a long series of investigations of the role of organic esters of phosphate in biochemistry which led to our current understanding of the central role of phosphoryl group transfer in biology. They realized that inorganic phosphate was necessary for glycolysis and that fermentation requires the presence of both a heat labile component they called "zymase" and a low molecular weight, heat stable fraction called "cozymase". (It was later demonstrated that zymase contains a number of enzymes whereas cozymase consists of metal ions, ATP, ADP, and coenzymes such as NAD.) In order to get the glycolytic pathway started, two molecules of ATP are utilized. This facilitates the addition of phosphate to the six-carbon sugar molecule. The addition of the first phosphate produces glucose phosphate and the addition of the second phosphate produces fructose diphosphate (the two molecules that Arthur Harden characterised). This six-carbon sugar diphosphate is then broken down (through a number of steps) into two three-carbon molecules. Each will add phosphate to two molecules of adenosine diphosphate (ADP) to make two molecules of ATP. In summary, two molecules of ATP are used to energize the process which will produce four molecules of ATP providing a net production of two molecules of ATP for each molecule of sugar that is broken down (Figure 9.2). Arthur Harden shared the 1929 Nobel Prize in chemistry for his work elucidating the glycolytic pathway. Building on these initial observations, the complete glycolytic pathway was elucidated by 1940, largely through the pioneering contributions of Gustav Embden, Otto Meyerhof, Carl Neuberg, Jacob Parnas, Otto Warburg, Gerty Cori, and Carl Cori. Glycolysis is also known as the Embden-Meyerhof pathway.

Glycolysis is a nearly universal process containing a sequence of reactions that metabolizes one molecule of glucose to two molecules of pyruvate

with the concomitant net production of two molecules of ATP. This process is anaerobic (i.e., it does not require O_2). The fate of its end product, pyruvate may vary in different organisms or even in different tissues of the same organism (Figure 9.3). In the absence of oxygen, pyruvate is converted or fermented into lactic acid within lactic acid fermentation or into ethanol within alcoholic fermentation. In the presence of oxygen, the most common situation in multicellular organisms (and many unicellular ones) is that pyruvate can be completely oxidized to carbon dioxide plus water via both the citric acid cycle and the electron transport chain generating much more ATP.

Fermentations yield only a fraction of the energy available from the complete combustion of glucose. Why such a relatively inefficient metabolic pathway is so extensively present is that cells can function in the presence or absence of oxygen. For many organisms and tissues this ability to survive without oxygen affords a host of living accommodations both for multicellular and unicellular organisms. They are considered facultative anaerobes which can function in the presence or absence of oxygen.

Glycolysis and cancer

Otto Heinrich Warburg (1883-1970), a German physiologist and medical doctor was one of the first who associated the type of metabolism to the critical changes in the transition from normal to cancer cells. While working at the Statione Zoologica, the Marine Biological Station in Naples (Italy), Warburg performed research on oxygen consumption in sea urchin eggs after fertilization. He proved that upon fertilization, the rate of respiration increases by as much as sixfold. Interested in medical problems, Warburg investigated the metabolism of tumors (especially within the respiration of cancer cells). In 1924, he hypothesized that cancer is caused by tumor cells that primarily generate energy by non-oxidative fermentation (breakdown) of glucose by the process called glycolysis. This is in contrast to "healthy" cells which mainly generate energy from oxidative breakdown. In 1924, Warburg published his findings in *Über den Stoffwechsel der Tumoren* (*On Metabolism of Tumors*). Remarkably, at that time, the chemistry of glycolysis, although confusing, was beginning to be better understood. The chemistry explaining how enzymes work in respiration was still a secret.

For several decades subsequent to 1924, discussions regarding the special relationship between tumor development, glycolysis and anaerobiosis were very confusing leading to many speculations regarding the role of oxygen deprivation in carcinogenesis. In the 1920's to 1960, a few remarkable findings suggested such a relationship. The fundamental facts that tissue culturing and a very low oxygen pressure are both carcinogenic were unexpectedly discovered. Warburg reported these data in 1927 suggesting that

respiration (but not fermentation) can maintain and create such a high degree of differentiation. However, scepticism remained for a long time.

In this respect, the famous discoveries of Dean Burk and colleagues at the National Cancer Institute (Bethesda, USA) must be mentioned. In 1941, Burk contributed (in a symposium on respiratory enzymes) his view *On the Specificity of Glycolysis in Malignant Liver Tumors as Compared with*

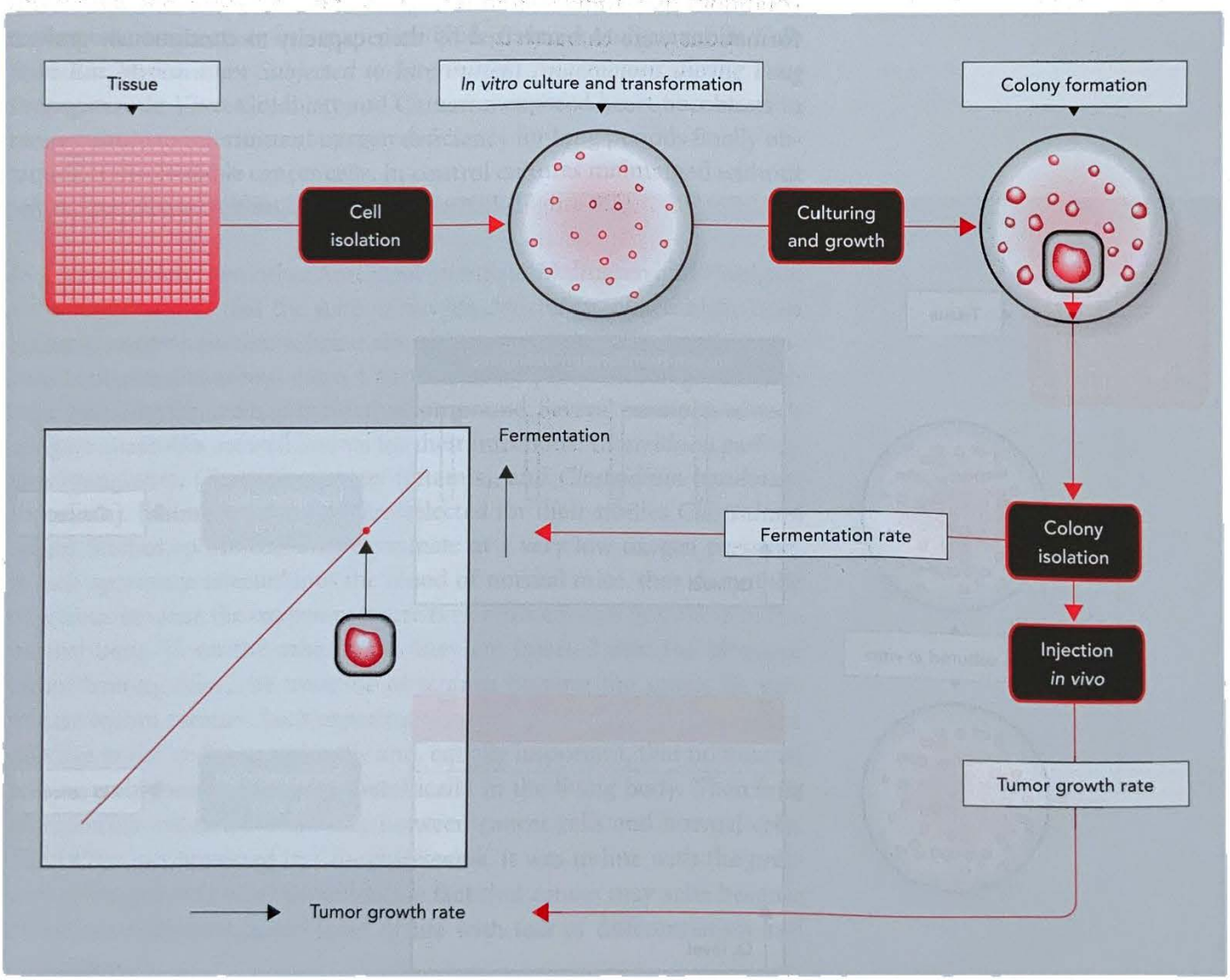


Figure 9.4
Glycolysis and cell transformation. Cells from tissue are isolated and seeded (at very low density). Individual cells are followed during their cell division and colony formation. Colonies with different growth were isolated and used for A. injection *in vivo* animals to determine the growth rate of the tumor, and B. estimation of the fermentation rate. Data show the relationship between malignancy and fermentation rate.

Homologous Adult or Growing Liver Tissues. He demonstrated that cancer metabolism has specific properties differing from the metabolism of regenerating liver (which grows more rapidly than most tumors). Burk's subsequent discoveries (published in 1956 and 1959) documented that he could isolate normal differentiated cells and maintain such cells in culture for longer periods of time under sterile conditions using appropriate culture media.

This work with normal differentiated cells opened all types of new possibilities. For instance, it opened the avenue for culture research regarding conditions that stimulate transformation of these normal cells. Such transformations were characterized by their capacity to continuously grow. It

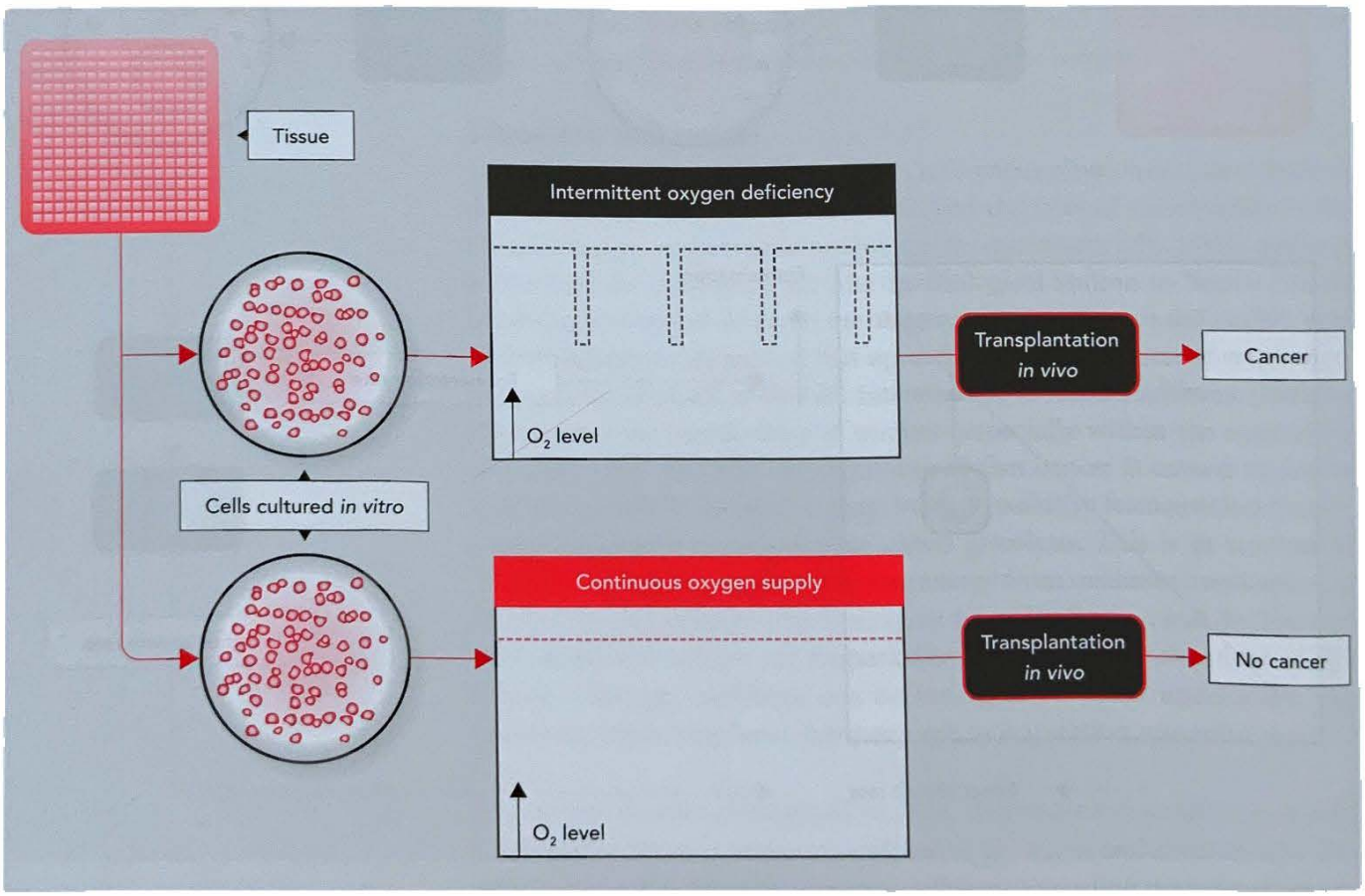


Figure 9.5
Intermittent oxygen deficiency in cancer development. Cells from tissue are isolated and propagated *in vitro*. During culture, the cells are subjected to intermittent deficiency or (as a control) maintained without any oxygen deficiency. Intermittent oxygen deficiency finally results in transplantable cancer cells. In control cultures no cancer cells result.

was even possible to seed the original normal cells at low densities and then observe which cell, after treatment, were able to divide rapidly and develop definite small colonies (Figure 9.4). Burk used such techniques to isolate cancer cells that originated *in vitro* from a single normal cell. A culture of those cells was then injected (*in vivo*) resulting in the development of a tumor in the animal. He was also able to estimate the growth rate of the tumor and compare it with the fermentation rate of the tumor. Such data clearly demonstrated that the more malignant the tumor (more rapidly leading to death), the higher the fermentation rate.

In 1953, the Americans Goldblatt and Cameron further confirmed the cancer/hypoxia connection in a paper entitled, *Induced Malignancy in Cells from Rat Myocardium Subjected to Intermittent Anaerobiosis during Long Propagation In Vitro.* Goldblatt and Cameron exposed heart fibroblasts in tissue culture to intermittent oxygen deficiency for long periods finally obtaining transplantable cancer cells. In control cultures maintained without any oxygen deficiency, no cancer cells resulted (Figure 9.5).

In a different way, two other American scientists, Malmgren and Flanigan, presented evidence that the state of oxygen deficiency (anaerobiosis) can become cancerous in tissue. It had already become known that some organisms (obligate anaerobes) cannot survive in the presence of O_2. For such organisms, oxygen is a highly reactive compound. Several examples of such obligate anaerobes are well known for their infections: *Clostridium perfringens* (gangrene), *Clostridium tetani* (tetanus), and *Clostridium botulinum* (botulism). Malmgren and Flanigan selected for their studies *Clostridium tetani.* Tetanus spores can only germinate at a very low oxygen pressure. If such spores are injected into the blood of normal mice, they do not die of tetanus because the oxygen pressure is not low enough anywhere in the normal body. If, on the other hand, they are injected into the blood of tumor bearing mice, the mice die of tetanus because the spores do germinate within tumors. Such experiments proved conclusively that cancer cells live in the body anaerobically and, equally important, that no normal cells or embryonic cells grow anaerobically in the living body. Therefore, anaerobiosis makes the difference between cancer cells and normal cells. The 1955 study appeared in *Cancer Research.* It was in line with the presence of oxygen deficiency as well as the fact that cancer may arise because of the anaerobiosis induced form of life with loss of differentiation and respiration.

Warburg also determined the growth rate of ascites cancer cells of mice at different oxygen pressures. When the oxygen pressure was lowered from 20 to 0.4% of an atmosphere, the respiration decreases to 1/10 of the normal respiration. No change of the growth rate was observed after this great decline in respiration. However, if the oxygen was completely removed, the

growth rate of the cancer cells was reduced to zero. He compared it with the metabolism of 10-day old chicken embryonic cells during growth. He discovered that such cells, growing at low oxygen pressures for 48 hours, change quantitatively and indefinitely to cancer metabolism (just like anaerobic cells). If high oxygen pressure is applied during growth, the cancer metabolism disappears and the strictly aerobic metabolism of the embryonic cells returns. In longer duration experiments with embryonic mice cells, it was observed that the transformation to cancer metabolism was completely irreversible. Thus, the low oxygen pressure during growth produced cells that grew irreversibly as facultative anaerobes. The intermediates between embryonic cells and cancer cells could arise when the oxygen pressure during growth was low. Their growth rate was not diminished by those changes of the metabolism. However, if the oxygen was removed, growth stopped completely and no change to cancer metabolism occurred. These were remarkable facts on how oxygen creates differentiation and how fermentation destroys it.

Finally, it was possible to grow cancer cells (such as Ehrlich ascites cancer cells of mice) indefinitely (*in vitro*) in manometric vessels provided free amino acids and coenzymes are supplied to the medium. They grew in those vessels even if the oxygen pressure was so low that 95 percent of the energy was supplied by fermentation. This was duplicated for the Ehrlich ascites cancer cells growing in the living animal. However, if a Beckman oxygen microelectrode was inserted into the ascites tumor of a living mouse, the measured oxygen pressure is found to be so low that 95 percent of the required energy had to be supplied by fermentation. Moreover, when a tumor grew in the body, the oxygen pressure in the tumor decreased as the tumor grew larger. If the oxygen pressure approached zero, the cancer cells (like most facultative anaerobes) were harmed. Some died and others separated from the tumor where they settled as metastases elsewhere in the body (growing until they reached their maximum size). Thus, the alternation cycle between aerobiosis and anaerobiosis was secured.

The above facts suggested to Warburg that differentiation is a process which, from thermodynamic reasons, is unlikely to proceed by itself. It can proceed only if energy is supplied. There are two kinds of energy available in life, the energy of respiration and the inferior energy of fermentation. Respiratory energy creates and maintains a high differentiation of body cells. Fermentation energy can only maintain a low differentiation. It follows that if respiration is replaced by fermentation, high differentiation must disappear. "Indeed, cancer cells derived from liver or kidney or muscle have lost all their specific functions and possess only the now useless capacity for the synthesis of nucleic acids and proteins."
The above understanding solved many of the riddles about cancer such as

why so many non-physiologic substances and actions could cause cancer. Cancer is the thermodynamic equilibrium state of all body cells. Almost every non-physiologic substance or action depresses respiration and increases fermentation. The consequence is that differentiation stops and eventually evolves to a complete dedifferentiation equilibrium state of the body cells. Warburg did not consider this answer useless speculation. The thermodynamics was established vis-à-vis many experiments.

According to Warburg, in a lecture at the meeting of the Nobel Laureates on June 30, 1966 (Lindau, Germany) regarding *The Prime Cause and Prevention of Cancer*, he stated that: "Cancer, above all other diseases, has countless secondary causes. But, even for cancer, there is only one prime cause. Summarized in a few words, the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar." Hence, and according to Warburg, cancer should be interpreted (in modern terminology) as a mitochondrial dysfunction. Warburg continued to develop the hypothesis experimentally. Meanwhile, cancer cell glycolysis became the basis of positron emission tomography, a medical imaging technology.

Conclusion

Basically, it was a long journey to get (at least part of) his ideas accepted. When frustrated by the lack of acceptance of his ideas, Warburg was known to quote an aphorism he attributed to Max Planck that science progresses not because scientists change their minds, but rather because scientists attached to erroneous views die and are replaced. He was convinced of the accuracy of his conclusions. Warburg expressed dismay at the "continual discovery of cancer agents and cancer viruses" which he expected to "hinder necessary preventative measures and thereby become responsible for cancer cases" (1956).

Not much has changed since he made this statement. Even recently most medical institutions continue to look for the answers in the wrong areas. In his 1998 book, *One Renegade Cell: How Cancer Begins*, Weinberg (Professor of biology at MIT and former director of the Oncology Research Laboratory at the Whitehead Institute in Cambridge, Massachusetts) presented his view regarding previous decades in the fight against cancer. "The problem with modern cancer researchers' utter failure to find the prime cause of cancer or a valid means of preventing either the initial inception of the disease or a recurrence after remission has been their gradual shift from concentration on practical research to exploring academic and theoretical questions. Many of today's cancer researchers seem to live in a dream world where pet theories may be explored for years without leading to any real solutions to disease".

Regarding the huge effort to explain cancer with genetics, Weinberg stated, "... Something was very wrong. The notion that a cancer developed

MORE ABOUT...

BOX 9.1

■ THE GLYCOLYSIS AND RESPIRATION SWITCH IN MICROORGANISMS: PASTEUR EFFECT AND CATABOLITE REPRESSION

Warburg frequently referred to Louis Pasteur's (1822-1895) description of the influence of oxygen on yeast growth and structure. Pasteur was one of the most important founders of medical microbiology. He discovered that microorganisms cause fermentation and disease. In 1854, Pasteur was appointed Professor of Chemistry at the University of Lille (France) where he was asked to help solve problems related to alcohol production at a local distillery. At that time, fermentation and putrefaction were still often perceived as being spontaneous phenomena, a perception stemming from the ancient belief that life could generate spontaneously. During the 18th century, the idea of spontaneous generation of life forms was pursued by the English naturalist John Turberville Needham and the French naturalist Georges-Louis Leclerc. However, among others, Lazzaro Spallanzani (as discussed in Chapter 2) maintained that life could never spontaneously generate from dead matter. In 1859, Pasteur decided to settle this dispute. He attacked the problem by using a simple experimental procedure. He demonstrated that beef broth could be sterilized by boiling it in a "swan-neck" flask. It has a long bending neck that traps dust particles and other contaminants before they reach the body of the flask. The boiled broth, if never reexposed to air, remained sterile. These experiments not only seemed to settle the philosophical problem of the origin of life but also placed, on solid ground, the new science of bacteriology (which relied on proven techniques of sterilization and aseptic manipulation). He, thus, began a series of studies on fermentative processes. His efforts proved successful in unravelling many of the aspects of fermentation (including the production of compounds such as lactic acid that are responsible for the souring of milk). Such studies led Pasteur to the unexpected discovery that the fermentation process could be arrested by passing air (that is, oxygen) through the fermenting fluid. This aeration of broth stimulates yeasts cell growth to increase. This inhibiting effect of oxygen on the fermentation process became known later as the Pasteur effect. It was the beginning of the unravelling of the glycolysis.

During his famous experiments on anaerobiosis, Pasteur observed that growing yeast anaerobically leads to degeneration in structure if periods of aerobiosis are not interspersed. This was the first hint that oxygen might be involved in the creation of structure or, in other words, in the creation of differentiation. Pasteur laid much stress on his observation. He suggested that it might be one of the great hidden mysteries of life. However, he concluded: "I do not dare to insist upon it today." This was in the year 1876. During the following 90 years, many facts were discovered that strengthened Pasteur's prophecy. In 1967, Warburg wrote: "Pasteur's forecast received even more convincing confirmation in recent years. When Pasteur noted the influence of oxygen on the structure of yeast, it was still unknown that cancer cells are facultative anaerobes similar to yeast. Cancer arises because of a lack of oxygen (or respiratory enzymes) which then produces fermentation in body cells. That leads to a destruction of the differentiation of these cells. Indeed, cancer cells derived from liver, kidney, or muscle have lost all of their specific functions and possess only the now useless capacity for the synthesis of nucleic acids and proteins."

The metabolic shift in yeast

The yeast is a facultative anaerobe and can produce energy using two different metabolic pathways. When oxygen concentration is low, the product of glycolysis (pyruvate) is turned into ethanol and carbon dioxide. The energy production is low (2 moles of ATP per mole of glucose). If the oxygen concentration grows, pyruvate is converted into acetyl CoA that can be used far more efficiently (in the citric acid cycle) which increases efficiency in energy production (to 38 moles of ATP per mole of glucose).

In the Pasteur effect, there is more than "inhibition of fermentation by respiration". There is also a slower rate of glucose uptake by the cell when aerobic is compared with anaerobic conditions. Something must happen in the sugar phosphorylation

pathway to account for this fact. Studies of metabolite levels by Feodor Lynen (1911-1979) and others have consistently demonstrated that a significant increase in hexosemonophosphates accompanies the Pasteur effect. While studying intermediary metabolism in cells in which energy is extracted from cellular nutrients and used to build cellular components, Lynen pointed out that not only the aerobic level of hexose 6-phosphates was higher, but also, that fructosediphoshate was lower than the anaerobic level and that it was obvious that something happened at the stage of the phosphofructokinase reaction. Lynen was awarded half the 1964 Nobel Prize in Physiology and Medicine.

End product inhibition of phosphofructokinase by ATP is obviously a key factor in the regulation of glycolysis. Nevertheless, by itself, it cannot account for the Pasteur effect in yeast because the level of ATP in anaerobically fermenting yeast does not increase when passing to aerobic conditions. This search was continued by the teams of H. Holzer, B. L. Horecker and A. Sols. It was found that an additional feedback inhibition involving citrate may account for the aerobic regulation of phosphofructokinase activity in yeasts. Citrate in glucose utilizing yeast increases in aerobiosis to levels that are inhibitory to phosphofructokinase. The inhibition appears to be cooperative with that of ATP (Figure B.9.1.1).

The above evidence finally led to the conclusion that the normal Pasteur effect in yeast depends on the involvement of a series of feedback mechanisms along the main series of pathways of glucose degradation that afford a good and fast adjustment to the metabolic requirements for both energy and biosynthetic processes.

In addition to these factors that can regulate enzyme activity, aerobic growth may also affect fermentation by changes in the levels of certain glycolytic enzymes. This type of regulation is of particular interest for our understanding of the metabolic main switch in tumor development.

Regulation of enzyme synthesis: substrate induction and catabolite repression

Bacteria such as *E. coli* usually rely on glucose as their source of carbon and energy. However, when glucose is scarce, *E. coli* uses lactose as their carbon source even though this disaccharide does not lie on any major metabolic pathway. An essential enzyme in the metabolism of lactose is β-galactosidase which hydrolyzes lactose into galactose and glucose. An *E. coli* cell growing on glucose contains fewer than 10 molecules of β-galactosidase. In contrast, with lactose as the sole carbon and energy source, the same cell will contain several thousand molecules of the enzyme. The large increase in the amount of β-galactosidase is elicited by the synthesis of new enzyme molecules rather than by activating a pre-existing but inactive precursor. Also, in the presence of both carbon sources, glucose suppresses the synthesis of β-galactosidase until the glucose is totally consumed. Clearly, it would be wasteful to synthesize this enzyme when glucose is abundant.

The synthesis of many, though not of all inducible metabolic enzymes, is repressed by glucose. In 1942, Epps and Gale named

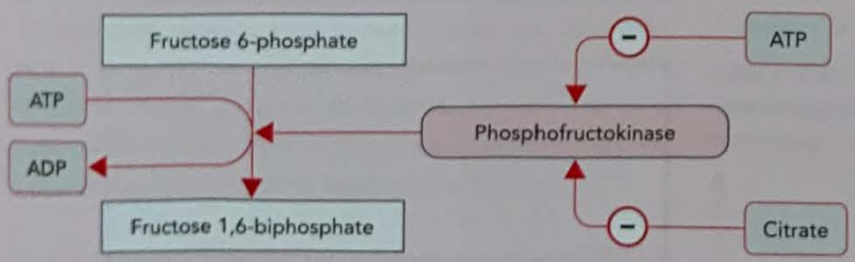


Figure B.9.1.1
The Pasteur effect: energy metabolism is regulated through regulation of enzyme activity. The inhibition of glycolysis was discovered by Pasteur. The consumption of carbohydrate is about sevenfold lower under aerobic conditions than under anaerobic ones. The regulation at the level of phosphofructokinase accounts for most of the Pasteur effect. Phosphofructokinase is the key enzyme in the regulation of glycolysis. The enzyme phosphofructokinase is inhibited by ATP and citrate.

this phenomenon the *glucose effect*. The glucose effect is not limited to bacteria. The same repression in yeast by glucose has been described in the 1950's. The glucose effect proved to be non-specific. It is produced to some extent by any substance which the cell can use as a source of carbon. Observations of this kind have led Mandelstam and Magasanik (in 1961) to suggest that it is some catabolite rather than glucose itself which acts as the repressor. Since then, the effect is often referred to as *catabolite repression*.

Many research teams in the 1960's focused on catabolite repression with reference to a.) poly- and disaccharide splitting

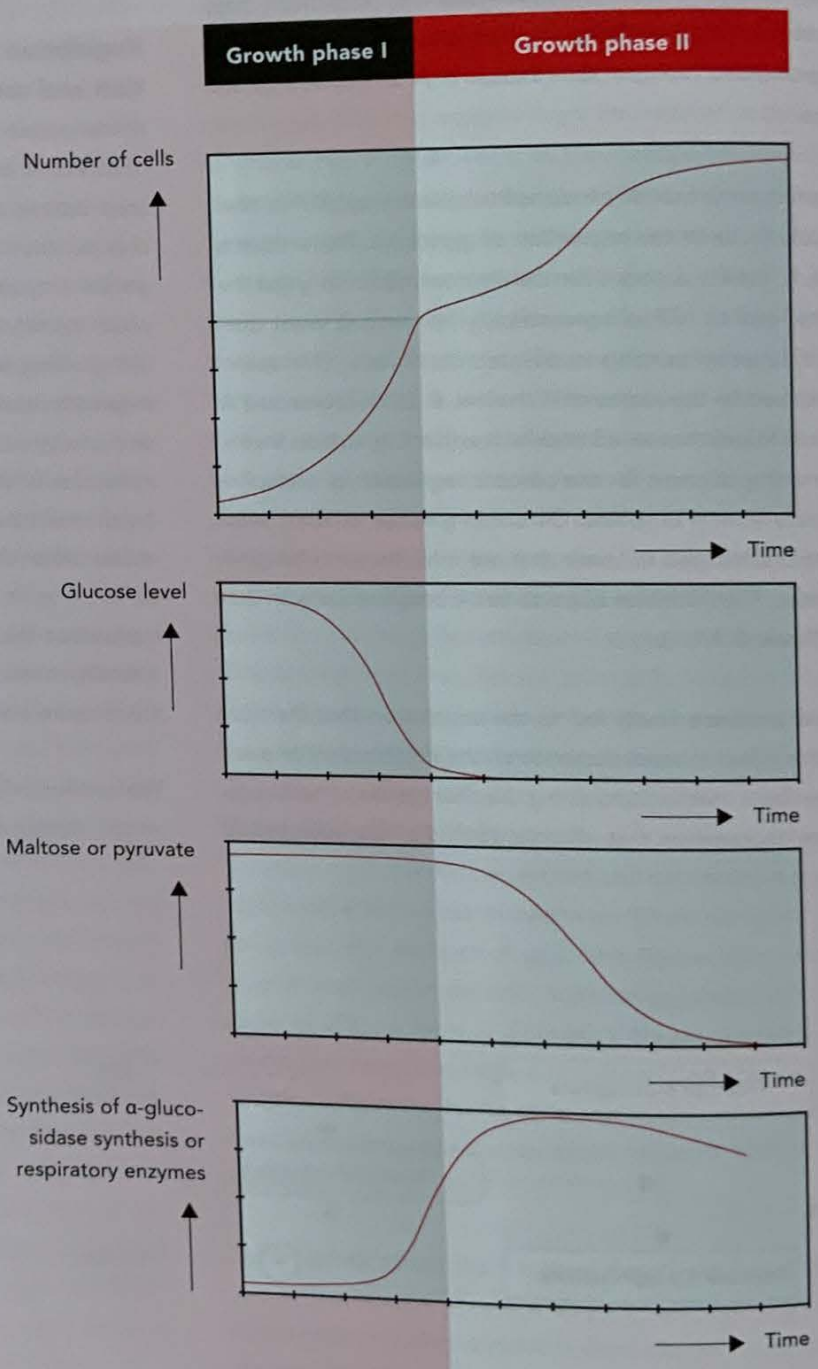


Figure B.9.1.2
Regulation of energy metabolism by catabolite repression of enzyme induction. In a combination of sources of energy, such as glucose and maltose, or glucose and pyruvate, glucose is the preferred carbon source. Only after glucose is exhausted, the enzymes for disaccharide utilization (for instance α-glucosidase) or for oxidative breakdown by the citric acid cycle and respiratory chain are synthesized. This temporary catabolite repression results in a biphasic growth curve, also called diauxie. The secondary lag during diauxic growth represents the time required for the loss of glucose repression and the induction of α-glucosidase or mitochondrial enzymes.

enzymes and b.) respiratory enzymes (Figure B.9.1.2). For example, the emphasis was first placed on the disaccharide splitting enzyme, α-glucosidase. The enzyme α-glucosidase splits maltose into 2 glucose molecules. Studies on inducible α-glucosidase synthesis in *Saccharomyces carlsbergensis* and *Saccharomyces cerevisiae* were started in the early 1960's by Konigsberger, De Kloet and Van Wijk. The yeast *S. carlsbergensis* has very low levels in the absence of maltose and it is able to synthesize α-glucosidase at a 250 x increased rate in the presence of the substrate maltose. The team studied the effects of glucose at various concentrations regarding this inducible α-glucosidase synthesis in the presence of the inducer maltose. In addition, the synthesis of α-glucosidase was estimated in cells which were grown on other monosaccharide sugars besides glucose (fructose, mannose, or galactose). Similar studies were also performed in *S. cerevisiae*. That yeast demonstrated higher enzyme levels in the absence of maltose (whereas the induction by maltose was correspondingly less). That situation was labeled semi-constitutive enzyme synthesis. The researchers concluded that repression of α-glucosidase synthesis was strongly dependent on the concentration as well as the nature of the carbon source. It corresponded with Mandelstam's 1962 data. That is, the "better" carbon sources (i.e., carbon sources on which a higher growth rate is observed) demonstrated a more pronounced inhibitory effect.

The other example of enzyme synthesis in yeast being affected by catabolite repression is the group of inducible enzymes involved in the respiratory activity of the yeast cell (i.e., enzymes of the citric acid cycle and of the respiratory chain). The effects of O_2 and catabolite expression on respiratory enzymes in yeast were studied by a number of authors. To name a few: Slonimsky, Strittmatter, Tustanoff, Bartley, Polakis, Meek, and Görts. Generally speaking, they observed that both anaerobiosis and growing on glucose initiated a repression of the synthesis of the respiratory enzymes and a correspondingly low respiratory activity. Using different types and concentrations of carbon sources for growth, they also demonstrated the occurrence of a correlation in the regulation of levels of some individual enzymes in the yeast cell and its respiratory activity.

The challenge to compare the catabolite repression of a disaccharide splitting enzyme (α-glucosidase) and that of some enzymes involved in oxidative carbohydrate metabolism (respiration) in yeast (both under aerobic and anaerobic conditions) were scientifically researched by Görts (regarding *S. cerevisiae*) and by Van Wijk (regarding *S. carlsbergensis*). They documented that a decrease in glucose concentration at the end of the logarithmic growth phase coincides with a derepression of α-glucosidase synthesis and in a strongly correlative fashion, with the synthesis of a number of citric acid cycle enzymes and respiratory activity.

It was concluded that the effect of the sugars appears to be qualitatively the same on both α-glucosidase synthesis and on citric acid cycle enzymes.

The role of cyclic AMP

Very little, if anything, was known about the nature of the metabolite acting as the active repressor. In studies on the repressing effect of various carbon courses and the high degree of correlation with regard to the synthesis of different enzymes, Magasanik, McFall and Mandelstam suggested (in 1963) that the active compound involved in catabolite repression is something metabolically closely related to the end product formed by action of the enzyme (of which the synthesis is being repressed). In this case, it should be related to energy metabolism in general. It was completely unexpected, however, that catabolite repression is due to the ability of carbon sources to lower the intracellular concentration of cyclic 3',5'-AMP. At that time, cyclic 3',5'-AMP was known to function as a mediator of hormone induced changes in the metabolism of vertebrates. Van Wijk and Konijn suggested a regulatory role for cyclic 3',5'-AMP in yeast based on its cell level under various conditions of catabolite repression. Since chemical techniques were not sensitive enough to detect very low levels of cyclic 3',5'-AMP, they used a sensitive microbiological assay based on the attraction of amoebae of the cellular slime mold *Dictyostelium discoideum* by cyclic 3',5'-AMP. They concluded that the intracellular cyclic 3',5'-AMP levels of yeast cells corresponded with the different degrees of catabolite repression. Later on, it became apparent that cyclic 3',5'-AMP plays a crucial clue in the mechanism of gene regulation. It functions in close cooperation with one of the DNA binding proteins that regulate transcription.

through the successive activation of a series of oncogenes had lost its link to reality". He details failure after failure of cancer researchers to find cancer's cause or cure. The prime cause of cancer is not "genetic". In the section, "Conquering Cancer by Preventing It", Weinberg states: "We must address the ultimate roots of cancer before we make substantial reductions in cancer incidence. Genes and proteins will help us little here."

Here lies the cry to look elsewhere than genetics for the roots of cancer. The key is glycolysis. There is no cancer cell whose cell respiration is intact. All cancer cells share this unique characteristic. And, if it is so much decreased that the oxygen transferring enzymes are no longer saturated with oxygen, respiration can decrease irreversibly and normal cells can be transformed into facultative anaerobes. And, once sufficient oxygen deficiency damage is done to a cell, it cannot ever be repaired. There is a "point of no return".

The mysterious interplay between glycolysis and respiration was not limited to animal cancer cells. In the 1950's a similar switch between glycolysis and respiration became apparent in microorganisms. Unlike animal cells, most microorganisms demonstrate the conditions that regulate this switch.

Unfortunately, cancer research in the field of oncology had hardly any attention for this phenomenon. It did not contribute to the hot dispute about the prime cause of cancer. However, the next step in the biochemical research arena was already active. The research on transplantable hepatomas rapidly developed and opened further insights into both dualities "differentiation/proliferation" as well as "respiration/glycolysis".

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10

More about Glycolysis and Respiration vis-à-vis Cancer as a Model and its Relationship to Proliferation

During carcinogenesis the organ undergoes extensive morphological and biochemical alterations which culminate in the development of cancerous tissue. Comparing many of the tumors that originated in this way demonstrated that tumors in the same organ could strongly differ in histological structure, differentiated biological behavior, and growth rate. To study this process, several research groups (e.g. Novikoff, Morris, etc.) searched for possibilities to have each tumor available in its specific individual state of differentiation and growth. If a specific tumor could be maintained, it should allow a.) a systematic study of the metabolic lesion(s) underlying the morphological and biological properties and b.) a comparative study of both the qualitative and quantitative alterations that emerge.

This became possible by utilizing the technique of "transplantation" of a tumor from one animal to another. Tumor tissue was isolated and divided into very small pieces. A small amount of tumor tissue was then injected subcutaneously. The tumor cells divided and the tumor increased in size concomitantly with the induction of a blood supply. In rats such tumors may reach a diameter of a few centimetres. Prior to the next transplantation, the tumor is isolated again, necrotic tissue is removed and the tumor tissue divided into small pieces which again are then injected subcutaneously into new animals. By utilizing such a transplantation procedure, a specific tumor can continuously be grown in animals for many generations. Most of the chemically induced, transplantable solid tumor lines revealed remarkable histological properties of stability, growth rate, and permitted good reproducibility of the studies in many laboratories.

Glucose metabolism

Brain, muscle, adipose tissue, kidney, and liver possess unique differentiation and metabolic profiles. Each organ is strikingly different in morphology and physiologic functioning. The metabolic patterns also differ in their use of fuels to meet their energy needs. In the 1950's (with reference to transplantation) much attention was focused on the metabolism of liver and its changes during the process of hepatocarcinogenesis.

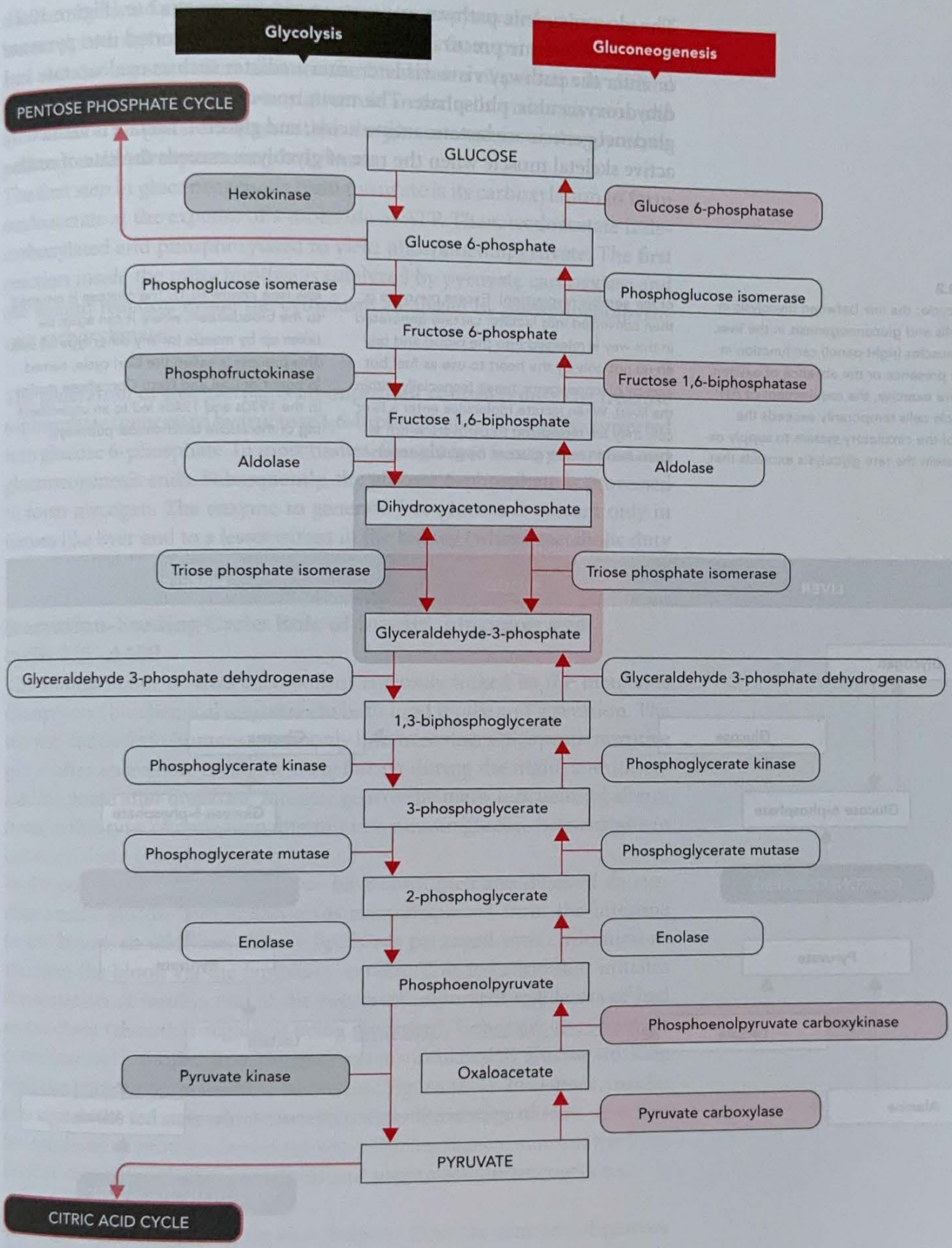
The liver (which can be 2% to 4% of body weight) is an organism's meta-

Figure 10.1 ►
Pathway of glycolysis (left) and gluconeogenesis (right). These pathways have nine intermediates and seven enzyme catalyzed reactions in common. The three essentially irreversible reactions of the glycolytic pathway (in gray shading) are circumvented in gluconeogensis by four bypass reactions (in red shading). The connection to two other main pathways is indicated. On top is the connection to the pentose phosphate shunt (important for the production of RNA and DNA). At the bottom is the connection to the citric acid cycle (important in oxidative energy production).

bolic hub. The metabolic activities of the liver are essential for providing fuel to the brain, muscle, and other peripheral organs. Most compounds absorbed by the intestine first pass through the liver which is able to regulate the level of many metabolites in the blood. With reference to our previous interest in sugar metabolism (Chapter 9), we must first consider how the liver metabolizes carbohydrates (Figure 10.1).

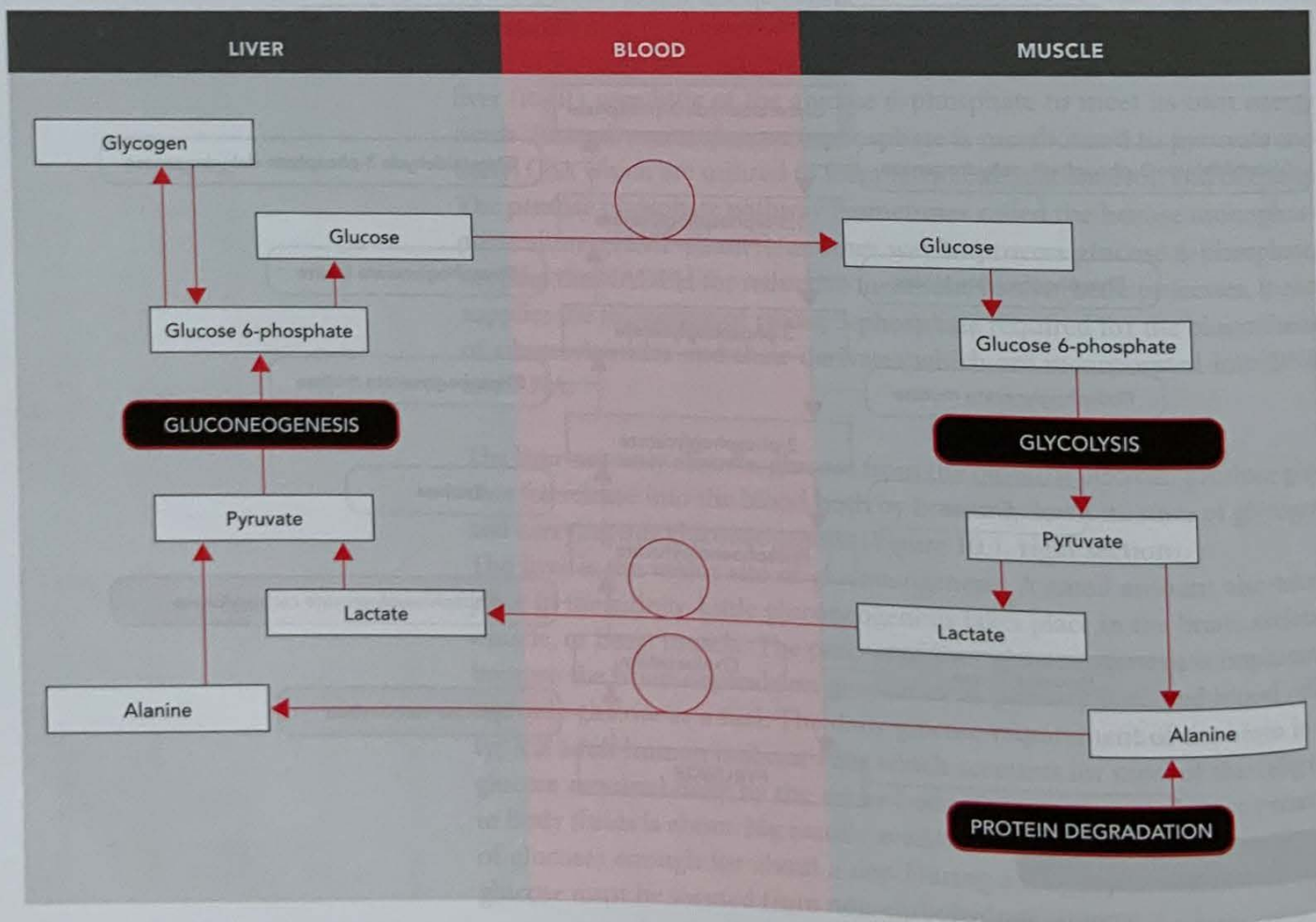
The liver removes two-thirds of the glucose and all of the remaining monosaccharides from the blood. Some glucose is left in the blood for use of other tissues. The absorbed glucose is converted in the liver into glucose 6-phosphate utilizing hexokinase and the liver-specific glucokinase. However, the liver (itself) uses little of the glucose 6-phosphate to meet its own energy needs. Instead, excess glucose 6-phosphate is metabolized to pyruvate and acetyl CoA which are utilized to form fatty acids, cholesterol, and bile salts. The pentose phosphate pathway [sometimes called the hexose monophosphate shunt (HMP-shunt)], another way to process glucose 6-phosphate, supplies the NADPH for reductive metabolic biosynthetic processes. It also supplies the formation of ribose 5-phosphate required for the biosynthesis of ribonucleotides and their derivates which are incorporated into DNA and RNA.

The liver not only absorbs glucose from the blood, it also can produce glucose for release into the blood both by breaking down its store of glycogen and carrying out gluconeogenesis (Figure 10.1, right section).
The liver is the major site of gluconeogenesis. A small amount also takes place in the kidney. Little gluconeogenesis takes place in the brain, skeletal muscle, or heart muscle. The process of liver gluconeogenesis is important because the brain depends on glucose as its primary fuel. Red blood cells use only glucose as a fuel. The daily glucose requirement of the brain in a typical adult human is about 120g which accounts for most of the 160g of glucose required daily by the entire body. The amount of glucose present in body fluids is about 20g readily available from glycogen (a storage form of glucose) enough for about a day. During a longer period of no eating, glucose must be formed from non-carbohydrate sources


Glycolysis
Gluconeogenesis
PENTOSE PHOSPHATE CYCLE
GLUCOSE
Hexokinase
Glucose 6-phosphatase
Glucose 6-phosphate
Phosphoglucose isomerase
Phosphoglucose isomerase
Fructose 6-phosphate
Phosphofructokinase
Fructose 1,6-biphosphatase
Fructose 1,6-biphosphate
Aldolase
Aldolase
Dihydroxyacetonephosphate
Triose phosphate isomerase
Triose phosphate isomerase
Glyceraldehyde-3-phosphate
Glyceraldehyde 3-phosphate dehydrogenase
Glyceraldehyde 3-phosphate dehydrogenase
1,3-biphosphoglycerate
Phosphoglycerate kinase
Phosphoglycerate kinase
3-phosphoglycerate
Phosphoglycerate mutase
Phosphoglycerate mutase
2-phosphoglycerate
Enolase
Enolase
Phosphoenolpyruvate
Phosphoenolpyruvate carboxykinase
Pyruvate kinase
Oxaloacetate
Pyruvate carboxylase
PYRUVATE
CITRIC ACID CYCLE

The gluconeogenic pathway converts pyruvate into glucose (Figure 10.2). Non-carbohydrate precursors of glucose are first converted into pyruvate or enter the pathway vis-à-vis later intermediates such as oxaloacetate and dihydroxyacetone phosphate. The main non-carbohydrate precursors for gluconeogenesis are lactate, amino acids, and glycerol. Lactate is formed by active skeletal muscle when the rate of glycolysis exceeds the rate of oxida-

Figure 10.2
The Cori cycle: the link between glycolysis in muscle cells and gluconeogenesis in the liver. Skeletal muscles (right panel) can function in either the presence or the absence of oxygen. With severe exercise, the requirement of ATP from muscle cells temporarily exceeds the capacity of the circulatory system to supply oxygen (wherein the rate glycolysis exceeds that of the aerobic respiration). Excess pyruvate is then converted into lactate. Lactate generated in this way is released into the blood and taken up not only by the heart to use as fuel but also by gluconeogenic tissue (especially within the liver). When lactate molecules enter a liver cell, they are reoxidized to pyruvate which is then used to make glucose by gluconeogenesis (see Figure 10.1). The glucose is returned to the bloodstream where it can again be taken up by muscle (or any other type of) cells. This process is called the Cori cycle, named in honor of Carl and Gerti Cori whose studies in the 1930s and 1940s led to an understanding of the above biochemical pathways.



tive metabolism. Lactate is readily converted into pyruvate by the action of lactate dehydrogenase. Amino acids are derived from proteins in the diet and also during starvation vis-à-vis the breakdown of proteins in skeletal muscle. The hydrolysis of triacylglycerols in fat cells yields glycerol and fatty acids. The glycerol enters either the gluconeogenic or the glycolytic pathway at dihydroxyacetone phosphate.

The first step in gluconeogenesis from pyruvate is its carboxylation to form oxaloacetate at the expense of a molecule of ATP. Then, oxaloacetate is decarboxylated and phosphorylated to yield phosphoenolpyruvate. The first reaction inside the mitochondria is catalyzed by pyruvate carboxylase and the second reaction inside the cytoplasm catalyzed by phosphoenolpyruvate carboxykinase.

The generation of free glucose is an important control point. The fructose 6-phosphate (generated by fructose 1,6-biphosphatase) is readily converted into glucose 6-phosphate. In most tissues, free glucose is not generated and gluconeogenesis ends. Subsequently, the glucose 6-phosphate is processed to form glycogen. The enzyme to generate free glucose is present only in tissues like liver and to a lesser extent in the kidney (whose metabolic duty is to maintain blood glucose homeostasis).

Starvation-Feeding Cycle: Role of insulin, glucagon and cyclic 3',5'-AMP

The multipotency of liver metabolism is closely linked to the metabolic changes and biochemical responses to both food intake and starvation. The starved-fed cycle in humans commonly has three stages: the postabsorptive phase after an evening meal, the early fasting during the night, and the refeeding phase after breakfast. A major goal of the many biochemical alterations in this type of cycle is an attempt to maintain glucose homeostasis (a constant blood glucose level).

In the postabsorptive state (after we have consumed and digested an evening meal), glucose and amino acids are transported from the intestine to the blood. In addition, dietary lipids are packaged into chylomicrons reaching the blood via the lymphatic system. This fed condition initiates the secretion of insulin, one of the two most important regulators of fuel metabolism (the other regulator being glucagon). Generally, the secretion of the insulin by the β-cells of the pancreas is stimulated by glucose working with the parasympathetic nervous system (Figure 10.3). In essence, insulin also signals the fed state which then stimulates the storage of fuels as well as the synthesis of proteins in various ways. During this fed state, in the liver, insulin stimulates glycogen synthesis and suppresses gluconeogenesis.

During this time of plenty, the liver helps to limit the amount of glucose in the blood by storing it as glycogen so that in the future it may be able to

quickly release glucose in times of scarcity. Hence, insulin accelerates the uptake of blood glucose into the liver. Then, the level of glucose 6-phosphate in the liver rises. The increase in glucose 6-phosphate coupled with insulin action leads to a buildup of glycogen stores. The hormonal effects on glycogen synthesis and storage are reinforced by a direct action of glucose itself. Thus, glucose shifts the glycogen system from a degradative to a synthetic mode. The high insulin level in the fed state also promotes the entry of glucose into muscle and adipose tissue. The action of insulin also extends to amino acid and protein metabolism. Insulin promotes the uptake of branched chain amino acids (valine, leucine, and isoleucine) by muscle and stimulates protein synthesis which favors a building up of muscle protein. In addition, it inhibits the intracellular degradation of proteins.
In an early fasting state, several hours after a meal, the blood glucose level begins to drop leading to a decrease in insulin secretion and a rise in glucagon secretion (Figure 10.3). Glucagon is secreted by the α-cells of the pancreas in response to a low blood sugar level in the fasting state. Just as insulin signals the fed state, glucagon signals the starved state. It serves to mobilize glycogen stores when there is no dietary intake of glucose. The main target organ of glycagon is the liver. Glucagon stimulates glycogen breakdown and inhibits glycogen synthesis. Glucagon also inhibits fatty acid synthesis by diminishing the production of pyruvate and by lowering the activity of acetyl CoA carboxylase. In addition, glucagon stimulates gluconeogenesis in the liver and blocks glycolysis by lowering the level of fructose 2,6-biphosphate.

All of the known actions of glucagon are mediated by cyclic 3',5'-AMP. The activation of the cyclic 3',5'-AMP dependent protein kinases results in a rapid mobilization of glycogen. The large amount of glucose formed by the hydrolysis of glucose 6-phosphate (derived from glycogen) is then released from the liver into the blood. The entry of glucose into muscle and adipose tissue decreases in response to a low insulin level. The diminished utilization of glucose by muscle and adipose tissue also contributes to the maintenance of the blood glucose level. The result of a depletion of the liver's glycogen stores is that gluconeogenesis from lactate and alanine replaces glucose in peripheral tissues. Moreover, the brain oxidizes glucose completely to CO_2 and H_2O. Thus, for the net synthesis of glucose to occur, another source of carbons is required. Glycerol released from adipose tissue vis-à-vis lipolysis provides some of the carbons. The remaining carbons are present via the hydrolysis of muscle proteins.

In the starved-fed cycle, the biochemical response to refeeding (breakfast) is slightly different from the normal fed state. Glucose is not processed exactly as it is processed in the normal fed state. The liver does not initially absorb glucose from the blood, but rather leaves it for the peripheral tis-

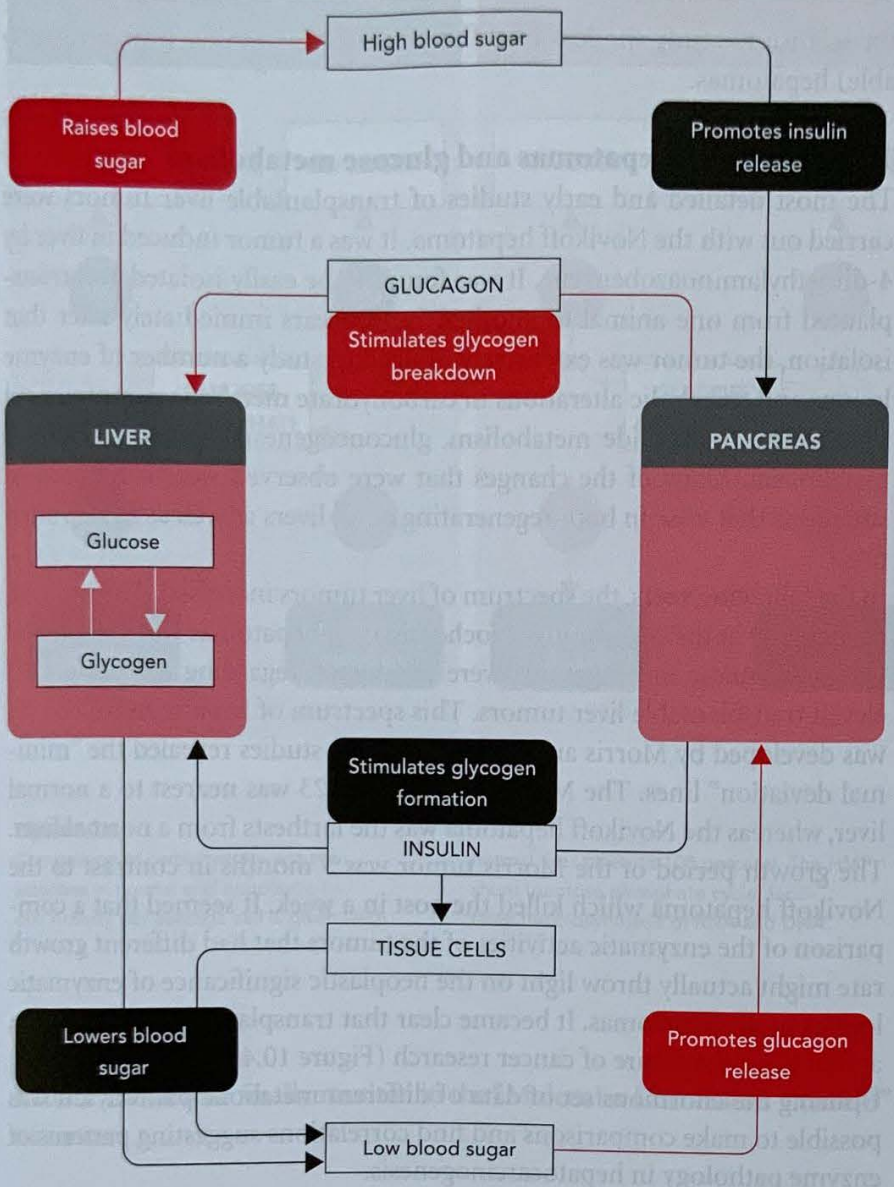


Figure 10.3
Insulin and glucagon in glucose metabolism. Insulin and glucagon have opposite effects on liver and other tissues for controlling blood glucose level. The pancreas has many islets containing insulin-producing β-cells and glucagon producing α-cells.

sues. Moreover, the liver remains in a gluconeogenic mode. Now, the newly synthesized glucose is used to replenish the liver's glycogen stores. As the blood glucose levels continue to rise, the liver completes the replenishment of its glycogen stores and begins to process the remaining excess glucose for fatty acid synthesis.

It is evident that liver, in utilizing its differentiated functioning to maintain glucose levels via glycolysis and gluconeogenesis, is an interesting model

for science to study the loss of differentiated functioning in (transplantable) hepatomas.

Transplantable hepatomas and glucose metabolism

The most detailed and early studies of transplantable liver tumors were carried out with the Novikoff hepatoma. It was a tumor induced in liver by 4-dimethylaminoazobenzene. It was found to be easily isolated and transplanted from one animal to another. In the years immediately after that isolation, the tumor was extensively utilized to study a number of enzyme lesions and metabolic alterations in carbohydrate metabolism, amino acid metabolism, nucleotide metabolism, gluconeogenesis, and phospholipid metabolism. Many of the changes that were observed were compared to situations that arise in both regenerating adult livers as well as in newborn rats.

In the following years, the spectrum of liver tumors increased. The interest, particularly in the comparative biochemistry of hepatomas was heightened when enzymatic investigations were conducted regarding a spectrum of eleven transplantable liver tumors. This spectrum of hepatomas of the rat was developed by Morris and colleagues. Such studies revealed the "minimal deviation" lines. The Morris hepatoma 5123 was nearest to a normal liver, whereas the Novikoff hepatoma was the farthests from a normal liver. The growth period of the Morris tumor was 3 months in contrast to the Novikoff hepatoma which killed the host in a week. It seemed that a comparison of the enzymatic activities of the tumors that had different growth rate might actually throw light on the neoplastic significance of enzymatic lesions in the hepatomas. It became clear that transplantable tumors were, at that time, the future of cancer research (Figure 10.4).

Utilizing the enormous set of data of different metabolic pathways, it was possible to make comparisons and find correlations suggesting patterns of enzyme pathology in hepatocarcinogenesis.

Molecular Correlation Concept

In 1959 and 1961, the first correlation studies were published by George Weber and colleagues at the Laboratory for Experimental Oncology, Indiana University School of Medicine. It was evident that three main propositions were considered: 1.) The biochemical strategy of the genome could be identified by elucidating the pattern of gene expression as revealed in the activity, concentration, and isozyme programs of key enzymes; 2.) Activities of key enzymes in metabolic pathways and concentrations of strategic metabolites were stringently linked with neoplastic transformation and progression. Those not stringently linked did not yield a pattern; 3.) The linking of enzymic and metabolic imbalance with transformation and progression could be identified in a spectrum of tumors that would include graded malignancy plus proliferative rates, and degrees of differen-

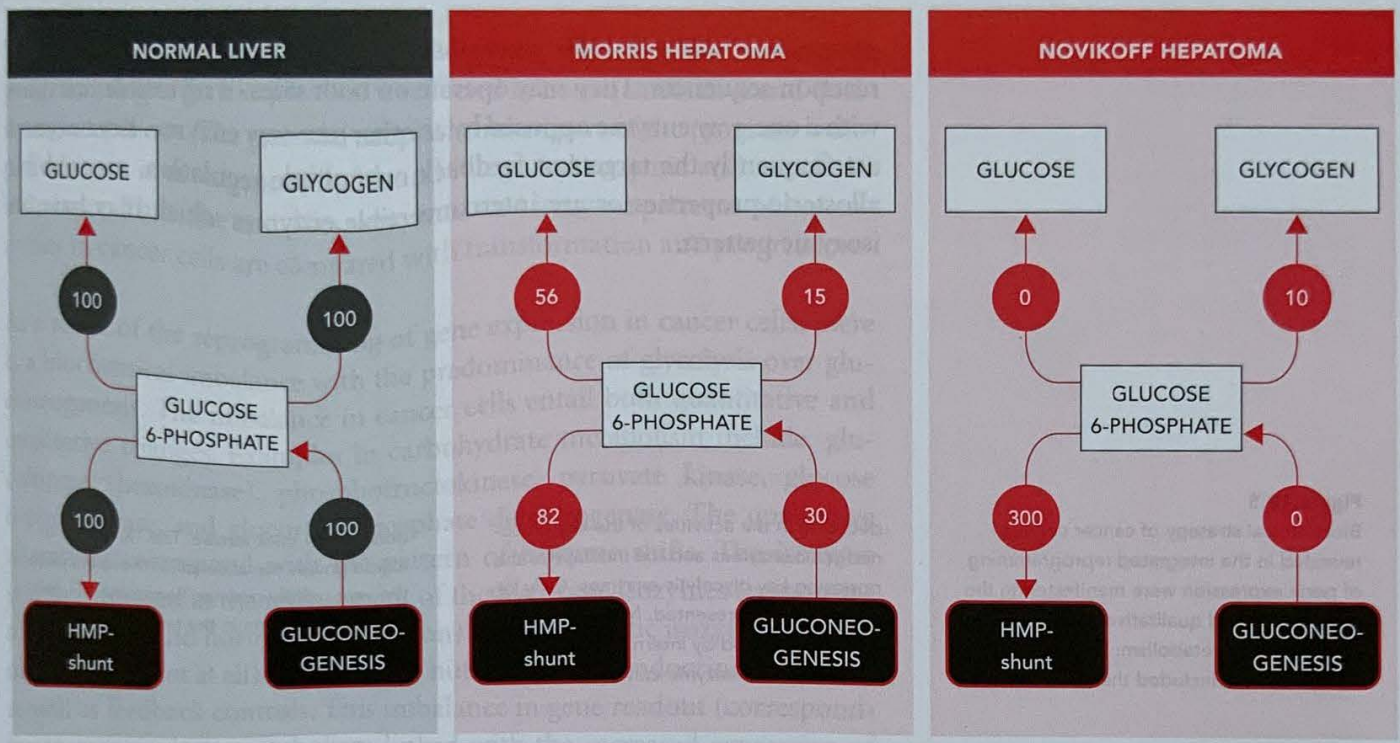


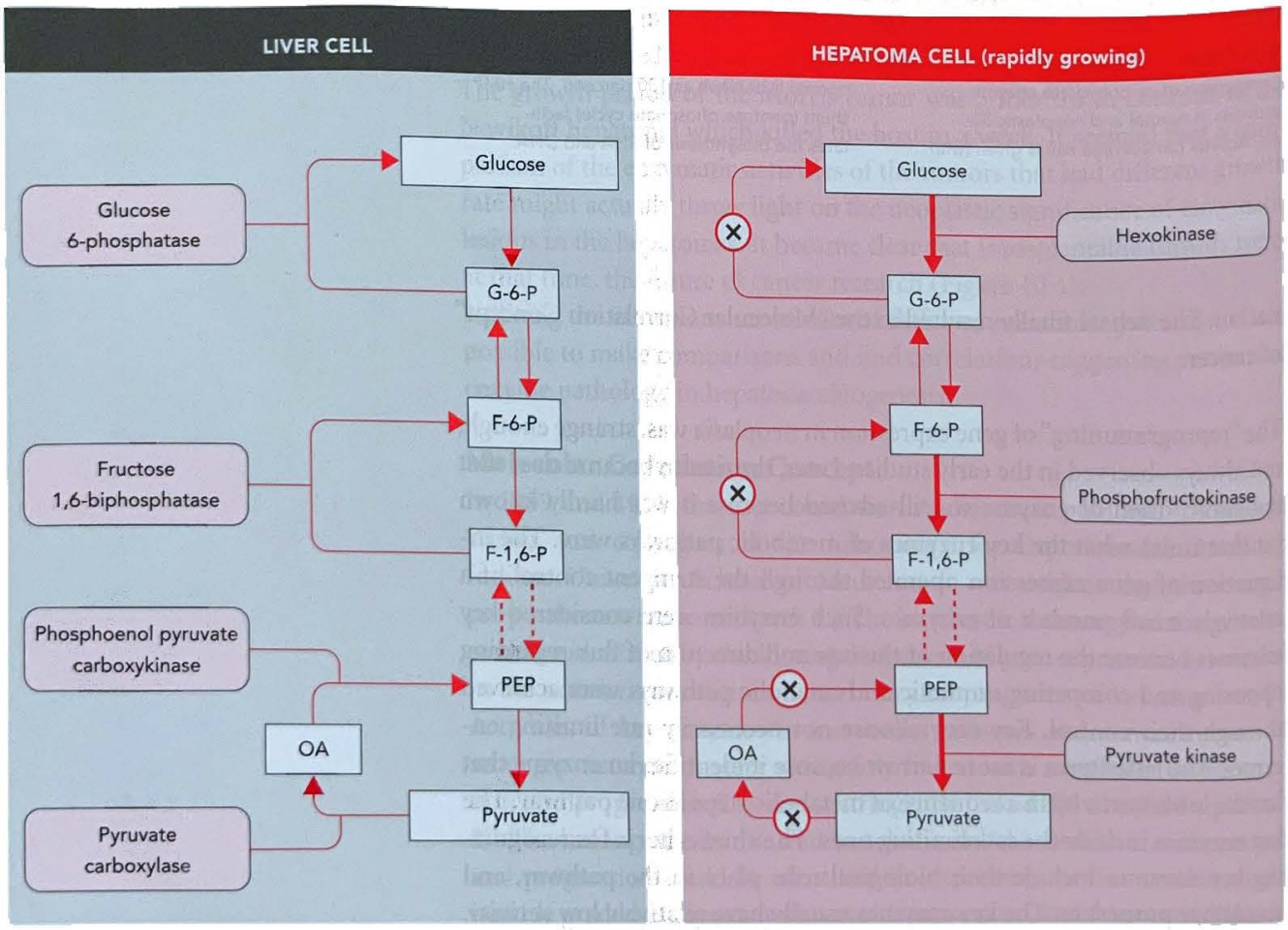
Figure 10.4
Comparison of carbohydrate enzyme activities in normal and neoplastic livers. Activity per average cell is given (with normal liver taken as 100 percent). The HMP shunt (pentose phosphate cycle) facilitates the biosynthesis of RNA and DNA.

tiation. The debate finally resulted in the "Molecular Correlation Concept" of cancer.

The "reprogramming" of gene expression in neoplasia was, strange enough, not always observed in the early studies. Later, the reason became clear that the choice then of enzyme was ill-advised because it was hardly known (at that time) what the key enzymes of metabolic pathways were. The integration of gene expression operated through the stringent control of a relatively small number of enzymes. Such enzymes were considered key enzymes because the regulation of the rate and direction of flux regarding opposing and competing synthetic and catabolic pathways were achieved through their control. Key enzymes are not necessarily rate limiting enzymes. The latter term is more narrow because it identifies an enzyme that has the lowest activity in a sequence of metabolic steps in one pathway. The key enzymes include the rate limiting ones. The chief criteria for recognizing key enzymes include their biological role, place in the pathway, and regulatory properties. The key enzymes usually have relatively low activity,

govern one-way metabolic reactions, and frequently are the first or last in reaction sequences. They may operate on both sides of reversible reactions with a one-way enzyme opposed by another one-way enzyme. Key enzymes are frequently the targets of feedback or multiple regulation, may exhibit allosteric properties or are interconvertible enzymes which may have an isozyme pattern.

Figure 10.5
Biochemical strategy of cancer cells as revealed in the integrated reprogramming of gene expression were manifested in the quantitative and qualitative imbalance in carbohydrate metabolism. The quantitative alterations included the decrease in the decrease in the activities of the key gluconeogenic enzymes and the increase of the opposing key glycolytic enzymes. Only key enzymes were presented. Non-key steps were represented by interrupted arrows. The increase in enzyme concentration was denoted by thick arrows. The "X" in the arrows signifies the absence of those enzymes in the very rapidly growing hepatomas. Further discussion is given in the text and references.



By focusing on the key enzymes and isozymes, it was evident that the reprogramming of gene expression is reflected in both quantitative and qualitative alterations. The essential neoplasia linked part of the program is displayed in an ordered, meaningful, and integrated enzymic and metabolic imbalance. Thus, the activities, amounts, and isozyme patterns of key enzymes in cancer cells are compared with transformation and progression.

As a result of the reprogramming of gene expression in cancer cells, there is a biochemical imbalance with the predominance of glycolysis over gluconeogenesis. The imbalance in cancer cells entail both quantitative and qualitative changes. Examples in carbohydrate metabolism include: glucokinase (hexokinase), phosphofructokinase, pyruvate kinase, glucose 6-phosphatase, and glucose 6-phosphate dehydrogenase. The qualitative alterations correspond with the pattern of isozyme shifts. The isozyme shift is expressed in the replacement of the liver type isozymes (responsive to nutritional and hormonal regulation) by non-hepatic isozymes that are much less (or not at all) responsive to nutritional and endocrine regulation as well as feedback controls. This imbalance in gene readout (corresponding to carbohydrate metabolism linked with the increased expression of malignant properties) confers selective advantages to cancer cells.

Regulation of DNA and RNA metabolism

A similar situation was observed in another part of the metabolism regulating DNA and RNA synthesis (Figure 10.6). Examples in the pyrimidine metabolism are: carbamoylphosphate synthetase II, CTP synthetase, ribonucleotide reductase, DNA polymerase, and dihydrothymidine dehydrogenase. Examples in the purine metabolism are: amidophosphoribosyltransferase, xanthine oxidase, and IMP dehydrogenase. Further studies demonstrated an increase in the activity of the enzyme transaldolase which channels fructose 6-phosphate to ribose 5-phosphate (R-5-P) production. R-5-P is the precursor of phosphoribosylpyrophosphate (PRPP) which is an indispensable substrate for both de novo and salvage pathways of purine and pyrimidine biosynthesis (and, correspondingly, the biosynthesis of RNA and DNA).

As in the pyrimidine metabolism, there was also a clear-cut imbalance in purine metabolism in the activities of the antagonistic key enzymes as well as in the opposing synthetic and degradative pathways. The activities of the key enzymes in the de novo and salvage pathways of purine metabolism (as in the pyrimidine metabolism) as well as the activities of the catabolic enzymes (nucleotidase, inosine phosphorylase, xanthine oxidase, and uricase) had much higher activities than those of the rate limiting and subsequent synthetic enzymes of the de novo pathway (glutamine PRPP amidotransferase, and amidophosphoribosyltransferase). In the utilization of IMP, the central purine metabolite for ATP biosynthesis, the activities

Figure 10.6 ►
Integrated reprogramming of gene expression manifested in the imbalance of key enzymes of biosynthesis, degradation, and utilization of pyrimidine and purine biosynthesis involved in DNA synthesis. Names of enzymes are omitted. Enzyme activities that are increased with hepatoma growth rate are denoted by thick arrows. In each pathway of a distinct deoxyribonucleotide, specific key enzymes have been induced. The breakdown pathways starting from uracil and thymidine (as part of the pyrimidine pathway) and from inosine (as part of the purine pathway) are presented by downward arrows. The nucleotide type of intermediates are presented as abbreviations. Further details are in the text and references.

of the synthetic enzymes (adenylosuccinase, and adenylosuccinate synthetase) were markedly elevated. In the de novo biosynthesis from IMP to GTP, the rate limiting enzyme, IMP dehydrogenase had the lowest activity among all the enzymes of purine biosynthesis. The activities of this enzyme and the subsequent GMP synthetase were markedly increased. The highest activities observed were those of ADP and GDP reductase which synthesize the dADP and dGDP leading to the production of dATP and dGTP. Since the activities of the powerful enzymes of degradation markedly declined, the imbalance favors the purine biosynthetic activities.

Typically, this change in the imbalance of the pyrimidine and purine pathways can best be understood from the analysis of the balance of opposing pathways and key enzymes of synthesis and degradation. The key enzymes in the de novo and salvage pathways of pyrimidine and DNA biosynthesis increase while the opposing catabolic enzymes decrease in parallel with tumor growth rates. To this end, the rate of proliferation of these tumors is quantified by several independent techniques: biological (size or volume of tumor), cytological (percentage of cells in mitosis), histological (degree of differentiation), and biochemical (percentage of thymidine incorporating cells). Ranking of malignancy of the tumors are based on the time required for the neoplasm to reach a uniform diameter of 1.5 cm. All the methods yielded the same ranking order of the tumors. Consequently, a progressively expressed imbalance emerges in the tumors between the capacities of the synthetic and catabolic enzymes and also between the overall activities of the opposing pathways of anabolism and catabolism. As a result, the ratios of thymidine incorporation into DNA and the rate of thymidine degradation to carbon dioxide yield the closest correlation with tumor growth rates among all the biochemical factors (Figure 10.7). The transformation/progression linked imbalance in pyrimidine and DNA metabolism provides an increased synthetic capacity for the tumor cells while the sharply curtailed catabolic potential confers selective reproductive advantages to the cancer cells.

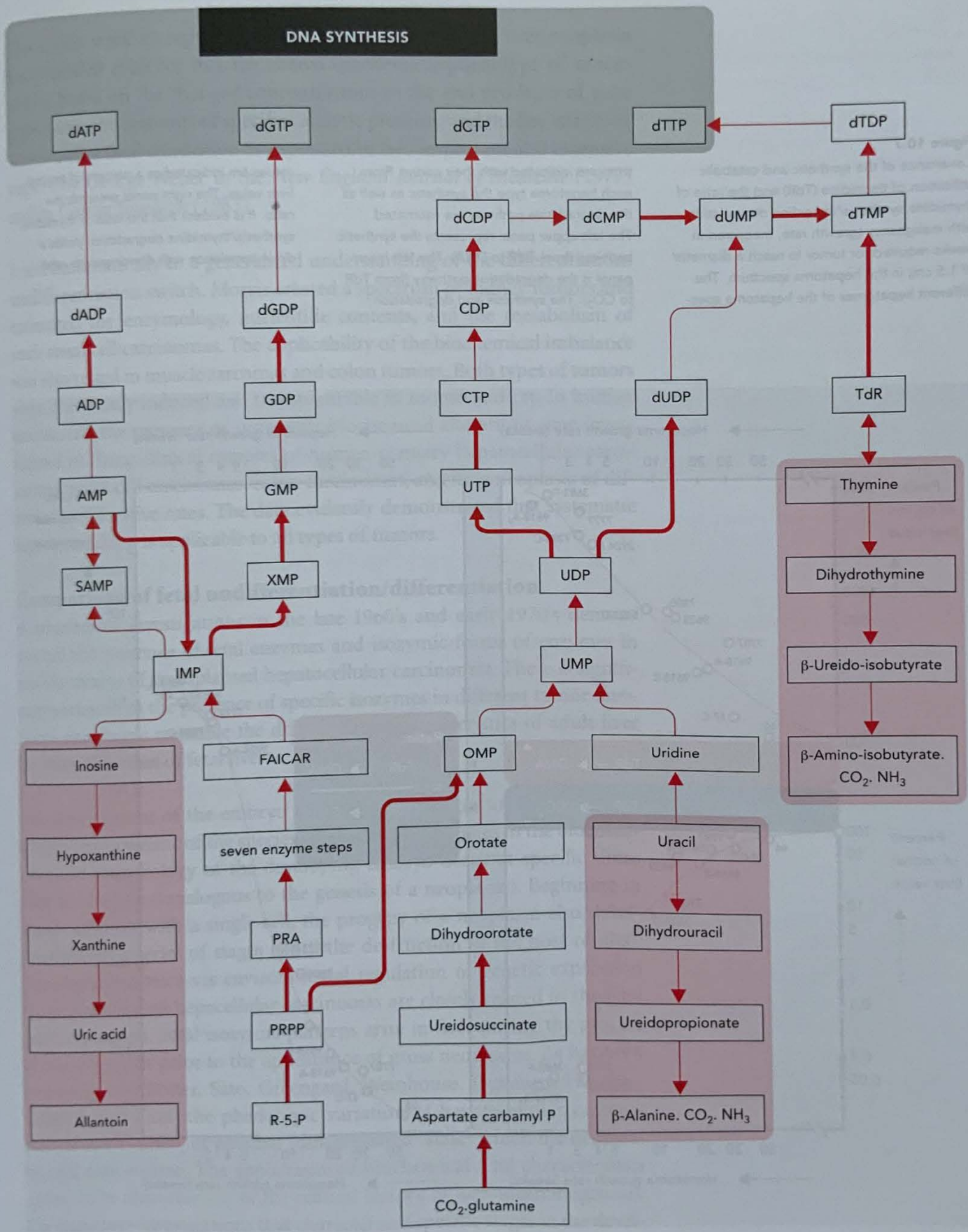
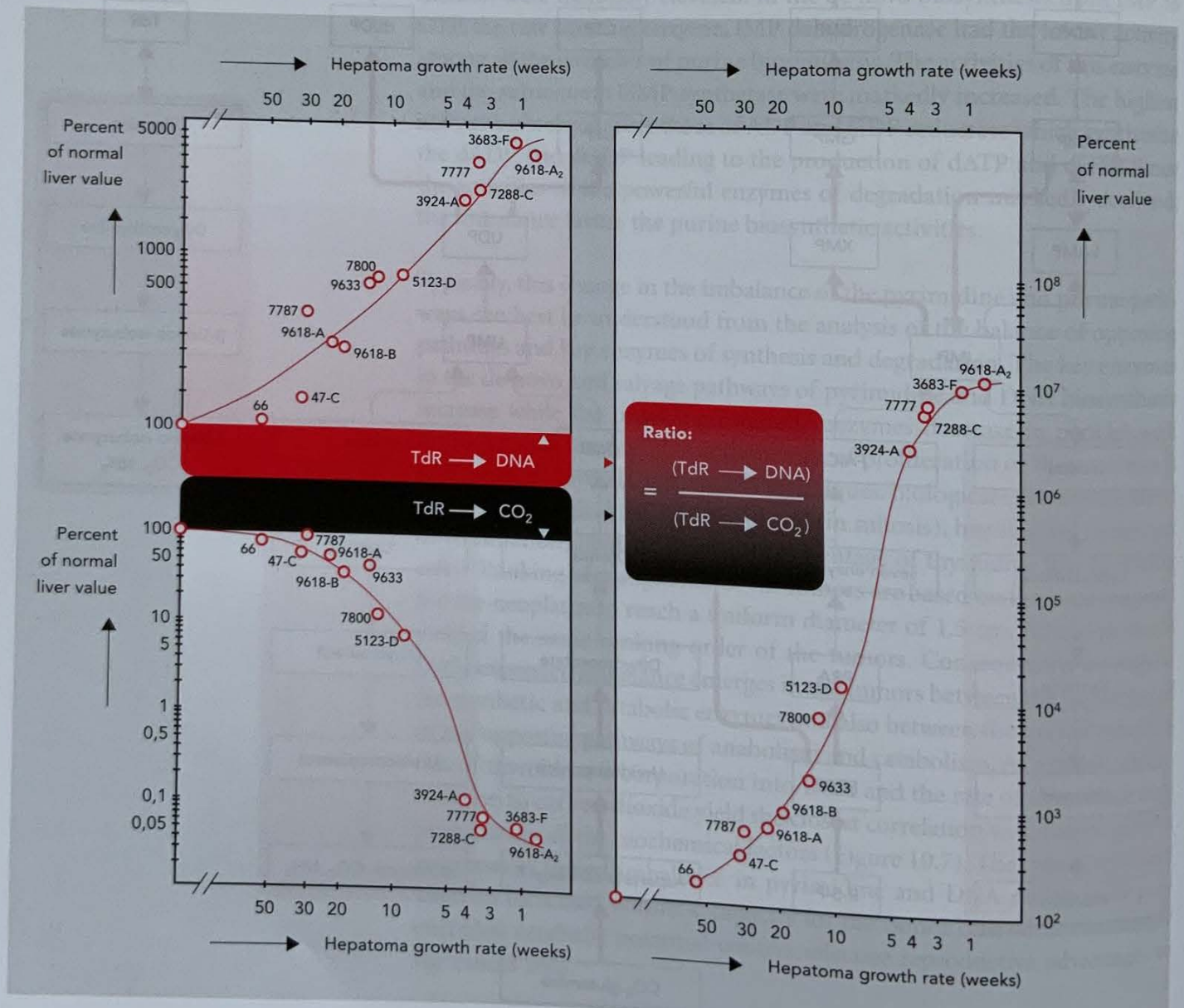

DNA SYNTHESIS
dATP
dGTP
dCTP
dTTP
dTDP
dCDP
dCMP
dUMP
dTMP
dADP
dGDP
CDP
ADP
GDP
CTP
dUDP
TdR
AMP
GMP
UTP
Thymine
SAMP
XMP
UDP
Dihydrothymine
IMP
UMP
β-Ureido-isobutyrate
Inosine
FAICAR
OMP
Uridine
β-Amino-isobutyrate. CO_2. NH_3
Hypoxanthine
seven enzyme steps
Orotate
Uracil
Xanthine
PRA
Dihydroorotate
Dihydrouracil
Uric acid
PRPP
Ureidosuccinate
Ureidopropionate
Allantoin
R-5-P
Aspartate carbamyl P
β-Alanine. CO_2. NH_3
CO_2.glutamine

Figure 10.7
Covariance of the synthetic and catabolic utilization of thymidine (TdR) and the ratio of thymidine synthesis/thymidine degradation with malignancy (growth rate, measured in weeks required for tumor to reach a diameter of 1.5 cm) in the hepatoma spectrum. The different hepatomas of the hepatoma spectrum are indicated with their codes. From each hepatoma type the synthetic as well as the degradative pathway are estimated. The left upper panel represents the synthetic pathway (from TdR to DNA). The left lower panel is the degradation pathway (from TdR to CO_2). The synthesis and degradation values are indicated as a percent of normal liver value. The right panel presents the ratio. It is evident that the ratio of thymidine synthesis/thymidine degradation yields a close correlation with tumor growth rate.



This entire work on reprogramming of gene expression in liver neoplasia has provided evidence that the altered biochemical phenotype of cancer cells is based on the changed concentrations in the end products of gene expression, the amounts of specific catalytic proteins, and the key enzymes. This was the final conclusion as expressed in two important and extensive papers by George Weber in the *New England Journal of Medicine* (1977) and in *Cancer Research* (1983).

It led automatically to a generalized understanding of the differentiation/undifferentiation switch. Morris created a spectrum of kidney tumors and estimated the enzymology, nucleotide contents, and the metabolism of such renal cell carcinomas. The applicability of the biochemical imbalance was also tested in muscle sarcomas and colon tumors. Both types of tumors were chemically induced and transplantable in mouse and rat. In human neoplasms, the presence of systematic biochemical alterations were investigated utilizing clinical samples of human primary hepatocellular carcinomas, renal cell carcinomas, colon carcinomas, and lung neoplasia of different proliferative rates. The data evidently demonstrated that systematic reprogramming is applicable to all types of tumors.

Comparison of fetal undifferentiation/differentiation

A number of investigations in the late 1960's and early 1970's demonstrated the presence of fetal enzymes and isozymic forms of enzymes in a wide variety of transplanted hepatocellular carcinomas. There is significant variation in the presence of specific isozymes in different tumor lines. Some neoplasms resemble the differentiated characteristics of adult liver far more than that of fetal liver.

The development of the embryo throughout gestation follows a detailed format characteristic of the species. Characteristic changes in the biochemistry and morphology of the developing embryo occur at specific times after fertilization (analogous to the genesis of a neoplasm). Beginning in many instances with a single cell, the progeny of a neoplasm also develops through a series of stages (until the destruction of the host results). The alterations vis-à-vis environmental regulation of genetic expression characteristic of all hepacellular carcinomas are closely related to the fetal genic expression. Fetal isozymic patterns arise in liver during the process of carcinogenesis prior to the appearance of gross neoplasms. As has been pointed out by Potter, Sato, Greengard, Weinhouse, Dunaway, Hatzfeld, Goldfarb and Pitot, the phenotypic variation of hepatocellular carcinomas reflects a variety of possible "differentiated" states which the neoplastic cells may assume. The appearance of biochemical fetal characteristics appear to be characteristic of the natural history of hepatocarcinogenesis. The numerous investigations that characterized specific stages in the devel-

opment of neoplasia led Potter (1972) to the statement, "The development of a neoplasm may result from blocked ontogeny".

Conclusion

During carcinogenesis, the organ undergoes extensive morphological and biochemical alterations. Comparing many of the tumors in the same organ has demonstrated that they could strongly differ in histological structure, differentiation, biological behavior and growth rate. The program of the differentiation of an embryo with its characteristic changes in molecular composition, biochemistry, and morphology is analogous to the genesis of a neoplasm. The appearance of fetal characteristics is a characteristic of the process of carcinogenesis. Both in embryonic development and carcinogenesis, the alterations in metabolic functioning and growth are highly linked to each other. At the molecular level, the Molecular Correlation Concept demonstrated the coordinated reprogramming of gene expression in cancer cells, both in the biochemical imbalance in organ functioning (i.e., predominance of glycolysis over gluconeogenesis) and in the metabolism regulating growth (i.e., enhanced DNA and RNA synthesis). Such alterations are also linked to a cell's response to hormones and subsequently to the functionality of the organ in the interplay between the diverse organs (i.e., liver functioning and pancreatic hormones). This entire work on reprogramming confirms the major coordinate switch between the two states (i.e., the state of undifferentiation and growth, as well as the state of differentiation and non-growth). However, this work does not give an answer as to what factor(s) may be responsible for this switch and how it is related to photon emission. This will be the subject of the next chapter.

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11

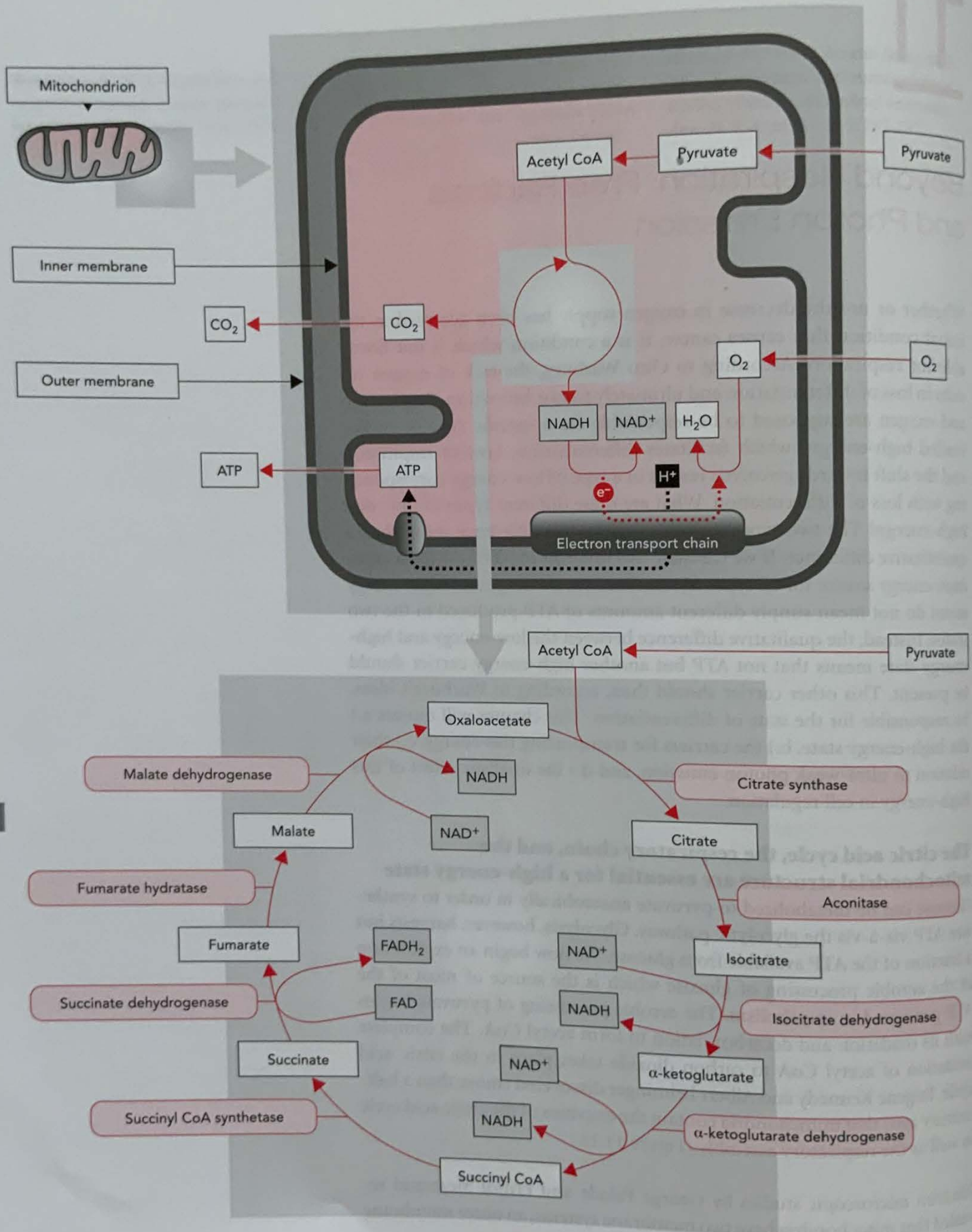
Beyond Respiration: Free Radicals and Photon Emission

Whether or not the decrease in oxygen supply has been accepted as the initial condition that causes cancer, it is a condition which is not favorable for respiration. According to Otto Warburg, the lack of oxygen results in loss of differentiation and ultimately tumor formation. Respiration and oxygen are supposed to be responsible for a specific type of energy (called high-energy) which facilitates differentiation. Loss of respiration and the shift towards glycolysis results in a type of low-energy corresponding with loss of differentiation. What are these different types of low- and high-energy? The two types include a qualitative difference more than a quantitative difference. If we translate this in terms of ATP (the most common energy source for all types of reactions), low-energy and high-energy states do not mean simply different amounts of ATP produced in the two states. Instead, the qualitative difference between the low-energy and high-energy state means that not ATP but another high-energy carrier should be present. This other carrier should then, according to Warburg's ideas, be responsible for the state of differentiation. This chapter will discuss a.) the high-energy state, b.) the carriers for transporting this energy, c.) their relation to ultra-weak photon emission, and d.) the dualistic effect of this high-energy in cell regulation.

The citric acid cycle, the respiratory chain, and the mitochondrial structure are essential for a high-energy state

Glucose can be metabolized to pyruvate anaerobically in order to synthesize ATP vis-à-vis the glycolytic pathway. Glycolysis, however, harvests but a fraction of the ATP available from glucose. We now begin an exploration of the aerobic processing of glucose which is the source of most of the ATP generated in metabolism. The aerobic processing of pyruvate begins with its oxidation and decarboxylation to form acetyl CoA. The complete oxidation of acetyl CoA to carbon dioxide takes place in the citric acid cycle. Eugene Kennedy and Albert Lehninger discovered (more than a half-century ago) that mitochondria contain the enzymes of the citric acid cycle as well as the respiratory assembly (Figure 11.1).

Electron microscopic studies by George Palade and Fritjof Sjostrand revealed that mitochondria have two membrane systems, an outer membrane


Mitochondrion
Inner membrane
Outer membrane
Acetyl CoA
Pyruvate
Pyruvate
CO_2
CO_2
O_2
O_2
NADH
NAD^+
H_2O
ATP
ATP
e^-
H^+
Electron transport chain
Acetyl CoA
Pyruvate
Oxaloacetate
Malate dehydrogenase
NADH
Citrate synthase
Malate
NAD^+
Citrate
Fumarate hydratase
Aconitase
Fumarate
$FADH_2$
NAD^+
Isocitrate
Succinate dehydrogenase
FAD
NADH
Isocitrate dehydrogenase
Succinate
NAD^+
α-ketoglutarate
Succinyl CoA synthetase
NADH
α-ketoglutarate dehydrogenase
Succinyl CoA

◄ **Figure 11.1**
Schematic overview of the mitochondrial structure and the citric acid cycle. The upper left panel presents the basic structure of a mitochondrion. Its processes involving an outer and inner mitochondrial membrane.
The upper right panel is an enlargement of the mitochondrion summarizing the energy generating metabolism in mitochondria. Pyruvate enters the mitochondrion (right) and is broken down to acetyl CoA, which is then metabolized by the citric acid cycle. This reduces NAD^+ to NADH (and FAD to $FADH_2$). High-energy electrons from NADH (and $FADH_2$) are then passed along the electron transport chain in the inner membrane to oxygen (O_2) resulting finally in the production of ATP.
The lower panel demonstrates the intermediates and enzymes of the citric acid cycle. The reaction of acetyl CoA with oxaloacetate starts the cycle by producing citrate. In each turn of the cycle, two molecules of CO_2 are produced, plus three molecules of NADH, and one molecule of $FADH_2$.

and an extensive highly folded inner membrane. A typical mitochondrion is about the size of a bacterial cell with a diameter of 0.2 to 0.8 μg and a length of 0.5 to 2 μm. The two membranes have markedly different properties. The outer mitochondrial membrane has few proteins, the major protein forms channels through this membrane allowing free diffusion of ions and small water soluble metabolites. The inner mitochondrial membrane is very rich in protein. This membrane is permeable to uncharged molecules such as water, O_2, and CO_2. However, it is a barrier to protons and larger polar and ionic substances which cross this membrane only via specific transporters.

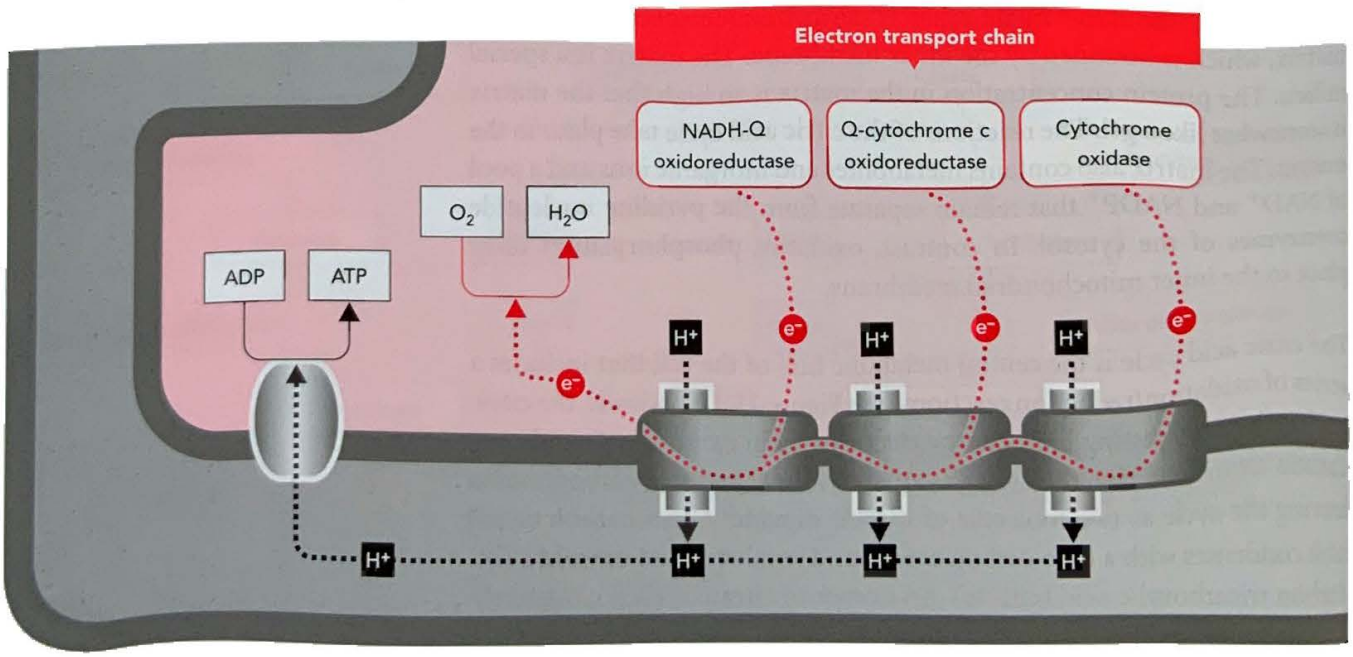
The inner membrane is folded into a series of internal ridges called cristae. Hence, there are two compartments in mitochondria: 1.) the space between the outer and the inner membranes (intermembrane space) and 2.) the matrix, which is bounded by the inner membrane. The matrix is a special milieu. The protein concentration in the matrix is so high that the matrix is somewhat like a gel. The reactions of the citric acid cycle take place in the matrix. The matrix also contains metabolites and inorganic ions and a pool of NAD^+ and $NADP^+$ that remain separate from the pyridine nucleotide coenzymes of the cytosol. In contrast, oxidative phosphorylation takes place in the inner mitochondrial membrane.

The citric acid cycle is the central metabolic hub of the cell that includes a series of oxidation/reduction reactions (see Figure 11.1). Basically, the citric acid cycle is harvesting high-energy electrons from carbon fuels with two carbon atoms entering the cycle (as an acetyl unit) and two carbon atoms leaving the cycle as two molecule of carbon dioxide. A two-carbon acetyl unit condenses with a four-carbon compound (oxaloacetate) to yield a six-carbon tricarboxylic acid (citrate). An isomer of citrate is then oxidatively decarboxylated. The resulting five-carbon compound (α-ketoglutarate)

also is oxidatively decarboxylated to yield a four-carbon compound (succinate). Oxaloacetate is then regenerated from succinate. In this process, three hydride ions (hence, six electrons) are transferred to three molecules of nicotinamide adenine dinucleotide (NAD^+). One pair of hydrogen atoms (hence, two electrons) is transferred to one molecule of flavin adenine dinucleotide (FAD). Thus, the citric acid cycle removes electrons from acetyl CoA and uses these electrons to form NADH and $FADH_2$. The high-energy electrons and redox potentials are of fundamental importance in oxidative phosphorylation in which O_2 is the acceptor of these electrons.

Oxidative phosphorylation is the culmination of a series of energy transformations called cellular respiration or simply respiration. It starts with the formed strong reducing agents NADH and $FADH_2$ that have the ability to yield electrons with high transfer potential. Then, this electron motive

Figure 11.2
The chemiosmotic theory of oxidative phosphorylation. In the process of oxidative phosphorylation, high-energy electrons from NADH (and $FADH_2$) are passed along the electron transport chain in the inner membrane. Some of the energy is used to drive the three respiratory enzyme complexes to pump H^+ out of the matrix. The high-energy electrons are finally passed to oxygen (O_2). The resulting electrochemical proton gradient across the inner membrane drives H^+ back through a transmembrane protein complex. This energy is used to synthesize ATP from ADP and P_i in the matrix.



force is converted into a proton motive force and, finally, the proton motive force is converted into phosphoryl transfer potential (ATP formation). Before considering the oxidative phosphorylation and release of electrons in the reoxidation of NADH and $FADH_2$, we now examine the chemiosmotic theory. How is the energy that is stored in a proton concentration gradient serving as the energy reservoir for driving the formation of ATP in mitochondria? The concept was originally formulated by Peter Mitchell in the early 1960's (Figure 11.2).

The conversion of electron motive force into proton motive force is carried out by three electron driven proton pumps (NADH-Q oxidoreductase, Q-cytochrome c oxidoreductase, and cytochrome c oxidase). These large transmembrane complexes contain multiple oxidation/reduction centers that include quinines, flavins, iron-sulfur clusters, hemes, and copper ions. The flow of electrons from NADH or $FADH_2$ to O_2 through these protein complexes in the mitochondrial inner membrane leads to the pumping of protons out of the mitochondrial matrix. The resulting uneven distribution of protons generates a pH gradient and a transmembrane electrical potential that creates a proton motive force. Finally, these protons flow through ATP synthase to generate ATP from ADP and inorganic phosphate. The electrons at the end of the electron transport chain are accepted by oxygen. Under normal aerobic conditions, the vast majority of energy used by cells is provided by the citric acid cycle in conjunction with oxidative phosphorylation. Based on the proton motive force that powers the generation of ATP, it has been estimated that oxidative phosphorylation generates 26 of the 30 molecules of ATP that are formed when glucose is completely oxidized to CO_2 and H_2O. The total understanding of the mechanism of oxidative phosphorylation has been one of the most challenging problems of biochemistry. Mitchell was awarded the Nobel prize in 1978 for this work.

The efficiency of the citric acid cycle is very high and this may be, at least in part, due to the physical arrangement of the constituent enzymes. These enzymes interact with one another to facilitate substrate channeling. Molecular oxygen is the ideal terminal electron acceptor because its high affinity for electrons provides a large thermodynamic driving force. The reduction of O_2 requires the transfer of four electrons providing two molecules of H_2O. Such a complete strategy for the full reduction of O_2 is accomplished vis-à-vis the catalyst cytochrome c oxidase which does not release partly reduced intermediates by holding O_2 tightly between Fe and Cu ions. If this protection of O_2 is not present, an only partial reduction of O_2 would be generated (Figure 11.3). The transfer of a single electron to O_2 results in a superoxide anion whereas the transfer of two electrons yields peroxide.

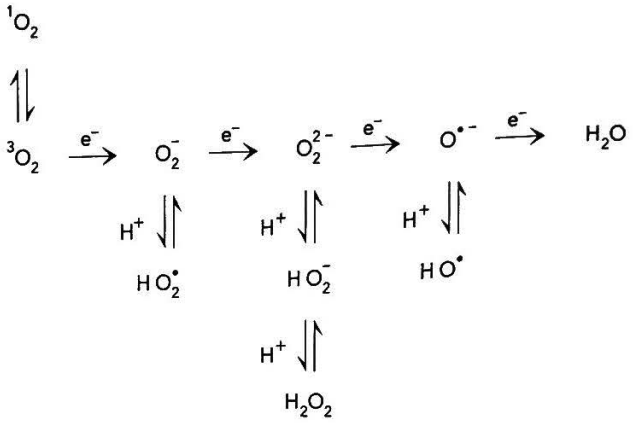


Figure 11.3
Stepwise reduction of O_2 to H_2O. For safe reduction of O_2, cytochrome c oxidase does not release reduced intermediates. If the enzyme does not offer sufficient protection for the process partially reduced intermediates are released. Transfer of a single electron to O_2 results in a superoxide anion. The transfer of two electrons yields peroxide.

Although cytochrome c oxidase is remarkably successful in keeping the release of intermediates at a minimum, small amounts of superoxide anion and hydrogen peroxide are unavoidably formed. Approximately 1-4% of the total oxygen utilized by the mitochondria is converted to superoxide. Superoxide, hydrogen peroxide, and species that can be generated from them (such as the hydroxyl radical $OH^{\bullet}$) are collectively referred to as reactive oxygen species (ROS). Here, we find the roots for the high-energy excited states and the difference between the two kinds of energy available in life (ROS and ATP).

Free radical metabolism

In the mid-1950's, the presence of free radicals in biological materials was discovered. That may account for the presence of gross cellular damage. In 1954, Gerschman and Gilbert proposed that most of the damaging effects of elevated oxygen concentrations in living organisms might be attributed to the formation of free radicals. Soon thereafter, in 1956, Denham Harman hypothesized that oxygen radicals may be formed as byproducts of enzymatic reactions *in vivo*. Harman proposed the "free radical theory of aging" which suggested that free radical damage on cellular macromolecules is responsible for the aging process. This idea did not capture the interest of many biologists and clinicians until the discovery by McCord and Fridovitch (in 1969) of the enzyme superoxide dismutase (SOD) which catalytically removes a specific free radical. At that point in time they were able to convince most colleagues that free radicals are important in biology. Notwithstanding their efforts, many scientists in the 70's and 80's, unfamiliar with free radicals, regarded the field as irrelevant to mainstream biology, biochemistry and medicine. However, experimental data in the 1980's and 1990's finally emphasized that the reactive species actually do attack biomolecules producing alterations in DNA, proteins, and lipids. They are

implicated in the pathogenesis of age-related diseases. Let us, therefore, begin with a more systematic approach of the characteristics to this particular part of metabolism and its bioenergetic aspects.

Molecular oxygen (O_2) is a triplet in its ground state because it contains two unpaired electrons within its outer orbits having parallel or unpaired spins. Therefore, molecular oxygen can accept a total of four electrons to form water. A few percent are converted to the free radical superoxide and released from the mitochondria. In tissues such as muscle (which increase their oxygen uptake during exercise) they also increase the generation of superoxide. Increased superoxide in tissues appears to also be produced during transition periods such as during anoxia/ischemia and in ischemic/reperfusion.

A "free radical" is defined as any atom, group of atoms or molecules containing one unpaired electron within an outer orbit. Strictly speaking, the free radical theory is only concerned with free radicals such as superoxide (O_2^-). Subsequently, it has since been expanded to encompass oxidative damage from other reactive oxygen species such as H_2O_2, $OH^\bullet$, as well as singlet oxygen. The latter, by definition, is not a free radical. Both electrons occupy the same orbit while the electron spins are paired.

Superoxide is not a highly reactive species. However, hydrogen peroxide (H_2O_2) may be formed by the spontaneous dismutation of superoxide. The latter reaction occurs very rapidly. Consequently, the production of O_2^- *in vivo* is generally accompanied by the production of H_2O_2. The hydroxyl radical ($OH^\bullet$) is the key radical species in damaging tissue. The hydroxyl radical ($OH^\bullet$), formed by the interaction of O_2^- and H_2O_2 (Haber-Weiss reaction), is highly reactive with almost every type of molecule (e.g., sugar, amino acid, phospholipids, nucleotides, and organic acids). In association with the process of lipid peroxidation, lipid hydroperoxides may be formed in the presence of some metals. Lipid hydroperoxides may also be cleaved to form more free radicals and thus accelerate peroxidation of membrane lipids.

Apart from the mitochondrial respiratory chain, many other enzymes (e.g., monooxygenases and several dehydrogenases) normally generate radicals. The body produces radicals purposefully in cells designed to be active during immune and inflammatory responses. Certain white blood cells (phagocytes) produce and make use of superoxide anions. Phagocytes engulf foreign material such as bacterial cells and destroy them using proteolytic enzymes and oxygen metabolites. When stimulated, phagocytes undergo a burst of respiratory activity increasing their oxygen intake up to 50-fold. At the same time, they rapidly metabolize glucose by the pentose phosphate pathway to produce NADPH. An NADPH dependent enzyme in the plasma membrane of the phagocytes catalyzes the reduction of

molecular oxygen to form superoxide. Superoxide anions are converted to hydrogen peroxide. These two oxygen species are the starting materials for the hydroxyl radicals that inactivate bacterial cells.

Because radicals and their products are continuously generated and are so reactive chemically, they must physiologically be closely controlled to avoid damage of vital components. To maintain cell and tissue integrity, the "antioxidant system" checks and balances the production of free radicals. In their definition of antioxidant, Halliwell and Gutteridge state, "Any substance that, when present at low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate". The antioxidant system includes a number of (protective) enzymes and low molecular weight compounds, many dependent on essential nutrients including vitamin E (tocopherol), vitamin C (ascorbic acid), beta-carotene, zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), and selenium (Se). The metals exert their action as antioxidants primarily via incorporation into specific enzymes. The accumulation of the superoxide anion is also prevented by enzymes called superoxide dismutases which contain manganese or copper-zinc at their active site (Figure 11.4). Hydrogen peroxide is decomposed to H_2O by catalase and a variety of peroxidases. Glutatione peroxidase (GSHPx) has been the most intensely studied enzyme of this group. Hydrogen peroxide is a weak oxidizing agent. However, it can inactivate sulfhydryl enzymes.

The half-life times of the major reactive oxygen species are vastly different. The hydroxyl radical has the shortest and therefore, its reactions are diffusion limited (i.e., they take place practically at the site of generation). In contrast, other radicals are relatively stable. Such molecules may diffuse away from their site of generation and transport the radical or oxidant function to other target sites. This situation does not automatically mean that they are less dangerous. Hydrogen peroxide is such example. It is not very reactive, and, it is able to cross biological membranes. However, the possible involvement of hydrogen peroxide in the generation of hydroxyl radicals places hydrogen peroxide in a more prominent role to initiate cytotoxicity than its chemical reactivity indicates.

Properties of an ideal free radical scavenger can thus easily be summarized as: a.) it must be present in adequate amounts in the body; b.) it must accumulate within compartments where a need for protection exists; c.) it must be versatile in order to combine with a wide variety of free radicals (e.g., SOD lacks versatility). It can interact with only one substrate); and d.) it might be suitable for regeneration (i.e., it must have a biologically convenient reducing mechanism).

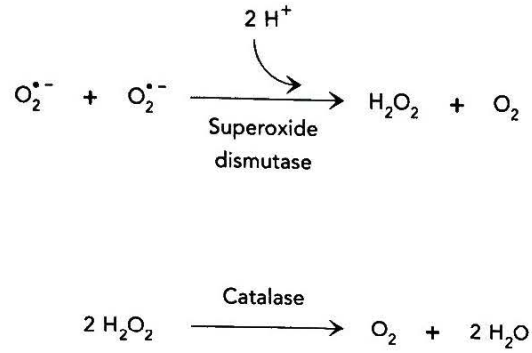


Figure 11.4
Major enzymes in the scavenging of free radicals: superoxide dismutase and catalase.

Bioenergetics: excited states and photon emission

In this paragraph, we will consider the role of high-energy excited states in the generation of ultra-weak photon emission. This subject has been discussed by many biochemists and biophysicists involved in the world-wide expansion of ultra-weak photon research in the late 1970's and 1980's (Chapter 8). Several teams during that period helped to understand what types of chemiexcitation reactions fulfill the requirements for photon emission. Major reviews came from Cilento (1982), Slawinski and Slawinka (1983, 1985) and Zhuravlev (1983). Generally stated, three criteria must be satisfied to achieve chemiluminescence: a.) an efficient chemical pathway leading to the formation of product molecules with adequate electronic structure facilitating electronic excitation, b.) a high efficiency in liberating sufficient energy in one single step, and c.) the ability to emit sufficient quanta per number of excited molecules (quantum yield). The major reaction types that fulfill the requirements for photon generation are: a.) dismutation of peroxyradicals leading to the formation of excited carbonyl compounds (>C=O*) and singlet (excited) molecular oxygen, b.) electron transfer reactions, and c.) the synchronous cleavage of weak C–C-bonds and O–O bonds in peroxides.

To understand the operation of these reactions in biological systems, we must again focus on the bioenergetics of living systems. The stepwise release of energy during the oxido/reductions of the citric acid cycle substrates and consecutive e^-/H^+ -carriers, (e.g. NAD/NADH, FAD/FADH) as well as the H^+ - e^- -processing within the inner mitochondrial membrane liberates small portions of energy. This is adequate for the biosynthetic and homeostatic requirements of the cell but *too small* for direct electronic excitation. Instead, the primary events leading to high-energy chemiexcitation are initiated by the electron leakage from the e^- -respiratory chain and

the consecutive formation of active species such as O_2^-, $OH^\bullet$, $^1O_2$, H_2O_2, and related excited species.

Dismutation reactions of peroxyradicals and the cleavage of peroxides produce the most common emitters of ultralow luminescence: carbonyl compounds (>C=O) in the excited singlet and triplet states as well as excited dimers (dimols) of the singlet molecular oxygen $^1O_2$ (Figure 11.5). Emission from excited carbonyl groups occurs at wavelengths in the 350-500 nm range. Excited singlet oxygen molecules can emit in the 780 nm. Other singlet oxygen emissions lie even further into the infrared. The excited singlet oxygen molecules may also excite a secondary emission by reacting with unsaturated lipids to produce excited carbonyl groups which can then emit as before (where the emission wavelengths are in the same vicinity as described above). Another emission band arises after dimerization of excited singlet oxygen (the dimer emits two broad bands centered on 634 and 703 nm). This emission from excited oxygen dimers is most compatible with the red emissions observed from the different organisms. Excited carbonyl groups may be responsible for the blue green components of the emissions. It is worthwhile to emphasize that recombination reactions of certain radicals release up to 480 kJ/mol energy. Such energy is sufficient to generate UV-photons of about 230 nm. Figure 11.5 highlights the many energy levels of the electronic states of O_2 and its dimole (excimers) with reference to their radiative transitions.

Such biochemical data link radiation to oxygen, respiration as well as the so-called "e^- -leakage" theory to the specific high type of energy and ultra-weak photon emission. This may be relevant to the earlier spectral distribution of mitogenetic radiation recording in the spectral range 190-350 nm. However, we must return to the other major question. Is such high-energy linked to differentiation? (differently stated, whether or not a decrease in oxygen supply results in loss of differentiation and ultimately tumor formation).

Free radicals in hypoxia, ischemia and reperfusion

Based on research begun in the 80's, free radicals were implicated in ischemic/reperfusion damage and pathogenesis of cancer. Oxygen free radicals are important mediators of hypoxic or anoxic cell death in heart, lung, kidney, gastrointestinal tract, and the brain. Hypoxic injury can occur during respiratory failure, systemic hypotension and regional hypoperfusion of organs. A simple model of hypoxia may exist in cell cultures wherein ATP depletion and the stress of hypoxia is mimicked by exposing cells to inhibitors of mitochondrial respiration and glycolysis, cyanide, and iodoacetate. Hepatocytes under the impact of such metabolic inhibition generate hydroperoxides and other reactive oxygen species (both during hypoxia

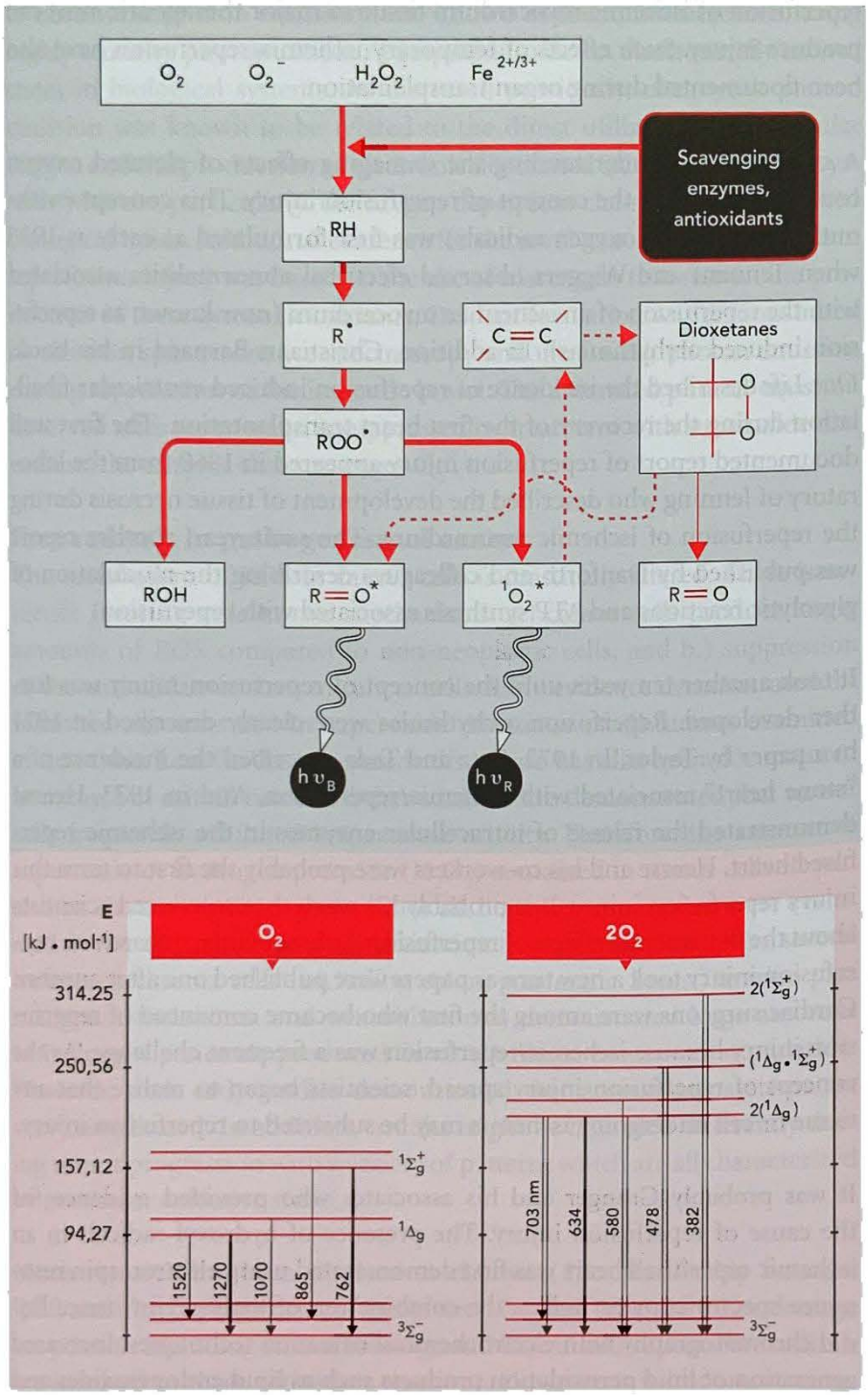


Figure 11.5
Excited states, energy levels and photon emission. Upper panel illustrates radical reactions involving unsaturated fatty acids and other susceptible compounds (RH) leading to the generation of electronically excited products: carbonyl compounds (>R=O*), singlet molecular oxygen ($^1O_2$*) and alcohols (ROH, not electronically excited). $^1O_2$* can oxidize unsaturated compounds >C=C< such as lipids, phospholipids, etc, that produce dioxetanes that decompose with photon emission. The excited species decay with emissions in the blue ($h\nu_B$) and red ($h\nu_R$) part of the spectrum. The rate of oxidative chain reactions is controlled *in vivo* by scavenging enzymes and antioxidants such as α-tocopherol. Lower panel depicts the electronic and vibronic energy levels of the ground state as well as excited oxygen molecules and their excimers (excited dimoles). Double and thicker arrows symbolize simultaneous and more probable transitions, respectively.

and before the onset of cell death). In this model, the loss of viability was delayed by antioxidants in an oxygen dependent manner. During severe hypoxia or ischemia, the reperfusion of the ischemic tissue can suffer additional injury. For instance, in the treatment of acute coronary thrombosis,

reperfusion of ischemic myocardium tissue (a major therapeutic aim) can produce injury. Such effects of temporary ischemia/reperfusion have also been documented during organ transplantation.

A major role in understanding the damaging effects of elevated oxygen concentrations was the concept of reperfusion injury. This concept (without understanding oxygen radicals) was first formulated as early as 1935 when Tennant and Wiggers observed electrical abnormalities associated with the reperfusion of an ischemiac myocardium (now known as reperfusion induced arrhythmias). In addition, Christiaan Barnard in his book, *One Life* described the incidence of reperfusion induced ventricular fibrillation during the recovery of the first heart transplantation. The first well documented report of reperfusion injury appeared in 1960 from the laboratory of Jenning who described the development of tissue necrosis during the reperfusion of ischemic myocardium. The same year, another report was published by Danforth and colleagues describing the modulation of glycolytic reactions and ATP synthesis associated with reperfusion.

It took another ten years until the concept of reperfusion injury was further developed. Reperfusion arrhythmias were clearly described in 1971 in a paper by Taylor. In 1972, Katz and Tada described the incidence of a "stone heart" associated with ischemia/reperfusion. And in 1973, Hearse demonstrated the release of intracellular enzymes in the ischemic reperfused heart. Hearse and his co-workers were probably the first to term this injury reperfusion injury. It is probably his work that convinced scientists about the detrimental effects of reperfusion. Subsequently, research on reperfusion injury took a new turn as papers were published one after another. Cardiac surgeons were among the first who became convinced of reperfusion injury because ischemic/reperfusion was a frequent challenge. As the concept of reperfusion injury spread, scientists began to realize that any tissue or cell undergoing ischemia may be subjected to reperfusion injury.

It was probably Granger and his associates who provided evidence for the cause of reperfusion injury. The presence of hydroxyl radicals in an ischemic reperfused heart was first demonstrated using electron spin resonance spectroscopy as well as the combination of high-performance liquid chromatography with electrochemical detection techniques. Increased generation of lipid peroxidation products such as lipid endoperoxides and hydroperoxides (specifically malonaldehyde) plus the formation of conjugated dienes supported the free radical hypothesis (see Figure 11.5). Most of the techniques to record free radicals were invasive. Such research required the destruction of living cellular or tissue structures to estimate either specific ROS species or products derived from reactions between ROS and macromolecules (mostly lipids). To circumvent this, attention was drawn

to ultra-weak photon emission (also known as chemiluminescence) using highly sensitive photomultiplier equipment to detect electronically excited states in biological systems. As discussed previously, ultra-weak photon emission was known to be related to the direct utilization of molecular oxygen and the production of electronically excited states in biological systems wherein, particularly, the oxygen dependent chain reactions involved biological lipids. Additional evidence for the involvement of free radicals in reperfusion injury was based on the use of scavengers that restrict the production of these cytotoxic oxidants. Oxidant formation was demonstrated by electron spin resonance spectroscopy and ultra-weak photon emission during reperfusion of the ischemic bowel. The enhanced photon emission observed after reperfusion is suppressed by treatment with superoxide dismutase, the enzyme that scavenges O_2^-.

Free radicals in pathogenesis of cancer

The metabolism of ROS in cancer cells is drastically altered. There is evidence favoring at least two mechanisms: a.) cancer cells produce larger amounts of ROS compared to non-neoplastic cells, and b.) suppression of the antioxidant system in cancer cells. Early evidence demonstrated diminished amounts of Mn superoxide dismutase of all tumors examined at that time. Less Cu/Zn superoxide dismutase has also been documented in many, but not all tumors. Other studies have demonstrated that tumor cells frequently exhibited low catalase activity. Therefore, the amount of superoxide or hydrogen peroxide (H_2O_2) contained in tumor cells should also be elevated. Indeed, most, if not all, hepatic tumours that were evaluated *in vivo* did exhibit more peroxidation than normal livers. Also, several human carcinoma cells (including colon, pancreatic, breast, ovarian plus malignant melanoma and neuroblastoma) demonstrated large amounts of hydrogen perioxide produced *in vitro* without exogenous stimulation. However, such early studies demonstrated that antioxidant systems are very complicated. In addition, circuits might be regulated differently during tumor progression with a variety of patterns which are all characterized by persistent oxidative stress.

Much research has been directed at clarifying the relationship between ROS and the development of neoplasias. Several types of DNA damage are possible including strand breakage and base modification. Free radicals are involved in these activation reactions. However, it is important to remember that highly reactive free radicals are essentially trapped in the immediate vicinity of their formation. Their radius of diffusion is frequently small from a cellular perspective. Therefore, the issue of location has led to the hypothesis that most cancer may originate in the mitochondria rather than in the cell nucleus. Mitochondria are self-regulating and contain their own DNA that directs the synthesis of some of the mitochondrial pro-

teins. Mitochondrial DNA is a single, circular molecule, much less protected than the coiled and chromatin packaged nuclear DNA. This became evident when we learned that mutagens bind to mitochondrial DNA up to 1,000 times more strongly than to nuclear DNA whereas the mutation rate in mitochondria is 10-20 times that of the nuclear DNA. Also, DNA repair mechanisms are much less efficient in mitochondria. Thus, both mitochondrial DNA and the organelle's inner and outer membranes (high in polyunsaturated fatty acids) are susceptible to attack by free radicals despite the impressive multilayer free radical defence system. The mutations in mitochondria could lead to defects in energy production and to increased production of ROS by faulty electron transport. The relationship between mitochondrial mutation and ROS in tumor development has become a new focus for research. Such studies are consistent with the ROS hypothesis regarding cancer development. However, we still encounter the problem of the anoxia/ischemia conditions that may lead to free radicals in mitochondria. Can we understand why free radicals may be formed? Is there a hypoxia (ischemia)/reperfusion phenomenon involved?

Almost every cell in almost every tissue of a vertebrate is located within 50-100 μm of a capillary. The vascular system in a tissue, once it has matured, turns over very slowly with an endothelial cell lifetime ranging from a couple of months (in liver and lung) to years (in brain and muscle). Endothelial cells not only repair and renew the lining of established blood vessels, but they also create new blood vessels. They do this in normal adult tissues to support recurrent cycles of remodeling and reconstruction as well as in damaged adult tissues to support repair. In such circumstances, they can be stimulated to proliferate with a doubling time of just a few days. Wounding, for example, induces a burst of capillary growth in the neighborhood of the damage to satisfy the high metabolic requirement of the repair process. Is such a situation also characteristic of loss of differentiation and tumor development? What mechanism ensures that the oxygen supply is adjusted to the local needs of the tissues, not only during normal development but also in all sorts of pathological circumstances?

As part of an answer, we start with the estimation that in each human, as many as 350 billion mitosis occur every day. It is "chance" where these divisions take place and whether existing patterns (on a microscale) are being (temporarily) deformed. However, there always is the potential that pockets are formed that may become limited in oxygen (Figure 11.6). Such pockets may become capable of forming tumors. However, the large majority will be not able to form observable tumors. The reason is that solid tumor, like any other rapidly dividing tissue, needs oxygen and nutrients to survive. Without a blood supply, potential tumors either die or remain dormant. Such "microtumors" remain as a stable cell population wherein dying cells are replaced by new cells.

The anoxic pocket of cells requires the vascular bed to supply the interior of its mass with nutrients and oxygen. The initiation of "microtumor" growth depends on the release from the anoxic cells of diffusible factors that stimulate the directed migration of the capillary endothelium from the surrounding normal tissues into the anoxic region. The signals are complex. The key is played by a protein known as vascular endothelial growth factor (VEGF) (Figure 11.6). A shortage of oxygen in practically any type of cell causes an increase in the intracellular concentration of the active form of a gene regulatory protein called hypoxia inducible factor I (HIF-I). HIF stimulates transcription of the VEGF gene. The VEGF protein

Figure 11.6
The mechanism controlling the sprouting of blood vessel relative to the tumor cell's need for oxygen. A microtumor may grow and form a pocket that becomes limited in oxygen because of its distance to the blood vessel system (see panels A and B). These anoxic cells release diffusible factors (marked by dots) including vascular endothelial growth factor (VEGF). The secreted VEGF protein acts on nearby endothelial cells stimulating the formation of capillary sprouts (panel C). Sprouting comes to a halt as the oxygen concentration is raised and VEGF production stops. It allows an increase in tumor cell proliferation until a new situation of anoxia occurs. Sprouting is then induced again and a new cycle of proliferation is started (panel D).

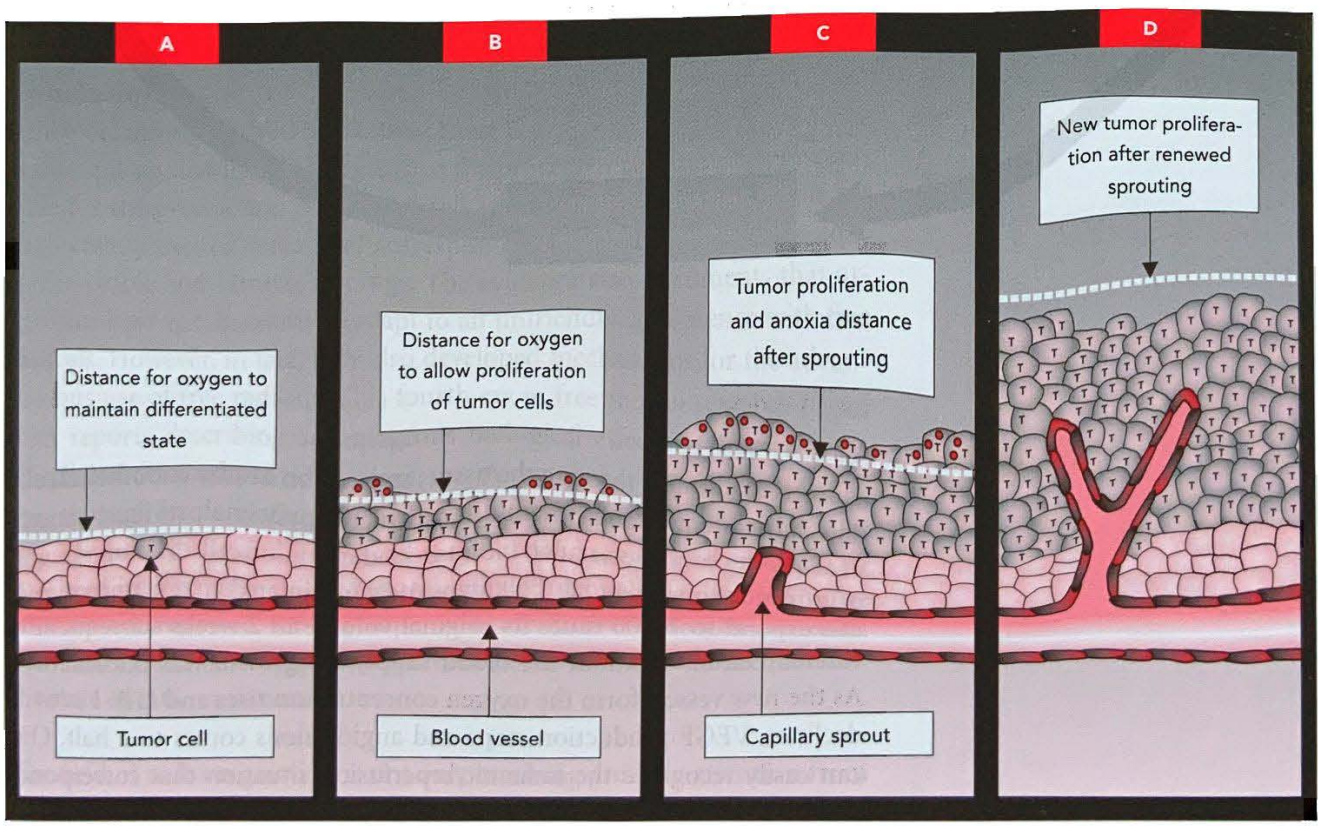
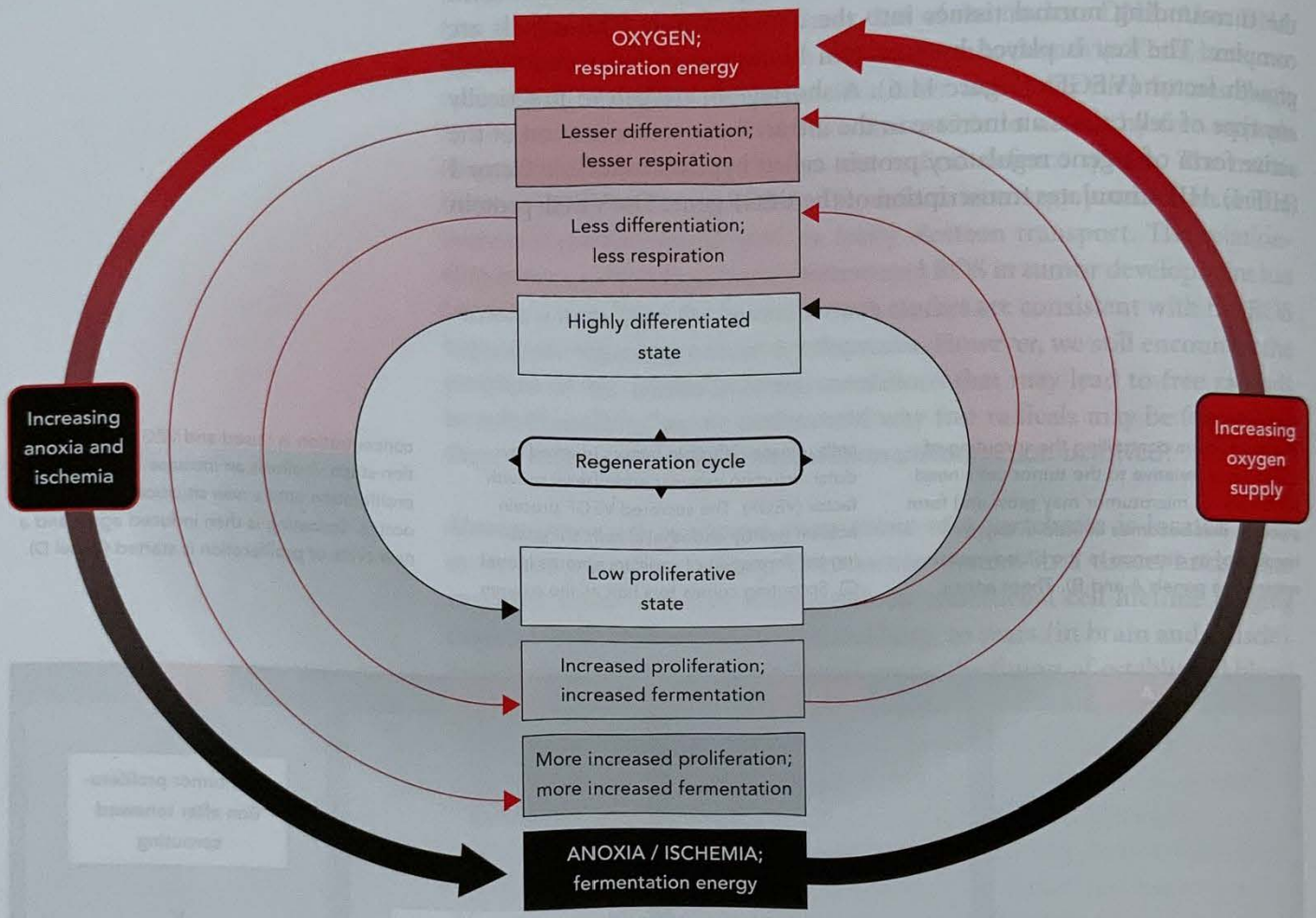


Figure 11.7
A model of cancer development based on the differentiation/proliferation cycle under conditions of cyclic fluctuations in oxygen.



is secreted, diffuses through the tissue, and acts on nearby endothelial cells. The subsequent establishment of a vascular supply in a previously anoxic area produces a spectacular increase in the rate of cell division. In case of a tumor in situ, Judah Folkman (1974) estimated that a microtumor can expand to 16000 times its original volume in 2 weeks subsequent to vascularization. Without the blood supply, no growth was documented. As the new vessels form the oxygen concentration rises and HIF-I activity declines, VEGF production stops and angiogenesis comes to a halt. One can easily recognize the ischemic/reperfusion situation that corresponds with the repeated cycle of growth: increasing anoxia/stimulation of vascu-

lar system to reperfusion/growth, etc. (Figure 11.7). The other remarkable fact is that each cycle at a specific location results in the above described ischemia/reperfusion damage due to free radical formations. Historically, this is not the end of the story.

A third era began in 1981 when Reimer and his associates published their revolutionary paper describing the induction of a resistance mechanism to such physiological fluctuations. Hearts subjected to several short durations of reversible ischemia would 1.) become more tolerant to ischemia/reperfusion and/or 2.) the onset of reperfusion injury would be delayed. These authors' observations were based on the pathologic evaluation of the heart. Similar pathologic observations followed in the 1980's and early 1990's. This phenomenon was termed "preconditioning". Finally, a number of scientists working on this phenomenon met in Sapporo, Japan vis-à-vis a satellite conference of the International Society of Heart Research. They discussed preconditioning in a more cohesive manner. Their reports resulted an issue of *Cardiovascular Research* (Vol. 27, April 1993). Now it is generally believed that preconditioning reduces post ischemic dysfunction and makes the heart more tolerant to ischemia. To what extent the same phenomenon is active during the development and progression in dedifferentiation and tumor formation is not yet known (Figure 11.7). However, since the early observations in cell culture (Chapter 9) such was considered as an important cause of tumor development.

Conclusion

At the beginning of the 21st century, there is now a large body of evidence documenting that in living organisms the process of respiration is responsible for the production of free radicals which in turn are responsible for high-energy excited states and correspondingly, the emission of photons in the visible and ultraviolet range. The evidence also documents that organisms have mechanisms to adapt to an unfriendly coexistence with free radicals. However, in fact, they also developed mechanisms for the advantageous use of free radicals. This fourth era in free radical research began with reports describing advantageous biological effects of free radicals. Mittal and Murard provided suggestive evidence that the superoxide anion, through its derivative, the hydroxyl radical, stimulates the activation of guanylate cyclase and formation of the "second messenger" cyclic GMP. This is of interest since this points to a link with growth regulation. Similar effects were reported for the superoxide derivative hydrogen peroxide. Therefore, these data suggest that critical levels of free radicals maintain the state of differentiation.

The field of redox regulation is not only receiving growing attention from clinical colleagues in view of the role that oxidative stress plays in cancer,

but also in numerous disease conditions as well. An excessive and/or sustained increase in ROS production has been implicated in the pathogenesis of diabetes mellitus, atherosclerosis, neurodegenerative diseases, rheumatoid arthritis, ischemia/reperfusion injury, obstructive sleep apnea, and other medical conditions.

These pathological situations demonstrate the biological relevance of redox regulation. As an important aspect of life, the biological "redox regulation" has now become a rapidly growing field of science that has impact on diverse disciplines including physiology, cell biology, and clinical medicine. In such scientific endeavors, ultra-weak photon emission contributes as an interesting and highly relevant tool for the analysis of redox regulation.

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Conclusion Part C

The third journey has entered the field of biochemistry and how it is related to the process of tumor formation and development. Subsequently, we learned about new, major physiological discoveries.

At the beginning of the 20th century, biochemistry and the physiologic understanding of both glucose fermentation (glycolysis) and respiration began. Unraveling all parts of such metabolism took almost a half century including the combined efforts of many scientists. As early as the 1920's, Otto Warburg studied oxygen dependency of glucose metabolism and its association to the critical transition from normal cells to cancer cells. Tumor growth is primarily associated with cells generating energy via glycolysis which is in contrast to "healthy" parental tissue cells which primarily generate energy from oxidative breakdown of pyruvate. In the 1920's to 1960's, more research continued to suggest such a relationship between a greatly diminished oxygen pressure and the development of the cancer cell.

Simultaneously with the above discoveries in the fields of metabolism and energy production, research also focused on the differentiation/proliferation duality in the areas of carcinogenesis. Major revelations were discovered utilizing liver tumors and their transplantation facilitating continuous growth of stable tumor tissues *in vivo*. Many of those liver tumors demonstrated different histological structures, biological behaviors, biochemical processes and growth rates. The data of so many hepatomas made it possible to find excellent correlations between metabolic alterations eventually resulting (around 1960) in the "Molecular Correlation Concept" of cancer. It included "reprogramming" of gene expression in tumors as reflected in the biochemical imbalance between key glycolytic, respiration and gluconeogenic enzymes. It also became clear that key enzymes active both in the synthesis and degradation (in the pyrimidine and purine metabolic pathways) are involved in RNA and DNA synthesis and consequently cell growth.

The progress in knowledge of enzymatic processes in respiration initiated studies about another unknown part of metabolism: free oxygen radicals, a class of highly reactive molecular species. Demonstrating how free radicals could originate during the respiratory process was a key finding. Such compounds are formed during an insufficient electron transfer and are released as high-energy oxygen species. They are very active vis-à-vis reacting with many types of macromolecules, especially, during lipid peroxidation when the high-energy of these oxygen species is emitted as photons in the spectral range from red to ultraviolet.

The interest in what physiological conditions favor this free radical production and simultaneous photon emission started in the 1970's. Equally important was the question if such physiologic conditions could be a major determinant in the transformation of a normal differentiated cell to a cancer cell. As progression of a tumor proceeds and oxygen becomes more limited, the metabolism becomes anaerobic. When blood supply is unchanged, "microclumps of cells" without oxygen will stop growing because oxygen and nutrient supply (and therefore vascularization) become the critical condition for growth. The process of ischemia induced vascularization was unraveled both at anatomic and biochemical levels. The elaboration of substances from ischemic cells which induce angiogenesis and vascularization are one part of the regulation. Its reverse regulation, bringing the angiogenesis to a halt, was also elucidated. As new vessels bring blood to the tissue and the tumor renews its growth, one can observe this part of aerobic and anaerobic cyclic conditions.

The irregular anaerobic/aerobic fluctuation within the tumor makes the tumor highly sensitive to oxygen. It can be considered as a special example of reperfusion of previously ischemic tissue which is associated with exacerbation of cellular injury. The concept of reperfusion injury has been accepted since the beginning of 1980's. Reperfusion injury is mediated, at least in part, by the formation of reactive oxygen metabolites. The oxidizing formation has been demonstrated during reperfusion by its concomitant ultra-weak photon emission. The result of oxygen radical formation is a damage to an entire array of biomolecules including nucleic acids, membrane lipids, and proteins. In particular, the damage in mitochondria may be related to the progress of tumor development and finally to the irreversibility of the tumor development. The idea that a tumor may be considered as a special type of mitochondrial dysfunction has recently drawn the attention of researchers.

Summary and Perspective

The discoveries in the field of metabolism and energy production as well as in the differentiation/proliferation duality have been a fruitful new avenue to approach carcinogenesis. The association of cancer with loss of differentiation has very slowly over a hundred years pointed to a similarity between the neoplastic process and embryonic development. It will also influence the fields of embryonal development and regeneration biology.

Although ultra-weak radiation (photon emission) has now been linked to a special part of metabolism (high-energy excitations in redox research), is this biological radiation functionally connected with growth and differentiation processes? So far, the data are indirect. We need to make one further

step. Does this radiation contain information about the formative process? We will therefore, during our fourth journey, focus on this issue.

PART D

From Bio-Electromagnetism to the Photon Field Concept

Introduction Part D

Iron-filings scattered on a card held over a magnet arrange themselves in the pattern of "lines of force" of the magnet's field. If the filings are thrown away and fresh ones are scattered, the new filings will assume the same pattern as the old. Something like this, may happen in the cell or the body accounting for the forces that mobilize molecules of different kinds into patterns. Molecules and cells are constantly being torn apart and rebuilt with fresh material. The optical photon emission window may, then, offer a view vis-à-vis the electromagnetic interaction field if a body forms and then continually maintains its shape by constantly reshifting its material. Perhaps, it may even direct the pattern development of chemical "morphogens" (which are then interpreted into body patterns by something else, such as, special genes).

From field theory to bioelectromagnetism

The idea of a controlling electromagnetic field arranging the new molecules and cells in the same pattern as the old ones was already proposed in the 1920's. With this in mind, cancer might be labeled as "electromagnetic disturbance of the cell". The measurement of such tiny electrical voltages in biological organisms began in the 1930's. It was concluded that wherever there is life there are voltage gradients. Various physiological processes in animals and man were found to have electrometric correlates. A particular focus of Burr was wound healing. The electrometric studies of the healing wound reflected the growth and structural replacement of tissue in an electromagnetic field. It led to the proposition of the electrodynamic theory of development.

Biological semiconductor concept

In the late 1950's, Russian reports regarding currents of injury gave Western scientists a new incentive to research bioelectromagnetism. With improved equipment, complex fields were reported that followed the arrangement of the nervous system. Studying injured limbs as their fractures healed demonstrated the presence of a highly negative potential that corresponds with the all important blastema. Utilizing solid state electronics, accumulation of knowledge in bone research documented, in the early 1970's, that the origin of the injury current was semiconducting. Solid-state devices were only beginning to hit the market in the mid-1960's and, unfortunately, the scientific community was not ready for biological semiconduction.

Electromagnetic fields and life

A little more accepted in the early 1960's were the diverse electromagnetic oscillatory systems of the brain and regulation systems that depended on

electromagnetic oscillations for their functioning. This included an increasing amount of vibrational processes at the molecular levels and their role in muscle contraction, cell division, glycolytic metabolism, etc. In the 1970's, biological influences of electromagnetic fields including visible light were established independent of simple thermal effects. Such interconnections were differentiated with regard to specific "working" frequencies, intensity, ranges, and methods of coding that include not only the interactions between cells but also specific interactions between macromolecules.

Towards integration and internationalization: revival of the photon field concept

Research on electromagnetic fields and photon emission in living systems called for multidisciplinary collaboration. It resulted in the (German) symposium on *Electromagnetic Bio-Information* (1977), the first international symposium on *Photon Emission from Biological Systems* (1986), and the first international school on *Biological Luminescence* (1989). Further internationalization began when the editors of *Experientia*, an interdisciplinary journal for life sciences asked for multi-author reviews regarding *Biophoton Emission* (1988) and *Biophoton emission, stress and disease* (1992). These activities began to discern the richness of information which can be retrieved from measurements of photon emission.

12

Field Theory and Bioelectromagnetism

How can a form exist in time with all its richness and complexity? When we meet a friend we have not seen for six months there is not one molecule in his face which was there when we last saw each other. Modern research with "tagged" elements has revealed that, in fact, all the protein in the body is "turned over" every six months and, in some organs such as the liver, the protein is renewed more frequently. In fact, it is common place that living creatures maintain a certain consistency in structure even though there are continuous changes in their physical makeup. The traditional modern doctrine (that chemical elements determine the structure and organization of the organism) failed to explain why such a structural constancy persists despite continuous metabolism and chemical flux. Driesch postulated an organizing "field", Child introduced a hypothesis of a physiological gradient, and Weiss postulated a "biological field". All of those concepts have certain "descriptive" validity. However, with regard to the most fundamental problem (organization), it appears that the difficulty was *not* in the failure of any possible theory, but rather, in the adequacy of scientific analysis proving its possibility.

Gurwitsch's Field Theory presented an adequate description of regulation of growth. However, biologists continued at a loss to explain how the forces in our bodies "keep our shape" through the ceaseless metabolism and changes of material. At this point in time, an important concept in biology evolved called the electromagnetic field.

Electromagnetism: Basics

Basically, everything electrical stems from the phenomenon of charge. No one knows exactly what this is, except to say that it is a fundamental property of matter that exists in two opposite forms (or polarities) which we arbitrarily call positive and negative. Protons, which are one of the two main types of particles in atomic nuclei, are positive. The other nuclear particles, the neutrons, are so named because they have no charge. Orbiting around the nucleus are electrons of the same number as protons inside the nucleus. Although an electron is 1836 times less massive than a proton, the electron carries an equal but opposite (negative) charge. Because of their lightness and their position outside the nucleus, electrons are much more easily dislodged from atoms than are protons. A flow of electrons is called

Figure 12.1 ►
Electric and magnetic field lines and the propagation of electromagnetic waves. Upper panel: Electric field lines for several charge distributions: From left to right: a single positive charge; two charges of equal magnitude and opposing sign (an electric dipole); and two equal positive charges. In general, the magnitude of E is different at different points along a given field line. Middle panel: Magnetic field produced by an electric current. From left to right: magnetic field lines around a straight current; magnetic field around a circular current; magnetic field lines around a coil. Lower panel: Linkage of electric and magnetic alternating fields which leads to the propagation of electromagnetic waves.

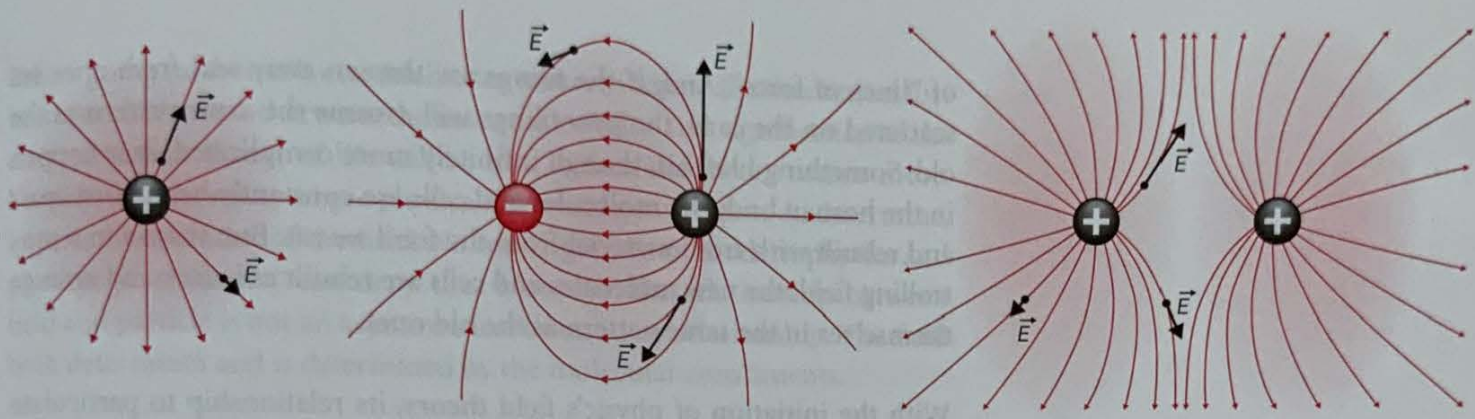
a current. It is measured in amperes, units named for an early nineteenth century French physicist, André Marie Ampère.

A current has another characteristic, its electromotive force. This can be visualized as the "push" behind the current. It is measured in volts (named for Alessandro Volta). A current flows only when a source of electrons is connected to a conductor that contains relatively fewer free electrons through which the electrons can flow. If there is no conductor, and hence no circuit, there is only a hypothetical charge of flow (called electric potential) between the two areas. The force of this potential is also measured in volts.

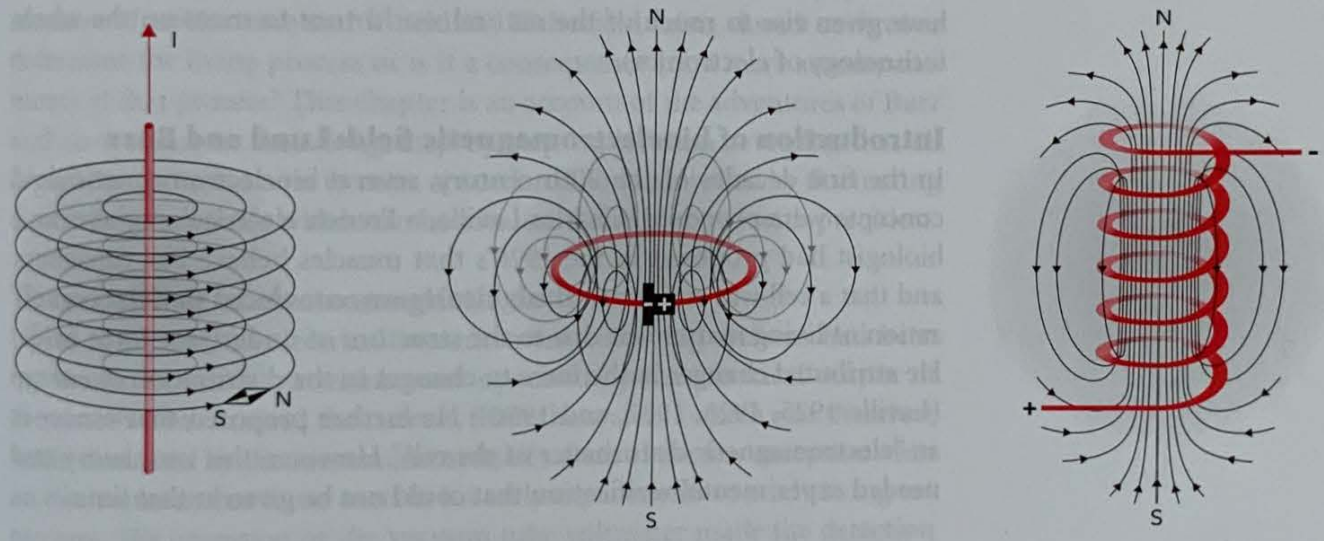
An electric field forms around any electric charge. This means that any other additional charged object will be attracted (if the polarities are opposite) or repelled (if they are the same) within a certain distance around the first object. The field is the region of space in which an electrical charge can be detected. It is measured in volts per unit of area.

An electric field must be distinguished from magnetic fields. Similar to charge, magnetism is a dimly understood intrinsic property of matter that manifests itself with two polarities. Any flow of electrons is accompanied by a combined electric and magnetic field around the flow of current. This, in turn, affects other electrons nearby. Just as a current produces a magnetic field, a magnetic field, when it moves in relation to a conductor, induces a current. The magnetic field is measured in gauss, units named after the nineteenth-century German pioneer who studied magnetism, Karl Friedrich Gauss.

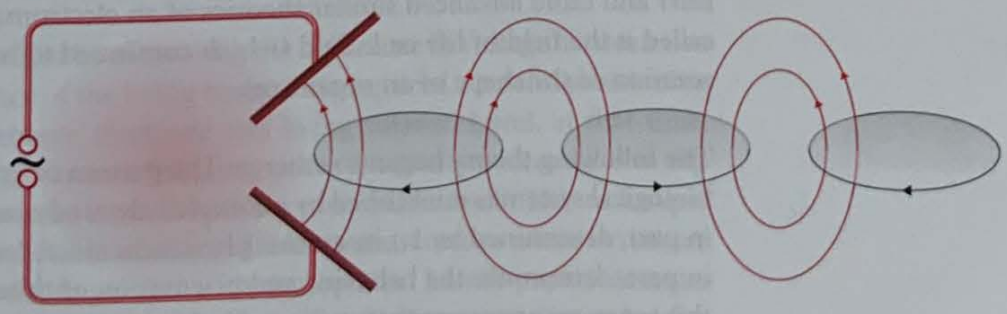
Both electric and magnetic fields are represented by lines of force that indicate its direction and shape (Figure 12.1). Both kinds of fields decline with distance. However, their influence is technically infinite. Magnetism is often associated with the observation that, if iron filings are scattered on a card held over a magnet, they will arrange themselves in the pattern



Electric field lines



Magnetic field lines



Propagation process

of "lines of force". And, if the filings are thrown away and fresh ones are scattered on the card, the new filings will assume the same pattern as the old. Something like this, though infinitely more complicated, may happen in the human body. Its molecules and cells are constantly being torn apart and rebuilt with fresh material from the food we eat. But, thanks to a controlling field, the new molecules and cells are rebuilt as before and arrange themselves in the same pattern as the old ones.

With the initiation of physic's field theory, its relationship to particulate matter in biological theory may become clear. It is important to know that the complexity of electromagnetic energy exists in a spectrum of wavelengths that include cosmic rays, gamma rays, X rays, ultraviolet radiation, visible light, infrared radiation, microwaves, and radio waves. Together, electromagnetic fields and energies interact in many complex ways that have given rise to much of the natural world (not to mention the whole technology of electronics).

Introduction of bioelectromagnetic field: Lund and Burr

In the first decades of the 20th century, several bioelectromagnetic field concepts were proposed. Charles Laville, a French electrical engineer and biologist had proposed in the 1920's that muscles behave like capacitors and that a cell was an electrical engine. He was convinced that the organization of living matter was due to the structure of underlying force fields. He attributed changes in the force to changes in the distribution of energy (Laville 1925, 1928, 1932, and 1950). He further proposed that cancer is an "electromagnetic disturbance of the cell". However, this was theory and needed experimental verification that could not be given at that time.

Another line of research began with Lund's papers. His research indicated that *Hydras* were electrically polarized. Stimulated by these papers, Harold Saxton Burr of Yale University began putting electrodes to all kinds of creatures. Burr was lucky enough to have a forum for this work. He edited the *Yale Journal of Biology and Medicine* where many of his reports appeared. Burr and Lund advanced similar theories of an electromagnetic field. Burr called it the field of life or L-field (which continued to maintain the organization of the shape of an organism).

The following theory began to emerge: The pattern or organization of any biological system is established by a complex electrodynamic field which is, in part, determined by 1.) its atomic physicochemical components and 2.) in part, determines the behavior and orientation of those components. In this sense, we are presently familiar with the reciprocal interaction between particle (molecule, etc.) and field.

Before going further, it is essential, once more, to repeat the theoretical formulation because, otherwise, the essential novelty and significance of the electrodynamic theory of life will be lost. In fact, the theory means more. Namely, that the microscopic physical/chemical constituents do determine, in part, the character of the field. No one cognizant of modern physics and physiologic chemistry can deny this. However, the relationship between field and particle is not an asymmetrical or one-way relationship. The field both determines and is determined by the molecular constituents.

However, at the beginning of the 20th century, a general statement about the field theory required answers to major questions: 1.) Are there both potential and polar differences in living systems? 2.) Do these potential differences exist in an organized fashion or are they chaotic and indeterminate? 3.) Are the electrical measurements recorded in the laboratory a valid measure of an electrodynamic field? 4.) If the field exists, does it control or determine the living process or is it a consequence (or a mere accompaniment) of that process? This chapter is an account of the adventures of Burr and co-workers in their long, step-by-step exploration revealing that all organisms, from bacteria to man, are ordered and controlled by fluctuating electrodynamic fields which can be measured and mapped with precision.

The recording of electromagnetic data

Up to that time, all electrical measurements of living systems had been made with instruments which were operated by the electrical output of the system. This drained the current from the system (which was therefore badly disturbed by the measurement itself). One of the few exceptions was an electrometer that Lund used in his early experiments to study living organisms. His invention of the vacuum tube voltmeter made the detection of the little differences (of the L-fields) possible because it required virtually no current for its operation. Everyone who worked at that time with this type of instrument knew that it was both difficult to set up and also to keep it in good operating condition for sufficiently long periods to permit careful studies of the living organism.

The field was examined by measuring the difference in voltage between two points on the surface of the living form. This required a careful positioning at the interface between electrode and living surface. Lund, at that time, made another great advance by using either zinc sulfate electrodes in tap water or cell sap to make contact with the protoplasm itself. This technique avoided the unpredictable and unreliable measurements made by others with metal electrodes in contact with protoplasm. Lund's papers demonstrated how much of an incredible rigorous technique was demanded for his studies. He not only utilized an electrometer (which draws no current from the system measured) but also reasonable reversible electrodes. Many

of his earlier experiments were carried out under stable environmental conditions with controlled stimuli for calibration.

Burr considered it desirable to construct an instrument easier to use and more reliable over long periods. In the early days of his research, Burr spent three years developing his instrument. The original instrument, built in the 1930's, following the design of Cecil Lane of the Physics Department of Yale University, utilized a then readily available vacuum tube. However, in those days, such tubes were in the process of development and it was difficult to get two tubes that were reasonably balanced. The tubes were connected in such a way as to form two arms of a Wheatstone Bridge. The other two arms were ordinary ohm resistance. Tube number one received the potential to be measured. Number two acted as a dummy [the function of which was to balance out the steady plate current of the input tube so that (with no potential difference impressed on the first tube) no current flowed through the galvanometer in the plate circuit of the output tube]. When imposing a potential difference upon the first tube, the effective resistance of this arm of the network changed proportionally and a deflection of the galvanometer emerged.

With this device, Burr had a reliable instrument to measure very minute electrical voltages. However, for living systems, the problem of connecting the instrument to the system was still insufficiently resolved. The solution devised by Burr (with the aid of a physical chemist, Leslie E. Nims) was to create electrodes (fine silver wire dipped into molten silver chloride) in order to bridge the gap between protoplasm and the amplifier. For their experiments, pairs were selected to have a minimum of self-potential. Thus, with Burr's instruments (consisting of a high impedance amplifier and silver-silver chloride electrodes working through a salt bridge in contact with living systems) reliable results were obtained. However, what was recorded was not specifically a value that, in itself, was important. Rather, it was the relationship between any two values measured by the instrument: one value dealt with relatedness rather than absolute values. With this, it soon became clear to Burr and his co-workers that every living system possesses an electrical field of great complexity. This field could be measured with considerable certainty and accuracy.

In their systematic studies, the first thing that they did was to determine (by measuring a variety of systems) whether there always were electrometric properties in a living organism. A large variety of living organisms were studied (some in more and others in less detail). The organisms ranged from bacteria to man. Based on that information, it was concluded that there is unequivocal evidence that wherever there is life, there are electrical properties. However, again, it must be stressed that those electrical proper-

ties (measured under his research conditions) were voltage gradients, not current movement nor changes in resistance to the passage of current.

Human subjects

If there are electrical (voltage) gradients in living systems, what are their essential characteristics? Burr proposed that they must have magnitude and, since they are electrical, they must have directional properties (polar properties with a positive and a negative disposition). To learn more about these gradients, one of the first things undertaken in Burr's laboratory was to measure human beings. With the advice of Leslie Nims, a very simple approach was adopted for such purpose. They positioned the subject's left index finger into a left-hand cup (functioning as an electrode) and simultaneously positioned the index finger of the right hand into a right-hand cup. Immediately, in the first experiments, it was clear that there was a voltage gradient between the left finger and the right finger. The data was carefully checked by moving the right-hand finger into the left-hand cup and vice versa. Moreover, such measurements were repeated (at least ten times or more) until the researchers substantiated reproducible results. Then, the question arose whether there was any significant difference between human beings in those measurements.

The first series of recordings was performed on male subjects. The data indicated that the magnitudes of the potentials were rarely less than two millivolts and often many times higher. At the other extreme, individuals were documented with voltage gradients between the right and left forefingers of about ten millivolts. These voltage gradients were quite consistent during the period of measurement and, what was more remarkable, they were quite consistent with the passage of time. Burr and colleagues suggested that there might be an electrical difference between males and females. Hence, the measurements were made on female members of the laboratory group, day after day, week after week, and month after month. They documented that during the course of a month's measurements that the female voltage gradients revealed remarkable increases (a sharp rise over a period of twenty-four hours). Discussions with endocrinologists suggested that the rise might be associated with ovulation. A comparison of women during the menstrual cycle (in the non-fertile period) compared to those in menopause revealed no such electrometric changes with the passage of time. This was true whether the menopause was natural or resulting from surgery.

In that same period, Burr (with the cooperation of Luther Musselman, Dorothy Barton, John Boling, and Vincent Gott) initiated parallel studies on rats, rabbits, cats, and other animals. Various stages of the estrous cycle in animals were found to have electrometric correlates which indicated

physiologic processes during the estrous cycle. The work on the bioelectric potentials of animals (and plants) demonstrated that all polar systems are surrounded by and possibly possess interpenetrating electric fields generated by each one of the constituent cells in order to maintain electrical correlation within the system.

Such research resulted in the electrodynamic theory of life. Developed by Burr and F. S. C. Northrop, it was first published in their joint paper (1935). A summary of the hypothesis by Burr and Nortrop is present in the following quote: "The pattern of organization of any biological system is established by a complex electrodynamic field, which is in part determined by its atomic physical-chemical components and which in part determines the behavior and orientation of those components. This field is electrical in the physical sense and by its properties it relates the entities of the biological system in a characteristic pattern and is itself in part a result of the existence of those entities. It determines and is determined by the components. More the establishing pattern, it must maintain pattern in the midst of a physicochemical flux. Therefore, it must regulate and control living things, it must be the mechanism the outcome of whose activity is "wholeness, organization and continuity". The electrodynamic field, then, is comparable to the morphogenetic field of Gurwitsch, and to the entelechy of Driesch.

Animals as subjects

To further test their hypothesis, Burr and co-workers explored the electrodynamic fields in other forms of life. In particular, they searched for types of organisms that could assure that such fields are involved in "Form", and "Formation", or "Becoming". The choice was the salamander. It changes in its form as it grows and its development can be observed with great accuracy. They are vertebrates and, therefore, have a head and tail axis with a bilateral symmetry (a right side and a left side as well as a dorsal and a ventral side). This is the most elementary aspect of the organizational pattern of the vertebrate nervous system. As we have seen before (Chapter 2), salamanders are admirably suited to the experimental analysis of growth and development. Moreover, by using microsurgical methods, it was possible to remove parts of the salamander and to observe the consequences during its development.

Beginning with the embryo, Burr and colleagues initially determined whether or not there were any significant electrometrics. They made potential measurements in the embryo from a point in the cephalic region and another in the caudal region. These were studied over periods of time demonstrating characteristic changes with the growth and differentiation of the embryo itself. They concluded that there is an electrometric correlate of the longitudinal axis of the salamander nervous system. There is also a

bilateral symmetry between the right side and the left side of the axis. Turning their attention to the salamander egg (a sphere with little or no differentiation except the difference between the animal and vegetal pole) they measured (in the unfertilized egg) between the animal pole and four points on the equator. From these measurements, they found that there was one point on the equator which revealed a marked increase in the voltage drop between the reference electrode and that point. When they marked the latter spot with Nile sulfate and followed it through the subsequent periods of development, they found that the point with the greatest voltage drop from the animal pole marked the head end of the developing salamander.

A further finding disclosed such that as the micropipette electrodes were pulled away from the surface of the embryo, the voltage gradient could still be recorded. In fact, they observed that the drop-off of voltage gradient continued as far as one or one and a half millimeters from the embryo. This made clear that the field properties of the embryo radiate through the medium of the liquid environment in which the embryo lives. The observation that direct contact with the living organism is not necessary to measure a voltage gradient made it possible to explore the field properties of a small part of a living system. Subsequently, Burr and Alexander Mauro developed a rigorously controlled experiment to measure fields accompanying the neural impulse travelling along the sciatic nerve of the frog.
The question behind the experiment addressed the nature of the field in living systems. It became increasingly clear that such fields are in fact quasi-electrostatic fields. This also changed the name of the field. Originally, the term "electrodynamic field" was used to describe, in the most general way, the nature of the fields in living organisms. However, henceforth, it was possible to give a more precise definition. The measurement of such fields indicated that forces exist not only in but also outside of the nerve during excitation. So, these fields may have direct consequences for processes in living systems.

Slime mould as a subject

It seemed reasonable to Burr and colleagues that the relationship of entities within living organisms may be measured by field properties. The simple hypothesis was that the electrostatic fields do not exist in the absence of charges nor do charges exit in the absence of fields. They are both fundamental properties of matter. Therefore, chemical components in living organisms, wherever they possess charges, cannot exist without fields and fields cannot be found without the presence of charges. Simultaneously, living is not a static affair. Rather, it is a moving, dynamic process. Therefore, energy is required. It has been proposed that the chemistry of biological systems provides that energy. To experimentally approach this phenom-

enon, Burr addressed fields related to the simplest living organization. He examined the electrical properties of a very simple protoplasmic system, the slime mould (*Physarum polycephalum*). This mould is a syncytium of protoplasm with no cell boundaries and with many nuclei. Its protoplasm is in constant movement oscillating from one end to the other. This streaming of protoplasm is well known to biologists. Under the microscope, it was demonstrated that every sixty or ninety seconds the protoplasm in the veins reverses the direction of flow. Burr and co-workers correlated the electrical recording from the vein with the moving picture. This revealed that in the majority of instances, polar reversal of the voltage occurs before there is a directional change of the plasma flow. In addition, there were many instances where the changes in both phenomena occurred simultaneously. It suggested that changes in the electrical environment may have an effect upon the flow properties of protoplasm. To find further experimental evidence, they imposed an external electrical field on the primitive protoplasm, leading to a change in the flow towards the negative side of the imposed electrical environment. So, in the slime mould, changing the electrical environment can, under certain conditions, modify the direction in the protoplasmic streaming.

Towards wound healing as a subject

Such documentation reinforced the concept that the electrodynamic field could serve as a signpost for a variety of physiological conditions. Subsequently, Burr focused on the peripheral nervous system, particularly on the relationship between the electrometrics and the physiological state of the nervous system. Collaborating with R. G. Grennel, Burr studied the surface potentials in peripheral nerve injury. To do so, they excluded the influence of blood flow. They confirmed that rapid closure of the blood-flow in the forearm and hand vis-à-vis a blood pressure cuff (as well as sudden opening) did not significantly alter the potential difference. In other words, altering the normal functioning of the vascular bed did not affect the standing potential. Furthermore, Burr and colleagues confirmed that the microvoltmeter was relatively unaffected by changes in resistance of the skin being measured (e.g., sweating). Since the latter process is mediated by the sympathetic nervous system, the role of the sympathicus in potential differences seemed unlikely. Nevertheless, when unilateral sympathectomy was performed, there was a difference in the standing potential on the operated and unoperated sides. This suggested a relationship between potential and nerve injury.

Simultaneously, Samuel Harvey and Max Taffel were studying wound healing in experimental animals and man. They called attention to the fact that the strength of a healing wound was probably caused by proliferation of fibroblasts. Moreover, an increase of tensile strength must involve at least

two processes: one of cell proliferation and the other cell differentiation. In other words, it is not just the proliferation, but also the increased process of collagen formation which led to increased tensile strength. In fact, the fibroblast slowed down the process of proliferation and instead, enhanced collagen formation (a highly differentiated function of these cells). In normal development, the events of proliferation and differentiation do not occur in the same cell simultaneously. Each cell takes part in the generalized mitosis of a group of cells for a period of time and then, subsequently, with other cells, enters the stage of differentiation. The wound healing study focused, therefore, on potential measurements during this differentiation/proliferation cycle. This cycle occurred most frequently during the first eight or ten days following the research wound incision. The question that then arose was: what is happening within the electrometrics during this period.

The experimental animals were guinea pigs. After the preliminary measurements (as a baseline) of potential gradient between the normal skin and the targeted area of injury, the skin and subcutaneous fascia were incised and sutured. Subsequently, other sets of examinations were made. These were continued daily for the next two weeks (or until the wound healed). The data documented a marked change. For the first twenty-four to twenty-eight hours after the injury, the wound area was positive to the normal skin. The potential gradient rose during that time and then rapidly declined until the third or fourth day when the wound became negative to the normal skin. This negativity increased until the maximum was reached on the eighth or ninth day. Subsequently, the potential gradient drop was followed on the tenth or twelfth day by another rise in potential. On the fifteenth or sixteenth day, the gradients returned to approximately normal limits. These electrometric data regarding tensile strength demonstrated an interesting parallel with the curves obtained by Harvey and Taffel. They illustrated a rapid increase during the first eight days. The bioelectric measurements documented a similar change. After the eight day, the tensile strength seemed to approach a plateau.

In cooperation with Harvey and Taffel, Burr proceeded with a further experiment focusing on human wound healing. Approximately twentyfive operative procedures with uncomplicated healing of the wounds were selected for this study. Determinations of the potential gradient between two points (one in the immediate vicinity of the wound and another at some distance from it) were made each day beginning on the day following the operation and continuing until the patient was discharged from the hospital (usually after fourteen days). Basically, the data documented a definite trend. There was a positive four day potential corresponding to the lag in the tensile strength. Following this, the wound evolved into a proliferated

phase during which a negative potential was encountered reaching its zenith between the seventh and ninth days. At that time, the maximum negative voltage was reached at approximately the same time as the maximum tensile strength. At the end of the tenth day the wound usually healed and, from then to the end of the determinations, the voltage gradients gradually fell to the normal baseline. The observations of electrometric studies regarding wound healing in man paralleled those made in experimental animals. It was particularly important giving further weight to the concept that electrometrics data reflect the process of healing in man.

Cancer of the female genital tract as a subject

In 1947, a preliminary report of Langman in cooperation with Burr presented data on electrometric tests in 30 women. Approximately half of the cases were women with clinical evidence of malignancy, confirmed by pathological (histological) examination. All of the electrometrics documented a characteristic negativity of the cervix with respect to the lower abdominal wall. The remainder of the cases consisted of non-malignant conditions of the female generative tract, documenting the opposite. Two years later, the electrometric observations in 428 women were reported. In 75 patients with documented cancer of the female generative tract, 98.7% revealed the cervix to be consistently electronegative. In 353 patients suffering from non-malignant conditions, 289 showed the cervix to be positive (81.9%). The results of a continuation of this study extended to a group of 860 women. Data continued to document the electrometric difference between patients suffering from benign and malignant pelvic disease and suggested the possibility that this technique may be of value as a screening method in the detection of early malignancy in the pelvic organs of the female.

Figure 12.2 illustrates the observations of patients with pelvic malignancy. In all of these cases, the diagnosis of cancer was made only after histological studies. However, the electrometric positive diagnoses of cancer were primarily documented prior to the histological diagnosis of cancer. The significance of electrometric recordings as a screening method in large groups of normal women (better defined as women presenting no gynecological symptoms) becomes more apparent when analyzing the findings in women with no gynecological condition. In this group, 94.8% positive potentials were observed. This is in striking contrast to women with malignancies in whom 95.9% negative potentials were recorded. In benign conditions 20.6% negative potentials were recorded.

The findings in patients who have had follow-up electrometric studies have indicated that a reversal in polarity from negative to positive occurs after total hysterectomy for intra-epithelial carcinoma of the cervix. These data

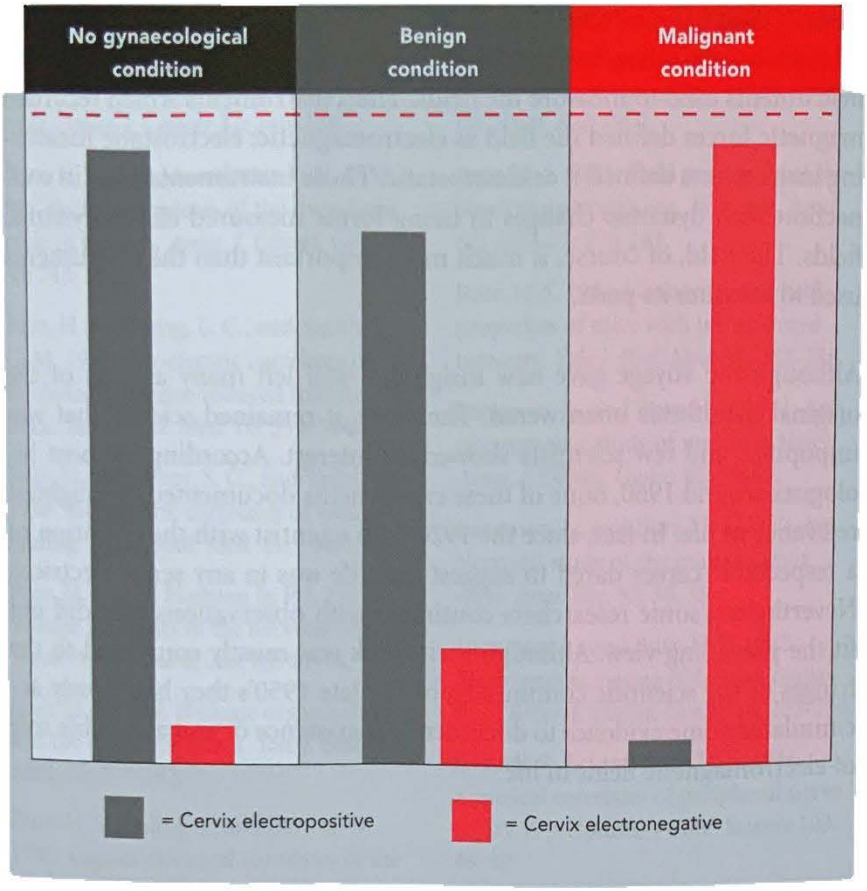


Figure 12.2
Electrometric tests in cancer of the female generative tract. The diagnosis of cancer was made by histological studies.

suggest that the electrometric correlates resulted from causes inherent in the tissue involved. If the involved tissue was removed in its entirety, reversal in potential occurs. Conversely, reversal did not occur when all the tissue involved was not removed by surgery.

Conclusion

Data collection illustrates the end of Burr's discoveries in cooperation with other scientists regarding the voltage gradient field. The voyage turned out to be lucky in the sense that their scientific efforts documented the patterns. The electrometric patterns may well reflect the growth and structural filling by matter in an electromagnetic field. In that respect, the original hypothesis of Burr and co-workers was experimentally close to the documented facts.

What regularly changed during their voyage was the name of the field. Burr referred to electromagnetic fields, electrostatic fields, and electrodynamic

fields. It used to be assumed that these were different and separate. But, this was not so, according to Burr. The various names were derived from the instruments used to measure the fields. Thus, instruments which recorded magnetic forces defined the field as electromagnetic; electrostatic measuring instruments defined it as electrostatic. Those instruments used in connection with dynamic changes in living forms measured electrodynamic fields. The field, of course, is much more important than the instruments used to measure its parts.

Although the voyage gave new insights, it still left many aspects of the original hypothesis unanswered. Therefore, it remained science that was unpopular and few scientists showed an interest. According to most biologists around 1960, none of these experiments documented the slightest relevance to life. In fact, since the 1920's, no scientist with the intention of a respectable career dared to suggest that life was in any sense electrical. Nevertheless, some researchers continued with observations that did not fit the prevailing view. Although their work was mostly consigned to the fringes of the scientific community, by the late 1950's they had slowly accumulated some evidence to document the existence of and a possible role of electromagnetic fields in life.

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13

Bone: Semiconductors as Biophoton Emitters

The fields that Burr and Lund found were far too simple to account for a salamander's limb or a human face. Biological knowledge of that time gave them no theoretical framework to explain where their fields came from. Burr's work was dismissed as "foggy vitalism". Lund suffered the same fate. However, one can disagree with a theory. In fact, no one bothered to see if the measurements they had made were valid. This was the situation in Western Europe and the United States. The situation in Eastern Europe and Russia, however, was different. Scientific interests in "electrical energy in life" remained. Even an influence of an artificially applied extra current on biological processes was part of their interest. That interest finally returned, although limited, to Western research.

Since the original Russian research was only published in Russian, Western knowledge was very limited. Only a small part reached Western scientists and such was severely "censured" by Western scientific forums. The electromagnetic field theory of living organisms returned vis-à-vis a Russian paper in *Biofizika*, the Soviet *Journal of Biophysiscs* entitled, *Nature of the Variation of the Bioelectric Potentials in the Regeneration Process of Plants* by A. M. Sinyukhin of the Lomonosov State University in Moscow. Sinyukhin reported his experiments regarding the severing of a branch from a tomato plant. He took electrical measurements both around the wound as each plant healed and as the plant initiated a new shoot near the incision. He found a negative current flowing from the wound within the first few days. This was similar to the "current of injury" as was previously published regarding imposed research wounds on animals. During the second week, after a callus had formed over the wound and the new branch had begun to form, the current reversed its polarity to positive and became stronger. Sinyukhin found a direct correlation between these orderly electrical events and biochemical changes. He then applied an extra current, using small batteries, to a group of newly cut plants which then augmented the regeneration current. These battery assisted plants restored their branches up to three times faster than the control plants. The currents used for stimulation were very small (only 2 to 3 microamperes for five days). Larger amounts of electricity destroyed the cells and also did not initiate a growth enhancing effect.

Becker follows Burr's work

Among Western scientists, it was Becker who put credence in Sinyukhin's report. He became further motivated by an even different Russian scientific report. In a government translation, Becker found a 1958 paper by A. V. Zhirmunskii of the Instutute of Cytology in Leningrad who studied the current of injury in the hind leg muscle of the bullfrog. Zhirmunskyi imposed a standard injury within each muscle, measured the current of injury, and then severed the nerves to those injured muscles (branch by branch) noting the effect on the currents. It decreased with each subsequent lesion. He concluded that the current of injury was proportional to the amount of nerves severed. For Becker, these pieces of Russian science were at that time enough to start working on the puzzle.

The first business of Becker was to repeat Burr's measurements on salamanders using modern equipment with higher sensitivity and less noise. He put a reference electrode at the tip of each animal's nose and moved the recording electrode point by point along the center of the body to the tip of the tail and then along each limb. He measured the voltages on the rest of the body and plotted lines of force connecting all the points where the readings were the same. Becker found a complex field that followed the arrangement of the nervous system. There were large positive potentials over each lobe of the brain and slightly smaller ones over the brachial and lumbar nerve ganglia between each pair of limbs. The readings grew increasingly negative as he moved away from these collections of nerve cell bodies. The hands, feet, and tip of the tail demonstrated the highest negative potentials.

Becker's next step was to find a way to relate inner currents to the outer potentials. By making an artificial (model) salamander, he first demonstrated that a direct current inside could produce the potentials he was getting outside. Becker argued that if the potential pattern was really a part of the nervous system, it should be widely distributed. So next, he surveyed the animal kingdom testing flatworms, earthworms, fish, amphibians, reptiles, mammals, and humans (Figure 13.1). In each species, the potentials on the skin reflected the arrangement of the nervous system. In the worms and fish, there was only one area of positive potential at the one major nerve ganglion, the brain. In humans, the entire head and spinal region (with its massive concentrations of neurons) was strongly positive. The three specific areas of greatest positive potential were the same as in the salamander: the brain, the brachial plexus (between the shoulder blades), and the lumbar enlargement at the base of the spinal cord.

The second topic that Becker researched dealt with injury and healing. He evaluated whether the surface potentials and the current of injury came

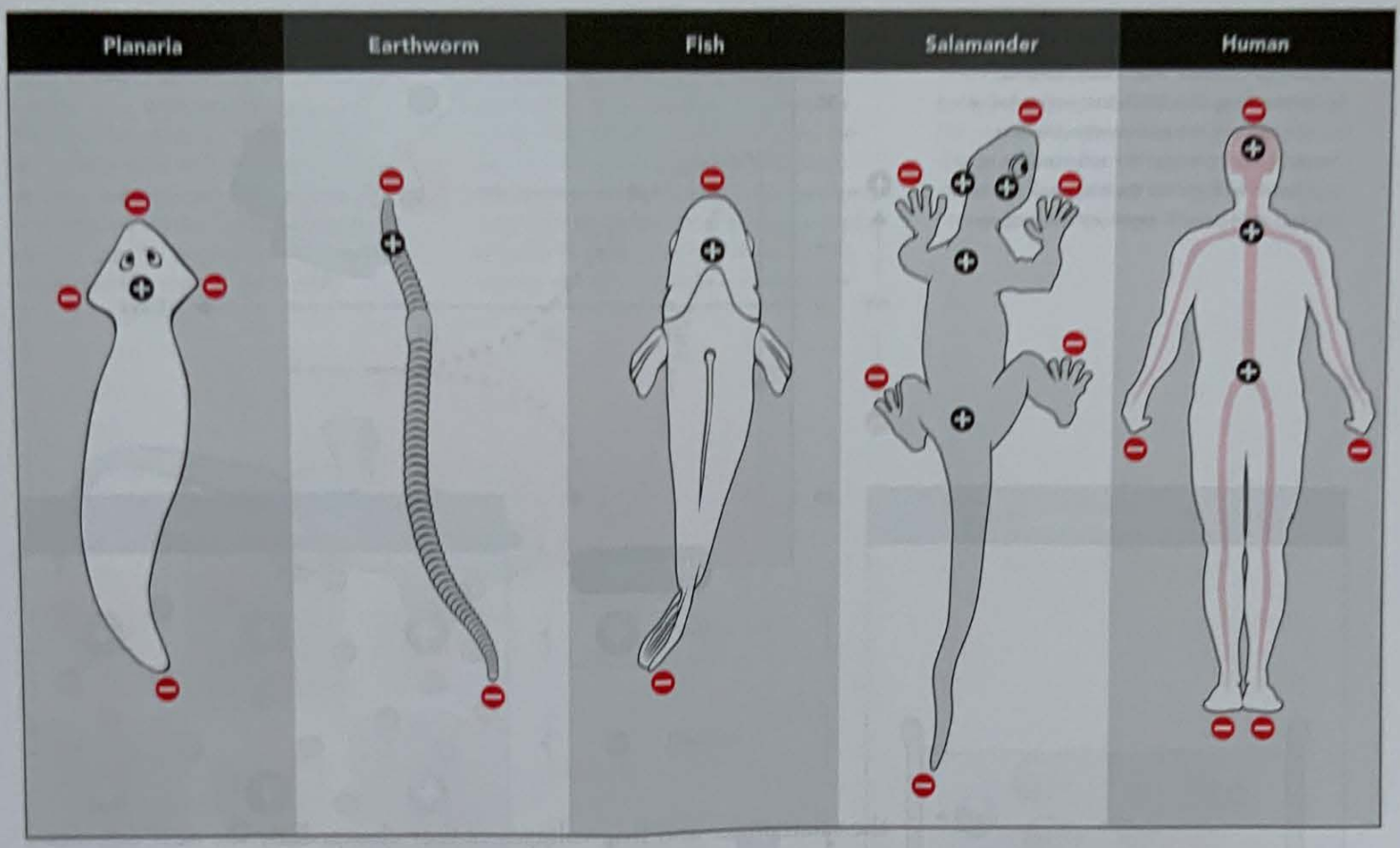
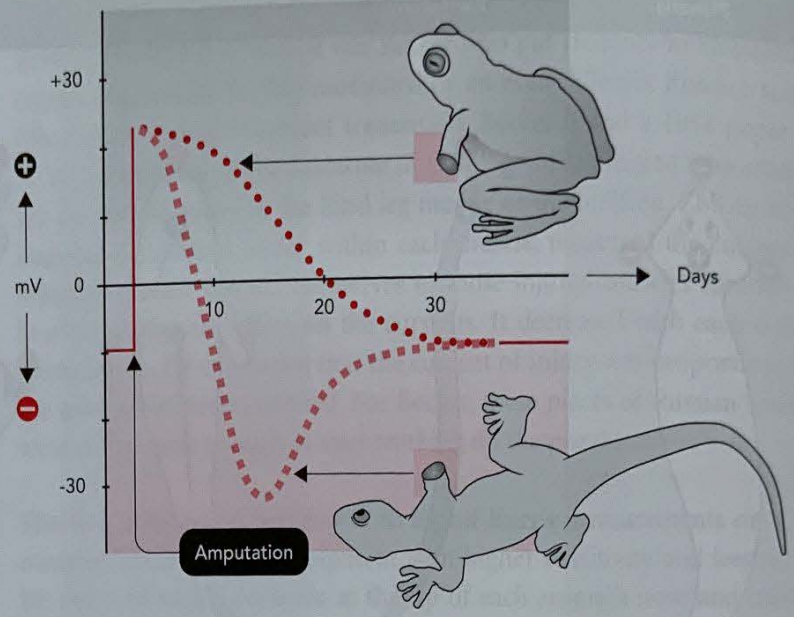


Figure 13.1
Electric field pattern of organisms: A DC field appears in all types of animals. Also, a complex field appears that follows the arrangement of the nervous system. The latter readings grew increasingly negative when moving away from the nerve centres. The hands, feet and tip of tail demonstrated highest negative potentials.

from the same source. He studied frogs and salamanders while their fractured limbs healed. Becker began the actual experiment by amputating (under anesthesia) the right forelimbs (between elbow and wrist) from fourteen *salamanders* and fourteen *grass frogs* (Figure 13.2). He took no special precautions against the bleeding since blood clots formed very rapidly. The wounds were left open, not only because closing the skin over the salamanders' amputation sites would have stopped regeneration, but also, because amputation is a "natural process". In the wild, both frogs and salamanders (favorite foods of the freshwater basins) had spontaneous injuries similar to the ones that were experimentally produced. Both healed without surgery. Becker found that the polarity at the stump reversed to positive immediately after the injury. He made measurements daily to observe

Figure 13.2
The voltage change after injury: salamander versus frog. The electrical properties of the two animals are profoundly different. The negative potential (for salamander) is considered specific for the blastema formation and subsequent regeneration process.

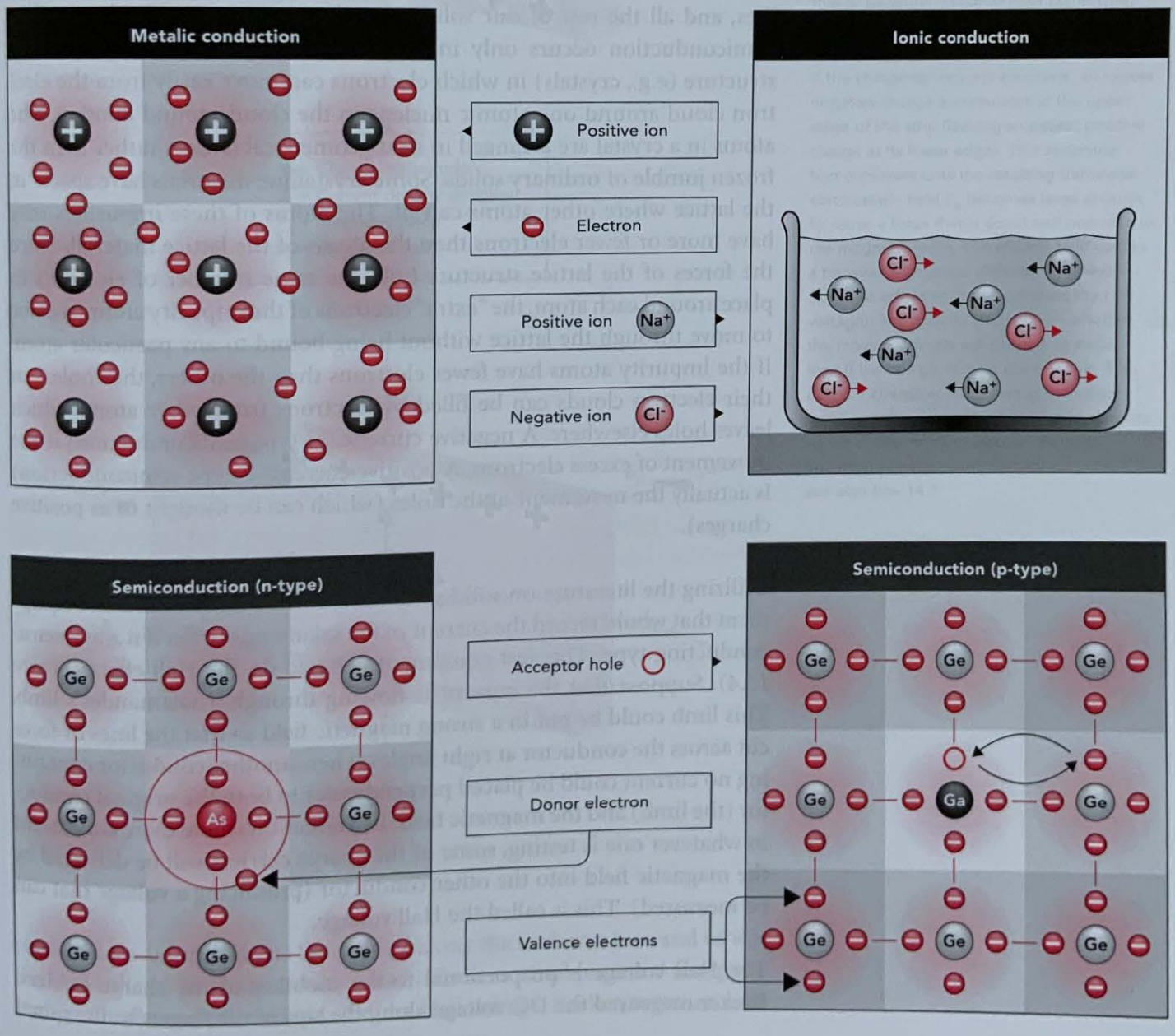


the salamander and frog voltages as they changed. By the next day it had climbed to over 20 millivolts, the same in both frogs and salamanders. The data demonstrated that the forces of current flowing from the salamanders' amputation sites dropped rapidly, whereas from the frogs' stumps stayed at the original level. Then, between the sixth and tenth days, the salamander potentials changed their sign again reaching a peak of more than 30 millivolts negative, just when the blastemas were emerging. As the salamander limbs regenerated and the frogs stumps healed over with skin and scar tissue (without regeneration), both groups of limbs gradually returned (from opposite directions) to the original baseline of 10 millivolts negative. Apparently, the opposite polarities indicated a profound difference in the electrical properties of the two species. Somehow, it might explain why only the salamander could regenerate. The negative potential corresponded with the all important blastema. However, where did the injury currents come from?

Injury current: towards semiconduction

Known at that earlier time, only three types of currents were considered. The most frequently known modes of current were ionic and metallic conduction. Metallic conduction was most easily visualized as a cloud of electrons moving along the surface of metal, usually a wire. This type of conduction is not present in living creatures. Ionic current is conducted in solutions by the movement of ions (atoms or molecules with a negative

Figure 13.3
Three models of current conduction: metallic, ionic, and semiconduction. The upper left panel is a representation of a metallic solid. One or more electrons are detached from each atom and are free to wander around the crystal forming the electron gas. The positive ions vibrate around fixed locations in the crystal. The upper right panel represents ionic conduction. As an example, it illustrates the situation of NaCl in water (forming the negative Cl^- ions and positive Na^+ ions). The lower panel demonstrates the two semiconductor types: the donor and the acceptor. A donor (N-type) impurity atom has a fifth valence electron that does not participate in the covalent bonding and is very loosely bound. An acceptor (P-type) impurity atom has only three valence electrons. It can borrow an electron from a neighboring atom to complete four covalent bonds. The resulting hole is then free to move around the crystal.



charge) because they either have more or fewer electrons needed to balance the positive charges of their protons. Since ions are much bigger than electrons, they move more laboriously through the conducting medium. Ionic currents die out after only short distances. They work well across the thin membrane of nerve fibers. However, it would be impossible to sustain an ionic current for long distances (even the shortest nerve).

The third kind of current is semiconduction. In the 1930's, this was a laboratory curiosity. Halfway between conductors and insulators, the semiconductors were inefficient (in the sense that they carry only small currents). However, they can conduct the currents with ease over long distances. Nowadays, we know, that without them, modern computers, satellites, and all the rest of our solid-state electronics would be impossible. Semiconduction occurs only in materials having an orderly molecular structure (e.g., crystals) in which electrons can move easily from the electron cloud around one atomic nucleus to the cloud around another. The atoms in a crystal are arranged in neat geometrical lattices rather than the frozen jumble of ordinary solids. Some crystalline materials have spaces in the lattice where other atoms can fit. The atoms of these impurities may have more or fewer electrons than the atoms of the lattice material. Since the forces of the lattice structure hold the same number of electrons in place around each atom, the "extra" electrons of the impurity atoms are free to move through the lattice without being bound to any particular atom. If the impurity atoms have fewer electrons than the others, the "holes" in their electron clouds can be filled by electrons from other atoms which leaves holes elsewhere. A negative current (N-type semiconduction) is the movement of excess electrons. A positive current (P-type semiconduction) is actually the movement of the holes (which can be thought of as positive charges).

Utilizing the literature on solid state electronics, Becker found test equipment that would record the current in the salamander even if it was a semiconducting type. This test equipment is based on the Hall effect (Figure 13.4). Suppose that the current is flowing through a salamander's limb. This limb could be put in a strong magnetic field so that the lines of force cut across the conductor at right angles. Then, another conductor containing no current could be placed perpendicular to both the original conductor (the limb) and the magnetic field. In a situation where there is a current in whatever one is testing, some of the charge carriers will be deflected by the magnetic field into the other conductor (producing a voltage that can be measured). This is called the Hall voltage.

The Hall voltage is proportional to the mobility of the charge carriers. Becker measured the DC voltage along the tips of the fingers to the spinal

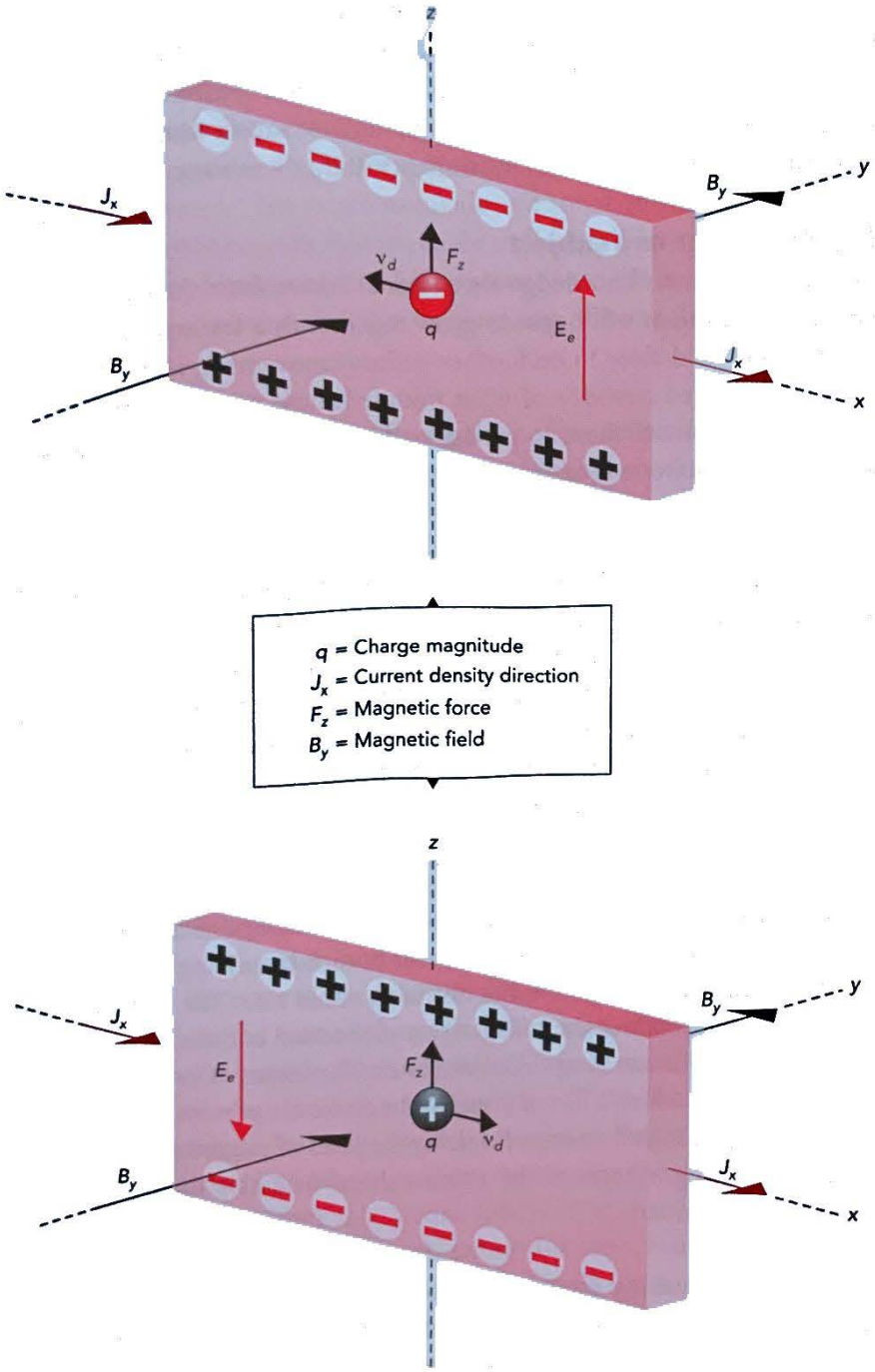


Figure 13.4
Forces on charge carriers in a conductor in a magnetic field: the Hall effect. The upper panel illustrates the case of negative charges. The lower panel highlights positive charges. Both panels consider a conductor in the form of a flat strip. The current is in the direction of the X-axis. In addition, there is a uniform magnetic field B perpendicular to the plane of the strip in the Y-axis. The drift velocity of the moving charge (charge magnitude q) has magnitude v_d.
In both cases, the magnetic force is upward. The magnetic force on the conductor is the same whether the moving particles are positive or negative. In either case the moving charge is driven toward the upper edge of the strip by the magnetic force $F_z = q \cdot v_d \cdot B$
If the charge carriers are electrons, an excess negative charge accumulates at the upper edge of the strip (leaving an excess positive charge at its lower edge). This accumulation continues until the resulting transverse electrostatic field E_e becomes large enough to cause a force that is equal and opposite to the magnetic force. This electric field causes a transverse potential difference between opposite edges of the strip (called the Hall voltage). The polarity depends on whether the moving charges are positive or negative. If the charge carriers are positive, then positive charge accumulates at the upper edge and the potential difference is opposite to the situation with negative charges.
For definitions of vector fields:
see also Box 14.1.

cord. In deep anesthesia, the DC voltage along the limb was zero and so was the Hall voltage. As the anesthetic wore off, the normal potential along the limb gradually reappeared, and so did a Hall voltage. Becker, together with

Charlie Bachman, then looked further for a direct (in standard engineering terms) test of semiconduction in living tissues. However, all the direct tests known at that time worked only with crystals. One needed a material that you can carve into blocks, something that did not squish when an electrode was put on it. For this reason, the only possibility was bone.

Repair of bone as a subject

Our most common knowledge about bones comes from the fact that they can break and then will begin to grow together in a few weeks as long as the ends are held close to each other without movement. However, in the 1950's one lacked any idea of what initiated these processes into motion (nor how to control them to produce a bone bridge). What was actually known was that bone is extraordinary in structure. It is stronger than cast iron in resisting compression.

The part that is actually alive, the bone cells, comprises less than 20 percent of the whole. The rest, the matrix, is not just homogeneous concrete. It is composed of two dissimilar materials: collagen (a longchain, fibrous protein that is the main structural material of the entire body) and apatite, a crystalline mineral that is mainly calcium phosphate. The electron microscope demonstrates that the association between collagen and apatite is highly ordered down to the molecular level. The primary feature of typical collagen is its long, stiff, triple-stranded helical structure in which three collagen polypeptide chains (called α-chains) are wound around one another in a ropelike superhelix. The collagen fibrils in adult bone are arranged in regular plywoodlike layers with the fibrils in each layer lying parallel to one another and at right angles to the fibrils on either side. The bone cells (osteocytes which produce the collagen) are embedded in these layers forming units a few millimeter long called osteones. The center of each osteone has a small canal in which a blood vessel and a nerve are present. The osteones, in turn, are organized so as to lie along the lines of maximum mechanical stress producing a bone of the precise shape best able to withstand the forces applied to it.

Bone has an amazing capacity to grow. In childhood, each long bone of the limbs has one or two growth centers called epiphyseal plates. Each is a body of cartilage whose leading edge grows continuously while its trailing edge transforms into bone. The process stops when the bone has the right length. Then, the remaining cartilage forms the bony knob (epiphysis) at the end of the bone. This "closure" of the epiphyses is an index of the body's maturation.

It is true that one can break a bone. However, bone cannot really "heal". That sounds like a conundrum. However, it is literally true. Fractures knit because new bone made from other tissues unites the fracture ends.

Although we sometimes speak of bone growth as part of fracture healing, the old, preexisting bone does not have the capacity to grow. Two tissues produce new bone at a fracture site. One is the periosteum, the bone's fibrous covering. The cells of the periosteum's innermost layer have the power of osteogenesis (bone formation). After a fracture, those cells are somehow activated. They begin to divide and some of their daughter cells turn into osteoblasts (cells that make the collagen fibers of bone). Apatite crystals then condense out of the blood serum onto the fibers. In this manner, such new osteoblasts build a swollen ring of bone (a callus) around the break.

Another repair operation exists inside the hollow center of the bone (the medullar cavity). In childhood, the marrow in this cavity actively produces red and white blood cells. In adulthood, most of the marrow turns into fat. However, some active marrow cells remain in the porous convolutions of the inner surface. Around the fracture, a new tissue forms from the marrow cells, most readily in children and young animals. This new tissue is unspecialized. Those particular marrow cells revert to a primitive, neoembryonic blastema state. They initially change into a type of primitive cartilage cells, then into mature cartilage cells, and finally into new bone cells in order to help heal the break on the inside. This process is true regeneration (following the same sequence of cell changes as in the regrowth of the salamander limb).

From a researcher's point of view, one question would be: What activates the cells of the periosteum and marrow? In the case of marrow, we can expect it to be the same factor that switches on the cells in a salamander's amputated leg. In the case of the periosteum, a process highly unique in the arena of bone growth needs attention. This process follows the Law of Wolff (named after the orthopedic surgeon, J. Wolff at the end of the nineteenth century). Basically, Wolff's Law states that a bone responds to stress by growing into whatever shape best meets the demands that its owner makes. When a bone is bent, one side is compressed and the other is stretched. When it is bent consistently in one direction, extra bone grows to shore up the compressed side while simultaneously some is absorbed from the stretched side. It is as though a pressure sensitive mechanism exists that can provide extra cables (on the pressure side while dismantling them from the other). This mechanism stimulates the periosteum to grow new bone at a surface where there is compression stress while simultaneously dissolving bone where there is tension stress.

Again, the question for researchers is: What turns on the periosteal cells? Becker and Andy Bassett, an orthopedic surgeon who was doing research at Columbia considered the possibility of piezoelectricity. The idea was

not completely new. As early as 1954, Iwao Yasuda, a Japanese orthopedist, demonstrated that bone was piezoelectric. He and Eiichi Fukada, a physicist, confirmed it a few years later (published in 1957 in Japanese). Simply put, piezoelectricity is the ability of some materials to transform mechanical stress into electrical energy (Figure 13.5). For example, if one

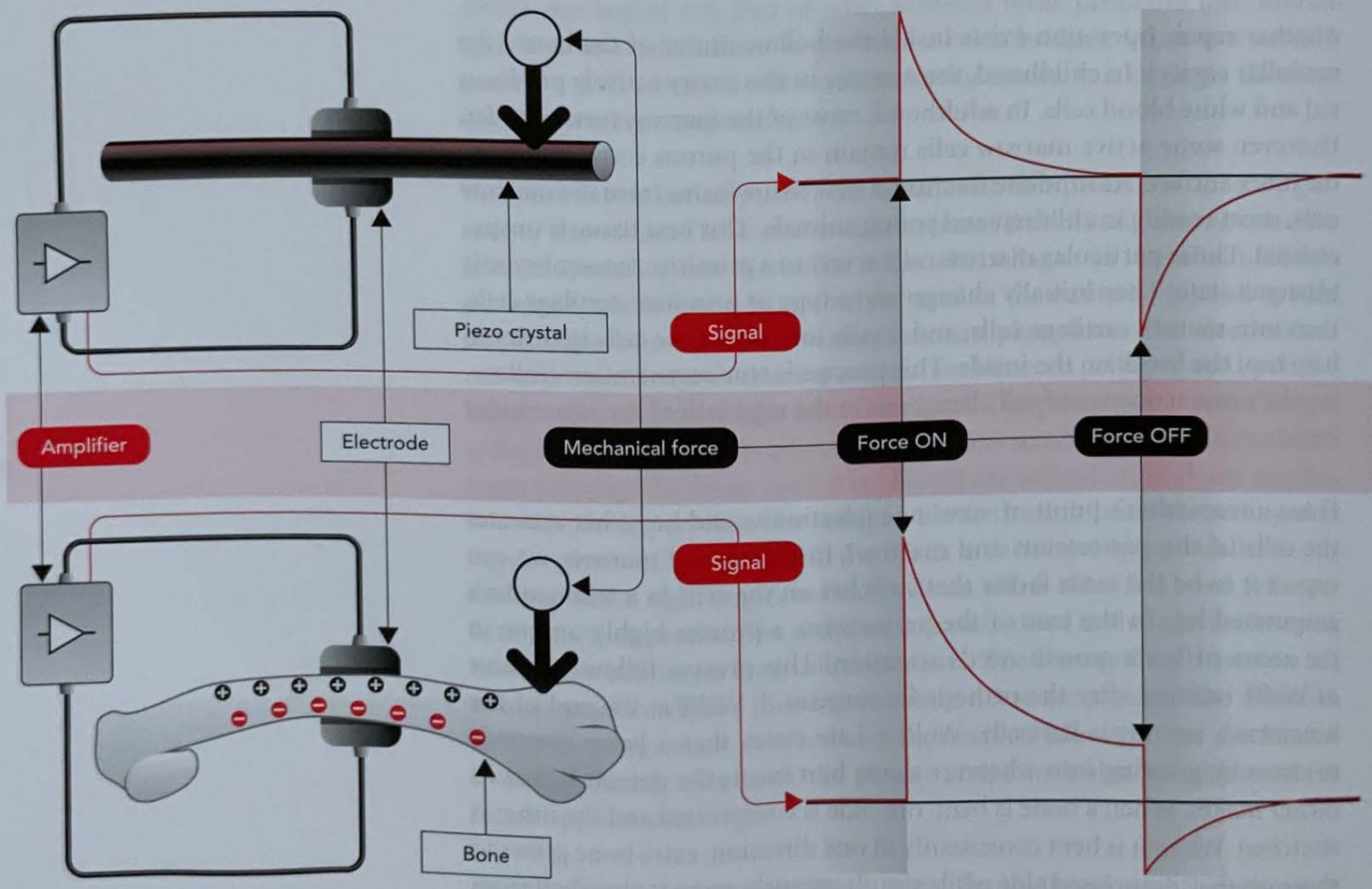


Figure 13.5
Piezoelectricity, particularly in bone. Piezoelectricity is the ability of some materials to transform mechanical stress into electrical energy. The upper panel illustrates that if force is put on a piezoelectric crystal hard enough to deform it, a pulse of current will appear. Electrons migrate toward the compression, so the charge on the inside curve of a bent crystal is negative. The potential quickly disappears if the stress is sustained. However, when released, an equal and opposite positive pulse appears as the electrons rebound while settling back into place. In bone, a similar phenomenon occurs. Bending on the compressed side elicits a negative charge; the stretched side becomes positive. However, the reversed potentials that appear when releasing the stress are not as large as the first. In an electronic sense, it suggests a solid-state rectifier (or PN junction diode).

bends a piezoelectric crystal hard enough to slightly deform it, a pulse of current runs through it. In effect, the squeeze pops electrons out of their places in the crystal lattice. They migrate towards the compression in such a way as to create a negative charge on the inside curve of a bent crystal. The potential quickly disappears if one sustains the stress. However, when it is released, an equal but opposite positive pulse appears as the electrons rebound while settling back into place. Bassett suggested that if bone was really piezoelectric, the negative charge from bending stimulates adaptive growth according to Wolff's Law. Becker and Bassett tested both living and dead bones from a variety of animals. They found, as expected, that bending produces an immediate potential (Figure 13.5). The compressed side becomes negative; the stretched side, positive. Furthermore, the reversed potentials that appear after releasing the stress were not nearly as large as the first potentials. In electronic terms, it has to be a solid state rectifier (PN junction diode). The name, rectifier, looks difficult. However, it is fairly simple. A rectifier is a filter that screens out either the positive (P) or the negative (N) part of a signal. As mentioned in Figure 13.5, current can flow through a crystal lattice either as free electrons or as "holes" that shift their positions. Since current can flow from a P-type to an N-type semiconductor (but not the other way), a junction of the two will filter, or rectify, a current.

Investigating the electrical properties of bone in more detail, Charlie Bachman and Becker postulated that the bone matrix is a biphasic (two-part) semiconductor. That is, either apatite or collagen is a N-type semiconductors. The other, then, is a P-type. Their connected surfaces form a natural PN junction diode that rectifies any current in the bone. During their experiments, they utilized bone removed from patients for medical reasons. By dividing that bone, they created several pairs of sample blocks. From one member of each pair, they chemically removed the apatite. The other of each pair was treated with a compound that dissolved the collagen. Then, they tested the samples for piezoelectricity in the same way that Bassett and Becker had previously tested whole bone samples. Collagen turned out to be a piezoelectric generator, while apatite was not. They now had the principle components of a PN junction: one an N-type and the other a P-type.

Bone as a light emitting diode

One has to imagine that within intact bone, collagen and apatite are joined together in a highly organized fashion. In this respect, the crucial part of the Bassett and Becker hypothesis was to test for rectification at the PN junction. It appeared that the amount of current they could put through the bone samples from a battery of constant voltage was much greater in one direction than in the other (Figure 13.6). Current flowing "uphill" (against the normal flow from P to N semiconductors) is called a reverse

bias current. It was commonly used to look for photoelectric effects. Many semiconductors absorb energy from light. If yes, current flowing through the material will get a boost. The question was: does bone have a photoelectric effect that is sensitive to the current's direction? Such is what one expects from bone containing a rectifier: the current in reverse bias should rise more with the same light intensity than the current in forward bias. The experiment confirmed the expectation (Figure 13.6).

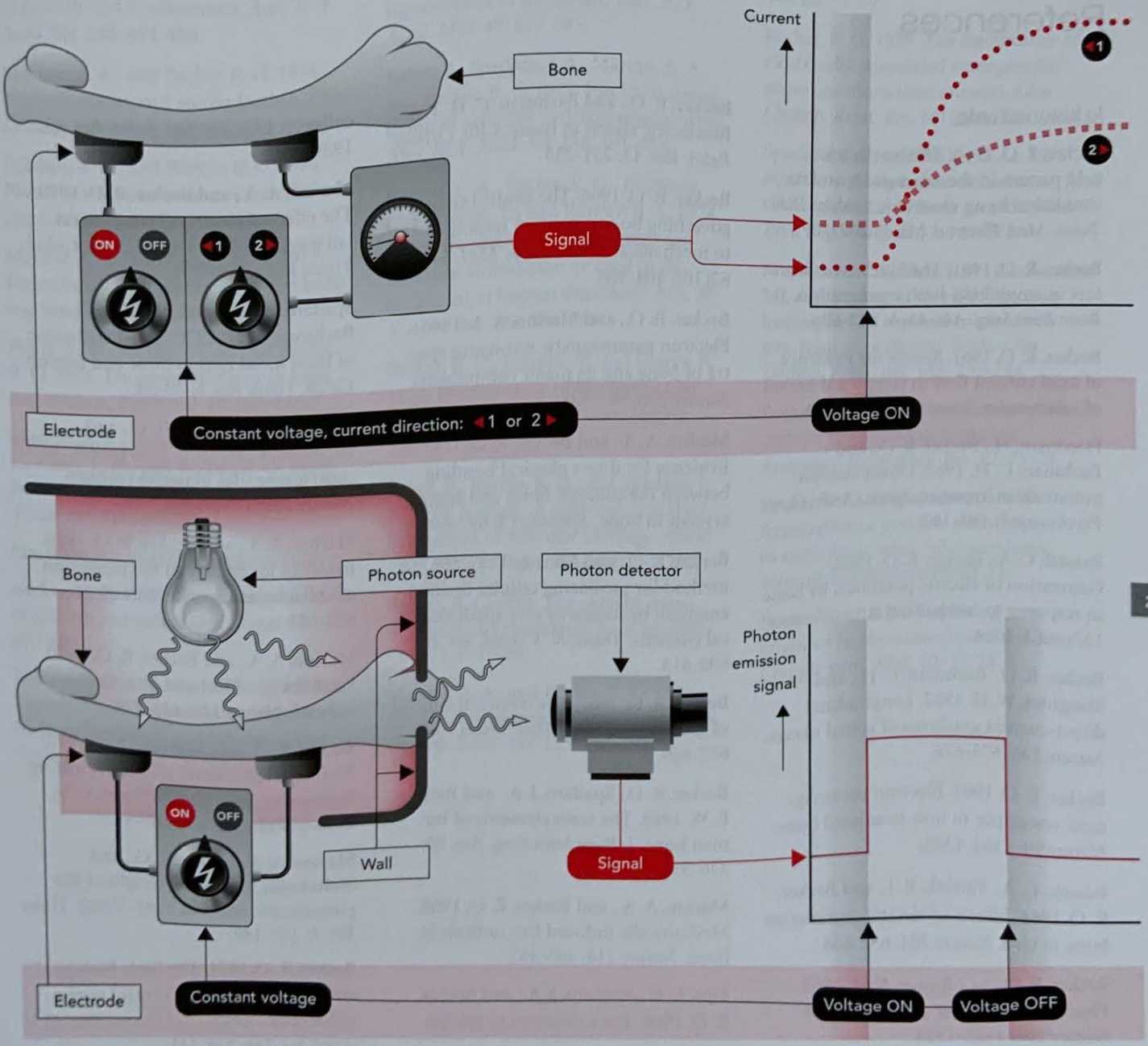
In line with these observations, Becker and colleagues found that bone was a light emitting diode (LED). Like many LED materials, it requires an outside source of light which facilitates the release of its own light. The light emitted is consistently an infrared frequency. It was known that some semiconductors fluoresced. That is, they absorbed ultraviolet light and emitted part of it as a lower frequency of visible light. Bone fluoresced a bluish ivory color. However, collagen yielded an intense blue and apatite a dull brick-red color. When fluoresced light from collagen was combined with that from apatite, it should have fluoresced light as whole bone. However, since it did not, it indicated that there was previously present some other material in the bone matrix (something that was washed out in the chemical separation).

At that time, based on new developments in solid-state technology, called doping, there arose a new experimental hypothesis. Within the process of doping, tiny amounts of certain minerals mixed into the semiconductor material could enormously change its characteristics. Such suggested that trace elements are also present within bone. It was already known that certain trace metals such as copper, lead, silver, and beryllium bind readily to bone. The metal responsible for the observed effect in bone was copper. It was deduced that each atom of copper fits into a little pit surrounded by a particular pattern of electric charges on the surface of apatite crystals and collagen fibers. The binding sites lined up to form one elongated cavity connecting the crystal and fiber. The electrical forces of this copper bond held the crystals and fibers together. The electrical nature of the hole suggested a PN junction at the atomic level.

Conclusion

By the early 1970's, Becker and colleagues had solid proof for nearly every detail of the control system within fracture healing in frogs (and by extension, probably in mammals as well). Like all other injuries, a fracture produces a current that originates from the nerves in and around the periosteum. At the fracture site, the bone generates its own current piezoelectrically due to the residual stress in the mangled collagen-apatite matrix. Such electrical signals combine to stimulate the cells that form new bone.

Figure 13.6
Two aspects of bone as a light emitting diode (LED). The upper panel demonstrates that at constant voltage, the current flowing in one direction 1.) is greater than in the other direction 2.) The lower panel demonstrates that bone (as many conductors) is able to absorb energy from light. Bone is a light emitting diode (LED) that requires an external source of light to release its own photons. If current flows through the material, photon emission gets a boost.



Unfortunately, the scientific community was not ready for biological semiconduction and the notion of diodes in living tissue seemed ridiculously far fetched to most of the people. In the mid-1960's, solid-state devices were only beginning to enter the market. Nowadays, various kinds of PN junctions (such as LEDs) are everywhere as digital readouts in watches and calculators. However, at that time, they were just laboratory curiosities.

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14

Electromagnetic Fields and Life: A more Comprehensive View

Bioelectromagnetism research within alive organisms steadily increased, perhaps implying that electromagnetic fields participate in informational connections. In a wide variety of organisms, regulation systems do depend on electromagnetic oscillations for their functioning. It had already been known that transmission of information along the nervous system vis-à-vis bioelectric pulses included a frequency spectrum extending to thousands of hertz. In addition, studies began to document long-range interactions of electromagnetic fields via very different frequencies which were present in electromagnetic relationships between macromolecules, in the synchronization of the electromagnetic vibrations within assemblies of macromolecules as well as groups of cells.

Electromagnetic relationships within living organisms

In the early 1960's, two important scientific books shed light on novel principles within peripheral nerve and central nervous system functioning. Eventually, such novelties became leading principles within the research field of electromagnetic regulation systems in living organisms. In addition, a major role was played by Nina Aleksandrovna Aladzhalova, a USSR scientist with impressive publications regarding brain research in the 1950's and 1960's. Those publications were summarized in her monograph appearing under the original title, *Medlennye Elektricheskia Protsessy Y Golovnom Mozge*, published by the USSR Academy of Sciences (1962). Fortunately, it was translated in Scripta Technica, Washington D.C., and published by Elsevier in 1964 under the title, *Slow Electrical Processes in the Brain* (*Progress in Brain Research*, Volume 7). It illustrated how, within the rates of regulation in the central nervous system of warm blooded animals, one can distinguish fast and slow control systems. The first control system included fast reactions to stimulation such as the orientation type of reaction. The slow system assesses, more or less systematically, influential environmental factors that alter the level of activity of the organism in connection with its regulation of homeostasis. A fundamental fact was established that the slow system acts on the parameters of the fast system. The generalizations and conclusions in Aladzhalova's monograph were even more worthwhile because of the categorization of the variety of structures containing self oscillations accompanied by ultra slow fluctuations of po-

MORE ABOUT...

BOX 14.1

■ BIOELECTROMAGNETISM

Bioelectromagnetism is the study of a.) the *action* of external electromagnetic fields and radiation upon *living* organisms, and b.) the generation of electromagnetic fields and radiation by the alive organisms themselves.

The kinds of influences that electromagnetic fields and electromagnetic radiation exert on an organism depends on whether it is alive or dead, a feature that, in itself, gives us some valuable clues concerning the organism's electromagnetic structure. The fact that there are any influences at all, empirically, implies that the organism must contain some aspects of *electromagnetic functioning* capable of coupling with an external electromagnetic field. On the other hand, the fact that *some* influences (generally the more interesting ones) occur only when the organism is alive indicates that only then are the relevant entities (bio-detectors) activated.

Electric fields couple to electric charges and electric dipoles. Magnetic fields couple with electric currents and magnetic dipoles whereas, electromagnetic *radiation* couples to electric charges and endogenous electrical *oscillators*.

Definition of fields

There are 4 vector fields: *E*, *D*, *B*, and *H*. They are defined, as follows:

—*E* is the *electric field*. This is defined in terms of the force exerted on an electric charge, q, irrespective of whether the charge is moving or not. The units of *E* are volts/meter (*V/m*, where *V=joule/coulomb, J/C*).

—*B* is the *magnetic induction*. This is also defined in terms of the force it exerts on an electric charge, q, but one that is *in motion*. The units of *B* are volt second/square meter ($V{\cdot}s/m^2$ = $J{\cdot}s/Cm^2$, *or tesla, T*).

—*D* is the *displacement field* whose source is the density of electric charge, ρ (in coulombs/cubic meter, C/m^2), the units of *D* being ampere.second/square meter ($A{\cdot}s/m^2 = C/m^2$).

—*H* is the *magnetic field* whose source is an electric *current density, J* (*in* A/m^2), the units of *H* being amperes/meter (*A/m*, where *A=C/s*).

Thus, *E* and *B* are "fields of force", whereas *D* and *H* are "fields of source".

Together, they constitute the *electromagnetic* field whose space and time evolution is given by the Maxwell Equations.

Classification

It is usual to classify electromagnetic fields according to frequency, as follows:

3 Hz - 3 kHz: Extremely Low Frequency (ELF)

3 kHz - 30 kHz: Very Low Frequency (VLF)

30 kHz - 300 MHz: Radio Frequency (RF).

300 MHz - 300 GHz: Microwave (MW)

tential. In addition to the cerebral cortex and the nuclei of the hypothalamus, the list included Paramecia and legume roots, the smooth muscles of invertebrates, the skeletal muscles of the frog, and the dendrites and ganglion cells of molluscs. Even at the level of the unicellular organism, she distinguished two control systems. As an example, the activity of the rapid system within Infusoria was documented vis-à-vis the beating of the cilia in response to random environmental factors and short-term influences. The activity of the slow system was manifest by a self oscillatory process on the cell wall, the excitability of the cells, and the coordination of the beating of the cilia varying in time with the rhythmic fluctuations of potential. The purpose of the slow system is, apparently, to ensure the stability of the entire organism and to preserve its state under the action of a wide class of external factors.

Another work by Dean E. Wooldridge appeared at approximately the same time. He received his doctoral degree in physics from Caltech (California Institute of Technology) and then joined Bell Laboratories becoming an expert in the theory of magnetism (basic to modern electronics). He later became director of TRW (a combination of Ramo-Wooldridge with Thompson Products formed in 1958) which was one of the world's largest high technology companies. After he retired in 1962, he devoted himself to scientific pursuits and writing especially in the physical and life sciences. He believed that there was a need to translate data obtained in one discipline into a language that is understandable to another. He did that with large success in *The Machinery of the Brain* (1963). In that publication, he summarized the discoveries and hypotheses stemming from research on the nervous system including electrical properties, peripheral data processing, the permanent wiring of behavior, and implications about emotion and consciousness. It is interesting to cite a few lines from his book with respect to the nervous system of lower animals. "The nervous systems of the lower animals supply many examples of configurations of neurons that produce periodic output signals for the control of rhythmic bodily functions. One interesting example of a neuronal oscillatory circuit is found in the lobster. It consists of a ring of nine interconnected neurons for the generation of the periodic electric impulses that control the heartbeat. The song of a cicada is originated by an oscillator in the insect's brain. Here an interesting new circuit refinement has been added: a sub-harmonic generator. The centrally originating frequency is 200 cycles per second whereas the neuronal arrangement in the sound muscles drives them at only 100 times per second. In many lower animals, neuronal oscillators are responsible for the rhythms involved in walking, swimming, or flying." These examples illustrate the developments.

MORE ABOUT...

BOX 14.2

■ FACTS ON THE ELECTROMAGNETIC NATURE OF THE ALIVE ORGANISM

Cell membrane field

Across the membrane of a living cell, ion pumping generates an electrical potential difference, ΔV, of magnitude $\Delta V \sim 100mV$. Assuming a separation, α, of 10^{-8} m between the inner and outer layers of the bilipid membrane, the magnitude, E, of the associated electric field is given by: $E = \Delta V/\alpha$ $(=10^7 V/m)$.

In *non-living* matter, an electric field of this magnitude is large enough to cause dielectric breakdown, suggesting that it plays a rather crucial role in the biophysics of *living* matter. One such role that can be immediately identified is that this large field protects the interior of the cell from external *DC* fields, but *not* technogenic *AC* (oscillatory) fields above a certain frequency (the value of which is determined by the electrical properties of the membrane).

Another physical consequence is that this enormous field results in a strong (possibly non-linear) polarization of any biomolecules (such as proteins) that find themselves in the field (resulting in the formation of *induced* electric dipoles).

Electromagnetic nature of the alive organism

Most familiar are the oscillatory electrical activities associated with heart beat and brain measured by the electrocardiogram (ECG) and electroencephalogram (EEG). The associated frequencies lie between 1 and 1.5Hz as well as between 1 and 35Hz, respectively (although much higher EEG frequencies have now been recorded using advanced techniques). Other electrical activities include those involved in nerve conduction and in the body's biochemistry which is controlled and regulated electrically (often in an oscillatory way).

Less well known, is an organized macroscopic electrical activity at cellular/sub-cellular levels based on the coherent oscillation of electric dipolar units of a given kind (the frequencies range from the microwave band to values in the terahertz region).

Further evidence of our electromagnetic nature (*when alive*) is the fact that our bodies radiate electromagnetically (thermal radiation, biophoton emission, and (nonthermal) microwave radiation).

Coherence

Electromagnetic radiation consists of a succession of wave packets within fields that undergo regular (sinusoidal) oscillations with a fixed phase and polarization. Consecutive packets do not, in general, connect "smoothly". However, they may at times be "out of phase" with one another. In addition, the planes in which the E and H fields oscillate (i.e., their *polarisations*) are also different. This destroys the "purity" of the macroscopic electromagnetic wave (in the sense that it is not perfectly characterised by a *single* frequency, ν.) Instead, it covers a *range*, $\Delta\nu$, of frequencies given by $\Delta\nu = h/\Delta t$, wherein Δt is the time duration of an individual wave packet and h is Planck's constant $(=6{,}626\times10^{-34}\ J.s)$.

Since within a packet there is no change in phase, Δt is a measure of the "coherence time", τ_c, or, equivalently, the degree of "temporal coherence". Associated with τ_c is the (longitudinal) coherence length, l_L $(= c\,\tau_c)$, which is a measure of the distance in the direction of propagation over which the radiation is phase stable (i.e., is coherent). In addition to the longitudinal coherence length there also exists the transverse coherence length, l_T. Clearly, unlike l_L, the value of l_T depends not only on the source but also on the distance of the field point from the source. Associated with transverse coherence length l_T and longitudinal coherence length l_L is the "coherence volume", V_c.

Some typical values of examples of τ_c and l_L are as follows:

	$\Delta\nu/\nu_0$	τ_c (s)	l_L
Sunlight	~1	10^{-15}	0.1 μm
Gas discharge lamp	10^{-6}	10^{-9}	0.3 m
Radio transmitter	10^{-9}	10^{-6}	300 m
Laser	10^{-12}	10^{-3}	3.10^5 m

It is scientifically understood that *all* radiation is actually coherent (but to differing degrees). Sunlight however, can be considered to be effectively and temporally incoherent. In the case of the laser, the long coherence time can be considered as a consequence of many successive wave packets being in phase.

In summary, the coherence of radiation provides both temporal and spatial information about the source of the radiation (although the latter depends on the field point).

More work on another phenomenon, "Attraction of frequencies" comes from the results of a harmonic analysis of the alpha rhythm of brain biopotentials. Wiener (1963) expressed the following hypothesis: "We think that the brain contains several generators with frequencies close to 10 Hz and, that within certain limits, these frequencies may attract one another. In such circumstances, the frequencies will probably collect into one or several groups, at least in certain regions of the spectrum." An illustration of such "attraction" of frequencies was provided by the results of experiments with isolated heart-muscle cells by Harary (1962). Harary found that while each cell exhibited its own individual rhythm of pulsation, the assembly of cells pulsed with the same frequency (set by a "leading cell" which had the highest pulsation rate). In addition, the discovery of new electromagnetic control systems and the theories relating to carefully investigated systems of such kind were further developed. For instance, Franke described (1962) that the human heart generates electrical oscillations in a much wider frequency range than was previously known (30 to 700 Hz). In the case of pathological changes (pathological ischemia), the most pronounced changes were observed in the high frequency part of the vibration spectrum. High frequency components (in the range 200-500 Hz) were also discovered in the electrical activity of the brain. Trabka described (1963) that these components were appreciable altered by barbiturate anaesthesia.

Searching for mechanisms

With respect to mechanisms of electromagnetic field mediated interconnections within organisms, the discussion should start at the "bottom floor" away from the interconnections between macromolecules. An important scientist in this discussion regarding the electromagnetic connection between macromolecules was Albert Imre Szent-Gyorgyi (1893-1986), a Hungarian born biochemist. He was the first to isolate vitamin C and his research on biological oxidation provided the basis for Krebs' citric acid cycle. His discoveries about the biochemical nature of muscular contraction revolutionized the field of muscle research. His later career

was devoted to studies in "submolecular" biology applying quantum physics to biological processes. He was especially interested in cancer and was one of the first to explore the connections between free radicals and cancer. Szent-Gyorgyi won the 1937 Nobel Prize in Physiology or Medicine for his work in biological oxidation and vitamin C. By the late 1950's, he stated that "...two molecules, the electrons of which are capable of a similar excitation, can thus act as coupled oscillators. In this case, it is not necessary to have a material connection between the two, for the electromagnetic field couples them, provided their mutual distance is not too great (small compared with corresponding wavelength)". In addition, as mentioned above, there are theoretical and experimental grounds for inferring an electromagnetic interaction between macromolecules which might be regarded as informational. An example would be dipole/dipole interactions between identical and similar macromolecules due to fluctuations of the distribution of the proton and electron charge in the molecules. This was already illustrated by the various types of resonance absorption of electromagnetic fields by protein molecules and their aggregates. Such ability usually involves the possibility of appropriate electromagnetic radiation. The above suggested the existence of interconnections between macromolecules vis-à-vis electromagnetic fields of different frequencies [most probably in the ultra high frequency and super high frequency ranges (and, possibly, also, in the low frequency range)].

In the 1960's, there was an increasing amount of experimental and theoretical information regarding vibrational phenomena at the molecular and supramolecular levels in chemical and biological systems. These questions were the topic of a special symposium on *Vibrational Processes in Biological and Chemical Systems* (1967). Many important contributions (e.g., by Frank-Kamenetskii, Shnol', Zhabotinskii, and Sel'kov) promoted discussions regarding models of vibrational processes and hypotheses relating to their mechanisms. The authors stressed that the investigation of such vibrational processes could play a very important role in the elucidation of the physical/chemical nature of several biological phenomena: muscle contraction, regulation of cell division, the mechanism of the biological clock, photosynthesis, glycolytic metabolism, various enzyme substrate reactions, etc..

At that time, it was an important statement that vibrations in chemical reactions and biological systems are always associated with electromagnetic vibrational processes (and in several cases are caused by these processes). There was no doubt that progress in the investigation of vibrational phenomena at the molecular level would greatly enlarge our view of the diversity of electromagnetic regulation systems and their interconnections in living organisms. However, progress was quite slow. The experimental

evidence regarding the sensitivity of organs, cells, and macromolecules to electromagnetic fields (of different frequency ranges, of the generation of such fields in these systems and, of some electromagnetic connections between them) finally indicated the correctness of the hypothesis of the existence of diverse interconnections mediated by electromagnetic fields in living organisms. It led to the speculation for an approach about the unexplained physical nature of special "radio communication" between different elements and systems (cells and macromolecules) within the organism. On the other hand, it also led to interesting hypotheses on the possible role of electromagnetic field mediated interconnections in important unresolved problems of biology.

It was speculated that in living organisms such electromagnetic field mediated interconnections are the explanation for the directional motion of cells in the body and selective interaction between like cells at a distance. This problem was examined in detail by Weiss asking the following questions: "...why a free cell, which can extend in many directions, often advances steadily in one direction to the exclusion of others?" Discussing the hypotheses regarding the role of possible "tropisms" and gradients in these effects, Weiss pointed out that "It has never been possible to demonstrate just how a cell could translate such directional cues into actual convention towards or away from the source." He referred to observations that the cells "recognize" each other and their surroundings and can find their proper destinations in the body even if they are deprived of their customary routes for getting there as well as the fact that cells are grouped in types and actively preserve this association (not only in the body but even in experiments *in vitro*). Referring to these facts, Weiss posed the question: "How do mixed cell populations achieve this orderly reassortment? Do like cells "attract" each other? Or do they "recognize" each other only after chance encounters?" Observing that there is still much that is obscure in the selective interaction between cells, Weiss concludes: "The only thing that seems clear is that these phenomena point to the same general area of specificity that covers immune reactions, enzyme-substrate interactions, pairing of chromosomes, fertilization, parasitic infection, and phagocytosis... So, from phagocytosis and the uptake of macromolecules, through cell-to-cell contacts with specific "recognitions", up to the problem of "selective" adhesion of a cell to its substratum, one deals with a continuous spectrum of problems of the same nature."

Influence on alive organisms of environmental electromagnetic fields

While the role of electromagnetic processes taking place within the vital activity of organisms became more important, simultaneously another, similar phenomenon developed: the effect of electromagnetic processes taking

place in the environment impacting the functioning of living organisms. In previous chapters we discussed that the origin of the above effects has a long history. Galvani's experiments in the 18th century initiated the development of electrobiology dealing with the reactions of living organisms to both environmental electrical stimuli and electrical phenomena within the organisms themselves. It was a form of electrophysiology (primarily in the low-frequency range within the emerging arena of contact application of electric energy). Galvani, however, was also the first to discover a long-distance electrical effect as he observed the contraction of the muscle in a frog (nerve/muscle preparation) situated at some distance from the spark of an electrostatic machine.

Such experiments on electrical stimulation of a nerve at a distance were not taken up again until the end of the 19th century. Experimental investigations and theoretical considerations suggest that external or environmental electromagnetic fields can have a significant biological action. The common opinion was that effects only occur when their intensity is fairly high and that such action was due to the process of conversion of the electromagnetic energy to heat. Yet, there appeared an increasing amount of reliable experimental data which indicate that electromagnetic fields can have nonthermal effects and that living organisms of diverse species (from unicellular organisms to man) are extremely sensitive to electromagnetic fields. Many of the discovered features of the biological action of electromagnetic fields clearly did not fit the Procrustean bed of the heat theory. These effects were observed with very weak natural electromagnetic fields.

These conclusions were easily extended to the broad regions of the electromagnetic spectrum that exist under natural conditions. It was assumed for a long time that such natural fluctuations have no influence on living systems. However, it was critically re-examined in the 1960's resulting in the observation that the effective spectral region extends from the super high radio frequencies, decreasing to and including essentially static electric and magnetic fields.

It was Aleksander Samuilovich Presman, the author of the monograph entitled, *Electromagnetic Fields and Life* (1970) who substantially contributed to this research. Presman completed his studies in physics at Moscow University in 1941. Following World War II, Presman engaged in the study of high frequency physics. In the 1950's and 1960's, he studied the effects of electromagnetic fields on living organisms at both the Institute of Labor Hygiene of the Academy of Medical Sciences of the USSR and the Central Institute of Health Resort Studies and Physical Therapy. Beginning in 1966, he then gave a special course on the above subject in the Department of Biophysics, Faculty of Biology, Moscow University. His teaching contrib-

uted not only to the extensive Russian literature, but almost equally to the comprehensive non-Russian literature dealing with biological influences of these fields. In contributing to the literature, Presman shed some light on possible theoretical foundations for these phenomena. He also put forth a novel and an imaginative, equally important general hypothesis in which he postulated that electromagnetic fields normally serve as conveyors of information from the environment to the organism, within the organism, and among organisms.

A substantial, rapidly increasing number of studies (in both many laboratories and countries) have now clearly established biological influences which are independent of the theoretically predictable, simple, thermal effects. Indeed, many of the effects are produced by field strengths very similar to those within the natural environment.
An analysis of such experimental data which indicated the appearance of these kinds of biological activities of external electromagnetic fields led (for some) to the conclusion that it is useless to attempt to seek the causes of such properties of life either at the molecular level or that of electromagnetic fields. However, it was found that a.) the totally intact, alive organisms are most sensitive to electromagnetic fields, b.) isolated organs and cells are less sensitive, and c.) solutions of macromolecules are even less sensitive. In other words, the property of receiving weak electromagnetic fields from nature (appearing only at the level of fairly complete organized biological systems) might be regarded as one of the manifestations of the specific nature of life, its "organization".

It was important to focus on practical investigations of such interaction (i.e., to follow the historical facts of the interaction of living organisms vis-à-vis any external factor beginning with the observation of the reactions of the entire, intact organism). Only after this might one then follow the simplest level of organization available where it is still possible to document "the" changes which are ultimately responsible for the particular reaction of the organism. The prime cause of the reaction need not necessarily be due to processes at the molecular level. Instead, it may be a feature of some macroscopic level of "organization".

With respect to the environment, it was evident that throughout the reviewable geological period, our biosphere was a region of electromagnetic fields and radiations of all the frequencies known to us (from slow periodic variations of the earth's magnetic and electric fields to gamma rays). Presman postulated that in the course of evolution, organisms had come to employ these fields in conjunction with well known sensory, nervous, and endocrine systems in order to initiate coordination and integration. However, at that time and even now, it is evident that "Only a few of the invisible

MORE ABOUT...

BOX 14.3

■ ENVIRONMENTAL ELECTROMAGNETIC FIELDS AND THEIR INFLUENCE ON THE ALIVE ORGANISM

Another indicator of the electromagnetic nature of living organisms is that their extreme sensitivities to external electromagnetic fields (both natural and technogical) whereby they can detect minute changes in their electromagnetic environments [even in the presence of much larger "irrelevant" signals ("noise")].

The natural environment

We are exposed to a variety of electric/magnetic fields and electromagnetic radiation [both ionising (e.g., cosmic rays) and non-ionizing]. The latter includes the following examples:

The *electrostatic* field of the Earth can range between 0.1 and 20 kV/m depending on atmospheric conditions. As a comparison, it may be noted that at 1m above ground beneath a 400 kV power line at 50 Hz, the electric field is about 5 kV/m (which will light up a fluorescent tube). In the animal kingdom, some species of fish can detect electric fields as low as 1 μV/m.

The *magnetostatic* field of the Earth is approximately a steady value of 50 μT. It includes a variable component of less than 2% of the steady value which is synchronized with the solar and lunar rhythm. Again, for comparison, the magnetic induction 1m above ground beneath a 400 kV power line at 50 Hz is about 12 μT. In the animal kingdom, again, striking sensitivities can be found in birds and bees which can detect changes in the Earth's magnetic field of a magnitude less than 1 nT.

Electromagnetic radiation from the Sun The black body spectrum of this radiation (which peaks in the *visible* part of the spectrum) results in an integrated intensity (power density) at Earth' surface of 1350 W/cm^2 (the so-called Solar constant). On the other hand, the human eye can detect a single light quantum (photon) corresponding to a threshold intensity of the order of about 10^{-15} W/cm^2.

Schumann radiation. This spatially coherent, non-ionizing electromagnetic radiation represents the transverse electromagnetic normal modes of the Earth's ionosphere cavity whose source is the totality of global lightening discharges. The structure of these resonant excitations at the Earth's surface principally consists of vertical *E* and horizontal *B* fields whose spectral field strengths are E_v ~(100-200) μV/m/Hz$^{1/2}$, B_h ~0.5 pT/Hz$^{1/2}$, respectively. The associated spectral power density being in the order of ~6×10^{-15} W/cm^2/Hz (and maximum at local noon). The Schumann power density is close to the threshold of the human EEG sensitivity. The lower frequencies correlate with those of the human EEG. Thus, 8 Hz is a mid α_1 band (7-9.5 Hz); 14 Hz is a mid β_1 band (12.75-18.5 Hz); and 20 Hz correlates with the β_2 band.

Isolation from the Schuman field (and also from the Earth's magnetic field) during space travel (beyond the ionosphere) correlates with the onset of certain neurological disorders in astronauts. To counteract this, Schumann simulators are now fitted in spacecraft.

Some technological electromagnetic radiation exposure to the alive human organism

Human EEG and behavior can be influenced in nonthermal frequency specific ways by exposure to external EM radiation of various kinds when its amplitude is modulated at frequencies close to those of the EEG.

In people who suffer from photosensitive epilepsy, exposure to a light flashing at a rate between 15-20 times/sec can provoke seizures. It is not the intensity of the light that triggers the seizure but rather, the fact that it flashes at the same frequency characterized by the electrical activity of the epileptic brain.

It has been known for over 30 years that microwave radiation, pulsed at certain ELFs (near those now used in GSM telephony) is psychoactive (i.e., has the ability to exert a variety of behavioral and psychological effects on humans, a fact that is now exploited in "*non-lethal*" weaponry. Thus, for example, it is possible to implant sounds and words into the brain which may excite a range of moods by exposure to certain low frequency modulations [such as depression (at 6 Hz) as well as manic and riotous behavior (at 11 Hz)].

radiations are known to us at present." The need by many to understand is difficult "by minds accustomed to other conceptions of the universe." "In fact, we are surrounded and penetrated (at all times and in all places) by eternally changing, combining and opposing radiations of different wavelengths (from ten millionths of a millimeter to several kilometers)".

Conclusion

All this indicated not only the necessity for a fundamentally new approach to the existence of biological electromagnetic fields, but also the need to reconsider the possible roles of external environmental electromagnetic fields on the vital activity of organisms. Every year, scientists are discovering more and more high frequency components of oscillations as well as new systems of "electromagnetic regulation" at the most diverse levels of organization in the organism (from the molecular level to the organism as a whole). A high sensitivity to electromagnetic fields of various frequency ranges is exhibited by all species of living creatures and at all levels of functioning of the organism. Interconnections may underlie not only the interactions between cells, but also specific interactions between macromolecules: enzyme and substrate, antigen and antibody, DNA and RNA.

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15

Revival of the Photon Field Concept: Towards an Integration of Sciences

Since Presman published his pioneer work entitled *Electromagnetic Fields and Life* (1970), it became more and more evident that biological systems can emit electromagnetic waves and also provide sensitive responses throughout the whole spectral range from the extremely slow fluctuations (with frequencies of the order of a few Hertz and below) up to the extremely short waves in the UV region (and even possibly above). A variety of experimental facts (documented over almost two decades) hinted at the existence of "biological resonances" that can give rise to sensitive reactions in biological systems under the influence of electromagnetic waves. It seemed that nature used such waves for regulatory processes, or more generally, for communication within living systems. It became evident that research on "electromagnetic fields and life" and "photon emission in living systems" calls for the collaboration of at least three sciences: mathematics (cybernetics and information theory) in regard to the term "communication", physics referring to "electromagnetic fields" and biology (biochemistry, quantum chemistry) with reference to "biological systems". This collaboration should then be considered as a composite field dealing with all of these phenomena and should be entitled, "Electromagnetic Bio-Information". At least this was the opinion of Fritz-Albert Popp who invited scientists (mostly German of diverse disciplines: biologists, biochemists, physicians, and physicists familiar with these problems) to meet in Marburg, Germany. The conference entitled, *Electromagnetic Bio-Information* took place on September 5, 1977. It was the first conference dealing in a multidisciplinary way with this subject.

Linking the photon field to biochemistry

At the conference, the topic of ultra-weak photon emission was intensively discussed. In particular, Popp promoted extending the concept of photon emission within the realms of cell communication and regulation (by referring to the possibility that chemical substances may influence cellular processes by interfering with a cell's electromagnetic field). Popp's major scientific interest was to study the chemical induction of carcinogenesis. Data had already documented both that cancer cells were intense radiation sources and proliferating cell cultures radiate more intensely than do those wherein growth has ceased. Such data corresponded with Popp's hypoth-

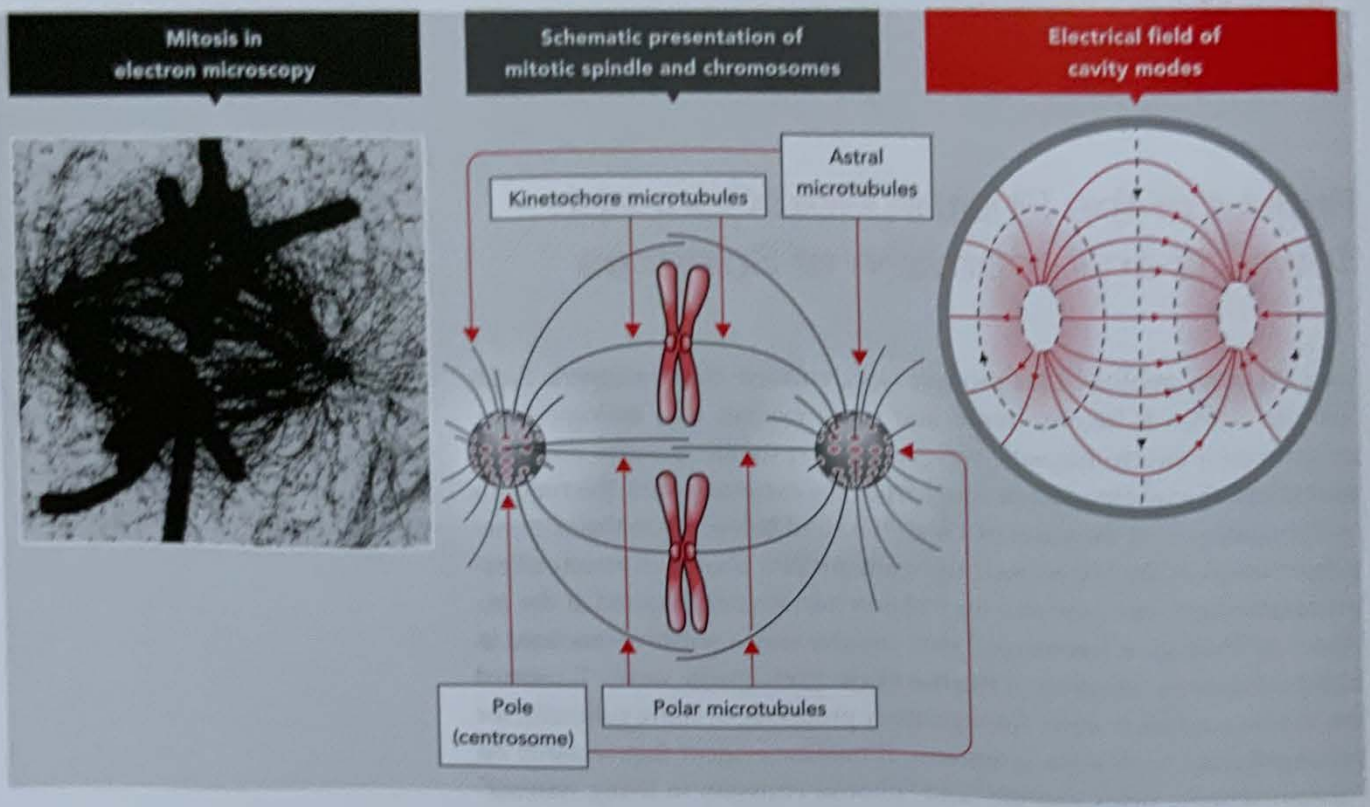


Figure 15.1

The similarity between the mitotic figures and one of the selected cavity resonator waves. The whole process of pattern formation during mitosis can be described by the slow change of superpositions of cavity resonator waves which are selected according to the boundary conditions within the cell. The left panel illustrates a mitotic apparatus in a metaphase mammalian cell visualized by electron microscopy. The middle panel is a schematic diagram corresponding to the cell on the left. That mitotic apparatus is organized into two parts: a central mitotic spindle and a pair of asters. An aster is a radial array of microtubules at each pole of the spindle. The spindle is a bilaterally symmetrical bundle of microtubules and associated proteins. It is divided into opposite halves at the equator by the metaphase chromosomes. In each half of the spindle, a single centrosome at the pole organizes three distinct sets of microtubules whose (-) ends all point towards the centrosome. One set, the astral microtubules, forms the aster. They radiate outwards from the centrosome towards the cortex. The other two sets of microtubules compose the spindle. The kinetochore microtubules are connected to chromosomes. Polar microtubules do not interact with chromosomes, but instead, overlap with polar microtubules from the opposite pole. They project towards the cell center with their distal ends overlapping. The right panel demonstrates the electrical field of TM11 cavity modes in a right circular cylindrical cavity. The comparison in pattern formation illustrates the long-lasting "storage of coherent modes within biological systems" as well as ample indications of stationary fields in the so-called "mitotic figures". These observations led Popp to postulate that biological systems generally have the capacity to store coherent photons arriving from the outside world. It has been speculated that a part of the excess electromagnetic energy of the non-equilibrium cell is distributed over the cell vis-à-vis the boundary conditions of the cell walls. Eventually, the cell slowly (via its own activity) changes the shape and probably the physical parameters of its membranes which in turn alters the boundary conditions (thus acting back on the field, etc.).

esis (formulated in the mid-1970's) regarding photon storage of coherent modes within biological systems as well as a role of photon fields in growth regulation (particularly in carcinogenesis). His interest was mainly theoretical (Figure 15.1).

Did the (chemical) induction of a cancerous state involve a disturbance of the intrinsic photon field? Such an approach demanded different questions. The first was related to the specific chemical property/properties responsible for carcinogenesis. We refer here to Percivall Pott reporting (1775) on a type of scrotum skin cancer of many chimney sweeps in London who regularly were exposed to coal tar. The cause was "a lodgement of soot in the rugae of the scrotum". The documentation that cancer could be initiated by an external agent began a search for the carcinogenic substance(s). Since the 1940's, a method utilizing rabbit and mice models was available to test for carcinogenicity by applying coal tar [its isolated constituents (including polycyclic aromatic hydrocarbons)] to their skin (Chapter 4). Having such knowledge, the question then arose if there is a relationship between the structure of a compound and its carcinogenic activity. This was not a new question at the time of the Marburg conference. Instead, such theoretical research on function/structure relationships of chemicals responsible for carcinogenesis at the very molecular level had, at least, begun with Otto Schmidt. He published a series of papers from 1938 to 1941. This German pioneer author (who probably died during World War II) hypothesized that pi electrons within the polycyclic aromatic hydrocarbons (PAH) (particularly in their charge density distribution within the molecule) might explain their carcinogenic behavior. The pi electron density is a measure of both nucleophilic reaction abilities and the free valence number as a measure of radical reaction ability (see also Box 15.1 and Box 15.2).

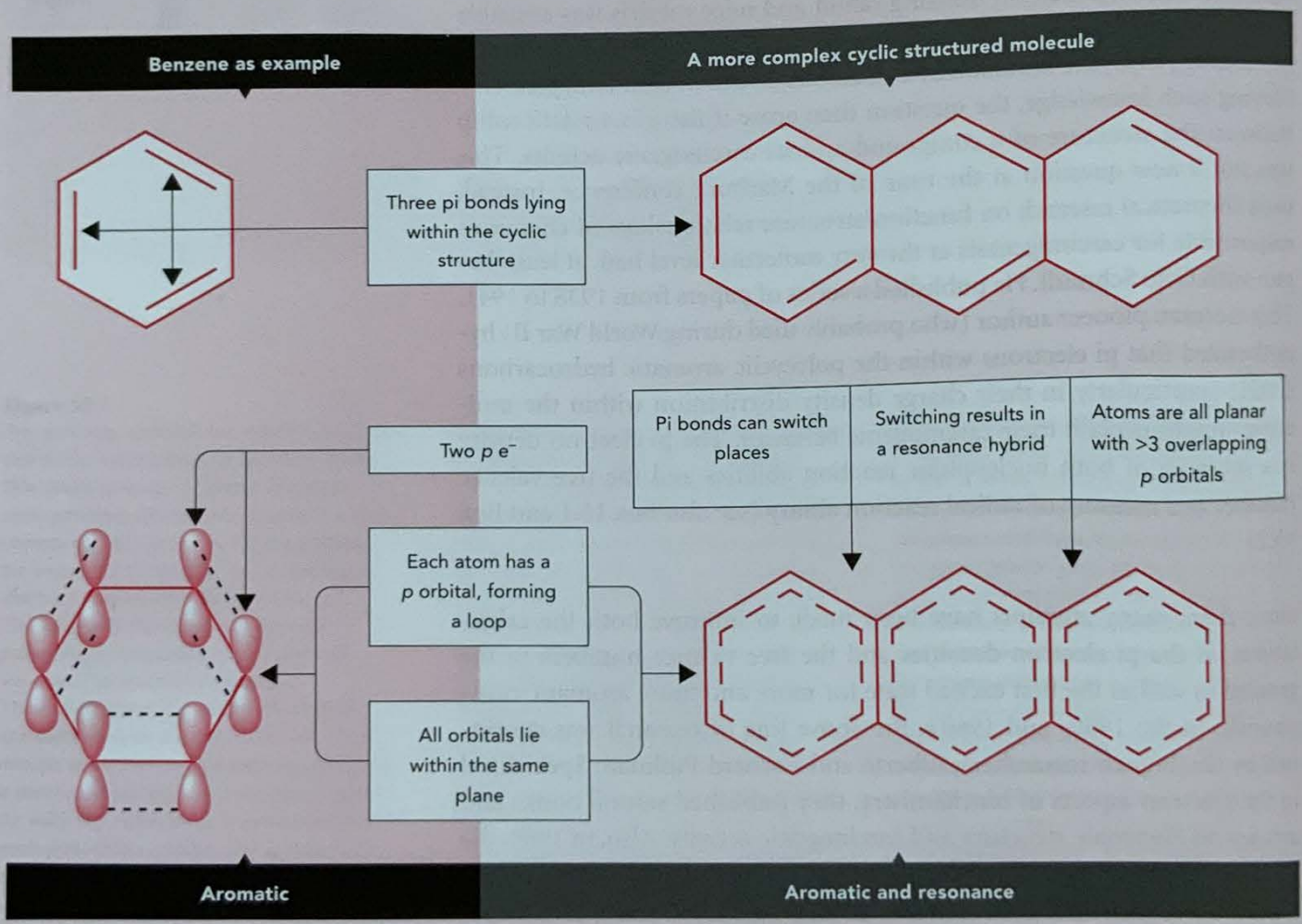
Since then, many attempts have been made to improve both the calculations of the pi electron densities and the free valence numbers in the ground as well as the first excited state for more and more aromatic compounds. In the 1950s and 1960's, the above line of research was continued by the French researchers, Alberte and Barnard Pullman. Specialized in the electron aspects of biochemistry, they published several books and articles on electronic structure and carcinogenic activity. Also, in 1969, the Japanese group of Kumaki and co-workers published regarding the correlation of certain biological activities (including excited state parameters) documenting that both certain positions of these aromatic compounds could be hydroxylated and that such hydroxylated positions are best explained by a nucleophilic reaction in the first electronic excited state. The hydroxylation of these positions was not explained on the basis of the electronic properties of the substrates within their ground state.

MORE ABOUT...

BOX 15.1

■ PI ELECTRONS AND AROMATICITY IN POLYCYCLIC AROMATIC HYDROCARBONS

Figure B.15.1.1
Pi electrons in relationship to resonance. The properties of aromaticity and resonance are illustrated utilizing an example of a benzene molecule and a more complex structure.



A pi electron is an electron involved in a pi bond. A pi bond is an overlap of two adjacent *p* orbitals between two atoms. Therefore, 1 pi bond contains 2 pi electrons. A double bond consists of 1 sigma bond and 1 pi bond, and therefore for each double bond there are two pi electrons present.

In aromaticity, pi bond of a molecule meet the following specific criteria: a.) they lie within a cyclic structure, b.) each atom of the cycle must have a *p* orbital forming a *p* orbital loop, c.) all *p* orbitals overlap and lie within the same plane, and d.) the orbital arrangements must result in a lowering of energy (i.e., the molecule follows Hückel's Rule).

Three or more adjacent, parallel, overlapping *p* orbitals provide a special stability. Aromaticity cannot exist without conjugation. Conjugation requires at least three, adjacent, overlapping *p* orbitals in the same plane such that electrons can be delocalized. The cyclic delocalization is a contributor to the molecule's extra stability.

As a result of electron delocalization resonance exists in a molecule. Different resonance patterns emerge as a result of structure and atom arrangement within a molecule.

(See also Figure B.15.1.1.)

To improve correlations between carcinogenicity and certain energetic aspects of pi electrons in the polycyclic aromatic hydrocarbon, the researcher Shou-Sin Sung of the Institut de Cancerologie et d'Immunogenetique de Villejuif (France) researched more polycyclic aromatic hydrocarbons. In cooperation with N. P. Buu-Hoï, they published (1970) a series of publications leading to some acceptance of the above approach. In later years, Sung argued that one needs as many polycyclic aromatic hydrocarbon compounds as possible. Thus, a theory cannot be considered valuable if it has not been applied with success to all cases known. In the past, researchers were often content with the comparison of a determined molecular property between a quite limited number of carcinogens and non-carcinogens and hence claimed the difference established as the cause of the carcinogenicity of the molecule. It was no wonder that this approach could lead to inevitable controversies (as it was easy to find exceptions if one tried to extend this conclusion to other cases). Sung made an effort to include in his analysis as many cases as possible for the calculation of charge density distributions. Although certain authors believed at that time that it was not suitable to deal with polycyclic hydrocarbons with more than 6 benzene cycles because calculations would be less accurate. Sung, however, had the opinion that such decrease in precision was gradual and no reason to limit the relationship study to a level of 6 cycles. He made a careful survey and used 62 non-substituted polycyclic aromatic hydrocarbons which had been hitherto experimentally studied as to their carcinogenic activity. He made the calculations and concluded (1975) that the degree of correlation between carcinogenicity and ortho-localization of energies was comparable to but not stronger than that between carcinogenicity and electronic excitation energies.

In this period, Popp initiated his Ph.D. research in carcinogenic compounds. In his opinion, the situation was far more complex and required more insight into the factors involved in the decomposition of high-energy states. In that respect, he suggested that a molecule should be more carcinogenic when a certain

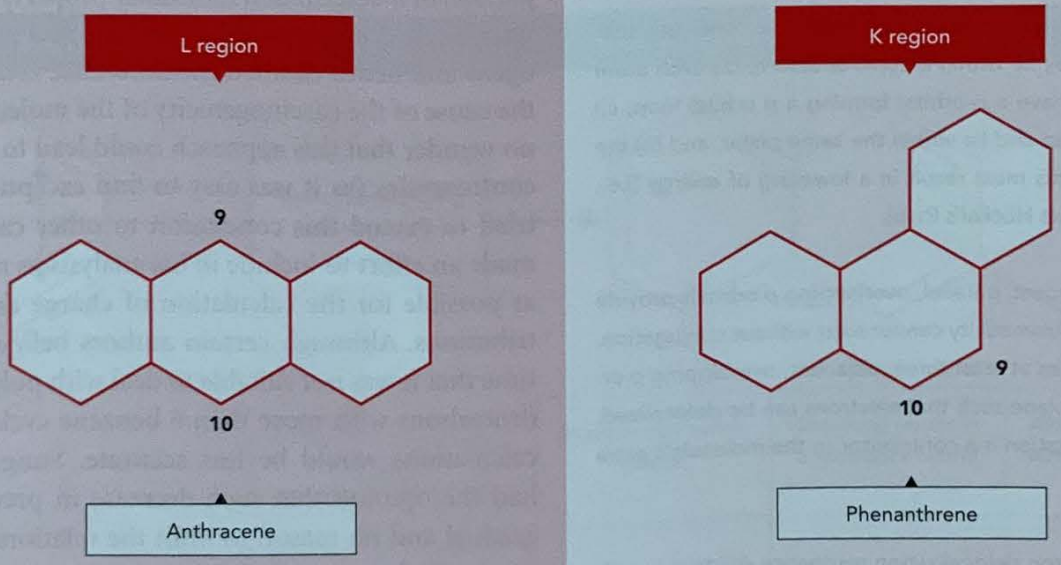
MORE ABOUT...

BOX 15.2

■ CHEMISTRY OF POLYCYCLIC AROMATIC HYDROCARBON, CARCINOGENICITY AND A POSSIBLE LINK TO THE PHOTON FIELD

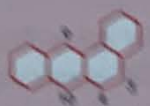
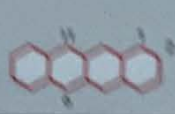
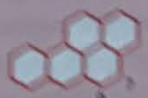
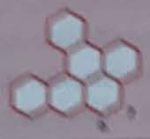
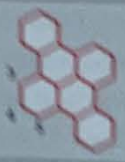
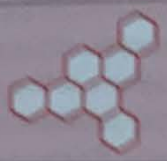
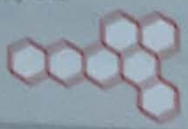
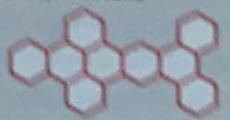
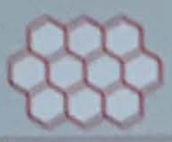
Figure B.15.2.2 ►
Polycyclic aromatic hydrobarbons (PAH) were studied for their charge density distribution in relationship to cancer inducing property. The figure presents PAH examples of different number of rings (1-10 rings), their carcinogenicity and the number of PAH's studied in each category.

Figure B.15.2.1
The K and L region of polycyclic aromatic hydrocarbons. The K region (illustrated by 9-10 bond in phenanthrene) and L region (illustrated by 9-10 bond in anthracene).



In 1930, dibenz[a,h]-anthracene (DBA) was the first carcinogenic polycyclic aromatic hydrocarbon (PAH) to be identified, whereas the picene was found to be carcinogenically inert. This important discovery led to both the synthesis and testing for carcinogenic properties of many new compounds and to the first attempt to formulate a quantum mechanical theory of carcinogenicity based on the fundamental proposition that the carcinogenic properties of PAH depend on a definite property of the carcinogenic molecule *in vivo*.

The famous K and L region theory constitutes a remarkable milestone in the evolution of theoretical research regarding chemical carcinogenesis. According to the K and L region theory, the appearance of carcinogenic properties depends on the occurrence of some additional reactivity at these regions. Originally, a K region in a PAH was the mesophenanthrene type bond (i.e., the 9-10 bond in phenanthrene). The L region consists of a pair of carbon atoms as typified by the 9-10 carbon pair in anthracene.

N° rings	Cancer (+)	Cancer (-)	N° tested
1		Benzene	1
2		Naphtalene	1
3		Anthracene	2
4	Benz 1,2-anthracene 	Naphtacene 	6
5	Benz 3,4-pyrene 	Benz 1,2-pyrene	15
6	Dibenz 1,2,3,4-pyrene 	Dibenz 1,2,6,7-pyrene 	18
7	Tribenz 1,2,4,5,8,9-pyrene 	Benz 1,2-(naphto-2',3')-4,5-pyrene 	10
8		Pyranthene	6
9		Tetrabenz 1,2,3,4,6,7,12,13-pentacene 	1
10		Ovalene 	1

Many electronic structural parameters have been used to cipher the reactivity of a PAH by its K and L regions. According to the quantum chemical concept (Box 15.1), each carbon atom should furnish two pi electrons to the mobile pi electron pool belonging to the whole framework of the molecule. Indices utilized (de-)localization energies in these regions based on pi electronic energies.

The essential hypothesis of the K and L region theory [as verbalized by Pullman and Pullman (1955)] was that the appearance of carcinogenic activity in a PAH is determined by the existence within its molecule of an active K region and, at the same time, an inactive L region. The meanings of the words "active" and "inactive" were, of course, understood to allude to the phenomenon of chemical reactivity. Popp calculated, based on research of 37 PAH's, that carcinogenicity corresponds with the pi electron excited state energy of a carcinogen which should be as near as possible to 3.25 eV. According to his theory, this specific value influences exciplex/excimer formation in DNA, thereby influencing the intracellular coherent ultra-weak photon field. Those data were published in 1977.

In more recent developments, it became evident that the approach had many pitfalls. Unsubstituted PAH preferentially underwent metabolic substitution and electrophilic substitution reactions precisely at the highly reactive L region positions. Over the years, many hypotheses or theories were devised which lent themselves to specific predictions offering a means of testing their validity. The advantage of many different hypothesis is that all sides of the question will be tested by experiments and the results discussed before reaching a definite conclusion. However, as time went by the Popp model has been almost forgotten.

probability (index P) was included whose decomposition factors concern the energies, the intensities, and decay constants of the 0-2 and 1-2 transition states. Popp listed these quantities for 37 polycyclic aromatic hydrocarbons and found a very satisfactory correlation to account for the carcinogenicity deduced from this analysis. The pi electron excited state energy of a carcinogen should be as near as possible to 3.25 eV. Those data were published in 1977. In a later analysis that Sung did on 59 polycyclic aromatic hydrocarbons, he further statistically supported the credibility of Popp's theory. Popp expected that this particular energy band interfered with the intrinsic photon field causing DNA damage.

The more fundamental role of DNA in the photon field is essential for understanding Popp's model for stress-perturbed biosystems. The model suggests that there is a negative feedback loop in living cells which couples together states of a coherent ultra-weak photon field and the conformational state of the cellular DNA. Popp assumed that photon energy transfer via radiationless chemical pumping from cytoplasmic metabolism resulted in changes of the DNA conformation via exciplex/excimer formation (see Chapter 16). Since the conformation of the DNA molecule is believed to be of importance for the regulation of the nuclear information transfer, such processes, in turn, influence the metabolic activity of a cell, thus closing the feedback loop. Popp's hypothesis also suggested that biophotons originate from a delocalized coherent field within living matter. The approach of Popp and his double resonance theory for carcinogenesis was highly interesting and can be followed in a list of his (mostly German) publications in the period 1972-1977.

Photobiochemistry without light: linking biochemiluminescence with biophotochemical reactions

Another field of interest was the existence of (at that time) a new type of coupled reactions in biology: simultaneous occurrence of a biochemiluminescence reaction and a biophotochemical reaction. A large body

of theoretical as well as experimental arguments converged on these new types of coupled reactions in biology. These new types of coupled reactions corresponded with the concept of "photobiochemistry without light" (already having been labeled for a few years based on the major work of Cilento in the early 1970's). Those studies bridged the gap between modern (in system terminology such as synergy, coherence, etc.) molecular aspects and the localized biochemical view point. His work demonstrated that in biological systems, excited molecules can transfer their excitation energy to other biomolecules and thereby trigger amplification mechanisms and/or promote photochemical processes in the dark (see also Box 15.3).

It was becoming more and more clear that living organisms are endowed with extremely complex, efficient, and indeed very ingenious devices for transforming and transmitting energy. They all are part of a continuous energy flow. In the course of this energy flow, numerous articulated chemical reactions of diverse types take place. It was known that thermodynamically endergonic reactions may occur simultaneously with exergonic reactions in the metabolic process. All of such ideas have a great heuristic value and open new horizons in the holistic interpretation of biological electromagnetic radiations. They support a possible triggering and regulating role of photons in biochemical activity.

All of the above led Popp to postulate that biological systems generally have the capacity to coherently store photons which come from the external world. To prove his ideas required the development of special equipment in combination with the photomultiplier tube. The equipment would need to facilitate the exposure of a biological system to a (light) radiation source. This may lead to a storage of photons in the system which, after exposure, start to emit. The emission is depended on the capacity of photon storage. Such storage capacity could be derived from the decay constant of the emission process. Popp's subsequent hypothesis emphasized that the emission of such biophotons is qualitatively and quantitatively linked to a changing metabolic state (Chapter 16).

Photons and information

Photon signals as such are not inconsistent with communication. Real communication, however, is only possible when the receptor cell is able to process signals. There are indications that this is indeed true. The most widely known examples of photon effects on physiological processes are photosynthesis and the vision process. But, enzymatic processes that are not connected with photosynthesis (as well as the vision processes) are also influenced by photons. At that time, it was already documented in the literature that photons could cause a change in the conformation of proteins. Since the biochemical activity of a protein is correlated with its conforma-

MORE ABOUT...

BOX 15.3

■ THE COUPLING BETWEEN PHOTOBIOLOGY AND BIOPHOTONICS

Photobiology and biophoton emission may be considered as the manifestation of the opposite or complementary aspects of a photon's complete life. This is evident from the two following equations describing basic photochemical and luminescent processes:

$A + h\nu \rightarrow A^* \rightarrow B + C$ (equation 1), and

$A + h\nu \leftarrow A^* \leftarrow B + C$ (equation 2),

wherein $h\nu$ is either the "substrate" of a photoreaction (photobiology) or the "product" of a chemiluminescent reaction. The arrows correspond to absorption, photoreactions, chemiexcitation, and emission processes.

Electronic excited states are generated in certain biochemical (enzymatic) reactions for non-emissive important purposes of

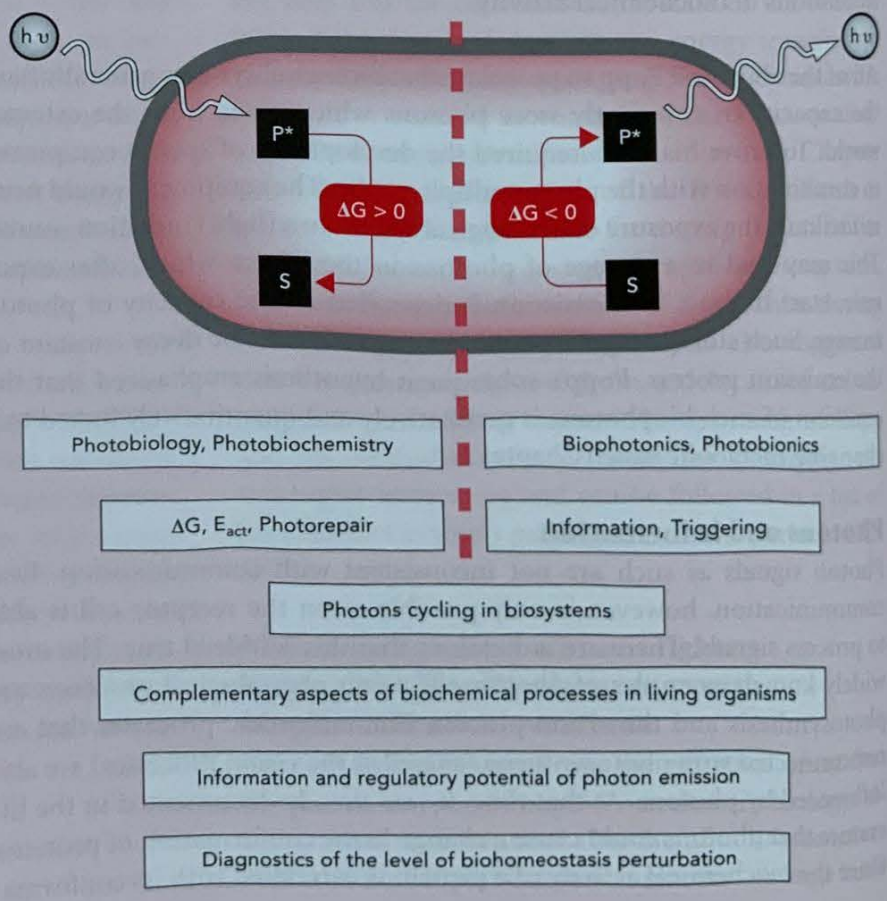


Figure B.15.3.1
Photons as driving force in relationship to products (P) of metabolic processes and sources of information in biosystems, as studied in different disciplines.

tion, it was not difficult to understand why photons influence or even regulate biochemical processes in enzymes. As to other reactions of live cells (when exogenously stimulated by light), the influence by those stimuli upon cell biological processes seem to be based on complicated mechanisms.

In the early 1960's, Arvanataki and Chalazonitis discovered excitatory and inhibitory photopotentials elicited by direct light stimulation at the axon of *Aplysia.* Later, Chalazonitis (1964), Brown and Brown (1973) and Henkart (1975) reported on light-induced changes of neuronal membranes of *Aplysia* (which do not belong to the optical sensory system). In addition, temporary changes in the ion permeability of optical nerve cells (initiated by light) accompany changes in their electric conductivity. Such phenomena were reported by Benolken (1961) and by Kikuchi (1962). In 1976, Oesterheld reported that photon impact on bacterial membranes (within milliseconds) was followed by changes in the membrane potential coupled with an ion transport. He interpreted this phenomenon as the effect of a proton pump powered by photons (Chapter 17).

In the light of the hypothesis (regarding cell biological communication vis-à-vis photons), we already discussed (Chapter 5) that Gurwitsch (1932) already suspected that photons play a part in the excitation and conduction in the nerves. At that time, a number of experimental investigations were initiated regarding the radiation of the nervus and the tractus optici as well as the lobi optici of the frog. At the Marburg Conference, Helmuth A. Fischer, researcher at the Max-Planck-Institute for Brain Research (Frankfurt a.M., Germany) presented his exploratory experiment utilizing photomultipliers to document the emission of photons via the stimulation of a guinea pig's bulbus olfactorius. He also confirmed previous USSR reports regarding the functioning of nerves (see also Chapter 7).

Most important, discussion began at the Conference about the regulatory role of photons, thereby indirect-

the cell's bioenergetics. The mechanisms and kinetics of these "dark" chemical reactions (photobiochemistry) as well as reversed photoreactions are subjected to spectroscopic selection rules proceeding via ground and excited states. This fact imposes an asymmetry on the photon circulation in living systems. In this sense, photochemistry neglects the (theoretical) possibility of regarding photons as conveyers or messengers of information.

Recent findings suggest that biophotons carry hidden information coded in the spatiotemporal parameters of the electromagnetic field. This aspect of biophoton's possible informational/triggering role of the regulation in real time of the life machine) must be exploited by biophotonics (Figure B.15.3.1). Emission might deliver important information about the onset and the end of life processes, the influence of internal and external stress factors and the adaptive capability to adverse situations and environmental conditions.

ly stimulating a revival of interest in the Gurwitsch concept.
The search for evidence of the "informational character" of ultra-weak photon emission in biological systems reappeared. How did this develop in the following decade?

Internationalization of new (informational) photon field research

It is the year 1985. Experimental activities in physics, biology and chemistry (regarding photon emission) developed during the last decade in many places of the world. Looking at the utilization of interdisciplinary communication, one cannot deny that it was indispensable in order to understand the complex problem of electromagnetic radiation in biosystems. Simultaneously, it still was very difficult, since a satisfactory comprehensive model of the phenomenon was still lacking and the mutual sharing of information between the Eastern and Western European scientific worlds continued to be absent. For the latter reason, several Polish researchers, in cooperation with the Polish Academy of Science, began to organize the *First International Symposium on Photon Emission from Biological Systems.* It was held in Wroclaw, January 24-26, 1986.

Many researchers were not willing to consider the aspect of informational potential of emitted radiation. The status of that research seemed only at the beginning stage. Two levels reflecting the research at that time were clearly based on a dichotomy of the conceptual approach to photon emissions: 1.) the biochemical level, claiming that biological luminescence is an adventitious emission associated with exergonic oxidation reactions and has no function in the energetics of biochemistry or regulation, and 2.) the biophysical level, assigning informative and regulative roles to the electromagnetic field within living organisms and interpreting the phenomenon in terms of non-linear thermodynamics and optics.

Janusz Slawinski symbolized the status of photon emission research as an iceberg. So far, one could not take into account all possible interactions of electronically excited states and photons within the components of a very complex biological system and medium. We still have a long way to go to develop our knowledge about cooperative, weak molecular interactions: 1.) how they contribute to the generation of excited states and photons and 2.) how they result from the action of endogeneous electromagnetic fields. Such limitations are also due to our lack of knowledge about cybernetic models which eventually might describe couplings, feedbacks, and account for the changes of the photon field within very dense, heterogeneous media (i.e., in cells). Therefore, today's status of this research is truly not more than the tip of an iceberg. The submerged portion represents the hidden part of the photon field and its properties. Ultimately, the convic-

tion evolved to the interdisciplinary communication between physics, biology, and chemistry of photon emission that it was indispensable to finally understand the complexity of electromagnetic radiation in biosystems.

The opportunity for such interdisciplinary communication was then further extended via an initiative of *Experientia*, an interpisciplinary journal for life sciences (in 1997 renamed, *Cellular and Molecular Life Sciences*). The editors of that journal, recognizing the developments (internationally) within that research arena regarding ultra-weak photon emission from living organisms, asked Popp (1987) to coordinate a multi-author review about *Biophoton Emission.* The review appeared July 15, 1988. Major subjects included 1.) low-level luminescence as a new discipline in photobiochemistry and consequently, the reactions and biochemical source(s) responsible for this phenomenon (J. Slawinksi); 2.) photobiochemistry without light radiation from (and within) biological matter, long-range interactions and energy dissipation (G. Cilento); 3.) coherent transfer of photon energy and its origin from a coherent electromagnetic field (F. A. Popp and K. H. Li and others); 4.) the experimental findings of correlations between photon emission and biological functions (R. Van Wijk, D. H. J. Schamhart, and W. B. Chwirot); 5.) technological aspects (H. Inaba); and 6.) the relationship between photon emission and models as originally proposed by A. G. Gurwitsch (A. A. Gurwitsch).

Meanwhile, the same organizers of the First International Symposium took the initiative to organize a second international meeting entitled, *The International School on Biological Luminescence.* It was held from June 20th to June 23rd, 1989, Wroclaw. The objective was to bring together more scientists from all of the interdisciplinary fields leading to many additional overviews of bioluminescence and photon emission. It considerably broadened the joint research within the fields of physics, chemistry, biology, biocybernetics, and medicine.

Since the second Polish meeting included many applications in medicine, a second multi-author review in *Experientia* was organized around several medical issues. Van Wijk coordinated the review entitled *Biophoton Emission, Stress and Disease.* It was published December 1, 1992. It focused on stress-perturbed systems, within bacteria to man, including disease states. Perturbations of the organisms were initiated by mechanical, temperature, oxidative, chemical and photochemical stress. In most instances, a perturbation of homeostasis gave rise to an increase in photon emission. The biological stressors included chemical, physical, emotional (psychological), and infections that cause an increase in endogenous free radical production. The stress related photon emission was reviewed for bacterial and low eukaryotic systems (R. N. Tilbury) and higher eukaryotic systems

(J. Slawinski). Photon emission in relationship to stress and disease was reviewed by E. M. Lilius and P. Marnilla. Van Wijk and Van Aken reviewed photon emission in the field of tumor biology. The more fundamental basis of the photonic response from stress-perturbed biosystems was presented by Popp utilizing his feedback model coupling radiationless chemical pumping of energy (from the cytoplasmic metabolism in DNA) with the DNA conformation dependent regulation of the metabolic activity of a cell (thus closing the feedback loop).

Conclusion

The growing research activities and international collaboration between scientists of different disciplines during the period 1977-1992 (plus the conferences and multi-author reviews in this period) can be seen as the beginning to discern the richness of information which can be retrieved from measurements of photon emission.

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Part I: Biophysical and biocybernetic:

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- A. I. Zhuravlev: *Spontaneous superlow chemiluminescence and creation of quantum biology* p. 19.
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Conclusion Part D

Iron filings scattered on a card held over a magnet arrange themselves in the pattern of "lines of force" of the magnet's field. If the filings are thrown away and new filings are scattered, they will assume the same pattern. In an abstract sense, something similar might actually happen in the cell or the body. Molecules and cells are constantly being torn apart and rebuilt with fresh material. Thanks to a controlling field, the new molecules and cells arrange themselves in the same pattern. Can such a field be documented? This idea was already proposed by Charles Laville in the 1920's. In his opinion cancer is an "electromagnetic disturbance of the cell". In 1932, Harold Saxton Burr observed the directional outgrowth of neural cells which led him to propose the electrodynamic theory of development. Burr began measuring the minute electrical voltages and concluded that wherever there is life, there are voltage gradients. Various physiological processes in animals and man were found to have electrometric correlates. A particular focus of Burr was wound healing. The electrometric studies of the healing wound reflected growth and structural replacement with tissue under the influence of an electromagnetic field. According to most biologists around 1960, these experiments had little relevance to life.

Among Western scientists, it was Becker who began to repeat the work of Burr. He improved the equipment and found a complex field that followed the distribution of the nervous system. Studying the injury of limbs as their fractures healed, he observed a highly negative potential that corresponded with the all important blastema. That eventually helped science to better understand that such currents functioned in a semiconducting manner (enabling them to conduct over long disctances). The proof materialized when he utilize solid-state equipment (based on the Hall effect) that records current only if it is semiconducting. By the early 1970's, Becker and colleagues had proof for nearly every detail of the fracture healing phenomenon. He documented that the current was derived from the nerves in and around the periosteum. At the same site, bone generated its own piezoelectrical current due to residual stress in the mangled collagen-apatite matrix. Such injury current signals stimulated the cells to form new bone. Unfortunately, the scientific community was not ready to completely accept the concept of biological semiconduction. Solid-state devices were only beginning to hit the market in the mid-1960's.

However, a little more acceptance at that time was already present due to the fact that transmission of information along nerves vis-à-vis bioelectric pulses included a frequency spectrum extending to thousands of hertz. In the early 1960's, research data was made available from both the USSR sci-

entist Nina Aleksandrovna Aladzhalova and the USA researcher, Dean E. Wooldridge regarding diverse electromagnetic oscillatory systems of the brain. In 1962, Harary pointed to the complex electromagnetic regulation system of the heart rate. It appeared that regulation systems depended on electromagnetic oscillations for their functioning. In addition, there was, in the 1960's, an increasing amount of experimental and theoretical information regarding vibrational phenomena at the molecular and supramolecular levels in chemical and biological systems. The vibrational processes played an important role in the elucidation of the physical/chemical nature of biological phenomena (e.g., muscle contraction, regulation of cell division, glycolytic metabolism, etc.). In 1970, Aleksander Samuilovich Presman contributed considerable research by evaluating the established biological influences of electromagnetic fields independent of the simple thermal effects. Presman differentiated such interconnections with regard to specific "working" frequencies, intensity ranges, and methods of coding all underlying not only the interactions between cells but also specific interactions between macromolecules.

Research regarding function/structure relationships of chemicals responsible for carcinogenesis began with Otto Schmidt in 1938. His attention was focused on the charge density of pi electrons. The French researchers, A. and B. Pullman undertook cancer research in the 1950's and 1960's demonstrating a correlation between cancer and several polycyclic aromatic hydrocarbons. Shou-Sin Sung extended the research of polycyclic aromatic hydrocarbons in his studies which, in the early 1970's, led to a generally increased scientific belief in this research approach, whereas the levels of correlation between carcinogenicity and electronic excitation energies continued to be the same. Popp provided some experimental data suggesting that an intrinsic photon field plays a role in carcinogenesis. He hypothesized that a specific chemical property might be responsible for its carcinogenic activity. In 1975, he published a very satisfactory correlation between the carcinogenicity and the degree to which excited state energy (of a carcinogen) is, as near as possible, to the energy range of the lowest triplet states of DNA (3.25 eV). In a subsequent analysis, Sung (1977) supported the credibility of Popp's resonance theory. It then stimulated Popp to postulate that biological systems generally have (according to Gurwitsch) a photon field. He suggested the possibility that if a carcinogenic compound is excited in the field, the absorption could lead to electromagnetic emission affecting DNA. To collect experimental evidence, it required both equipment to measure photon storage and a biological model that would include tumor cells and their normal counterparts.

Summary and Perspective

The discoveries in the field of bioelectromagnetism vis-à-vis functioning of physiological and biochemical processes stimulated the belief that cell regulation depends on electromagnetic oscillations including an intrinsic photon field. At the end of the fourth journey we can conclude that Gurwitsch's postulation of a photon field had been enhanced with amazing numbers of physiological, biochemical and bioelectromagnetic data. Those scientific developments finally opened the door that would help to both integrate such knowledge and to focus on the specific scientific optical window required to decipher the "language" of photon emission and on how to subsequently utilize the information to understand the organization of the living organism.

We will, therefore, during the last trip of our journey, learn how the late 1900's and early 2000's helped to better link photon emission to metabolism in health and disease.

PART E

Improving Understanding and Application of the Photon Field Concept in Health and Disease

Introduction Part E

The focus expands to international multi-disciplinary research regarding biophoton arenas moving from molecular to cellular to entire living systems.

Photon emission and storage capacity in tumor cell line model
Cilento contributed to the idea that photon trapping influences metabolic events (photochemical processes). The hepatoma cell model was the first to facilitate research regarding photon storage utilizing cells with varying degree of differentiation. It demonstrated that the state of a tumor corresponds with high short storage and low resonance within the photon storage system.

Multi-disciplinary photon field research
In the 1990's, the collaboration between physicists and biologists intensified focusing on "novel" aspects of ultra-weak photon research addressing transmission and transduction of high-energy (endogenous to the living system). The research covered energy transfer at the level of the individual protein, DNA and the entire organism. At the individual protein level, attention was focused on collective excitations resulting from the combination of intra-peptide vibrations [with or without a local deformation of the chain (solitons and excitons, respectively) and their photon excitation and emission properties]. The close connection of photon emission from DNA bases and mechanical vibrations of base dipoles within DNA were studied addressing the coupled states of photons (electromagnetic waves) and phonons (base vibrations or mechanical lattice vibrations) within the DNA molecular skeleton. It was a major challenge to explain the effects of weak external fields impacting pattern formation in developing organisms. Electromagnetic fields within the entire range of frequencies are intimately involved in biological functioning. It was the expectation that ultra-weak photon emission should be able to target the specific "optical window" of such an electromagnetic interaction field. The future detection of such emission would provide a unique "language" to facilitate more information about the biophysics of the living organization.

Human Photon Emission in Health and Disease
Human photon emission fascinated researchers for several decades. Since the mid-1990's, the technology finally captured accurate quantitative data regarding human body photon emission in Germany, Japan and South Korea. In addition, animal research demonstrated photon emission in diseased states utilizing both photomultipliers and two-dimensional imaging systems. Human subject protocols were developed in order to record the

body pattern of emission intensity, color spectrum, relationship to health and disease as well as mental influences on emission. Other research developments focused on the analysis of the language utilized to describe human light.

Imaging human photon emission

The development of a two-dimensional detection system began in the mid-1980's. Its sensitivity increased in the 1990's. In addition, a highly sensitive CCD camera system was developed for ultra-weak photon imaging. In the early 2000's, using special dark rooms, the human anatomical emission pattern was finally imaged. They demonstrated high and low emission areas. Most recently, attention had been focused on the dynamics of the emission pattern and its importance to better understand novel aspects of health and disease.

16

Photon Storage and Emission in Cancer Cells

The body of an animal or human operates as a society whose individual members are cells. The cells reproduce by dividing themselves (cell division). However, the anatomical organization of the adult body remains stable over time. Ultimately, all somatic cell lineages are committed to die dedicating their continued existence to the support of germ cells. In this society, each cell behaves in a social manner: dividing, differentiating or dying as needed for the good of the organism. Cancer does not observe the boundary laws of normal tissue. Its cells encroach imperialistically upon its neighbours. In the early stages of development, tumors are relatively benign. They grow slowly and are weak or noninvasive. With time, they enter a phase of increasingly rapid growth. Gradually, the organizational stability is disrupted by the invasive behavior of cancerous tissue (replacing adjacent normal tissues) thereby disrupting normal functioning of the afflicted organ. When a metastasis occurs, local dissemination of the malignant tissue (from the parent tumor mass) evolves spreading the malignant tissue to distant sites in the body.

Diversity and morphological heterogeneity of neoplasms, even derived from the same tissue or cell type, have been recognized from the very beginning of cellular pathology. In comparison with their "normal" precursors, the cancer cells are less differentiated (or less specialized). Apparently, uncontrolled proliferation goes hand in hand with a similar lack of control in the structural differentiated arrangement of the cells. This correlation was studied in a range of chemically induced tumors each having its specific stabile state of differentiation and growth. This became possible utilizing the technique of "transplantation" of a tumor from one animal to another in order to grow each specific tumor continuously for many generations. In the 1960's, such a tumor model system was developed for experimental tumor biology. It was a spectrum of transplantable rat hepatomas of different growth rates. Within the spectrum, not only did growth rate differ, but also exhibited heterogeneity at the biochemical level (see also Chapter 10).

The comparative biochemistry of this spectrum of liver tumors resulted in an enormous set of data regarding different metabolic pathways. It made it possible to find correlations suggesting patterns of enzyme pathology

MORE ABOUT...

BOX 16.1

■ THE FRANCK-CONDON PRINCIPLE

What happens to an atom or molecule that absorbs a photon? When light is absorbed, each quantum is taken up by a single atom and/or molecule. The entire energy of the quantum is communicated and the absorbing atom or molecule is lifted from its normal state of lowest energy (highest stability) to an excited, energy rich state. When a photon is absorbed, the molecule usually is not merely transferred into an excited electronic state, but also acquires some vibrational energy. This is the basis of the so called Franck-Condon principle.

The Franck-Condon principle is a rule in spectroscopy that explains the intensity of vibronic transitions. Vibronic transitions are the simultaneous changes in electronic and vibrational energy levels of a molecule due to the absorption or emission of a photon of the appropriate energy. The Franck-Condon principle is based on the original contributions of James Franck who shared, with Hertz, the 1925 Nobel prize in Physics for the experimental verification of the quantum theory. Franck studied the mechanisms of *photon-induced chemical reactions*. The presumed mechanism was the excitation of a molecule by a photon followed by a collision with another molecule during the short period of excitation. The question was whether it was possible for a molecule to break into photon products in a single step. In order for a molecule to break apart, it must acquire (from the photon) vibrational energy exceeding the energy to break a chemical bond. In examining how much vibrational energy a molecule could acquire when it is excited to a higher electronic level, Franck studied possible changes in energy between the lowest electronic state and higher electronic states. The changes in vibrational levels could be a consequence of both the instantaneous nature of excitation to higher electronic energy levels and a new nuclear interaction potential (because the relative momentum of the nucleus cannot be directly affected by an electronic transition). This transition promotes the electron from the lowest vibrational state of a molecule (within the ground state) to a higher (excited) vibrational state. The molecule in the excited state then dissipates immediately (within 10 to 100 femtoseconds) to some other energy (e.g., heat). The electron subsequently reaches the lowest vibrational level (of the excited state). When the molecule relaxes to the ground state it emits light (fluorescence). The emission generally occurs as a longer wavelength [compared to the absorbing wavelength (Rotverschiebung, The Red Shift)]. The phenomenon was shared with Condon who was then able to generalize Franck's ideas.

in hepatocarcinogenesis. The coordinate alternations in the tumor's metabolic pathways indicate that the tumor includes a multitude of stable states of cellular organizations that lead to the Molecular Correlation Concept in tumor biology.

The photon energy storage concept in tumor biology

Since the time that Presman published his work on *Electromagnetic Fields and Life* (1970), it became evident that biological systems not only emit electromagnetic waves but also respond to radiation from extremely slow fluctuations up to the extremely rapid short waves in the UV region. However, the possible use of electromagnetic radiation to regulate metabolic processes within the living cell was still a matter of speculation. The knowledge of what happens to a molecule that absorbs light was largely limited to photon-induced chemical reactions. It included the Franck-Condon principle stating that when a photon is absorbed, the molecule is not merely transferred into an excited electronic state but also acquires vibrational energy (see also Box 16.1).

In 1976, Cilento postulated the existence of a new type of coupled reaction in biology related to light excitation. The postulate was based on simultaneous occurrence of biochemiluminescence and biophotochemical reactions as well as the occurrence of excited electronic states in dark biological processes (photobiochemistry without light). It was quite analogous to the coupling of reactions that produce and utilize chemical compounds such as ATP or NAD(H). It eventually became clear that living organisms are, indeed, endowed with ingenious and efficient devices for transforming and transmitting energy. In the course of such energy flow, numerous chemical reactions of diverse nature take place. Since it is known that different states of organization also exist within tumors, it implies that they also function not only with shifts in energy flow patterns but also in the coupling of biochemiluminescence and biophotochemical reactions.

Effective intracellular photon trapping is expected to influence metabolic and cellular events. It has been hypothesized that the actual number of photons trapped (i.e., the efficacy of photon trapping) also actually continues to change within the process of carcinogenesis. In order to experimentally study these ideas, it was suggested to fill the photon stores by artificially illuminating the cells. If photons are actually trapped, the properties of the trapping mechanism(s) are reflected during delayed emission. Precise analysis of post irradiation photon emission should demonstrate photon storage characteristics of a biological system.

Testing the hypothesis required the development of special equipment. The important technique for estimating light-induced delayed emission

MORE ABOUT...

BOX 16.2

■ TYROSINE TRANSAMINASE: A KEY ENZYME IN THE DEGRADATION OF AROMATIC AMINO ACIDS TO GLUCONEOGENESIS

The liver absorbs the majority of amino acids leaving some of them in the blood for use by peripheral tissues. The priority use of amino acids is for protein synthesis rather than catabolism. However, when catabolism does take place, the first step is the removal of nitrogen. The α-ketoacids are then utilized for either gluconeogenesis or fatty acid synthesis.

The conversion pathways in amino acid degradation range from extremely simple to quite complex. The carbon skeletons of the diverse set of 20 fundamental amino acids are funneled into only seven molecules: pyruvate, acetyl CoA, acetoacetyl CoA, α-ketoglutarate, succinyl CoA, fumarate, and oxaloacetate. It is an example of the remarkable economy of metabolic conversions.

Amino acids that are degraded to either acetyl CoA or acetoacetyl CoA are labelled ketogenic amino acids because they can give rise to ketone bodies or fatty acids. Amino acids that are degraded to pyruvate, α-ketoglutarate, succinyl CoA, fumarate, or oxaloacetate are called glucogenic amino acids. The net synthesis of glucose from these amino acids is feasible because these citric acid cycle intermediates and pyruvate can be converted into phosphoenolpyruvate and then into glucose.

The enzyme tyrosine transaminase is specifically required in the degradation of phenylalanine (Figure B.16.2.1). The degradation of phenylalanine begins with its hydroxylation to tyrosine, a reaction catalyzed by phenylalanine hydroxylase. This enzyme is called a monooxygenase (mixed function oxygenase) because one atom of O_2 appears in the product and the other in H_2O. The reductant here is tetrahydrobiopterin, an electron carrier that is derived from cofactor biopterin. Biopterin is synthesized in the body. It is not a vitamin. The next step in the degradation of phenylalanine and tyrosine is the transamination of tyrosine to p-hydroxyphenylpyruvate.

Here we meet the enzyme tyrosine transaminase. For the further transformation to fumarate, several more steps are required. The α-ketoacid tyrosine then reacts with O_2 to form homogentisate. The enzyme catalyzing this complex reaction, p-hydroxyphenylpyruvate hydroxylase is called a dioxygenase [because both atoms of O_2 become incorporated into the product (one on the ring and one in the carboxyl group)]. The aromatic ring of homogentisate is then cleaved by O_2 which yields 4-maleylacetoacetate. This reaction is catalyzed by homogentisate oxidase, another dioxygenase. The product, 4-maleylacetoacetate is then isomerized to 4-fumarylacetoacetate by an enzyme that uses glutathione as a cofactor. Finally, fumarylacetate is hydrolyzed to fumarate and acetoacetate.

In this entire pathway, the tyrosine transaminase is a highly regulated enzyme. Famous studies have focused on the regulation of its synthesis by hormones.

was the double shutter system in combination with a sensitive photomultiplier tube. In Popp's laboratory, such equipment was initially constructed by Ruth. After excitation, a shutter imposed between a light source and sample is rapidly closed, while, a second shutter between sample and the photomultiplier tube is almost immediately opened. The time between closing the first and opening the second shutter is short, usually about 100ms indicating that the recording of delayed photon emission begins 100 ms after the end of illumination.

To study the photon trapping in tumor development, an experimental model (including cell lines with different degrees of differentiation) must be available in order to estimate photon storage within cells while utilizing the excitation of the cell's photon stores vis-à-vis artificially illuminating the cells.

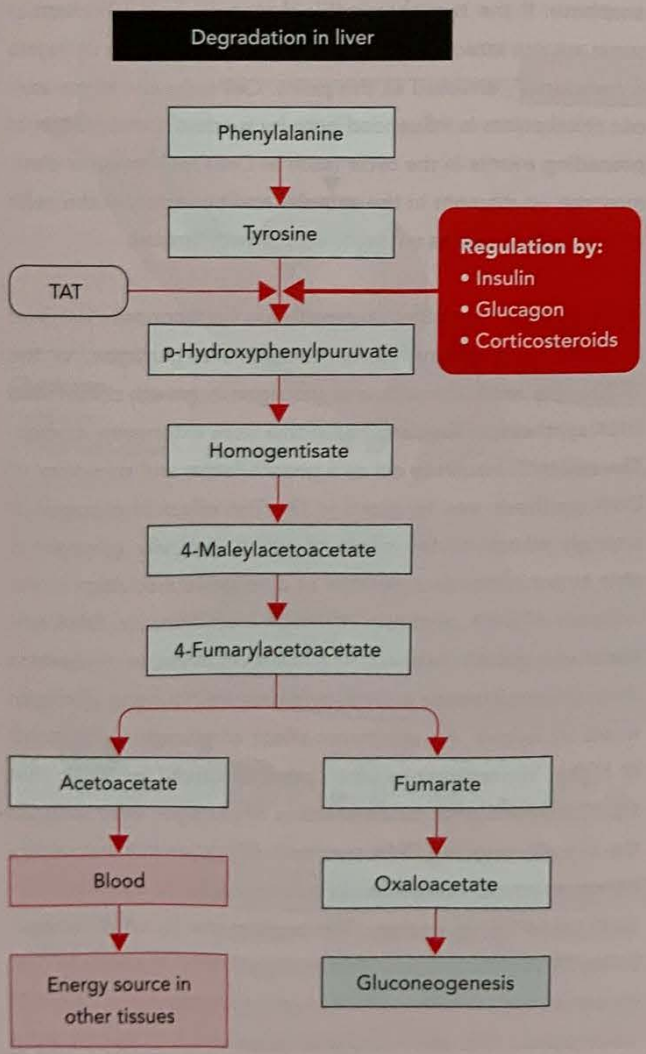
The experimental cell model

The extension of the Molecular Correlation Concept from *in vivo* transplantable hepatomas to *in vitro* cultured hepatoma cell lines was established in the early 1970's by Van Wijk and colleagues. Four hepatoma cell lines isolated in the late 1960's were utilized for comparative biochemical studies in order to estimate the degree of loss of differentiation. These cell lines are commonly named H35, HTC, MH_1C_1 and RLC.

The initial researched differentiated liver function was glucagon responsiveness as part of the regulation of key gluconeogenic enzymes, tyrosine transaminase (see Box 16.2) and PEP carboxykinase (see also Chapter 10). Since glucagon regulates vis-à-vis cyclic 3',5'-AMP (cAMP), it is utilized to replace the hormone in cell studies. The research demonstrated that H35 and MH_1C_1 exhibited a normal liver type of regulation of PEP carboxykinase and tyrosine transaminase, whereas HTC and RLC have lost such a type of regulation.

Other studies focused on growth rate and its hormonal regulation of these same cells in culture. The H35 and MH_1C_1 cell lines were relative "slow growers" (doubling

Figure B.16.2.1
Tyrosine transaminase (TAT) in liver: a key enzyme in the degradation of aromatic amino acids to gluconeogenesis. Aromatic amino acids may contribute to gluconeogenesis through degradation pathways. This contribution is regulated by hormones. Tyrosine aminotransferase is also such a hormone regulated key enzyme.



MORE ABOUT...

BOX 16.3

■ THE (LIVER) CELL CYCLE AND ITS REGULATION BY HORMONES

The most basic function of the cell cycle is to duplicate accurately the vast amount of DNA in the chromosomes and then segregate the copies precisely into two genetically identical daughter cells. These processes define the two major phases of the cell cycle (Figure B.16.3.1). DNA duplication occurs during S phase (S for synthesis). After S phase, chromosome segregation and cell division occur in M phase (M for mitosis).

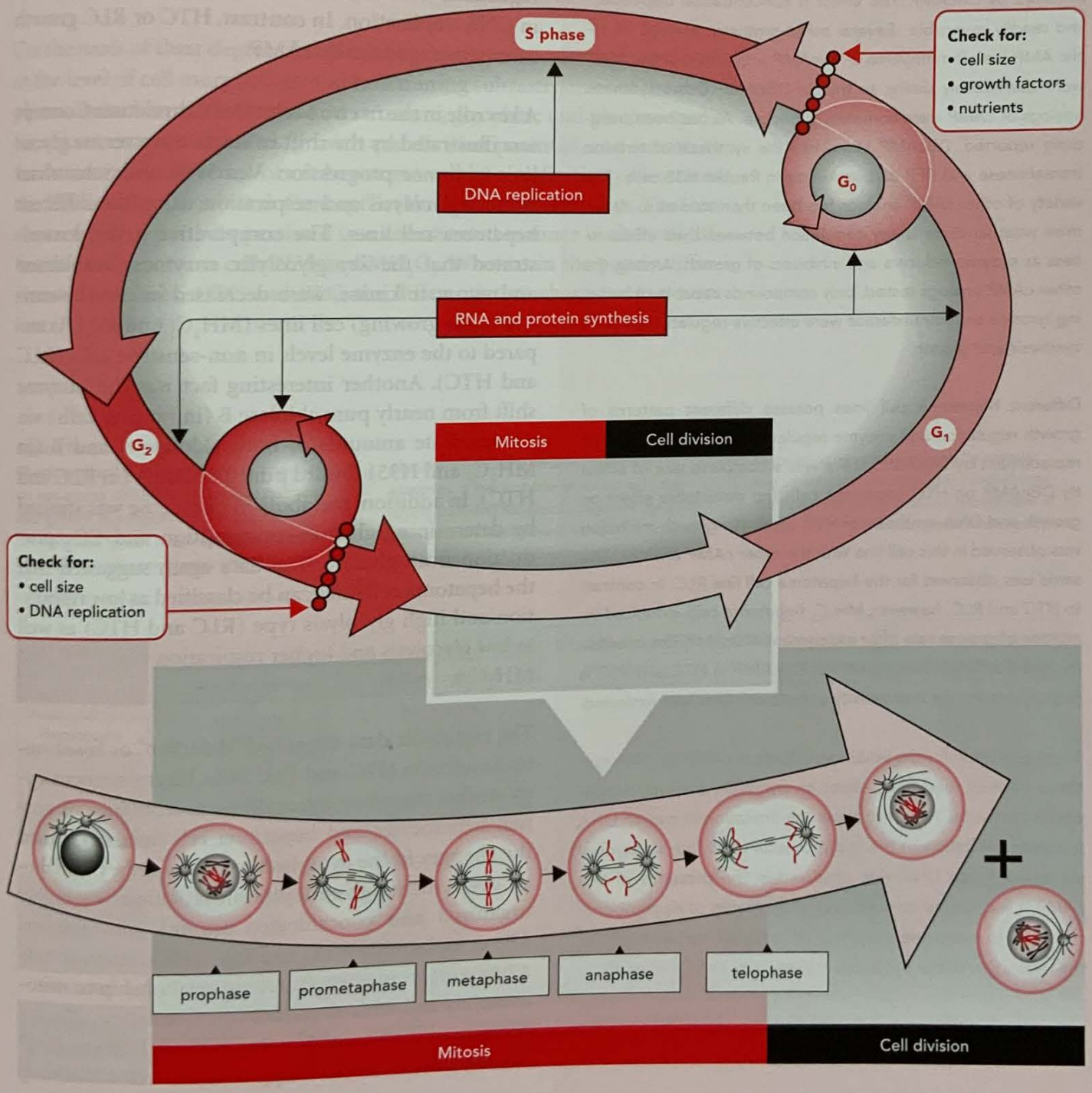
Mitosis begins with chromosome condensation. The nuclear envelope then breaks down and the replicated chromosomes (each consisting of a pair of sister chromatids) become attached to the microtubules of the mitotic spindle. As mitosis proceeds, the cell pauses briefly in a state called metaphase. This marks the beginning of anaphase (during which the chromosomes move to opposite poles of the spindle where they decondense and reform intact nuclei). The cell is pinched in two by cytoplasmic division (cytokinesis) and then, cell division is complete.

Most cells require much more time to grow and double their mass than they require to replicate their DNA and divide. To facilitate more time for growth, extra gap phases are inserted in most cell cycles (e.g., G_1 phase between M phase and S phase and a G_2 phase between S phase and mitosis. Thus, the eukaryotic cell cycle is traditionally divided into four sequential phases: G_1, S, G_2 and M. The two gap phases serve as more than simple time delays to allow cell growth. They also provide time for the cell to monitor the internal and external environment to ensure that conditions are suitable and preparations are complete before the cell commits itself to the major upheavals of S phase and mitosis. The G_1 phase is especially important in this respect. Its length can vary greatly dependent on external conditions.

Cultured cells stop or slow down the process of cell division when they run out of either nutrients or space. In all such cases, the cells are arrested in G_1. These finding suggest that passing from G_1 into S phase is a critical control point in the cell cycle. In animal cells, the G_1 checkpoint is called the restriction point. The ability to pass through the restriction point is controlled, to a large extent, by extracellular, growth signaling proteins. Cells that have successfully passed through the restriction point are committed to S phase, whereas those that have not passed this point can remain in G_1 (in the resting state called G_0). In addition to the G_1 checkpoint, another cell cycle check point has been characterized. At the G_2 checkpoint (located at the boundary between G_2 and M phase) proper completion of DNA synthesis is required before the cell can initiate mitosis. In certain cell types, the cell cycle can be arrested at this point and the cells enter a resting state analogous to G_0. The relative importance of the G_1 and G_2 checkpoints in regulating the rate of cell division varies with the organism and cell type. In mitosis, a third checkpoint is at the junction between metaphase and anaphase. If the two chromatids that make up each chromosome are not attached to opposite spindle poles, the cell cycle is temporarily arrested at this point. Cell behavior at the various checkpoints is influenced both by successful completion of preceding events in the cycle (such as DNA replication or chromosome attachment in the spindle) and by factors in the cell's environment (such as nutrients and growth factors).

In liver, major regulators of growth are the hormones involved in maintaining glucose levels: insulin and glucagon. In the 1970's, the roles of insulin and glucagon in growth control and DNA synthesis of liver and hepatoma were extensively studied. The ability of insulin to act as a growth factor and stimulator of DNA synthesis was localized in G_1. The effect of glucagon is strongly related to the effect of insulin. Basically, glucagon is able to act either as a positive or a negative modulator in the initiation of DNA synthesis. At a high level of insulin, DNA synthesis was greatly delayed. At a low level of insulin, however, a dose related increase in DNA synthesis was found as glucagon levels increased. An additional effect of glucagon was found at higher concentrations when present without insulin. In that situation, the higher concentrations of glucagon even reduced the actually ongoing DNA synthesis. Glucagon in a stimulatory hormone mixture could be completely replaced by cyclic 3',5'-AMP (cAMP) or its analogs. This positive role for cAMP in regulating DNA synthesis was already suggested in the early 1970's. However, the inhibiting effect of glucagon alone had its cAMP counterpart. This effect could be observed with the dibutyryl

Figure B.16.3.1
The events of eukaryotic cell division: the phases of the cell cycle and checkpoints. The cells grows continuously in interphase, which consists of three phases: DNA replication is confined to S phase; G_1 is the gap between M phase and S phase, while G_2 is the gap between S phase and M phase. In M phase, the nucleus and then the cytoplasm divide. In the figure two of the main checkpoints in the cell cycle are marked. These points determine whether or not the cell proceeds through the rest of the cell cycle. The determination is based on signals reflecting both the cell's internal state and its external environment. The two checkpoints are near the end of G_1 and at the end of G_2.



analog of cAMP (DBcAMP) (inhibiting both insulin stimulated and basal DNA synthesis).

In the early 1970's, Van Wijk and Wicks began studies regarding the hormone dependent growth regulation of liver tumor cells. The first hepatoma type was Reuber H35. Both growth rate and DNA synthesis of Reuber H35 hepatoma cells could be inhibited by DBcAMP. The effect is concentration dependent and readily reversible. Several other synthetic analogs of cyclic AMP (i.e., 8-methylthio 3',5'-cAMP and 8-thio 3',5'-cAMP) were qualitatively similar to that of DBcAMP. Other synthetic analogs of cAMP were completely inactive. As has been previously reported, DBcAMP stimulates the synthesis of tyrosine transaminase and PEP carboxykinase in Reuber H35 cells. The variety of other cAMP analogs has been then studies to determine whether there is any correlation between their effectiveness as enzyme inducers and inhibitors of growth. Among the other cAMP analogs tested, only compounds capable of inducing tyrosine aminotransferase were effective regulators of DNA synthesis and growth.

Different hepatoma cell lines possess different patterns of growth regulation and enzyme regulation by glucagon and its replacement by DBcAMP. There was a complete lack of effect by DBcAMP on HTC hepatoma cells (no detectable effect on growth and DNA synthesis of HTC cells). No growth inhibition was observed in this cell line with the other cAMP analogs. The same was observed for the hepatoma cell line RLC. In contrast to HTC and RLC, however, MH_1C_1 hepatoma cells exhibited inhibition of growth rate after exposure to DBcAMP. The possibility that a different metabolism of DBcAMP in HTC and RLC is responsible for the insensitivity of both cell lines was excluded.

The explanation for the inhibitory effects of cAMP on DNA synthesis (in H35 cells) is its effect on pyrimidin deoxyribonucleotides synthesis. This introduces the limitation in overall DNA synthesis. Inhibition of the incorporation of all four deoxyribonucleosides into DNA was observed in the presence of DBcAMP. It was further confirmed by the capacity of deoxyribonucleosides to overcome the effect of inhibition by DBcAMP. For the regulation of the pyrimidin metabolism: see Chapter 10.

times of 24-30 hr) whereas RLC and HTC are "rapid growers" (doubling times of 18-20 hr). Glucagon regulates (inhibits) liver growth. In 1972, Van Wijk and Wicks examined the effect of cyclic 3',5'-AMP on growth and DNA synthesis. They demonstrated that cAMP is able to inhibit the growth of H35 and MH_1C_1 cells. The effect on growth was due to cyclic 3',5'-AMP regulation of deoxyribonucleoside synthesis required in DNA duplication. In contrast, HTC or RLC growth cannot be regulated by cAMP.

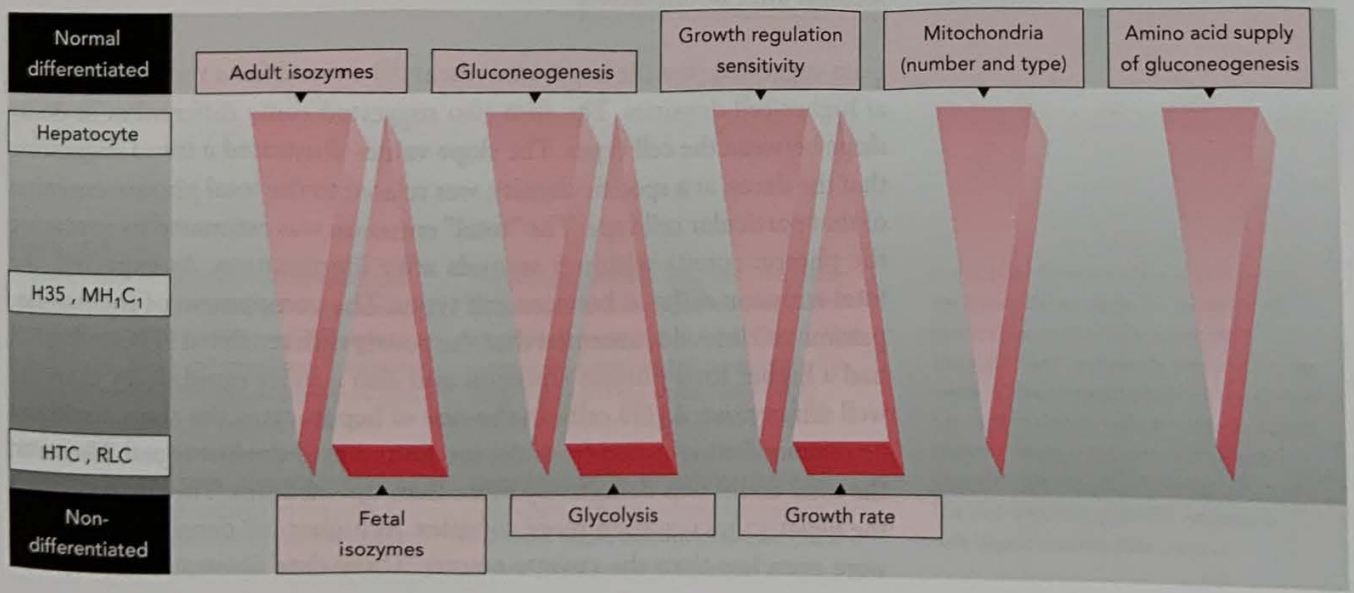
A key role in the *in vivo* Molecular Correlation Concept was illustrated by the shift in respiration versus glycolysis in tumor progression. Van Wijk and Schamhart studied glycolysis and respiration using the different hepatoma cell lines. The comparative study demonstrated that the *key* glycolytic enzymes, hexokinase and pyruvate kinase, were decreased in cAMP sensitive (slow growing) cell lines (MH_1C_1 and H35) compared to the enzyme levels in non-sensitive cells (RLC and HTC). Another interesting fact was the isozyme shift from nearly pure aldolase B (in rat liver cells) via intermediate amounts of both aldolase A and B (in MH_1C_1 and H35) toward pure aldolase A (in RLC and HTC). In addition, metabolism of glucose was studied by determining glucose consumption and CO_2 production from glucose. The data again suggested that the hepatoma cell lines can be classified as low respiration and high glycolysis type (RLC and HTC) as well as low glycolysis and higher respiration type (H35 and MH_1C_1).

The metabolic data suggested "defective" or fewer mitochondria in HTC and RLC cells. Electron microscopy studies regarding intracellular morphology of normal hepatocytes and hepatoma cell lines confirmed the changes in the cytoplasm of those cells. In the hepatocyte, the rough endoplasmic reticulum was highly organized and concentrated around mitochondria. The cytoplasm of HTC and RLC cells contained only a few rough cisternae (rarely in relationship to mitochondria and with abundant free ribosomes). The cytoplasm of the slower growing H35 cell line was intermediate. Most cisternae appeared as strands coursing

through the cytoplasm and around mitochondria. Free ribosomes were less numerous and more associated with the endoplasmic reticulum (as in the liver cells). In hepatocytes, mitochondria were in orthodox configuration with irregular, short cristae and a moderately dense staining matrix with granules. In H35, the mitochondria were relatively numerous and their fine structure resembled that of liver mitochondria. The organelles in HTC and RLC seemed less numerous and with an empty appearance of their matrices. Parallel running cristae transversed the mitochondria.

On the basis of their degree of differentiation systematically evaluated both at the level of cell morphology and functioning (ultrastructure of the cytoplasm, mitochondrial volume and structure, glycolytic and gluconeogenic functions, enzyme activities, growth and division potential, and sensitivity for hormones) the series of rat hepatoma cell lines definitively illustrated the "Molecular Correlation Concept" at the level of cells *in vitro*. The liver characteristics partially persisted in a coordinated manner in the well differentiated hepatoma cells H35 and MH_1C_1. Whereas in the poorly differentiated cell lines, HTC and RLC, the differentiation characteristics were lost (Figure 16.1).

Figure 16.1
Characteristics of rat hepatocytes and cultured rat hepatoma cell lines. Metabolic patterns and growth regulation were evaluated for different cell types. The metabolic patterns included key gluconeogenic (PEPCK) and glycolytic (pyruvate kinase, hexokinase) enzymes, a key enzyme of amino acid supplying pathway for gluconeogensis (tyrosine aminotransferase) as well as relative activities of adult and fetal isozymes (aldolase). Respiration was characterized by number and structure of mitochondria. Growth was characterized by its absolute growth rate as well as the sensitivity for hormonal regulation.



Degree of differentiation and photon storage capacity

This cell model system regarding loss of differentiation in tumor development was utilized to study the relationship between the state of differentiation and the photon storage capacity. This comparative study focused on three types of cells from fully differentiated (liver cells) to very poorly differentiated cells (HTC hepatoma cells) with the well differentiated H35 cells as an example of an intermediate state. In 1983, a long-term cooperation of the research groups of Popp (Kaiserslautern, Germany) and Van Wijk (Utrecht, Netherlands) began. The light-induced photon emission reflecting the photon storage capacity was studied after pre-illuminating the cells with visible light. The cells were incubated in suspension. Since hepatoma cells were continuously grown in a monolayer culture on a plastic surface, these cells were detached prior to their use. Hepatocytes were isolated from liver. The cells (in suspension) were incubated in a quartz cuvette placed in front of the photomultiplier tube. The quality of the quartz was important regarding the signal to noise ratio (since quartz has delayed luminescence). The research focused on a.) total number of photons emitted after pre-illumination and b.) the kinetics of the light-induced delayed photon emission. The studies were published in 1987-1990.

The kinetics of delayed luminescence were estimated using several cell densities. For all of these cell types, the decay curve of the delayed photon emission could best be described as hyperbolic rather than exponential. Such decay is illustrated in Figure 16.2. If recorded photon counts in time were plotted in a log time/log count number plot, the plot would be represented by a straight line. From the delayed emission estimated from cell suspensions as well as a cuvette (either empty or with medium without cells), it was concluded that almost all (>99%) photons were emitted within 5 seconds after illumination.

Data suggested (for the hepatoma research) a trend for a more rapid decay at higher cell densities. The data also suggested some differences in decay slope between the cell types. The slope values illustrated a trend suggesting that the decay, at a specific density, was related to the total photon emission of that particular cell type. The "total" emission was estimated by summing the photon counts within 5 seconds after illumination. As expected, the total emission differed between cell types. The comparison of the two hepatoma cell lines documented that the poorly differentiated HTC cells both had a higher total photon emission and also a more rapid decay than the well differentiated H35 cells. In the case of hepatocytes, the slope could not be reliably distinguished from the medium due to the low signal. However, the total count was definitively more than the medium. The situation with the hepatocytes was even more complex. At higher cell density, total counts were even less than the cuvette counts. These data illustrated basic prob-

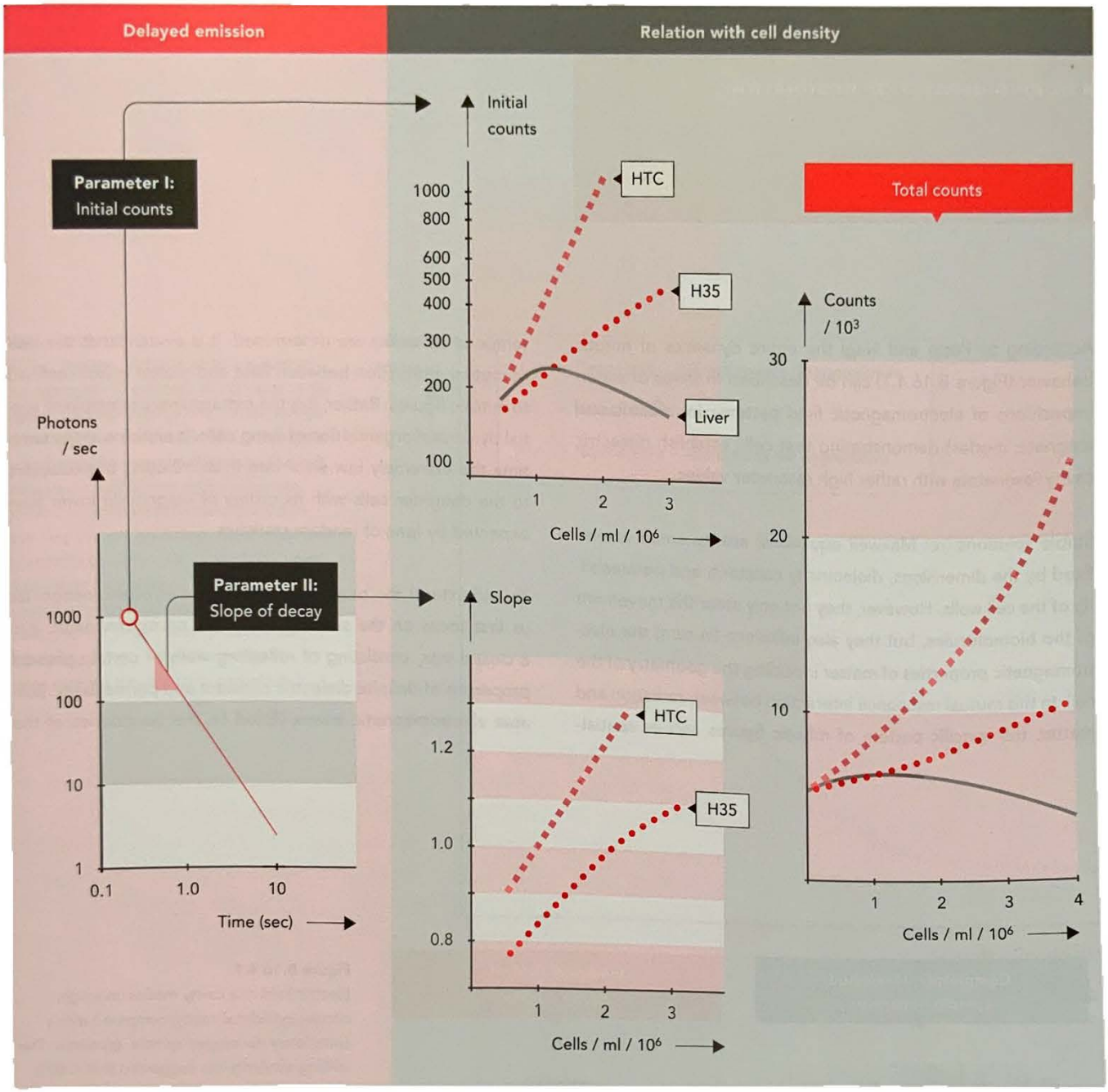


Figure 16.2
Characteristics of light-induced delayed photon emission of rat hepatocytes and cultured rat hepatoma cell lines H35 (well differentiated) and HTC (poorly differentiated) in relationship to cell density. The figure presents a schematic representation of experimental data. Left panel illustrates the basic estimation of delayed luminescence. After the end of irradiation of the cell suspension the cells emit photons with decreasing strength. Data are presented in a log time (X-axis) versus log photon counts (Y-axis) plot. The decay follows an almost straight line (representing a decay according to a hyperbole). That curve is described by two parameters: the initial value and the slope of the decay. The middle panel presents the values of both parameters for several cell densities (ranging up to 4 x 10^6 cells per ml). The upper part of the middle panel presents the initial values and the lower part of the middle panel presents the slope values. In the latter figure no data of liver cells are presented because the reliability of such data is rather limited (see text). The right panel represents the total photon count in relationship to cell density. Overall, normal hepatocyte cells are able to absorb photons whereas the poorly differentiated HTC hepatoma cells reflect photons. The well differentiated H35 hepatoma cells depict intermediate behavior.

MORE ABOUT...

BOX 16.4

■ POPP'S QUALITY OF RESONATOR

According to Popp and Nagl the entire dynamics of mitotic behavior (Figure B.16.4.1) can be described in terms of superimpositions of electromagnetic field patterns (of electric and magnetic modes) demonstrating that cells establish dielectric cavity resonators with rather high resonator values.

Stable solutions (re: Maxwell equations) are (to some extent) fixed by the dimensions, dielectricity constant, and permeability of the cell walls. However, they not only steer the movement of the biomolecules, but they also influence (in turn) the electromagnetic properties of matter including the geometry of the cell. In this mutual resonance interaction between radiation and matter, the specific pattern of mitotic figures and its spatial-temporal dynamics are determined. It is evident that this kind of mutual interaction between field and matter is not confined to mitotic figures. Rather, it is the extraordinary principle of spatial dynamical organization of living cells. It explains at the same time the extremely low error rate in distributing biomolecules to the daughter cells with its orders of magnitude lower than expected by laws of random statistics.

To understand the basic physics behind this phenomenon, let us first focus on the simplest case of a cavity resonator. It is a closed box, consisting of reflecting walls of certain physical properties of definite dielectric constant and permeability. Suitable electromagnetic waves (fitted to the boundaries of this

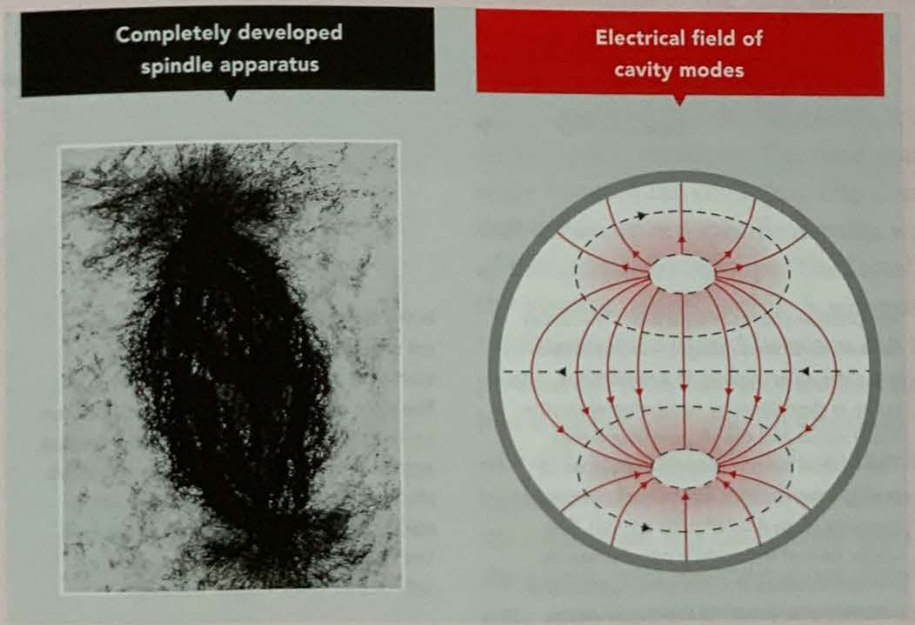


Figure B.16.4.1
Electric field of a cavity modes (in a right circular cylindrical cavity) compared with a completely developed spindle apparatus. The striking similarity has suggested that mitotic figures are examples of both a long-lasting photon storage and a coherent field within biological systems. (See legend of Figure 15.1.).

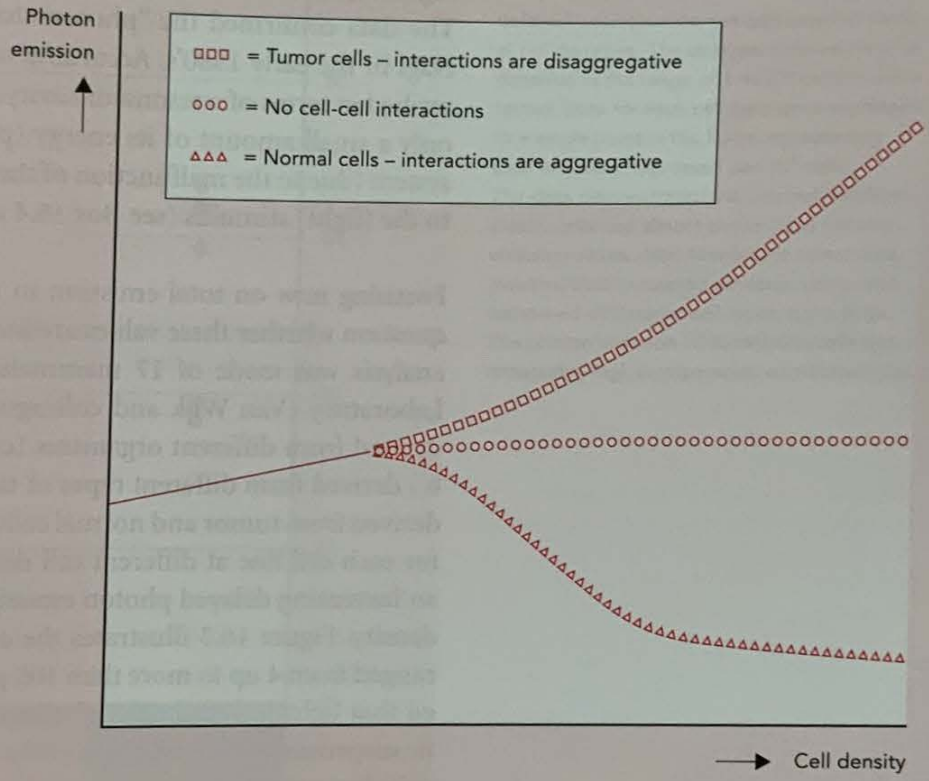


Figure B.16.4.2
Energy storage and Q in cells: a hypothetical approach. To estimate the Q value in the optical frequency range one can determine the intensity at the outside of a cell suspension in dependence of cell density. The cell densities in suspension experiments are rather low compared to that of a solid tissue. Any change would indicate that optical properties of living cells depend on mutual long-range interactions. This figure suggests that normal cells exhibit an increasing absorption of weak mutual photons with increasing density (while the malignant ones increase the reflection probability.)

box) can be stored over a definite time before the thermal loss considerably exceeds the initially stored energy. The storage capacity of a resonator (called resonator value Q) has a time dimension reflecting the lifetime of the stored electromagnetic wave within the cavity. It is evident that this definition of Q can be used for any kind of energy storage including the excitation of molecules, the detection of microwaves or radiowaves (cavities or antennae), or the number of reflections of a Laser mode between the mirrors before the mode is emitted.

Acceptable optical transitions have a Q value of nanoseconds. Forbidden optical transitions may reach values between 10 microseconds to milliseconds. Lasers display maximum Q vales as high as seconds. As a rule, one may keep in mind that the Q value can (but must not) increase the higher complexity and the "orderliness" of a system under investigation. Living systems should at least reach a Q value of one day in order to use (with sufficient efficiency) the photon storage of sunlight.

lems in studies with mammalian cells. Some cell types apparently have a very dim delayed photon emission (such as normal hepatocytes) compared to the cuvette. In that type of situation, the cuvette signal dominates that of the cell suspension and (as it is for hepatocytes) it is problematic to estimate exact decay values.

In the 1990's, more (non-hepatoma) mammalian cell lines were studied regarding their emission (i.e., decay dynamics and total photon emission). The data confirmed the "photon storage" model proposed by Popp and Nagl in the early 1980's. According to this model (which was initially described in terms of a resonator cavity), a high quality resonant system loses only a small amount of its energy (photons) per unit time. A less quality system (due to the malfunction of the feedbacks) will give a larger response to the (light) stimulus (see Box 16.4 and Figure B.16.4.2).

Focusing now on total emission in relationship to density as well as the question whether these values are indicative of cell transformation, a third analysis was made of 17 mammalian cell types present in the Utrecht Laboratory (Van Wijk and colleagues). The study included cell lines a.) derived from different organisms (cat, human, monkey, mouse, rat, etc.); b.) derived from different types of tissue (heart, kidney, liver, etc.); and c.) derived from tumor and normal cells. Total photon emission was estimated for each cell line at different cell densities. In all cases, the cells displayed an increasing delayed photon emission almost linear to the increasing cell density. Figure 16.3 illustrates the emission for the different cell types. It ranged from 4 up to more than 100 photons per 10^4 cells. The data suggested that light-induced delayed photon emission is a general phenomenon in suspensions of mammalian cells. The question was: why do differences exist between the cell types.

A relationship between delayed photon emission and species of origin was not observed. Instead, a relationship between emission intensity and a specific cell type was most obvious. Cells of mesodermal origin (fibroblast/endothelial) were, either obtained from primary cultures of dissociated cells or derived from monolayer cultures of established cell lines. The cells demonstrated the highest values. The other group of cell types (of non-mesodermal origin) included normal cells and tumor cells. In general, the lowest delayed emissions were observed from normal cells. Tumor cells demonstrated an intermediate range.

The data led to the conclusion that a.) tumor cells have higher delayed emissions than normal cells and b.) that a special class of cells exist (of mesodermal origin) that have an even higher emission than the tumor cells. Why did the tumor cells tend towards an energy balance which was

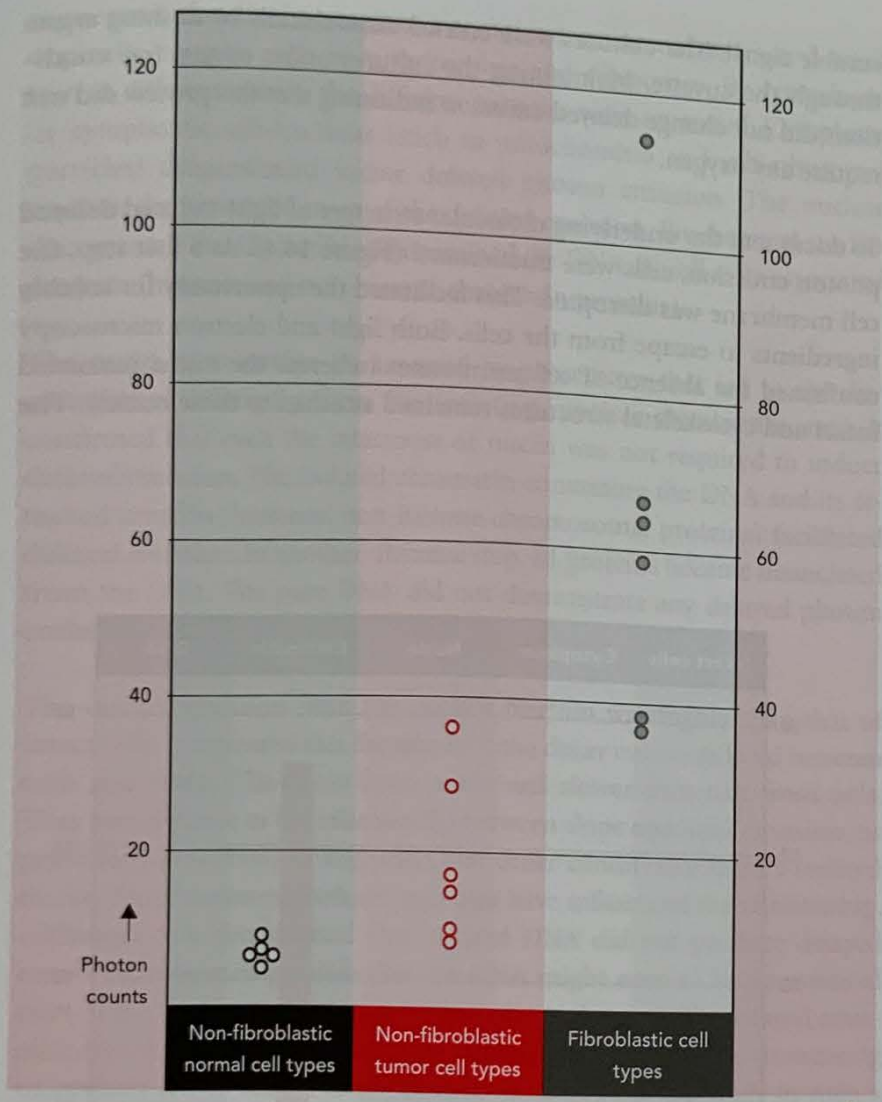


Figure 16.3
Light-induced photon emission of mammalian cells. A total of 17 cell types were studied including cells of fibroblastic origin (n=6), and non-fibroblastic cells (n=11). In the latter category both normal (n=5) and tumor cells (n=6) were studied. Normal cells and tumor cells were cultured in suspension under similar conditions and then utilized for the estimation of delayed luminescence. For each cell type the delayed luminescence was estimated at several cell densities. The delayed luminescence of densities in the range of 1-4x10^6 cells/ml were tested. Data for each cell type were expressed as a single point in the figure representing total emission (expressed per 10^4 cells).
The data demonstrated that normal non-fibroblastic cells had almost similar (low) photon emission values. Non-fibroblastic tumor cells revealed both increased emission values and compared with tumor cell types, quite large. The photon emission of fibroblastic cells was remarkably high (compared to non-fibroblasts).

expressed mostly by mesenchymal cells? This may be important to understand the underlying, cellular, and structural conditions.

Searching for molecular sources of photon storage and delayed emission

The process (or principle) leading to light-induced delayed photon emission was studied in several ways. One of the first was to check whether or not this emission was (like spontaneous ultra-weak photon emission) derived from reactive oxygen species that was oxygen dependent. The studies were performed with HTC cells because that line emitted a consistent mea-

surable signal. The cultures were created anaerobically by flushing argon through the cuvette. Maintaining the cultures under oxygen free conditions did not change delayed emission indicating that this process did not require any oxygen.

To document the underlying molecular structure of light-induced delayed photon emission, cells were fractionated (Figure 16.4). As a first step, the cell membrane was disrupted. This facilitated the opportunity for soluble ingredients to escape from the cells. Both light and electron microscopy confirmed the absence of cell membranes (whereas the nuclei remained intact and cytoskeletal structures remained attached to those nuclei). The

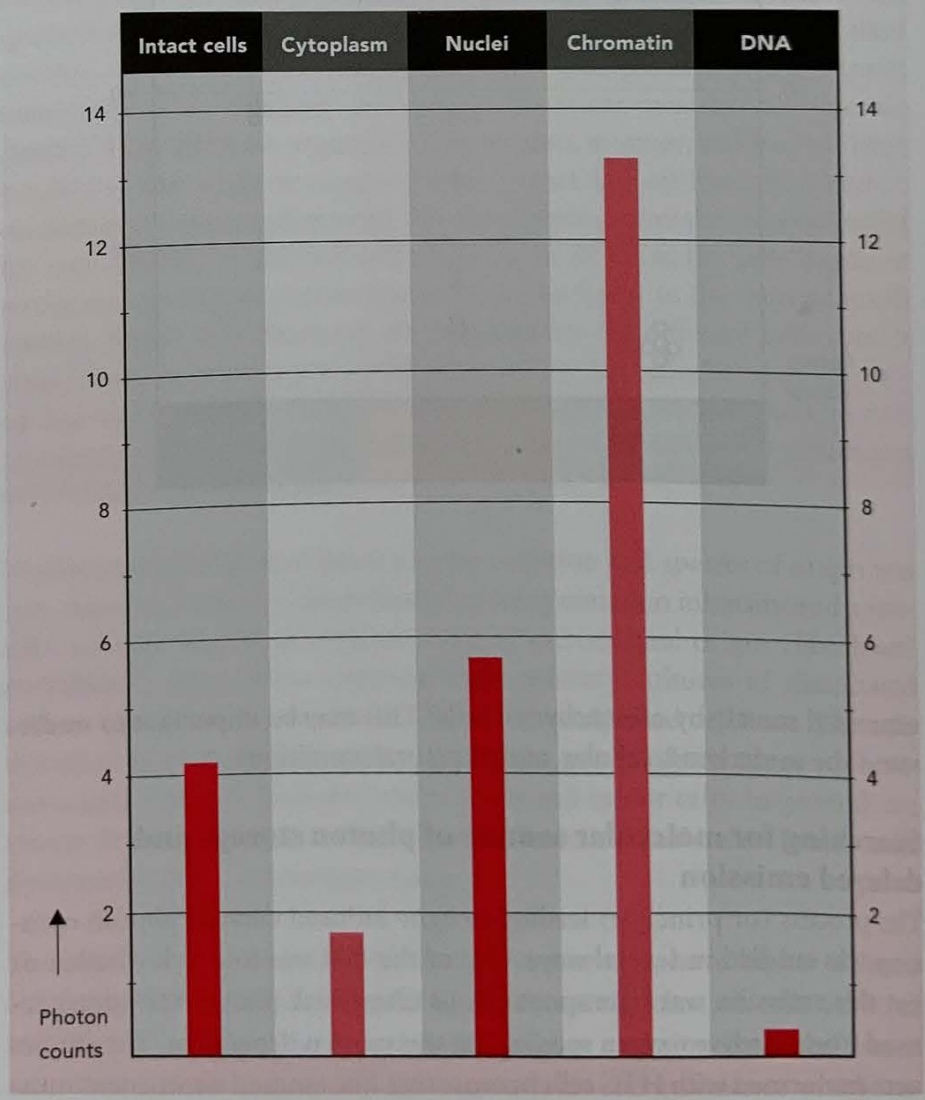


Figure 16.4
Delayed luminescence in cell fractions. The hepatoma cell line HTC with high delayed luminescence capacity was fractionated in order to estimate major sources of delayed luminescence. Cell fractions were measured at amounts that correspond with the original cell number. The total counts in the first 0.5 s per 10^6 cells is presented. Details of fractionation are in Chapter 16.

homogenization fluid (cytoplasm) contained macromolecules and small organelle particles such as mitochondria and ribosomes. By centrifugation at different speeds, the different particles were separated. Cytoplasm or cytoplasmic sub-fractions (such as mitochondria and sub-ribosomal particles) demonstrated minor delayed photon emission. The nuclear fraction demonstrated most of the delayed emission. Biochemical studies documented that the nuclei still contained the DNA as well as its associated proteins (histones and non-histone chromatin proteins).

The nuclei were further fractionated by procedures that led to the dissociation of different protein fractions (e.g., nuclear membranes, etc.). It confirmed that even the intactness of nuclei was not required to induce delayed emission. The isolated chromatin containing the DNA and its attached proteins (histones, non-histone chromosomal proteins) facilitated delayed emission. In another ultimate step, all proteins became dissociated from the DNA. The pure DNA did not demonstrate any delayed photon emission.

The delayed emission from the nuclear fraction was higher than that of intact cells. It appeared that the slopes of the decay curves differed between cells and nuclei. The decay from nuclei was slower than that from cells. This was opposite to the relationship between slope and total emission (as previously described). It suggested that other conditional factors (related to the fractionation procedure) may also have influenced the relationship. Although data documented that isolated DNA did not produce delayed emission, it seemed possible that the DNA might need to be an essential part of the nuclear protein complex in order to demonstrate delayed emission (see Box 16.5). The actual question then was: If a specific structuring of protein can be related to the delayed emission, can a change in such a structure influence delayed emission?

Theoretical considerations: Photon Storage in DNA

Ever since J. D. Watson and F. Crick created their classical model, biologists and biochemists have uncovered an extensive repertoire of DNA architecture. In this respect, it had been known for a long time that DNA molecules are luminescent materials. We know experimentally that the spectrum of fluorescence of DNA peaks around 350nm. However, to understand the photochemical events occurring in DNA, we must discuss that any excitation of an individual base must influence the other bases vis-à-vis electromagnetic interactions between charge distributions and delocalization of excited energies. Therefore, we must treat the emission from DNA differently from that of separate molecules radiating independently of each other. In such a situation, the emission rate is linearly proportional to the molecular concentration. When different bases cooperate, then the linear

law is broken. We must consider the exciplex formation (which is the consequence of DNA base excitation due to the stacking effect between base molecules). Experimental evidence for this behavior had been observed by different groups of DNA fluorescence researchers. Their data documented that the fluorescence quantum yield of DNA is 10 to 100 times lower than that of an equimolar mixture of the four nucleotides. The important process of base excimer (exciplex) formation was overlooked in all of the mentioned discussions. Li and Popp discussed the possibility of coherent emission by DNA molecules caused by the cooperative motions of DNA base molecules. According to Dicke's theory, the emission from DNA base molecules must be coherent due to the cooperation of the base molecules.

The necessity for dynamic considerations of DNA is obvious since life is a dynamic structure of processes far away from thermal equilibrium in physical space time. In reality, DNA is rich in movement. It vibrates, "breathes", and emits. The generation time of *Escherichia coli* DNA (with approximately $2x10^6$ nucleotide pairs) needs only 40 minutes for replication. It is clear that we ought to consider more correlations between individualities and collectivity.

Understanding the role of proteins utilizing milk as a model

The role of milk in nature is to nourish the mammalian young. It is a food with an approximate composition of water (87.3%), milk fat (3.9 %), and solids that are not fat (8.8%). The latter group consists of protein (3.25%), lactose (4.6%), minerals (0.65%), acids (0.18%), and very small amounts of vitamins. The properties of milk are not only determined by the composition, but also by the physical structure. The main "large" structural components of milk are fat globules and casein micelles. If raw milk is left to stand, it will separate. As fat globules cluster, their speed of rising increases and sweeps up the smaller globules with them. Other procedures also help to provide milk in a fat free form. The proteins in such milk include caseins (80%) and whey proteins (20%). The principal casein fractions are alpha(s_1) and alpha(s_2) caseins, beta casein, and kappa casein. The distinguishing property of all caseins is their low solubility at pH 4.6.

Most of the casein proteins exist in a colloidal particle known as the casein micelle (Figure 16.5). It is important that the casein micelle contains conjugated proteins (primarily with phosphate groups). The phosphate groups are essential to the structure of the casein micelle. Calcium binding by the individual caseins is proportional to the phosphate content. This is important for its biological function: both to carry large amounts of highly insoluble CaP to mammalian young in liquid form and to form a clot in the stomach for more efficient nutrition.

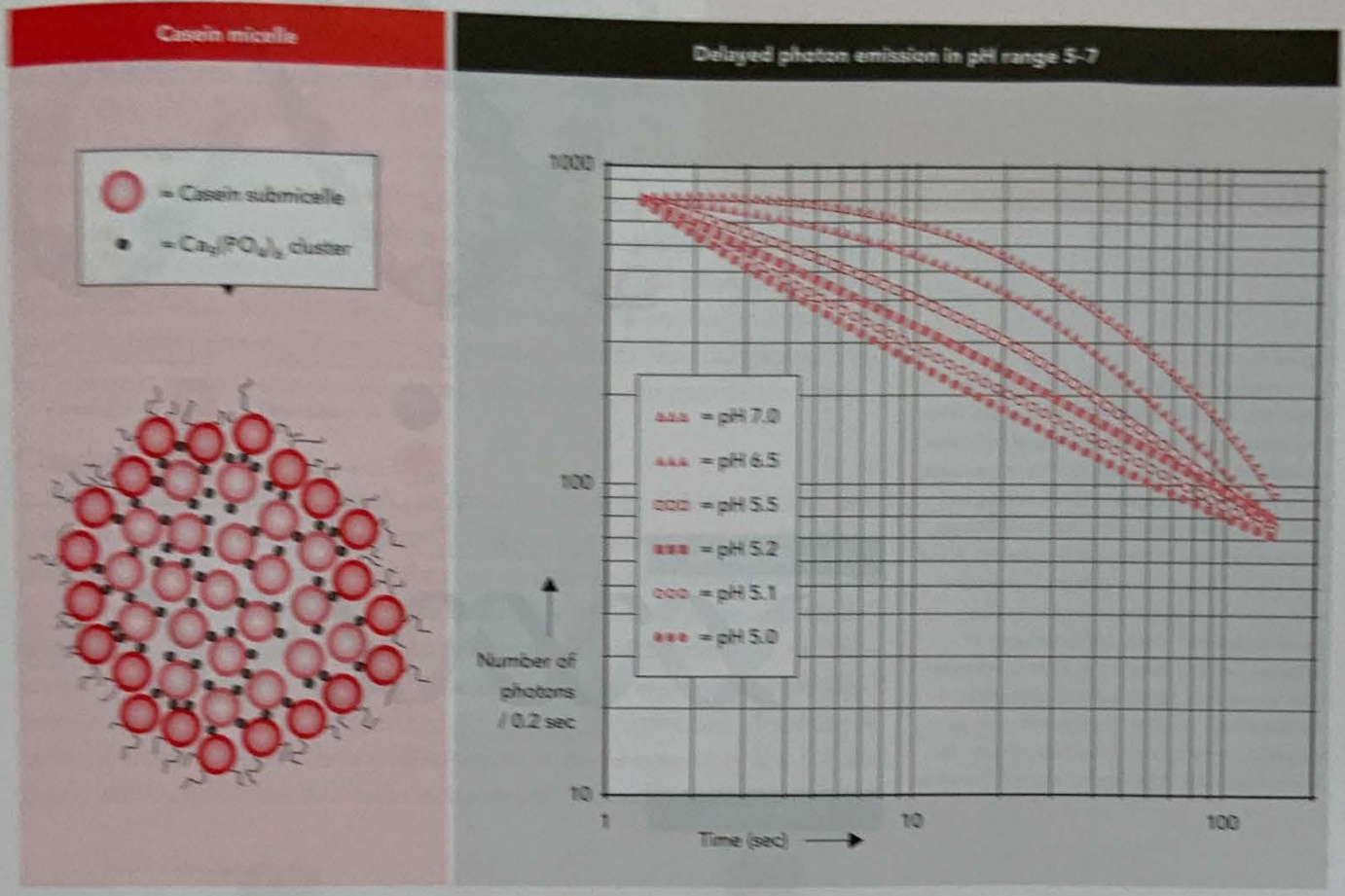


Figure 16.5
Light-induced delayed photon emission of the casein micelle system in fat free milk in the pH range 5-7. Delayed luminescence curves at different pH's are represented by different symbols (note that placement of symbols does not represent measurement times). The delayed emission depends on pH. When milk is acidified, the kinetics of delayed emission changes drastically. At pH closer to the isoelectric point the decay follows more of a hyperbolic decay.

The following factors are known to affect stability of a casein micelle: a.) Ca^{2+}, b.) hydrophobic interactions (caseins are among the most hydrophobic proteins), and c.) electrostatic interactions. Consequently, there are several factors that will affect the stability of the casein micelle system: a.) salt content, b.) pH (lowering pH leads to dissolution of calcium phosphate until, at the isoelectric point, all phosphate is dissolved and the caseins precipitate), and c.) heat treatment (whey proteins become adsorbed, altering the behavior of the micelle).

MORE ABOUT...

BOX 16.5

■ THE DNA DOUBLE HELIX AND ITS PACKAGING IN HIGHER LEVELS OF ORGANIZATION

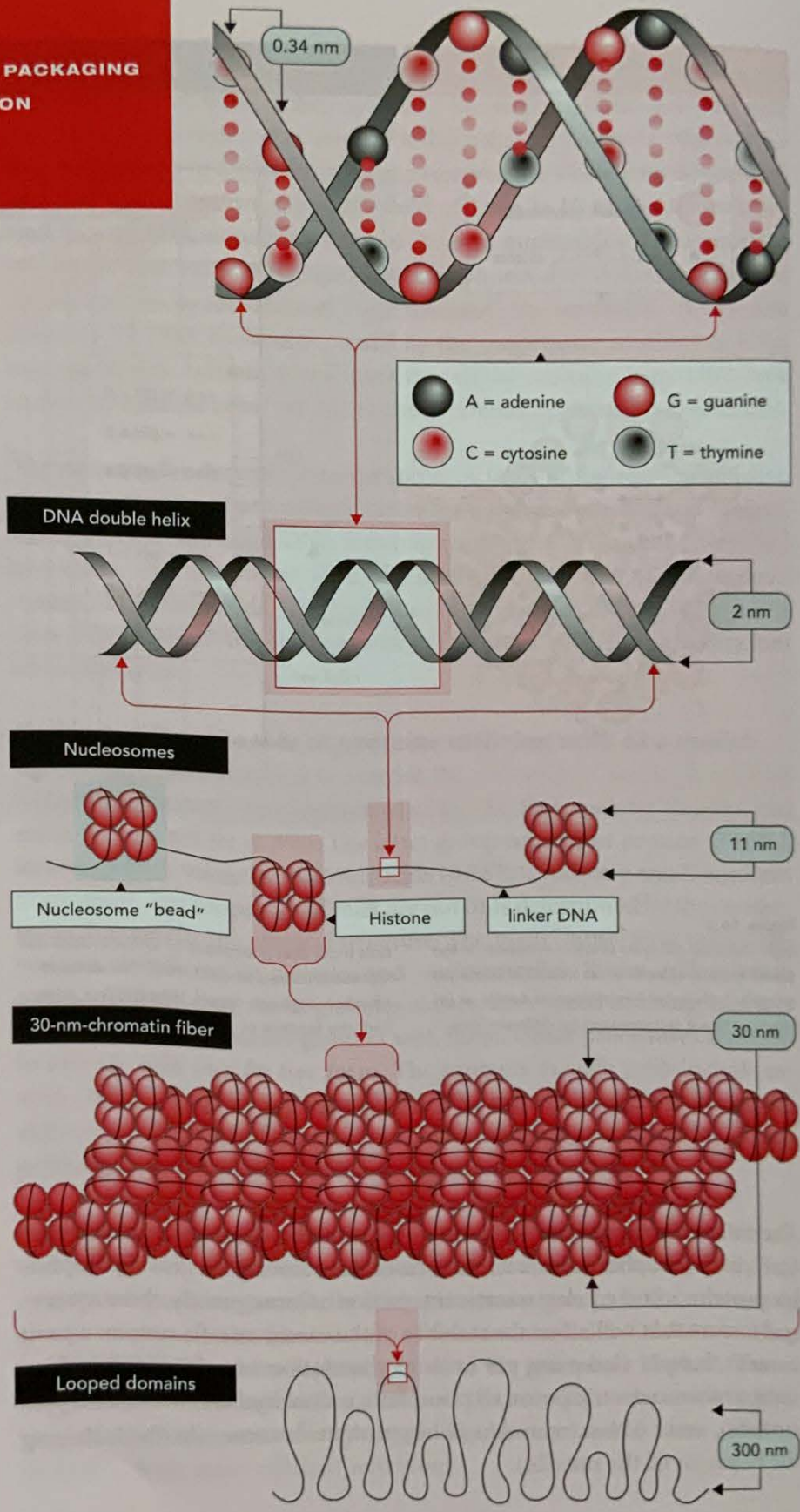


Figure B.16.5.1
The upper part of this schematic illustration shows the sugar-phosphate chains of the DNA backbone, the complementary base pairs and several important dimensions. The lower parts show progressive stages of DNA coiling and folding by interacting with proteins (histones and non-histone chromosomal proteins). A first step are the beads on a string: an extended configuration of nucleosomes formed by the association of DNA with four types of histones. Second step is the 30 nm chromatin fiber shown as a tightly packed collection of nucleosomes. The fifth histone, H1, may be located in the interior of the fiber. Finally, the 30 nm fibers are organized in looped domains.

The structure dependent energy dynamics of the micelle aggregates has been characterized utilizing light-induced delayed luminescence (Figure 16.5). After UV excitation, the delayed emission in the visible range can be recorded for several minutes. The delayed emission definitely confirms that experimentally, the delayed emission kinetics depend on pH and temperature. This means that the intrinsic (physical/chemical) structure is reflected in the energy dynamics of light-induced delayed photon emission. Therefore, it was concluded that 1.) delayed emission of protein structures (without DNA) is possible and 2.) the photon storage (and correspondingly delayed emission) can be influenced by changing the protein structure.

Conclusion

It became evident that research on "electromagnetic fields and life" and "photon emission in living systems" calls for the collaboration of at least three sciences: mathematics (cybernetics or information theory) in regard to the term "communication", physics referring to "electromagnetic fields", and biology (biochemistry, quantum chemistry) with reference to "biological systems". It is this type of discussion which will make it possible (in the future) to better understand the gradual progression in a state of differentiation and growth as well as the shift in the boundary law (of the body cells' society) that facilitates a cancer cell to develop.

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17

Photon Field Aspects in the 1990's

In Chapter 16 we ended with a major challenge. It was important to manage the interdisciplinary research in an orderly manner. The cooperation and the excitement of the researchers world-wide resulted in the production of a series of scientific books. The choice to write books instead of articles has sometimes been criticized. It was argued that information provided in books can have a smaller impact as supposed to materials published in peer-reviewed articles. However, the participating researchers were, almost without exception, professors, associate professors or senior scientists at distinguished universities. Therefore, books presented the possibility to strategically share important information publicly regarding the umbrella theme of "Life, Electromagnetic Fields and Light" (see Box 17.1). In this chapter, several subjects will clarify, in more detail, the development of a body pattern, the role of photon radiation therein, and the recognition by cells as well as molecular structures of oscillating electrical fields and their transmission either to coordinate or regulate chemical reactions.

The "photon sucking" phenomenon in early development

The challenge to understand how a body pattern is determined in development remained almost untouched since Hans Driesch and others introduced the concept of an "embryological field" that determines the body pattern. Such a field includes a pattern in early development that facilitates future differentiation. This domain is approximately 1 mm in linear dimensions in all animal and plants regardless of the adult size of the species. A rough estimation is that this domain contains approximately some 10^{20} types of molecules.

How does this macroscopic pattern arise in such a diverse molecular system? In 1952, Alan Turing's trend setting paper on chemical morphogenesis gave rise to a whole genre of reaction diffusion models wherein "pre-patterns" of chemical "morphogens" form (which are then "translated" into specialized genes of body patterns). However, such models had their limitations. Missing was an account of the *forces* that *mobilize* molecules into different kinds of patterns. Several models of morphogenesis have emphasized *physical* forces involved with patterning. The subject of such *physical* forces have already been discussed in Part D (suggesting that the morphogenetic field is electrodynamic). Since all molecular and intermolecular forces are electromagnetic, such forces are sufficient, by themselves,

MORE ABOUT...

BOX 17.1

■ THE SCIENTIFIC COOPERATION IN BIOPHOTON RESEARCH: LEADING CONFERENCES, CORRESPONDING BOOK VOLUMES, AND THE INTERNATIONAL INSTITUTUE OF BIOPHYSICS

Figure B.17.1.1

YEAR	LOCATION	TITLE	EDITORS	PUBLISHER
1986 ▶	Wroclaw, Poland	Photon Emission from Biological Systems	B. Jezowska-Trzebiatowska, B. Kochel, J. Slawinski, W. Strek	World Scientific, Singapore, 1987
1989 ▶	Wroclaw, Poland	Biological Luminescence	B. Jezowska-Trzebiatowska, B. Kochel, J. Slawinski, W. Strek	World Scientific, Singapore, New Jersey, London, Hong Kong, 1990
1992 ▶	Kaiserslautern, Germany	Recent Advances in Biophoton Research and its Applications	F.A.Popp, K.H.Li Q.Gu	World Scientific, Singapore, New Jersey, London, Hong Kong, 1992
1994 ▶	–	Bioelectrodynamics and Biocommunication	Mae-Wan Ho, F.-A. Popp, U. Warnke	World Scientific, Singapore, New Jersey, London, Hong Kong, 1994
1994 ▶	Moscow, Russia	Biophotonics. Non-equilibrium and Coherent Systems in Biology, Biophysics, Biotechnology	L.V. Beloussov F.-A. Popp	Bioinform Services Co., Russia, 1995
1995 ▶	Neuss, Germany	Current Development of Biophysics	Ch. Zhang, F.-A. Popp, M. Bischof	Hangzhou University Press, Hangzhou, 1996
1997 ▶	Ilmenau, Neuss, Gemany	Biophotons	J.-J. Chang, J. Fisch, F.-A. Popp	Kluwer Academic Publishers, Dordrecht, Boston, London, 1998
1999 ▶	Moscow, Russia	Biophotonics and Coherent Systems	L. Beloussov, F.-A. Popp, V. Voeikov, R. van Wijk	Moscow University Press, Moscow, 2000
1999 ▶	Neuss, Germany	What is Life?	H.-P. Dürr, F.-A. Popp, W. Schommers	World Scientific, New Jersey, London, Singapore, Hong Kong, 2002
2002 ▶	Catania, Italy	Energy and Information Transfer in Biological Systems	F. Musumeci, L.S. Brizhik, Mae-Wan Ho	World Scientific, New Jersey, London, Singapore, 2003
2003 ▶	Beijng, China	Biophotonics. Optical Science and Engineering for the 21st century	Xun Shen R. Van Wijk	Springer, New York, 2005
2003 ▶	Neuss, Germany	Integrative Biophysics, Biophotonics	F.-A. Popp, L. Beloussov	Kluwer Academic, Dordrecht, Boston, London, 2003
2004 ▶	Simferopol, Crimea, Ukraine	Biophotonics and Coherent Systems in Biology	L.V. Beloussov, V.L. Voeikov, V.S. Martynyuk	Springer, New York, 2007

to mobilize and organize the molecules (within the field) into a pre-pattern and subsequently into a more specific pattern.

Lev Beloussov at the Lomonosov Moscow State University focussed on photon emission in relationship to early development. He referred to the classic Gurwitsch data in his own theory of early development (wherein average intensity of spontaneous photon emission decreases). Simultaneously, the intensity of a degradational radiation increased (which might be initiated vis-à-vis slightly reversible damaging influences) suggesting the destruction within the collective states of each domain. Such domains are progressively generated and enlarged during development. Beloussov hypothesized that they have properties of "photon sucking". Along this line, it is of interest that already in the early 1990's, Mae-Wan Ho documented progressive decrease in spontaneous photon emission in *Drosophila* eggs. In 1997/1998 this same trend was also described by Beloussov regarding chicken embryos and fish (*Misgurnus fossilis*) eggs plus their embryos. In this description of the developmental changes, Beloussov utilized the Fourier spectra of photon emission to describe the progressive enlargement of the collective excitation domains (the increased interaction of coupled oscillators). Fourier spectra of the different stages of fish embryos significantly differ from each other.

Beloussov's studies of chicken eggs and their shells documented that a hen's egg, irrespectively of whether it contains a developing embryo (or was non-fertilized) did emit after it had absorbed daylight. Within many hours a considerable amount of photons appeared, primarily in the red range. The only significant emitter was the shell (both yolk and embryo emits two orders less whereas, egg whites do not emit photons at all). However, only in dead eggs and non-fertilized eggs, the rate of photon emission of a whole intact egg is approximately equal to that of its isolated shell. Whole, second day fertilized eggs primarily emit many more photons than their isolated shells. The 9 day fertilized eggs appear in a reverse manner. Hence, during early stages, an egg's interior stimulates, whereas, during later stages it suppresses its shell emission. This latter effect may be due to active absorption ("sucking") of photon energy from the shell by the embryo. By hyperbolic decay criteria emission was considered highly coherent. The obvious explanation is the crystalline structure of the shell. The same is not true for a fresh non-fertilized egg. Consequently, only a developing egg can support coherence of shell emitted light.

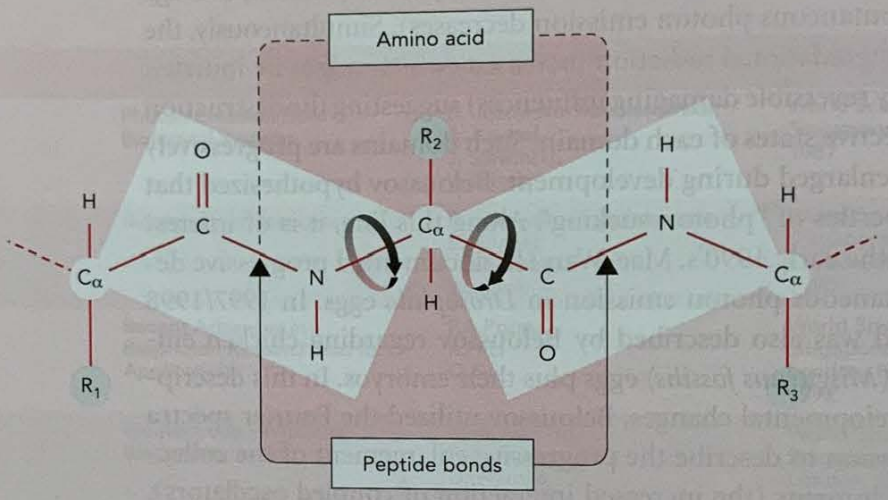
Similarly, non-additive relationships between the emission of developing embryos and egg membranes have been observed in amphibians. In the gastrula stage, the amount of photon emission of the isolated egg membrane is smaller than that of embryos plus their membranes. However,

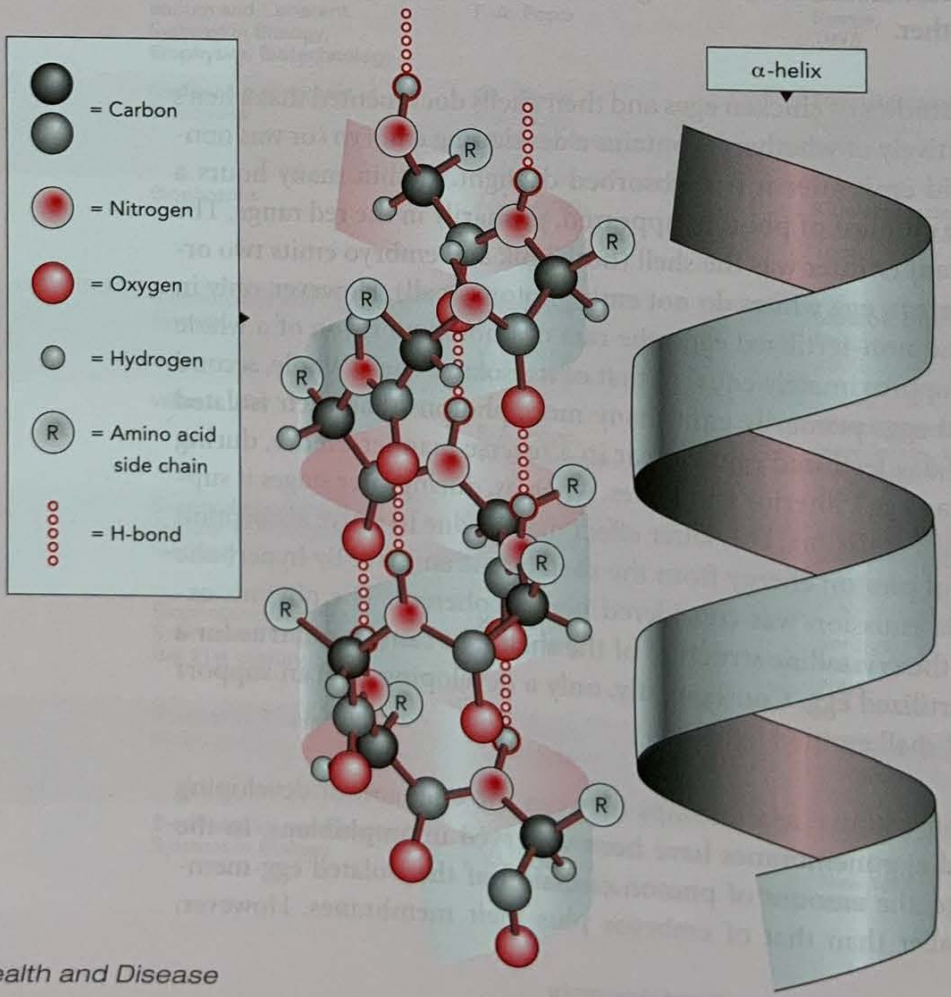
MORE ABOUT...

BOX 17.2

■ THE α-HELICAL PROTEIN STRUCTURE

Proteins are by far the most structurally complex and functionally sophisticated molecules. A protein consists of a polypeptide backbone with attached side chains. Each type of protein dif-





fers in its sequence and number of amino acids; therefore, it is the sequence of the chemically different side chains that makes each protein distinct. The amino acids are joined together by an amide linkage, called the peptide bond. The peptide bond consists of four atoms (C=O linked to N–H) that form a rigid planar unit. There is no rotation around the C–N bond. Between two peptide bonds two single bonds exist facilitating rotation so that long chains of amino acids are very flexible. Stretches of polypeptide chain could form the protein's secondary structure such as the formation of α-helices and β-sheets.

The regular conformation of the polypeptide backbone observed in the α-helix is presented in Figure B.17.2.1. The N–H of every peptide bond is hydrogen-bonded to the C=O of a neighboring peptide bond located four peptide bonds away in the same chain.

◄ Figure B.17.2.1
The α-helix in exciton-soliton discussions. A protein molecule is built from thousands of atoms linked together by precisely oriented covalent and noncovalent bonds. The amino acid sequence is currently recognized as the first level of organization. It is known as the primary structure. Each amino acid contributes three bonds to the backbone of the chain. The upper panel demonstrates the steric limitations on the bond angles in a polypeptide chain. The peptide bond itself is planar and does not permit rotation (presented in a box). By contrast, rotation can occur about the C_α–C bond, as well as the N–C_α bond. The lower panel demonstrates an α-helix as one of protein's secondary structures. It is based on the potentiality of hydrogen bonding between the N–H of every peptide bond and the C=O. In the α-helix, the N–H of every peptide bond is hydrogen bonded to the C=O of a neighbouring peptide bond located four peptide bonds away in the same chain. By omitting the individual atoms the α-helix structure of the peptide backbone can be highlighted (lower panel, right).

the advanced neurula stage embryos demonstrate the reverse. This, again, might point to an increase in "photon sucking" during development. Egg shells and membranes (besides their routine protective role) seem to act as storages and transformers of an external light.

By analyzing Fourier spectra of ultra-weak photon emission from different stages of fish embryos, Beloussov demonstrated significant differences. He interpreted such differences as follows: Immediately after fertilization a single powerful oscillator containing many harmonics becomes active. At the end of the cleavage period, the single oscillator exchanges to a large set of smaller ones which are, at first, non-correlated with each other. Later, these oscillators again become coupled, this time including only a few dominating frequencies. These latter events are then associated with the beginning of active behavior simultaneous to the functioning of a central nervous system.

Beloussov finally compared the Fourier patterns of ultra-weak photon emission from cell cultures of fibroblasts and cardiomyocytes. He demonstrated that in such cell cultures, important substances for growth and differentiation (e.g., fibroblast growth factor) could influence the cell Fourier spectra (although the average rates of photon emission remained the same). He also demonstrated changes in Fourier spectra utilizing substances and conditions that might influence cytoskeleton structures (i.e., cytochalasin, colchicin, and trypsin). In some instances, the intensity of photon emission increased suggesting the presence of entropy outflow and a decrease in the complexity of the cells. Beloussov also postulated a decrease in the formation of excitons with "photon sucking" properties. However, the situation was the opposite from the decrease of photon emission as development proceeded. This may have been associated with the formation of excitons with "photon sucking" properties. However, what is an exciton?

Excitons and solitons

An *exciton* is a collective of vibrational excitations.

MORE ABOUT...

BOX 17.3

■ EXCITONS AND SOLITONS

Excitons

The concept of excitons was first proposed by Yakov Frenkel (1931) when he described the excitation of atoms in a lattice of insulators. An exciton can be formed when a photon is absorbed. The photon excites an electron from the valence band into the conduction band. In turn, this leaves behind a localized positively charged hole. The electron in the conduction band is then attracted to the localized hole by the Coulomb force. Vis-à-vis the bound state of the electron, the exciton is an elementary excitation that can transport energy without transporting net electric charge. Frenkel proposed that this excited state would be able to travel in a particle-like fashion through the lattice without the net transfer of charge.

Alternatively, an exciton may be thought of as an excited state of an atom, ion, or molecule wherein the excitation wanders from one cell of the lattice to another. When a molecule absorbs a quantum of energy (photon) that corresponds to a transition from one molecular orbital to another molecular orbital, the resulting electronic excited state is also properly described as an exciton. The probability of the hole disappearing is limited by the difficulty of losing the excess energy. As a result, excitons can have a relatively long lifetime (up to several milliseconds) after which the ground electronic state is restored and the molecule undergoes photon emission. Molecular excitons have several interesting properties, one of which is energy transfer (resonance energy transfer) whereby, if a molecular exciton has proper energetic matching to a second molecule's spectral absorbance, then an exciton may transfer (hop) from one molecule to another. The process is strongly dependent on molecular rules.

The existence of exciton states may be inferred from the absorption of light associated with their excitation. When excitons interact with photons, a polariton (also called an exciton-polariton) is formed. These excitons are sometimes referred to as "dressed" excitons. Provided the interaction is attractive, an exciton can bind with other excitons to form a biexciton. If a large density of excitons is created in a material, they can interact with one another to form an electron-hole liquid, a state observed in some semiconductors.

Solitons

The soliton phenomenon was first described by John Scott Russell (1808-1882) who observed a solitary wave in the Union Canal in Scotland. He reproduced the phenomenon in a wave tank and named it the "Wave of Translation". In 1834, John Scott Russell described this "wave": "I was observing the motion of a boat which was rapidly drawn along a narrow channel by a pair of horses, when the boat suddenly stopped – not so the mass of water in the channel which it had put in motion; it accumulated round the prow of the vessel in a state of violent agitation, then suddenly leaving it behind, rolled forward with great velocity, assuming the form of a large solitary elevation, a rounded, smooth and well-defined heap of water, which continued its course along the channel apparently without change of form or diminution of speed. I followed it on horseback, and overtook it still rolling on at a rate of some eight or nine miles an hour, preserving its original figure some thirty feet long and a foot to a foot and a half in height. Its height gradually diminished, and after a chase of one or two miles I lost it in the windings of the channel. Such, in the month of August 1834, was my first chance interview with that singular and beautiful phenomenon which I have called the Wave of Translation."

It must be noted that "Translation" here means that there is real mass transport, although it is not the same water which is transported from one end of the canal to the other end by this "Wave of Translation". Rather, a fluid parcel acquires momentum during the passage of the solitary wave and comes to rest again after the passage of the wave. However, the fluid parcel had been displaced substantially forward during the process and a net mass transport was the result.

Scott Russell built wave tanks at his home and noticed the following properties: a.) The waves are stable and can travel over large distances; b.) The speed depends on the size of the wave and its width throughout the depth of the water; c.) The waves will never merge; and d.) If the wave is too big for the depth of water, the wave splits into two volumes, one big and one small. In 1895, Diederik Korteweg and Gustav de Vries published a theoretical document which provided what is now known as the Korteweg-De Vries equation.

Much experimentation has been done using solitons in fiber optic applications. A soliton's inherent stability facilitates long distance transmission possible without the use of repeaters. It could potentially double transmission capacity. Solitons may occur in proteins and DNA. They are related to the low frequency collective motion category.

The structure of an alpha helical molecule (Box 17.2) provides the opportunity for collectivization of excitations. In alpha helical proteins, the polypeptide chains are coiled into long helices. They are stabilized by hydrogen bonds. The energy of the hydrogen bond is considerably weaker than that of a peptide bond. Therefore, the displacement of peptide groups (situated at the hydrogen bonds) from their equilibrium positions occur much more readily than their displacements along the main polypeptide chain. However, a displacement depends on spreading of the excitation.

An exciton spreads along the chain with a high group velocity exceeding the velocity of longitudinal sound waves (see also Box 17.2). Owing to such rapid motion of an exciton, the local displacement (i.e., deformation of the chain) cannot keep up. Therefore, the stability of excitons is limited. They are described as wave packets diminishing with time. However, they are easily excited by light of a corresponding frequency because the transition into the excited state is not accompanied by the displacement from the equilibrium positions of the peptide groups. In fact, the study of infrared spectra (when excitons are generated in protein molecules) is an effective means to analyze protein structure.

Solitons in molecular energy transfer

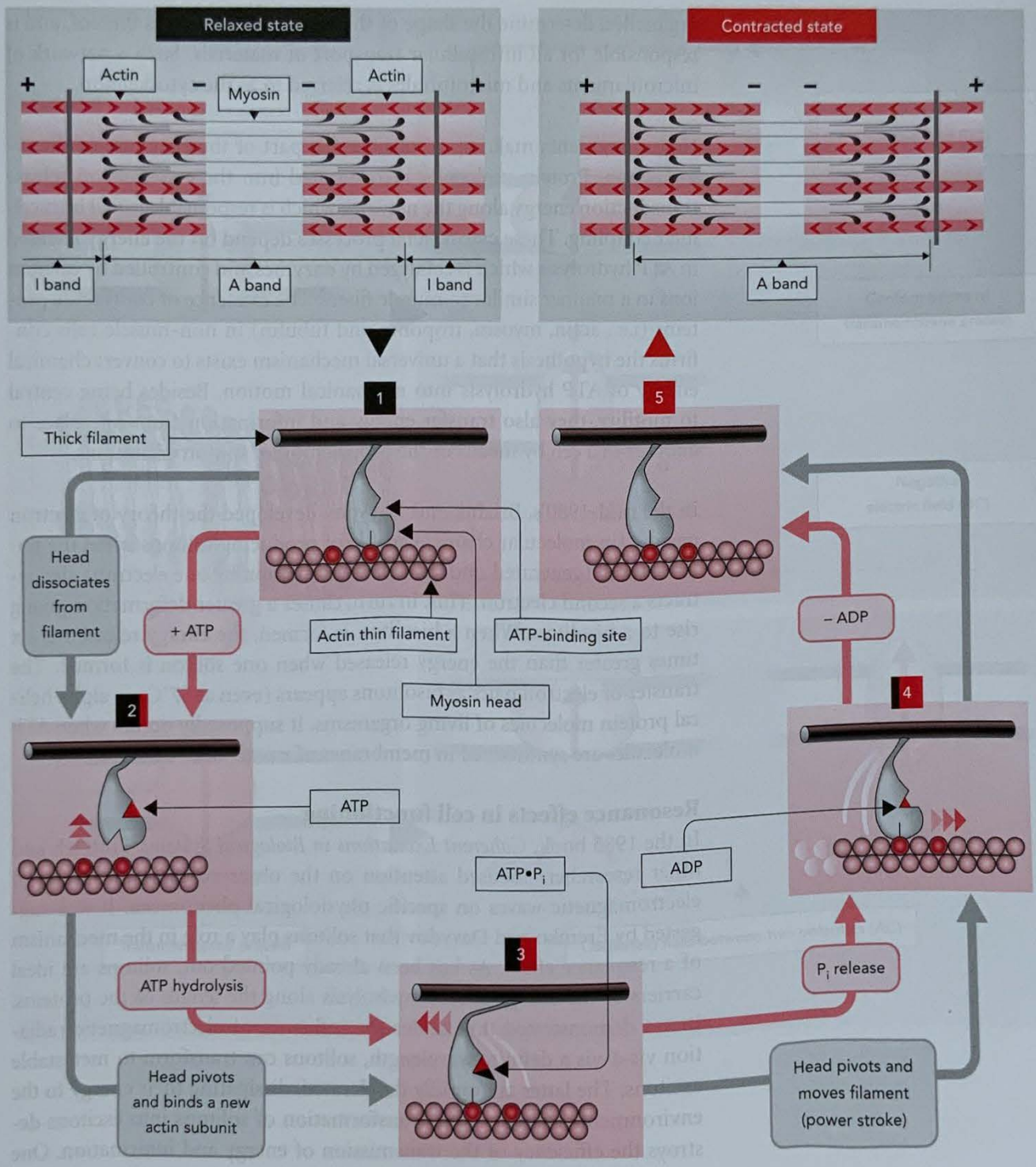
The exciton is different from another form of collective excitation (the soliton) which can be formed as a result of the combination of intrapeptide C=O vibrations with a local deformation of the chain (see Box 17.2). They always move with less velocity. In fact, the soliton is very stable. The interaction of electrons within the field of displacements in the alpha helical protein molecules stabilizes their motion. In contrast to excitons, the soliton excitation cannot be excited by light. Also, the probability of light emission by solitons is small [in line with the Frank-Condon principle (see Box 16.1)]. Instead, the solitons are excited by local external effects (i.e., by chemical reactions).

Important theoretical investigations on solitons were conducted in the late 1960's by A. S. Davydov at the Institute for Theoretical Physics in Kiev. They used

Figure 17.1 ►
Operational model for the sliding filament model of muscle contraction. The two arrangements (representing the relaxed state and the contracted state) are shown in the left and right boxes. The process of contraction is presented (by the cycle) in the middle. In the presence of ATP and Ca^{2+}, the myosin heads extending from the thick filaments walk towards the (+) ends of the thin filaments. Because the thin filaments are anchored at the Z disks, movement of myosin pulls the actin filaments towards the center of the sarcomere, shortening its length in the contracted state as shown in the lower box. The process of contraction is a repeating cycle of changes of the myosin II head that is part of a thick filament. The cycle includes four steps. In the absence of a bound nucleotide, a myosin head binds actin tightly in a "rigor" state. The binding of ATP opens the cleft in the myosin head, disrupting the actin binding site and weakening the interaction with actin. In step 2, when freed from actin, the myosin head hydrolyzes ATP, causing a conformational change in the head that moves it to a new position, closer to the (+) end of the actin filament, where it rebonds to the filament. In the next step as phosphate (P_i) dissociates from the ATP binding pocket, the myosin head undergoes a second conformational change – the power stroke – which restores myosin to its rigor conformation. Because myosin is bound to actin this conformational change exerts a force that causes the myosin to move the actin filament. Lastly, the release of ADP completes the cycle.

proteins that split an ATP molecule. Within the perspective of large protein dimensions, the site at which ATP hydrolysis occured is often separated by a considerable distance from the site at which the energy is used. Davydov used the concept of soliton propagation in alpha helical protein molecules to elucidate the mechanism of contraction of striated muscles at the molecular level (Figure 17.1). A muscle fiber is a bundle consisting of several thousand densely packed parallel myofibrils. Each myofibril consists of repeating segments (sarcomeres) separated by transverse membranes. From both sides of the transverse membrane, thin filaments of actin project into the sarcomere interdigitating with the thick myosin filaments situated in the middle of the sarcomere. Thin and thick filaments are bathed in the sarcoplasm which contains ATP molecules and Mg^{2+} ions.

According to Davydov's hypothesis, calcium ions (diffusing to the first series of myosin heads at the ends of thick filaments) initiate the hydrolysis of ATP molecules attached to them. The energy released generates (in the long helical parts of the myosin molecules) solitons which move from the heads of the molecules to their tails in the sarcomere center. The motion of a soliton is accompanied by a local bending and swelling of the molecule. The heads of myosin molecules attached to the thin filaments initiate a slight displacement of the latter in the direction of the motion of the solitons (towards the center of the sarcomere). Accordingly, the myosin heads detach themselves from the thin filaments. However, this movement is due, not to the lengthening, turning, or contraction of the head itself, but rather to the movement of a soliton inside the thick fiber accompanied by the motion of bend in the helical domain of the myosin molecule. In other words, the kinetic energy of solitons is converted into contraction energy (energy of tension) if a load is applied to the muscle. In this model, all parts of the myosin molecule (not only its head) are active contractile elements.



Other investigations have pointed to the role of solitons in cytoskeletal structures. The entire interior of the cell is crisscrossed by a network of protein microfilaments and microtubules which anchor all intracellular

organelles, determine the shape of the cell and any changes thereof, and is responsible for all intracellular transport of materials. Such a network of microfilaments and microtubules is referred to as the cytoskeleton.

Helical segments make up a considerable part of the structure of the cytoskeleton. Protein molecules incorporated into the cytoskeleton release transduction energy along the network which is responsible for all intracellular coupling. These cytoskeletal processes depend on the energy released in ATP hydrolysis which is catalyzed by enzymes and controlled by calcium ions in a manner similar to muscle fibers. The existence of contractile proteins (i.e., actin, myosin, troponin and tubulin) in non-muscle cells confirms the hypothesis that a universal mechanism exists to convert chemical energy of ATP hydrolysis into mechanical motion. Besides being central to motility, they also transfer energy and information from one place to another in a cell by means of the propagation of soliton excitations.

In the mid-1980's, Brizhik and Davydov developed the theory of electron pairing (in molecular chains) capable of producing solitons when the potential well (generated under a local deformation by one electron) also attracts a second electron. This, in turn, causes a greater deformation giving rise to a bisoliton. When a bisoliton is formed, the energy released is six times greater than the energy released when one soliton is formed. The transfer of electron pairs as bisolitons appears (even at 37°C) in alpha helical protein molecules of living organisms. It supposedly occurs when ATP molecules are synthesized in membranes of mitochondria.

Resonance effects in cell functioning

In the 1983 book, *Coherent Excitations in Biological Systems*, Fröhlich and other researchers focused attention on the observed resonant effects of electromagnetic waves on specific physiological phenomena. It was suggested by Eremko and Davydov that solitons play a role in the mechanism of a resonance effect. As has been already pointed out, solitons are ideal carriers of the energy of ATP hydrolysis along the length of the proteins. It was demonstrated that under the influence of electromagnetic radiation vis-à-vis a definite wavelength, solitons can transform to metastable excitons. The latter are rapidly decelerated, dissipating their energy to the environment. Therefore, the transformation of solitons into excitons destroys the efficiency of the transmission of energy and information. One can conclude, therefore, that the non-linear dynamics of solitons provides a key to understanding the mechanism of the transduction of information within the cell as well as in sensing (whereas excitons are easily excited by light of a corresponding frequency).

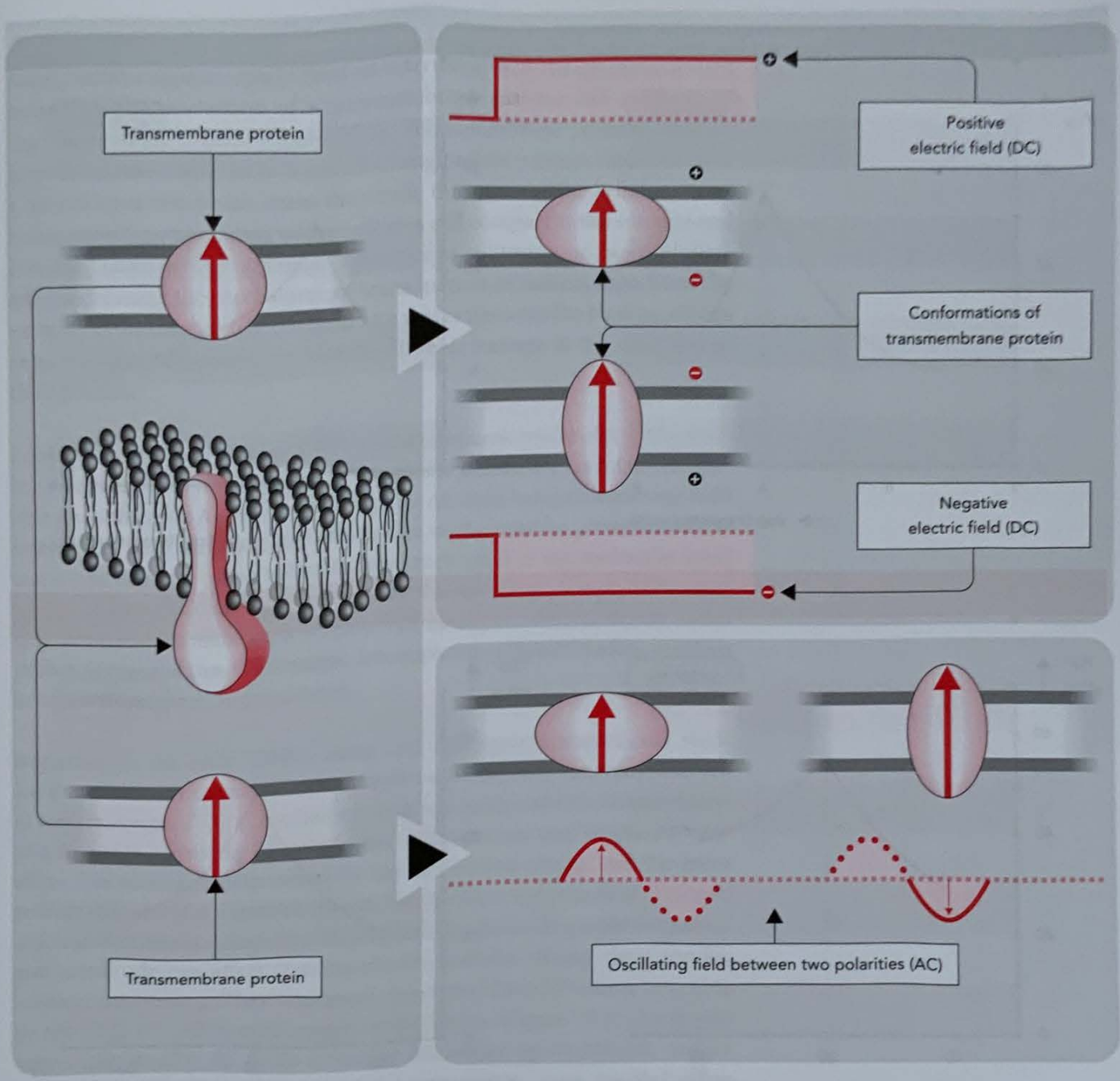


Figure 17.2
Electroconformational changes in a transmembrane protein.
At the left side, part of a cell membrane with a transmembrane protein plus a two-dimensional schematic view of the same situation with the protein being presented as a large open circle and its molar electric moment (arrow) pointing outwards. The interaction with an electric field is presented at the right side. The upper right box shows the interaction with either a direct electric field of positive or negative polarity. The DC field initiates the protein to undergo electroconformational changes. The lower box illustrates the interaction with an oscillating field between two opposite polarities. This AC field facilitates the protein to oscillate between the two conformations.

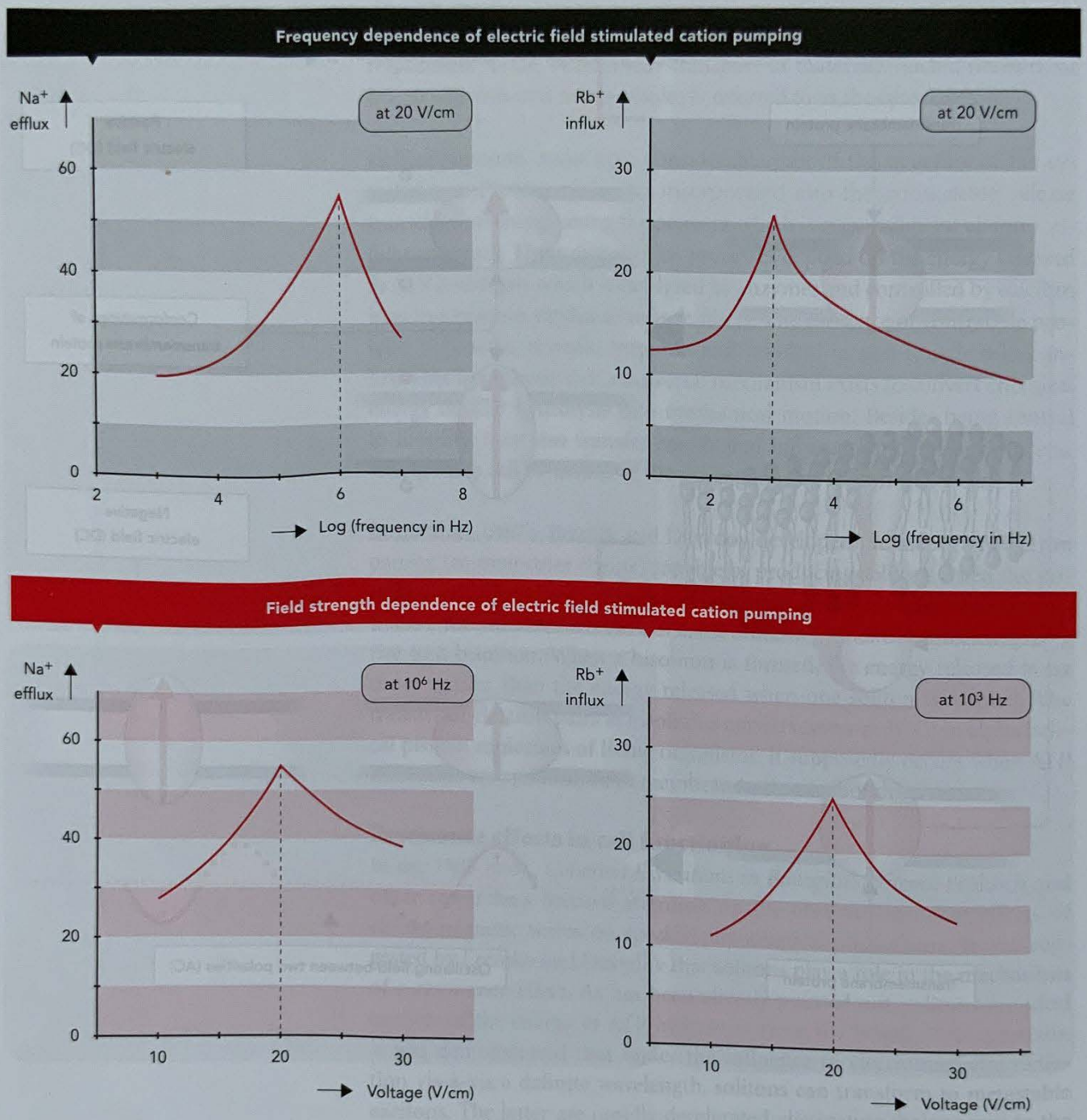


Figure 17.3

Electric field stimulated cation pumping. Specific frequency dependence for different functions of the same protein (according to Liu *et al*). The stimulation of Na^+ efflux and Rb^+ influx in erythrocytes was estimated at different frequencies and strengths of an AC field. The left part illustrates the Na^+ efflux data and the right part the Rb^+ influx data. The frequency dependence (both upper panels) of the electric field stimulated cation pumping with an AC of 20 V/cm demonstrates that optimal frequency of Na^+ efflux at 10^6 Hz, whereas it was at 10^3 Hz for Rb^+ influx. The field strength dependence was estimated at the maximum frequency and is illustrated in both lower panels. The data demonstrate that voltage dependence has a similar maximum (at 20 V/cm) for both fluxes.

Many potential mechanisms have already been evaluated wherein a cell can sense electromagnetic signals from an external source. An example of such research is the interaction of a transmembrane enzyme and an alternating electrical (AC) field (Figure 17.2). Transmembrane proteins play an important role in the life of a cell. The longitudinal protein fractions have a helical structure which spans the whole thickness of the cellular membrane providing a coupling of the cell (or a cell compartment) interior and exterior. Based on the nonlinear dynamics of solitons, one can undoubtedly understand the mechanism of transduction of information from the exterior to the interior of a cell (and vice versa) vis-à-vis the binding of ions (e.g., sodium, potassium, or chloride) in their passage in the direction of their gradient.

Looking carefully at the interaction of an anisotropic molecule (with a driving force that oscillates between two opposite polarities) reveals a remarkable phenomenon. A symmetrical, bipolar AC field has a time average field intensity of zero. However, the effects due to the positive phase and those due to the negative phase will not cancel each other at the molecular level. Special kinetic effects are able to transfer the energy in the applied field to specific forms of a membrane protein. The energy is then transiently stored in such forms and can, for example, be coupled to a ligand binding reaction in order to perform chemical work.

Beginning in the early 1980's, Tsong and colleagues systematically studied the effects of external electrical fields on membrane enzymes involved in ion transport. Typical oscillatory electrical fields exhibit characteristics of a language consisting of wave forms with intensity and frequency variables. The former is determined by the interaction energy and the latter reflects the rate of a chemical process being affected by the field. Tsong *et al* deciphered the language by studying how AC fields of specific frequency and amplitude can affect transmembrane proteins during their specific cellular functioning. They addressed AC stimulation of cation pumping by the Na^+, K^+ -ATPase of human erythrocytes (Figure 17.3). Such cells were stimulated by AC fields including intensities up to 30V/cm and by frequencies from 1Hz to 10MHz. In the experiments, both the Na^+ efflux as well as the K^+ influx (the latter by means of Rb^+ ions) were estimated. In such a systematic approach, several important features were recognized: an optimum frequency of 1 kH for Rb^+ pumping and 1 MHz for Na^+ pumping. However, both of the stimulated ion fluxes reached maximal values at a field strength of 20 V/cm. This meant that the two pumps of the same enzyme can function independently of each other (i.e., they could be uncoupled). At 1 kHz, the potassium pump would be activated but not the sodium pump. The converse is true when a 1 MHz AC field was utilized.

MORE ABOUT...

BOX 17.4

■ EARLY DEVELOPMENT OF *DROSOPHILA*

We know more about the genetics of *Drosophila* than that of any other multicellular organism. The zygote nucleus undergoes several mitotic divisions within the central portion of the egg: 256 nuclei are produced by a series of eight nuclear divisions averaging 8 minutes each. The nuclei then migrate to the periphery of the egg where the mitosis continues, albeit at a progressively slower rate. During the stages of nuclear division, the embryo is called a syncytial blastoderm (meaning, that all the cleavage nuclei are contained within a common cytoplasm). However, this does not mean that the cytoplasm is itself uniform. When the nuclei reach the periphery of the egg during the tenth cleavage cycle, each nucleus becomes surrounded by microtubules and microfilaments. Following division cycle 13, the oocyte plasma membrane folds inward between the nuclei. This first phase of blastoderm cellularization is characterized by the invagination of cell membranes between the nuclei to form furrow canals. This process creates the cellular blastoderm in which all the cells are arranged in a single layered jacket around the yolk core of the egg.

In *Drosophila*, the cellular blastoderm consists of approximately 6000 cells and is formed within 4 hours of fertilization. Gastrulation begins shortly after the mid-blastula transition. The first movements of *Drosophila* gastrulation segregate the presumptive mesoderm, endoderm, and ectoderm. The prospective mesoderm (about 1000 cells) constituting the ventral midline of the embryo folds inward to produce the ventral furrow. The prospective endoderm invaginates to form two pockets at the anterior and posterior ends of the ventral furrow. At this time, the embryo bends to form the cephalic furrow. The ectodermal cells (on the surface) as well as the mesoderm undergo convergence and extension (migrating towards the ventral midline) to

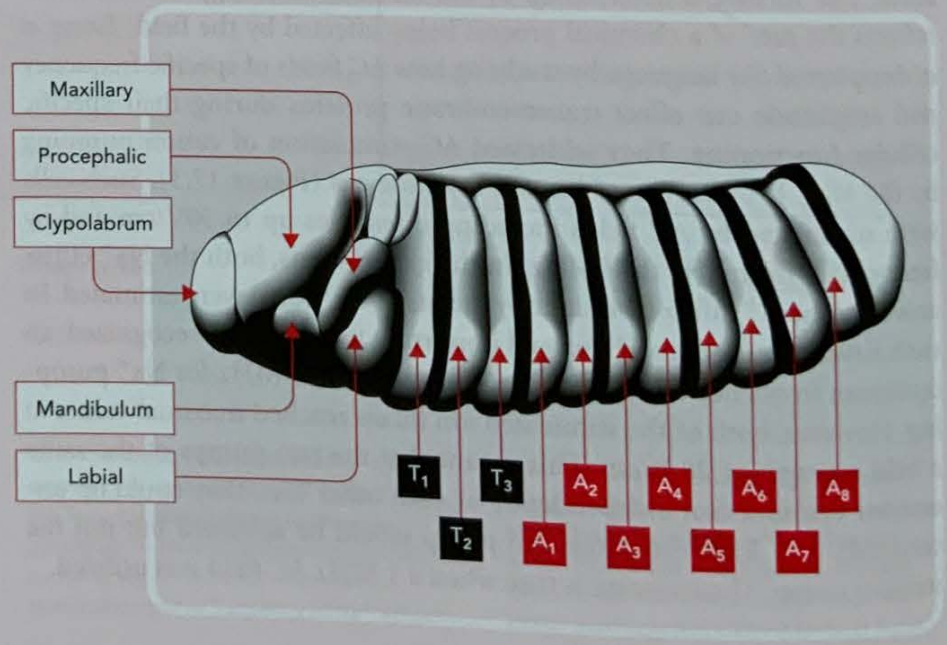


Figure B.17.4.1
Diagram of wild-type embryonic segmentation. This figure can be used to illustrate the abnormalities among hatched larvae exposed to static magnetic fields. The thorax segments T1-3 as well as the Abdomen segments A1-8 (and A9/10) are abbreviated in the picture. The four head parts develop into structures named in the figure.

There are many conditions necessary to satisfy before the pump can actually function. Among others, the turnover rate of the enzyme must match the frequency of the AC field. This last property of the system implies that a cell can recognize an electrical signal by the virtue of the kinetic characteristics of the transport system. It also means that there are at least three windows which are associated with coupling of electrical energy to chemical potential energy. These are the frequency window, the amplitude window, and the ligand concentration. All three have been documented. The optimal frequency, amplitude and ligand concentration required for the transduction of an electrical signal are not fixed for a transmembrane protein. Depending on the state and the environment of the protein, these windows can change. Such observations of amplitude and frequency windows are crucial for formulating enzyme models based on the concept of electroconformational coupling. By determining the position of the windows, thermodynamic and kinetic parameters of the enzyme catalytic reaction can be elucidated.

Such studies resulted in additional knowledge regarding the relationship between radiation and the biochemical electrodynamics (as well as in the relationship between radiation and the effects of weak, external electromagnetic fields) in early development with its mechanically stressed states and transitions.

External magnetic field effects in early development

Without focusing on the optimalization of frequency and amplitude, Mae-Wan Ho investigated the effects of weak external magnetic fields on pattern formation in the early development of *Drosophila melanogaster* (see Box 17.4). The development of this organism is extremely well characterized in terms of morphological changes as well as changes in gene expression. Pattern determination processes occur within the first three hours of development. During this time, the body pattern (in the ensuing larva) is sensitive to the perturbation from a wide variety of environmental agents. *Drosophila* embryos are also a good model in other arenas. It is relatively easy to obtain large num-

form the germ band (that includes all the cells that will form the trunk of the embryo). At this time, the body segments begin to appear (dividing the ectoderm and mesoderm).

The general body plan of *Drosophila* is the same as in the embryo, the larva, and the adult (each of which has a distinct head end and a distinct tail end between which are repeating segmental units). Three of these units form the thorax while another eight segments form the abdomen. Each segment of the adult fly has its own identity. The first thoracic segment, for example, has only legs, the second thoracic segment has legs and wings, and the third thoracic segment has legs and halteres (balancing organs). Thoracic and abdominal segments can also be distinguished from each other by differences in the cuticle.

A hierarchy of genes that establish anterior-posterior polarity and divide the embryo into a specific number of segments has been identified. This hierarchy is initiated by *maternal effect genes* that produce messenger RNA's that are placed in different regions of the egg. These messages encode transcriptional and translational regulatory proteins that diffuse through the syncytial blastoderm and activate or repress the expression of certain *zygote genes*. The first zygote genes to be expressed are called *gap genes* (because mutations in them cause gaps in the segmentation pattern). These genes are expressed in certain broad (approximately three segments wide) partially overlapping domains. Differing combinations and concentrations of the gap gene proteins regulate the transcription of *pair-rule genes* which divide the embryo into periodic units. The transcription of the different pair-rule genes results in a striped pattern of several transverse bands perpendicular to the anterior-posterior axis. The pair-rule gene proteins activate the transcription of the segment *polarity genes* whose mRNA and protein products divide the embryo into 14-segment-wide units establishing the periodicity of the embryo.

bers of embryos that are synchronous within one minute of development. This homogeneity of the system is important to obtain reproducible results which can then be further characterized according to developmental time.

Mae-Wan Ho's team utilized weak magnetic fields in order to avoid thermal and other effects unrelated to the electromagnetic nature of the disturbance. The experiments included a series involving a steady magnetic field as well as a second series involving 60Hz AC magnetic fields. A third (control) series was done using a "field-free" condition. The researchers placed the embryos in a moist, covered petri dish in the holder at the center of the coil before the preset field was activated. Exposure period was 30 min for all field strengths in the DC and AC series. The controls were placed in an identical petri dish on the bench sufficiently far from the coil at field strengths below 3.5mT in the DC and AC series. After exposure, both experimental and control embryos were returned to their rearing sites. They were allowed to develop for 24 h. Then, they were examined microscopically in order to recognize the abnormalities. Significant decreases in hatching rate occurred in treatments with both DC and AC fields. Field exposures also resulted in significant increases in overall patterning abnormalities. The abnormalities were classified into 8 categories [i.e., "Nonspecific early arrests" (empty vitelline membranes), "headless" (failed to form a head), "crooked" (ventral denticle bands crooked), "short-segments" (segments are truncated), "head and tail reduced", "twisted" (twistings of segments around the anteroposterior axis), "strong dorsal denticles" (thicker than normal), and "T1-reduced" (first thoracle denticle is reduced).

Although significant increases in overall patterning abnormalities occurred, the categories of abnormalities were quite different. A comparison of the DC and AC series demonstrated that the latter contained far fewer categories of certain abnormalities. Thus, the categories "twisted", "strong dorsal denticles", and to a large extent, "head/tail reduced" occurred fairly often in the DC series (but seldom in the controls or in exposures to AC fields). In general, the pattern defects resulting from both DC and AC magnetic field exposures were quite limited in kind and characteristically different from those obtained with other agents such as exposure to heat or cold shocks or even ether vapor. Gaps in segmentation that occurred in abundance as well as in diverse forms in the ether treatment were almost entirely absent with magnetic field exposure.

Conclusion

The idea gaining acceptance was that electromagnetic fields in the entire range of frequencies are intimately involved in biological functioning and that bioelectromagnetic phenomena may provide a key to understanding the organization of living systems. The electromagnetic fields connect bio-

logical systems. They are the basis of biological organization and, simultaneously, of "biocommunication". The term, "biocommunication" has the characteristics of a "language" wherein cells, organs, and organisms actually communicate within and amongst themselves. The electroconformational coupling in membrane proteins illustrates the possible effects associated with electrical fields within the cell as well as with fields outside the cell. It is just one example of biocommunication as the key to biological organization. An organic whole is a maximally transparent system wherein changes and adjustments simultaneously propagate "upwards", "downwards" and "sideways" in the maintenance of the whole. Biophotons indicate the "optical window" of the electromagnetic interaction field. Such a window has the advantage that (despite their low intensities), technically speaking, biophotons have a rather high signal to noise ratio. However, is one really able to experimentally retrieve the richness of information from measurements of photon emission that one might expect based on new insights into organization? This will be the topic of the last three chapters.

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18

Human Photon Emission in Health and Disease in the 2000's

In recent years, there has been increasing interest in human photon emission. This interest has fostered the development of a potential non-invasive diagnostic tool (photon emission from human ROS) which then began to be utilized to study life style and its subsequent consequences in the development of chronic pathology. The general role of ROS in disease development was discussed earlier. This chapter first reviews the historic development and current status of human photon emission recording techniques. In the second part, latest developments will be discussed regarding variation of the anatomical pattern of photon emission as well as the aspect of a photon "language" in its relationship to life style, health and disease.

Historical aspects

USA

The rise in USA photon emission research in the late 1970's (Chapter 8) was specifically focused on research regarding ROS related photon emission. It resulted in many publications in biochemical journals. It is little known that human photon emission had already fascinated USA researchers. An early publication in 1979 illustrated how Dobrin and colleagues entertained the possibility that sensitive photomultipliers may document "an envelope of radiation surrounding human organisms". They mounted a photomultiplier in a light-tight darkroom taking all types of precautions to avoid artifacts. After being in that room for at least one hour, the subjects stood naked from the waist up in front of the photomultiplier tube. The protocol avoided signals resulting from static electricity and fluorescence from dyes. They utilized a tube with maximal sensitivity at 400 nm and almost zero activity at 650 nm. The lack of sensitivity to infrared ruled out heat effects.

A major difficulty encountered in those early experiments was the inherent internal noise produced by the photomultiplier tube. The researchers reported an 11% increase of the signal above background noise. However, it was too small to study the color or nature of the photon emission. They did, however study different inanimate objects with emissions similar to that of the human skin (heated to varying temperatures from 30°C to 90°C). It did not increase the phototube output. Moreover, small fluctuations in room

temperature gave only a negligible increase in dark current. Those data strongly suggested the existence of measurable human body emission.

United Kingdom

A decade later, Edwards and colleagues, as part of the Dove Alliance Project in the United Kingdom, published a study addressing human body photon emission. The protocol included a photomultiplier mounted in a sealed housing with a quartz window maintained at a constant low temperature of -23°C. The cooling of the photomultiplier tube improved the signal to noise ratio. The photomultiplier was placed in a specially constructed darkroom with no leakage of light containing no phosphorescence of the construction materials. The improved signal to noise ratio allowed the researchers to study fluctuations in the photon signal. They studied the temporal emission variations of one hand of each human subject by recording photon emission every 1.5 h over a 28 h period. The subject maintained his/her usual eating and sleeping patterns. However, the data were insufficient to conclude about diurnal variation.

In addition to focusing on a single hand of each human subject, the research project also included a study of two other subjects emitting over five different areas of the body illustrating various emission patterns. The largest number and variance of emissions were over the forehead and palms. The common assumption that photon emission was oxygen dependent was also tested. When a tourniquet was applied at the upper arm, the photon emission of the palm was approximately 15% lower than the control. An initial spectral analysis of the hand emission was attempted. Kodak gelatin "Wratten" color filters were interposed between hand and photomultiplier dividing the visible spectrum into four bands approximating the following colors: blue (410-495 nm), green (495-540 nm), yellow (540-570 nm) and red (570-650 nm). The authors were unable to conclude whether or not the detailed shape of the spectral curve was due to temporal variation.

Japan

In the studies by Inaba and colleagues, attention was focused on emission from hand and finger acupuncture points. In 1998 and 1999 they reported that the insertion of a needle (or a laser beam) into an acupuncture point induced intensity enhancement of photon emission from other acupuncture points. In 2000, the intensity of acupuncture points was compared with that of non-acupuncture points. The paper documented that emission intensity of acupuncture points was, in general, higher than the intensity of non-acupuncture points. In 2000, Yanagawa and colleagues reported the effect of thermal stimulation by moxa, another method to influence skin acupuncture points. The influence of Qigong on emissions was studied by Nakamura and colleagues in 2000. A possible correlation between Qigong,

photon emission, and skin surface temperature were explored. Emission was measured from the tip of the right middle finger of a Qigong master. In the trial, Qigong and relaxation periods were alternated five times every sixty seconds (total trial was 10 min). The subject demonstrated increased emission during the Qigong and decreased emission during relaxation. In addition, temperature from the middle fingertip of the same subject changed during Qigong and relaxation. During the Qigong, temperature decreased. However, temperature rose during relaxation but the same did not occur with the other three subjects.

Germany

In Germany beginning in 1993, Popp and co-workers pioneered human photon emission research utilizing a cooled system with a photon detector head hanging from rails that could be positioned over any part of a research subject. From July 1994 to November 1995, the Popp device was utilized to record emission from 80 healthy as well as diseased subjects over various body areas. A systematic measurement schedule was not followed. The objective of this early period of study was to document features at specific body locations which could characterize states of health or disease. Despite the use of sensitive recording devices, a clear understanding of the intensity over various areas of the human body had not been accomplished.

In 1977, Cohen and Popp published a systematic single subject study addressing emission from both hands as well as the forehead utilizing the moveable photon detector in a light-tight dark room. They examined the subject daily during a 9 month period. The data demonstrated a clear preference for left/right hand correlation. In addition, the long-term spontaneous emission of biological rhythms of this one subject became evident vis-à-vis the use of Fourier analysis. The phases of the oscillations depended on the body location. Cohen and Popp also reported that a multiple sclerosis patient emitted more biophotons than healthy subjects. Such data introduced an additional parameter in disease. Namely, that left/right emission from hands was asymmetrical.

A similar device was utilized by Bieske and colleagues systematically recording emission from defined specific areas of the hands as well as the inside of the lower arm. Thirty subjects between the ages of 20 and 60 years participated. Nine subjects were women. Recordings of both right and left dorsal aspects of the hands plus the left and right lower arms were collected in the summer 1998 and the winter of 1998/1999. Spontaneous emission varied according to the location on the arms and hands. The emissions increased near the ends of the extremities. The data documented that the emission differences between the left and right of a subject's hand or arm were variable from one subject to another.

Three of the subjects took part in the measurements during both summer and winter. The subjects emitted less at all measurement points in the winter compared to the summer. In 1999, during that same period, Sauermann, *et al* investigated and published about the influence of age regarding photon emission of the hands of two different age groups. The intensity of emission from the hands of the elderly increased.

Developments in human photon emission research during the 2000's

All of the previous studies in the late 1990's and early 2000's clearly indicated that research on human spontaneous photon emission was a slow process. The resolution of human emission in time and space included not only technical difficulties but also requires special measurement protocols plus signal analysis techniques. Notwithstanding these difficulties, research predominantly continued to study health and disease. The collaboration between the research groups of Popp and Van Wijk resulted in a number of publications about photon storage in animal cells (Chapter 16) and then, subsequently focused on systematic multi-site recordings of human emission. Additionally, the South Korean research team headed by Kwang-Sup Soh contributed to the highly productive human biophoton research. Furthermore, the Japanese group of Kobayashi improved human photon research vis-à-vis their developments in imaging technologies (Chapter 19).

Multi-site registration of human body spontaneous emission

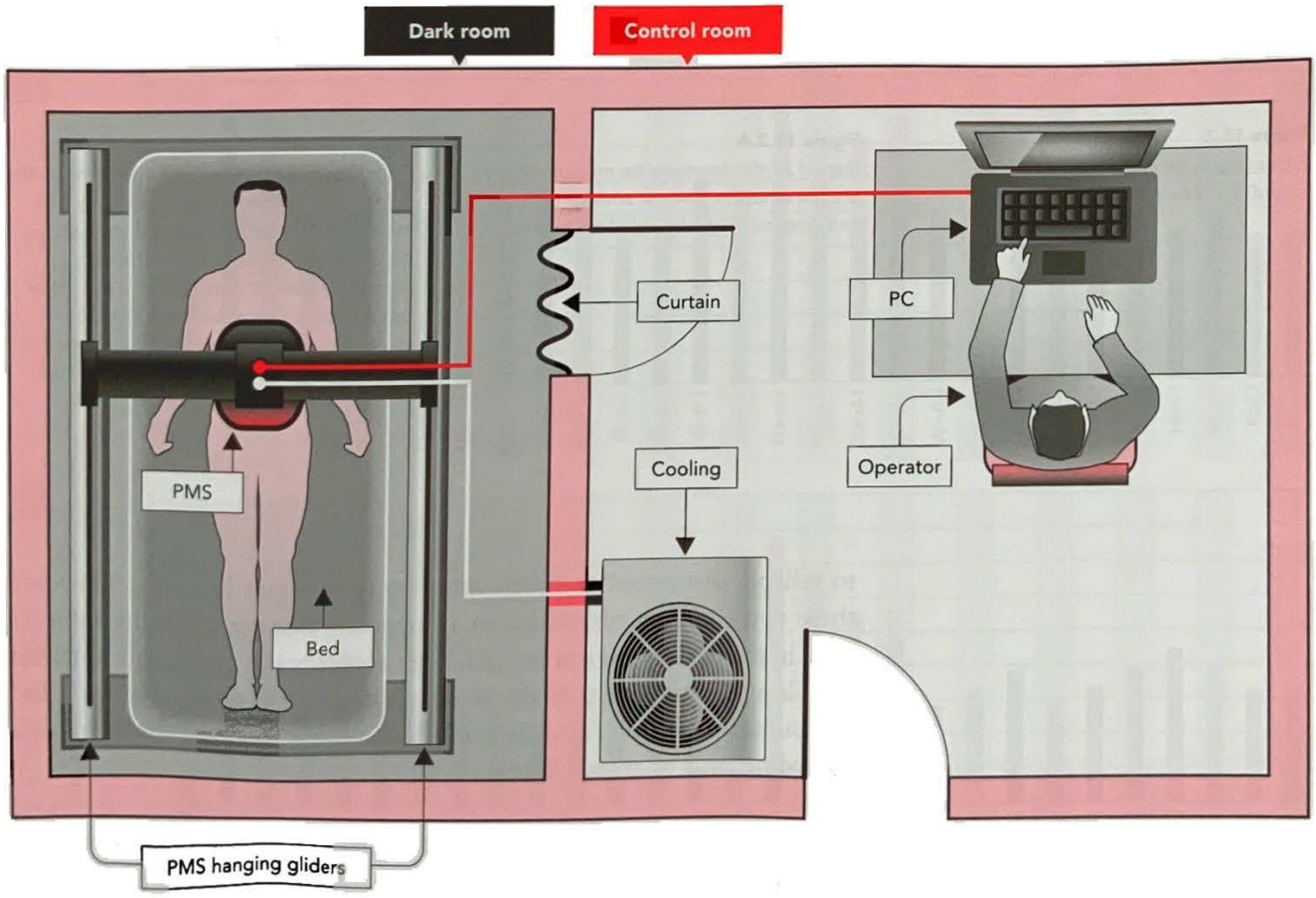
The major breakthrough in multi-site recording of spontaneous human emission was achieved by including a pre-measurement protocol, a very specialized recording device, and a measurement protocol. Popp's construction of a highly cooled photomultiplier tube facilitated manipulation in three spatial directions above the human body (Figure 18.1). A special dark room was utilized with a count rate of less than 0.1 photons s^{-1} cm^{-2}. The darkroom was not exposed to light sources. The walls and ceiling were covered with mat black paint. The inner size of the dark room was 2m x 1.5m x 2m and included a bed upon which subjects were comfortably recorded in lying or sitting position.

A special pre-measurement protocol was developed. Photon emission from skin can be due to previous exposure to light. Therefore, care must be taken to avoid such delayed emission which would interfere with the estimation of spontaneous photon emission. It was particularly important that the hands and face (frequently exposed to sunlight) be completely dark adapted (total darkness for 1 h) or under dark red light conditions prior to the measurement of photon emission. Subsequently, the subject was posi-

tioned in place for at least 10 min and then the photomultiplier tube was placed above the designated area of the skin.

The time slots for recording photon emission during multi-site registration was extremely important. To compare studies of spontaneous emission at different locations, a duration of recording had been selected that facilitated, on the one hand, registration of as many skin locations as possible within a reasonable time, and, on the other hand, a high accuracy

Figure 18.1
The special darkroom and moveable photomultiplier for multi-site recordings of spontaneous emission from the human body. PMS: Photomultiplier system. The computer controlled moveable PMS hung on runners. Subject in lying position on a bed below hanging runners.



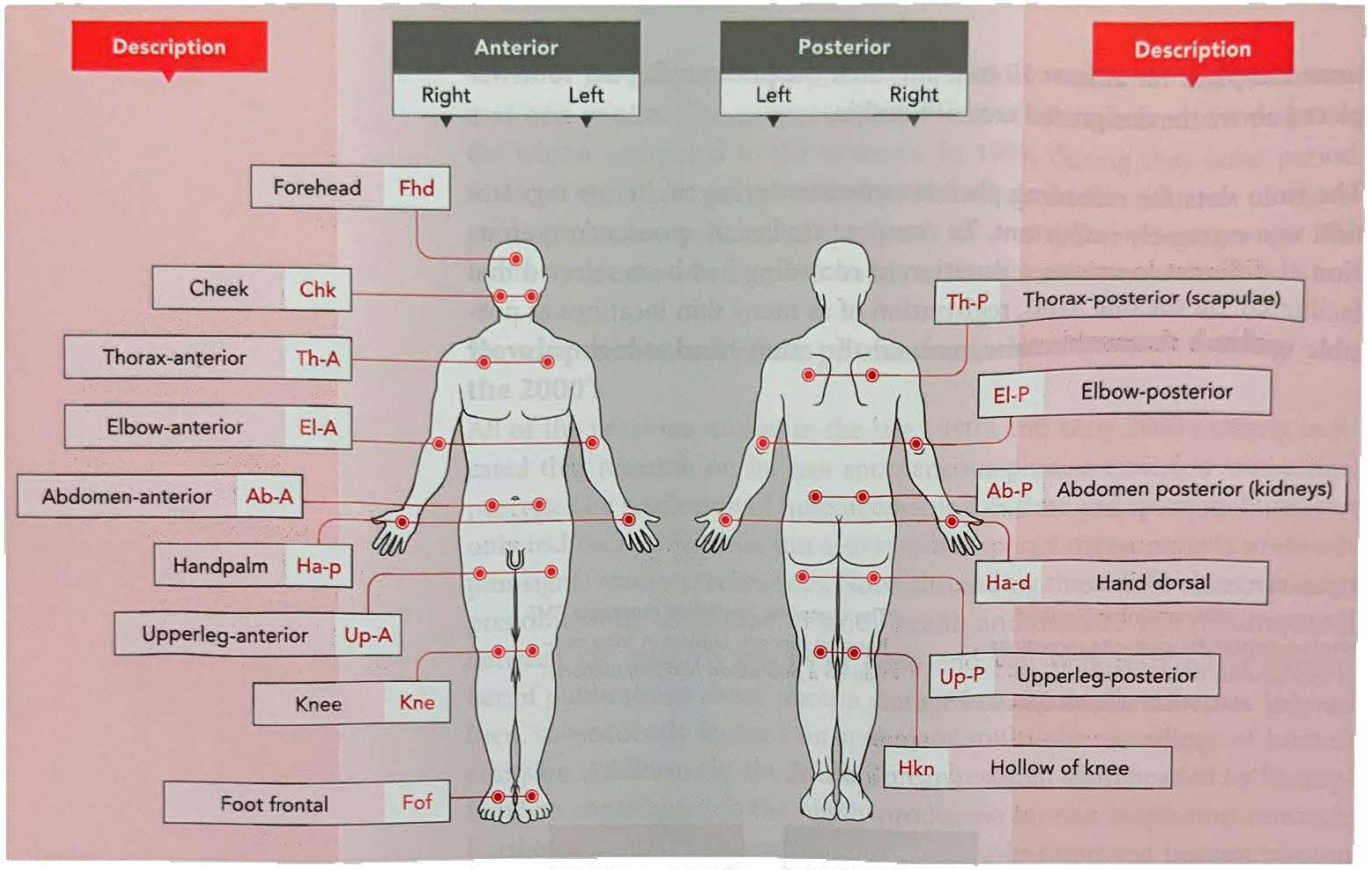


Figure 18.2
First protocol for systematic multi-site recordings of human photon emission.

Figure 18.2.A
Map of 29 skin locations for measurement of photon emission over the anterior (left figure) and posterior (right figure) of the human body.

Figure 18.2.B ►
Multi-site photon emission of 4 subjects (A-D) in the morning and the afternoon (gray bars are emission in the morning and red bars the additional emission in the afternoon). Each body site was recorded for 2 min. Total measurement time was 75 min. Dark room values were 6.0 ± 0.4 cps and are included in the presented data (indicated by a red line).

to reliably distinguish between intensity of different locations. However, these two variables responded in an opposite manner when the subject's time in the dark room was limited. High accuracy was commonly obtained with longer recording periods. When photon counts range between 6 and 40 counts per second (cps), for statistical analysis, it was advisable to use a recording time of at least 2 min.

The following section will summarize aspects of spatial intensity resolution, its relation to health and disease, the issue of color, and methods for

A
B
C
D
Photon counts (/s)
0
5
10
15
20
25
30
35
40
45
2
4
6
8
12
14
16
18
Fhd
Chk-l
Chk-r
Th-A-l
Th-A-r
Th-P-l
Th-P-r
Ab-A-l
Ab-A-r
Ab-P-l
Ab-P-r
El-A-l
El-A-r
El-P-l
El-P-r
Ha-p-l
Ha-p-r
Ha-d-l
Ha-d-r
Up-A-l
Up-A-r
Up-P-l
Up-P-r
Kne-l
Kne-r
Hkn-l
Hkn-r
Fof-l
Fof-r

analysis of the photon signal. The studies began in 2003 supported by The Samueli Institute for Information Biology.

Anatomic intensity

The question was asked whether photon emission over different skin locations reflects a symmetrical pattern and whether this pattern changes during the day. Figure 18.2 illustrates the locations on the skin selected for recording of photon emission. The locations were selected in such a way that the distribution of emission could be studied as right/left symmetry, dorsal/ventral symmetry, and the ratio between the torso and extremities. Such estimation required a lengthy measurement time frequently difficult for many subjects. Therefore, the initial attempt study began only with four subjects (colleague researchers). They were recorded at different times of the day.

Recording of 29 selected body sites demonstrated that emission over the body did demonstrate a systematic pattern. The thorax/abdomen region emitted the lowest and most constant intensity. In contrast, the upper extremities and the head region revealed the highest. Generally, a high degree of left/right symmetry was observed. Dorsal/ventral symmetry was less obvious.

Spectral analysis of human light

In 1989, Edwards, *et al* utilized color filters positioned between hand and photomultiplier dividing the visible spectrum into four bands: blue (410-495 nm), green (495-540 nm), yellow (540-570 nm) and red (570-650 nm). However, they were not able to describe the detailed shape of the spectral curve. Spectral analysis (with its many pitfalls) was described in more detail by Van Wijk and Van Wijk in 2005 (Figure 18.3). They analyzed the wavelength spectrum of the emission for ultraviolet and visible light (200-650 nm) of a human subject at body sites with high, intermediate and low intensity. Their procedure was based on the use of cutoff filters instead of bandpass filters. The cutoff wavelengths were at 320 nm, 360 nm, 420 nm, 470 nm, 530 nm, 570 nm and 630 nm. Photon emission was initially recorded in the absence of a filter and subsequently with the different filters in the sequence from 320 nm to 630 nm. The four skin areas selected for such analysis were: palm of left and right hand, forehead, and superior frontal aspect of the right leg. The different areas were characterized by different emission intensities: highest from the hands, lowest from the frontal section of the leg, and intermediate values from the forehead.

In general, emission was lower when the cutoff was close to the red part of the spectrum. The difference in body emission between two filters with successive wavelength cutoffs represented the emission at a particular wavelength range. The final estimation of the spectral distribution of the

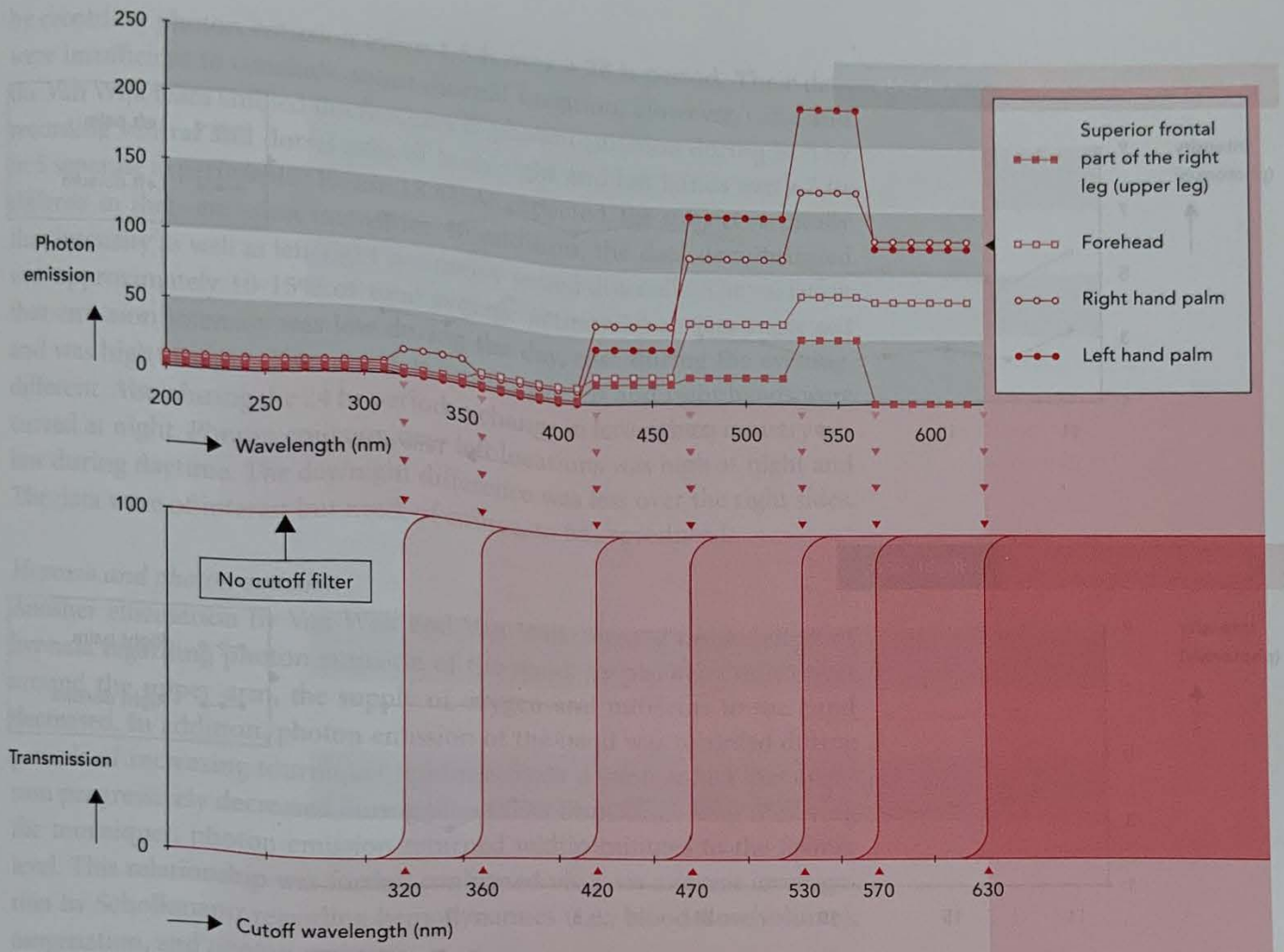


Figure 18.3:
Upper panel: Spectral distribution of photon emission at different body sites: superior frontal part of right upper leg, forehead, left hand and right hand. Lower panel: Emission was estimated utilizing different cutoff filters. The estimation of the spectrum utilizing such filters was as follows. The difference [(skin + filter) – filter] is the skin emission recorded with each filter. The difference in body emission between two filters with successive wavelength cutoffs represents the emission of a particular wavelength range. The differences between filters with successive wavelengths cutoffs were then combined with the technical specifications of the quantum efficiency of the photomultiplier tube. This resulted in the spectral distribution of the four body locations. At low emission sites, emission was largely in the 470-570 nm range. High emission sites (both hands) included a broader calculated spectrum. The broadening was most likely due to a better discrimination between emission values with subsequent filters. There was some indication that specific spectral differences were present between the two hands. The right hand demonstrated higher intensities in the short (< 360 nm) wavelength range. The left hand demonstrated higher intensity when recording was made with a 470 nm cutoff filter. The percentage was 83% and 57% for the left and the right hand, respectively. This meant that the left hand emitted relatively more red compared to the right.

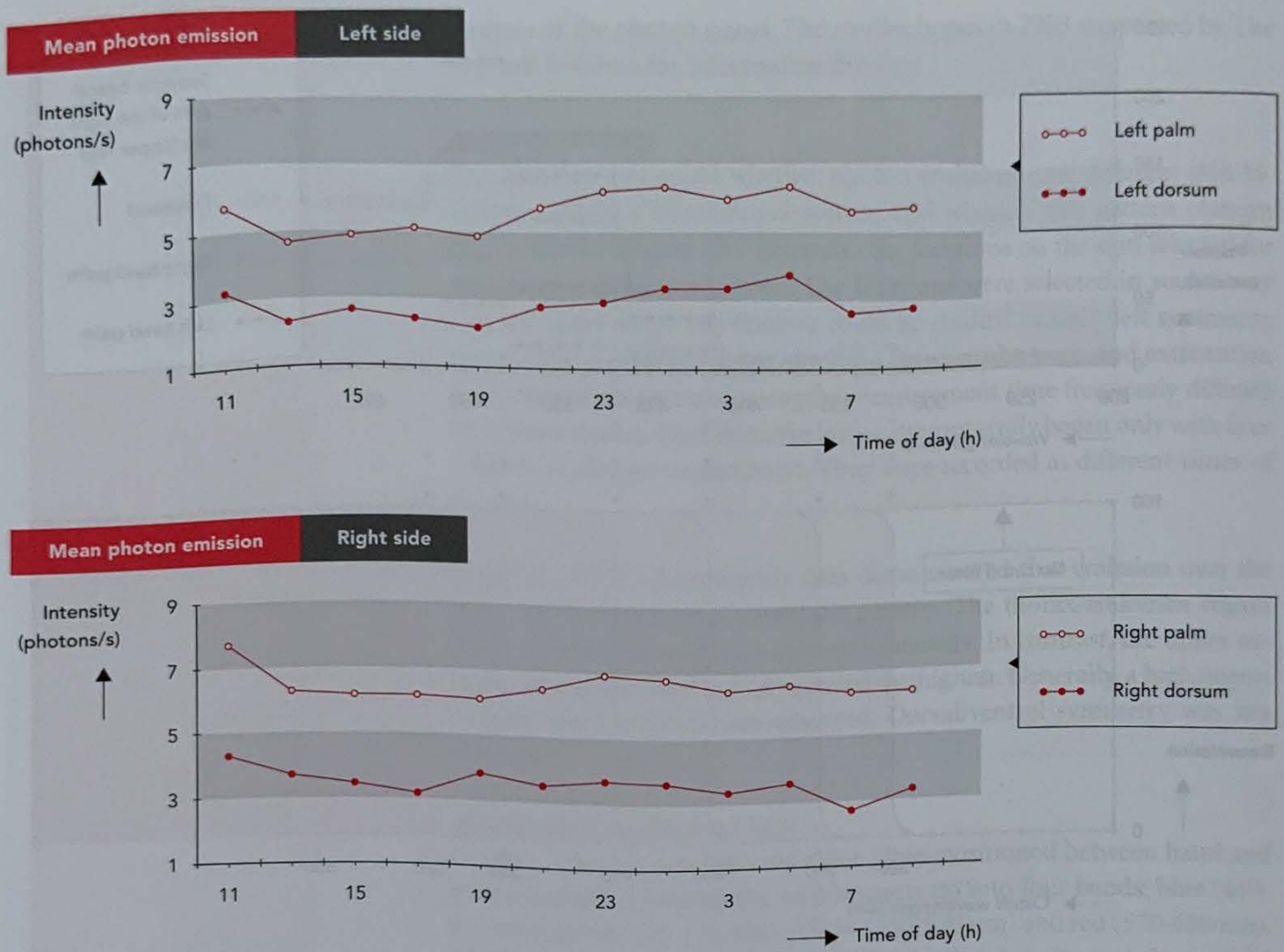


Figure 18.4
Fluctuation of photon emission regarding daily average. Upper panel: Photon emission of left palm as well as dorsal side. Lower panel: Photon emission of right palm as well as dorsal side for each time of a day. The curves present the average of 5 experiments.

four locations is shown in Figure 18.3. Emission was not detected in the 200-420 nm range at high and low emission sites. Emission was largely in the 470-570 nm range.

Time of day – Small (diurnal) fluctuations

Fluctuations that may occur during the day became the next piece of research. That question had been raised earlier in the 1989 U.K. study by Edwards *et al.* They studied the temporal emission variation of the hand

by recording photon emission every 1.5 h over a 28 h period. Their data were insufficient to conclude about diurnal variation. However, Cifra and the Van Wijk team studied fluctuations in photon emission during 24 h by recording ventral and dorsal sites of both right and left hands every 2 hr in 5 separate experiments (Figure 18.4). As expected, the subjects typically differed in their emission intensities. In addition, the data demonstrated that intensity as well as left/right symmetry varied diurnally. The variation was approximately 10-15% of total average activity. That data suggested that emission intensity was low during the day, rose during the evening, and was high at night. The time patterns for the left and right hands were different. Also, during the 24 hr period, a change in left/right symmetry occurred at night. Photon emission over left locations was high at night and low during daytime. The day/night difference was less over the right sides. The data were of interest but need, of course, to be reproduced.

Hypoxia and photon emission

Another elucidation by Van Wijk and Van Wijk focused on the effect of hypoxia regarding photon emission of the hand. By placing a tourniquet around the upper arm, the supply of oxygen and nutrients to the hand decreased. In addition, photon emission of the hand was recorded during periods of increasing tourniquet tightness. Data demonstrated that emission progressively decreased during blood flow limitation. After removing the tourniquet, photon emission returned within minutes to the former level. This relationship was further confirmed vis-à-vis a recent investigation by Scholkmann regarding hemodynamics (i.e., blood flow/volume), oxygenation, and photon emission.

Temperature and photon emission

Initially, the question was whether anatomic locations with different photon emission intensities reflected different skin temperature. Multi-site recordings of both photon emission intensities and skin temperature demonstrated that temperature at different anatomic locations ranged between 26° and 34°C. However, a relationship with photon emission was not observed. In a second study, both photon emission and temperature were recorded at the same body location in different subjects in order to evaluate whether differences between subjects correlated with temperature. Between subjects, temperature at any of the recorded anatomic sites was not correlated with the photon emission. It was concluded that differences between both anatomic locations and subjects could not be explained by differences in temperatures. At a given location, however, photon emission intensity may be temperature sensitive. If human photon emission was a product of biochemical processes, then, environmental temperature would be expected to both influence photon emission of anatomic locations (less than 37°C) and depend partially on environmental temperatures. Indeed, it was

observed that, as temperature of the hand declined, the intensity of photon emission decreased (whereas photon emission increased during a rising temperature). A repeat study led to a similar conclusion wherein subjects participated in cold exposure experiments after being dark adapted. They lay in supine position to stabilize their body temperatures at a room temperature between 17°-18°C. Subsequently, they disrobed for 30 min wearing only T-shirts, shorts and sport shoes. Both skin temperature and photon emission were recorded from several anatomic locations. Temperature decreased during the cooling process (roughly correlated with the photon emission of a specific location).

Pattern of photon emission and its application in meditation research

To further study the anatomical pattern, the researchers selected only 12 anatomical locations in order to evaluate a larger number of subjects (Figure 18.5). The reason for the reduced number was to limit to 1 h the duration inside the dark room. The first major challenge to perform a systematic, multi-site recording included a group of 60 healthy males. The research question in that systematic study was extended to the type of relaxation that subjects practiced in daily life.

It was, then, generally accepted that meditation, if practiced for a long time, induces a greater state of self awareness and inner calm in its practitioners. Techniques of meditation include attention to one's breath, repeating a mantra, and detaching from various thought processes in order to focus one's attention. The hypothesis asked whether or not "inner calm" might induce a reduction of stress which may have physiologic prophylactic and therapeutic health benefits. It suggested a possible link between meditation and a physiologic therapeutic role that initiates a decrease of free radical mediated oxidations. It proposed that oxidized lipids may physiologically reflect free radical damage contributing to pathophysiology. The hypothesis stimulated considerable curiosity in the scientific community. The measurement of serum lipid peroxide fluctuations did, in fact, indicate that chronic psychosocial stress probably does increase oxidative stress as opposed to the presence of lower lipid peroxide levels in the plasma of practitioners of transcendental meditation (TM), Zen meditation, and yoga practitioners.

The study in the early 2000's by Van Wijk and Van Wijk included 20 experienced male TM practitioners, 20 male experienced practitioners of other types of meditation, and 20 control subjects without experience of using any form of meditation. Each of the subjects in the TM group practiced meditation for at least 10 years. Meditation is a mental technique practiced for 20 min twice a day sitting relaxed with eyes closed. The technique was originally taught by Maharishi Mahesh Yogi. The TM practitioners in

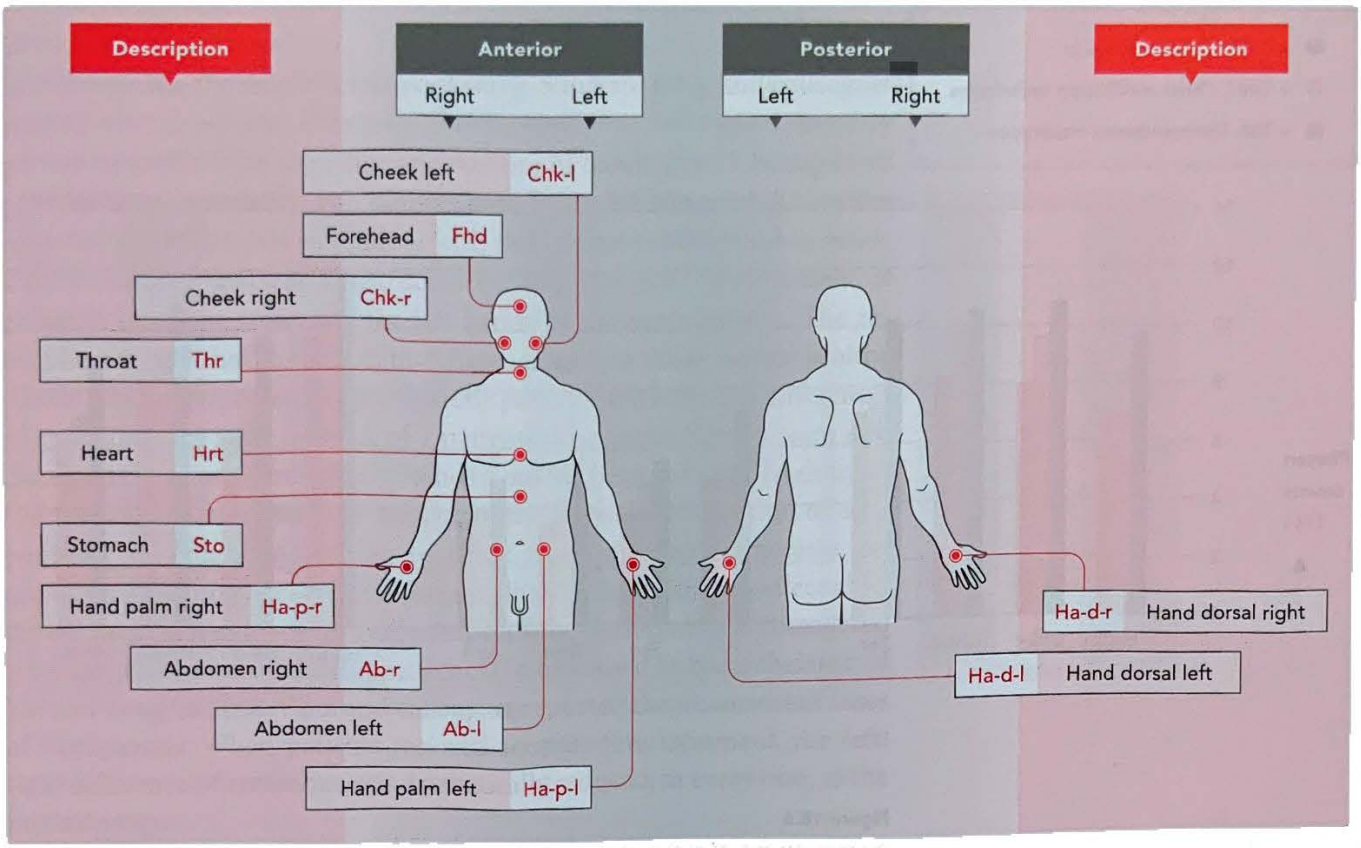


Figure 18.5
Second protocol for systematic multi-site recordings of human photon emission Map of 12 skin locations for measurement of photon emission over the anterior (left figure) and posterior (right figure) of the human body.

the research learned the technique from an authorized teacher under the auspices of the Maharishi's Global Administration Through Natural Law, Ltd. Subjects in the group using "other meditation techniques" were long-term practitioners in one of the following techniques: Zen, Yoga and other personal variations of mindfulness based relaxation techniques. The control and meditation subjects were, by self-report, healthy, non-smokers and free of medication. The study systematically examined the photon emission of 12 anatomic locations on the upper frontal torso, head, neck, and hands (Figure 18.6).

The comparison between the three groups of subjects demonstrated that the intensity of ultra-weak photon emission was 15-33% less for the TM

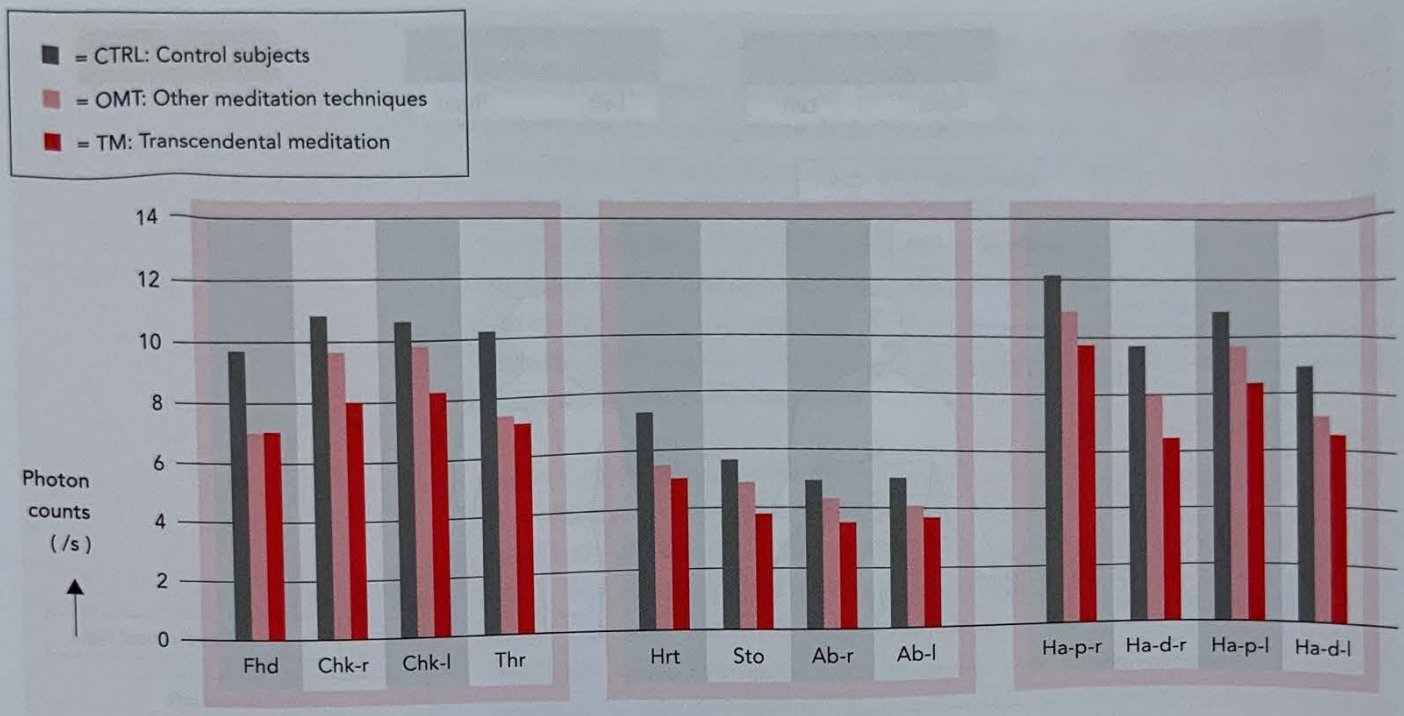


Figure 18.6
Average intensity of each of the 12 specific anatomic locations for the groups of TM, OMT practitioners and control subjects.

practitioners and 4-15% less for the other meditating practitioners (compared to the control group). All subjects demonstrated a high degree of symmetry. The data supported the conclusion that persistent meditation does influence metabolic activities responsible for photon emission. Emissions of the 12 locations were utilized to calculate average intensities of the specific anatomic locations. As in the previous studies, photon emission of the abdomen was lowest, values increased along the central axis rostally to the throat, highest values were observed over the cheeks, and emission again decreased at the forehead. For all subjects, data suggested that, generally, each individual anatomic position participated in the total emission with a predictable, constant percentage (independent of the total emission). The results also indicated that the pattern of emission did not change for each of the above two types of meditation (including the control group) and that the decreased emission during meditation was a systemic phenomenon.

The health/disease issue

In South Korea, the research team of Kwang-Sup Soh, Jung, and colleagues studied several aspects of human photon emission. Left/right symmetry photon emission from the palm and dorsum of hands from 7 hemiparesis patients were compared with similar data from the hands of 20 healthy subjects. Measurements were done with two photomultiplier tubes inside a dark chamber specially constructed for simultaneous measurement of emission from the right and the left hands of the same subject. The researchers found that the left/right difference among those twenty healthy people was not large. For the hemiparetic patients, the left/right differences of emission were large in 4 out of 7 patients. The South Korean team also discussed the photon emission of hands from the perspective of Traditional Chinese and Korean Medicine relative to the physiologic function of acupuncture meridians. According to those medical systems, diseases are caused by the imbalance of vital forces called Yin and Yang as reflected by the left and right sides of the body, respectively. Hemiparesis is considered (in these traditional medical approaches) to be caused by the imbalance of Yin and Yang. In 2003, Choi and colleagues reported the treatment of cases of hemiparesis. When patients received acupuncture treatment, the left/right difference of emissions was dramatically reduced, in every case, to the normal range.

The data, so far, initiated the possibility that changes in ultra-weak photon emission occur in the transition from a healthy to a diseased state. It highlighted very interesting issues. What is health? What type of design could be utilized to study three defined stages from health to disease: a.) healthy state, b.) early state of disregulation in which an impaired cell function is detectable, and c.) overt diseased state? How could these different populations of subjects be documented by complex laboratory equipment? Well defined markers for most chronic diseases are available only for the stage when the disease is overt (and the subject has contacted the medical system regarding specific symptoms). Relatively few markers are available for early stages. Another difficulty is that many specific ROS related chronic diseases are final manifestations of an early stage of a common disregulation (increased ROS activity).

With these limitations in mind, Van Wijk and Van Wijk began (as an initial approach) a descriptive, explorative photon recording study. The aim was to describe the photon emission of 150 subjects divided into three groups. The two extreme groups were the group of healthy young subjects without severe disease history as well as the group of chronically diseased subjects asking for complimentary alternative medical treatment after a long-term history of disease. The intermediate group were pre-diabetic subjects. The 50 healthy were assessed by questionnaires with ages between 20 and 30.

The 50 chronic diseased subjects (between 30 and 65 years of age) were under the supervision of a general practitioner. The pre-diabetic group was healthy (assessed by questionnaires and physical examination), ages between 30 and 65 years, a body mass index between 26 and 35 kg/m, and fasting glucose blood values between 6.0 and 7.0.

A new mobile device was constructed to study the above 3 groups because the original scientific equipment for human research was too large and bound to specific research sites. The new equipment was smaller and more appropriate for laboratories and clinical practices (Figure 18.7). It was built in such a manner that both hands could be recorded simultaneously, if required. The selection for hand recordings was based on data discussed above. Such data have demonstrated that ventral and dorsal surfaces commonly demonstrate high emissions compared to other anatomical locations. The data collection protocol facilitated analysis regarding the strength of a photon signal and its left/right symmetry (utilized as before). However, the data were collected in a manner such that other novel types of analysis could be explored equally well with the same data.

A novel development: the analysis of the human photon signal

The groundbreaking work by the Indian theoretical physicist, R. Bajpai began in 2000. In close cooperation with the Van Wijk's team, Bajpai applied his approach to human data regarding life style research addressing both healthy and diseased human subjects. It started from the perspective that the photon signal appeared to have linkages with some physiological, psychological, and pathophysiological processes. Such possible linkages implied the potentiality of actually identifying and then documenting quantitative specified data of the photon signal. His investigations attempted to identify the properties of a photon signal by analyzing its time series determined from a large number of measurements made with small measuring intervals. Such time series can be very long because of the development of rapid recording techniques. A registered time series of a photon signal, as such, cannot be utilized to obtain the properties because significant background noise. Bajpai developed various procedures to correct for this challenge.

Bajpai confined his investigations of statistical properties to the mean, variance, and probabilities when detecting different numbers of photons in a bin (i.e., in a measuring interval). He effectively discriminated between classical and quantum photon signals. A crucial step of the analysis was to calculate every statistical property (in many time series) of a signal utilizing different bin sizes in each of the series. The analysis of such quantities in sixty human photon signals revealed the existence of a few holistic attributes with characteristic universal features. The following categories utilized to describe photon "language" have only recently become availble:

Figure 18.7
The device for simultaneous recording of photon emission from two hands.

Signal strength —It is measured vis-à-vis the mean number of photons detected in a specific region of a bin. The signal strength of different regions is usually in a small range and appears to change without predictable patterns. The ongoing changes in signal strength probably reflect continuing variations of a subject's physiological state (whereas its range reflects restrictions in permissible physiological states probably imposed by homeostasis).

Fano Factor Curve —This is the curve that is situated around the ratio of variance and mean of a photon signal that fluctuates with bin size. Its shape is subject to specific situations. It begins with a value of approximately 1 in every region of signals which then changes (slowly and almost linearly up to a bin size of 5s) as a result of specific changing situations. The initial value indicates the quantum nature of the photon signal. The slope characterizes some aspects of the physiological state (which still needs to be unraveled).

Squeezed state index —establishes the quantum nature of photon signals and also separates photon signals into normal and abnormal types. It is a major categorization. Further sub-categorization can be expected when new indices for such can be constructed. The determination of the quantum state of photon signals kindles the hope that holistic properties of its

emitter will be expressible in terms of its squeezed state parameters. It has profound implications for understanding and managing holistic features that include health care.

Left/right correlation curve—The continuous cooperative behavior of molecular processes occurring at spatially separated locations in a human subject. It is a new holistic feature that probably reflects some aspect of spatial coordination. It is suspected that this holistic feature opens up a new field to explore and speculate.

Ongoing changes in the values of holistic attributes—Some holistic attributes change continually with time while others do not. The continual changes probably reflect the dynamism of human subjects. It is anticipated that in the future some combinations of measured attributes will be able to capture human psychological and physiological states.

Conclusion

The human subject is the most researched living species. Such may be due to the promising temporal measurement possibilities of photon emission in biomedicine. However, one important aspect is still missing: the spatial aspect of photon emission. So far, anatomical emission has been recorded only by systemic, multi-site recording utilizing a photomultiplier system. Eventually, knowledge will further increase once the spatial anatomical pattern of body emission can be imaged so that its dynamics can be studied. This will be the subject of the following chapter.

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19

Imaging Human Photon Emission

This chapter begins with a historic overview regarding the progression in sensitivity of the photon imaging technology. A few recent examples regarding human body emission demonstrate specific patterns. The data also include slow dynamic fluctuations. Those spatial photon emission patterns illustrate similarity to cutaneous patterns of energy described in traditional Chinese medicine (TCM).

Historic development

The interest in imaging ultra-weak photon emission increased in the mid-1980's. Spatial distribution of photon emission from living organisms offers information about emission within a specimen. The knowledge of such imaging began spreading with the 1984 publication by Tsuchiya, Inuzuka, Kurono and Hosoda of Hamamatsu Photonics, Inc regarding a Photon counting Image Acquisition System (PIAS). The system employs an imaging photomultiplier tube consisting of a photocathode and a position sensitive detector. A special research project utilizing two-dimensional photomultiplier imaging techniques in biology was initiated as part of the "Inaba Biophoton Project" in Japan (1986). The project was funded by the Exploratory Research for Advanced Technology (ERATO), a subsidiary of the Research Development Corporation of Japan (subsequently called, Japan Science and Technology Corporation). The imaging system was developed and first utilized by Inaba and co-workers addressing the two-dimensional detection of ultra-weak photon emission of soybean seeds and roots as described in various publications. The images of germinating soybeans were obtained after keeping specimens in complete darkness (in order to eliminate the possible effects of delayed luminescence). Remarkable emission was observed in the segment of the hypocotyl, the junctional region between radicle and plumule. It was concluded that emission is strongest in areas where active cell division and growth is taking place.

The two-dimensional photon imaging and counting system was subsequently utilized to clarify the emission of the seedling subsequent to physical injury. Suzuki and colleagues created lesions on the bean seedlings in the form of a cross (2-3 mm deep and 5-10 mm in length). The two-dimensional image of light from injured soybean seedlings revealed a 7-20 times higher intensity centered around the injury. The emission intensity was also higher at the root system (hypocotyl and the radicle) away from

the site of injury. The simultaneous increase of light emission at the root system far away from the injury site was very interesting and offered clues to the transfer of information within the living system. The same research team also utilized intact and injured soybean seedlings in order to demonstrate where emission intensity increases rapidly on the cotyledon as soon as H_2O_2 is applied. The emission intensity of the injured seedling was far stronger in the central part of the cotyledon than in the peripheral regions (in contrast with the intact controls). The authors noted that responsive emission also took place in the root system (distant from the injury). The data clearly demonstrated the additional value of such an additional type of imaging (compared to just basic photomultiplier data).

Beginning in 1991, Inaba and colleagues reported that a weak light emission from injured animals could also be imaged. They placed a lesion on the back of the animals in the form of a circle (10 mm in diameter). The intensity of the lesion increased from the injury. The image obtained immediately after creating the lesion did not show any clear pattern or localization. It became more clear 48 hours after the injury (resembling the shape of the wound), whereas the emission intensity continuously rose. The emission intensity was at its maximum between the third and the fifth day subsequent to the injury. Beginning with the sixth day from the injury, the emission intensity began to decrease. On the eighth day from the injury (when the wound had completely healed), the image no longer revealed any clear pattern.

In Inaba's laboratory, a special research line was developed to make images of human emission. The sensitivity of the system made it possible to image light emission from a human body surface when that surface was positioned close to the camera system (as is the case of hands and fingers). A two-dimensional image exhibited a characteristic pattern of intensity with the highest levels in the region of the index and middle fingers and the lowest intensity in the middle of the palm regions. Inaba's team studied the light emission from the fingers in relationship to the metabolic disease of hypothyroidism (a lower state of metabolic activity). In 1994, Inaba's team demonstrated that they could use the measurements from the middle and index fingers of the left hand to differentiate hypothyroidism from control subjects. They reported that the intensity of patients with hypothyroidism was always lower than the controls. The lower emission intensity was also observed in the case of patients whose thyroid glands had been removed. The researchers also compared the emission from the back of the left and right index fingers. It was observed that the emission intensity was greater from the right index finger and that the distribution of emission intensity was also different for the two fingers. Although the reason for such a difference was not clear, they speculated that it could be attributed to differences

in activity of the acupuncture points. Photon emission could also be measured at other regions of the human body surface. The researchers, however, limited their choice to human hands with a view in the future to develop a simple and easy non-invasive technique for diagnostic purposes.

In the mid 1990's, the video intensified measuring system of Hamamatsu Photonics was modified in Inaba's laboratories. The photons emitted from the specimen were imaged onto the detector assembly by a lens. The active surface of the photocathode was 15 mm in diameter and the surface under investigation was approximately 1 cm^2. Improved equipment was applied in a 1995 study targeting ultra-weak photon emission from mice with transplanted bladder cancer. A solution of bladder cancer cells was implanted into the left feet of the mice. The photon emission documentation was performed 2 weeks after implantation. The data from the left foot tumor and the control (right foot) were compared. Ultra-weak photon emission was evident only in the implanted tumor region containing actively proliferating cancer cells. The improvement of photon imaging technology continued and in 1996 Kobayashi and colleagues published about even newer technology. The novelty was to calculate the intensity kinetics at several regions based on continuous and simultaneous measurement of time series and position data of photoelectron pulses from two-dimensional photon counting tube. This approach (to characterize the dynamics of emission) was then utilized to explore the relationship between a physiological state and emission phenomena. The new technology was first used with soybean seedlings as a model. The photon emission image from a soybean seedling and its time course was determined at five different areas. The improved analysis clearly demonstrated the spatial dynamics. The kinetics of the emission intensity were characteristically different for each area. Utilizing this approach, the variation of emission at the excision of the root tip was also displayed. Emission intensity of the cut region increases and remained at a high level for 3h. Significant changes in photon emission intensity were observed at a distal position away from the injury site (a manifestation of response to injury that was reported earlier). In this same study, a typical result was also documented when a high concentration of H_2O_2 was added to the root tip. A remarkable enhancement of emission was observed at the hypocotyl region which is distant from the root tip.

In a 1997 paper, Kobayashi and colleagues reported the first images of a germinating seedling that was obtained utilizing a highly sensitive charge-coupled device (CCD) camera. They compared the performance of the CCD camera with conventional low level light detectors. They deduced that the signal to noise ratio of the CCD camera was superior to the conventional two-dimensional photon counting tube. Meanwhile, Kobayashi and colleagues continued the biological applications of the two-dimensional

photon counting tube technology. In 1999, they demonstrated (for the first time) images of ultra-weak photon emission from a rat's brain. The equipment utilized in these experiments consisted of a two-dimensional photon counting tube, a highly efficient lens system, and an electronic device utilized to record time series with spatial information. Ultra-weak photon emission was demonstrated from a rat's cortex *in vivo* without adding any chemical agent or employing external excitation. The researchers compared the image emission with another obtained after cardiac arrest. The intensity after cardiac arrest was depressed by approximately 60%. The regional properties of the time courses of emission intensity indicated the potential usefulness of mapping oxidative stress. The technology was further utilized to extract information from central nervous system activity. They reported the correlation of emission intensity with electroencephalographic activity that was simultaneously measured on the cortical surface. In those studies, the researchers estimated the θ-wave component of the EEG power spectrum. Temporal changes of the photon emission intensity were then compared with that of the θ-wave activity and found it to be comparable. They then analyzed the correlation between photon emission and θ-wave activity. It was evident that the imaging of ultra-weak photon emission from a brain constitutes a novel method with the potential to extract pathophysiological information associated with neural metabolism and oxidative dysfunction of the neural cortex.

Towards human body imaging

A recent step in human photon emission was initiated with cooperation between Kobayashi and the Van Wijk's team in their search to image the unique human anatomical emission pattern that was described in Chapter 18. The CCD camera system was utilized in the dark room of Dr. Kobayashi's laboratory. Subjects were measured in sitting position after dark adaptation. The study began with an emission image from the upper frontal torso, head, neck, and upper extremities of a single subject (Figure 19.1). Recording was continuous for 30 min with the camera at a distance of 100 cm. As illustrated in the ventral image of the superior part of the body, intensity around the face and neck was highest and gradually decreased both over the torso and the abdomen. A gradual decrease in intensity from the superior central torso to its lateral dimensions was recorded. Dorsally, the highest intensity was emitted from the neck. The image of the arm of the same subject illustrated how the low intensity of the body extends over a large part of the arm and then increased over the hand. These images corresponded with the intensity pattern data of the same subject obtained with the moveable photomultiplier for those parts of the body. Images of hands (both palm and dorsal sides) revealed that (in particular) the dorsal side has a marked increase in intensity at the end of the fingers.

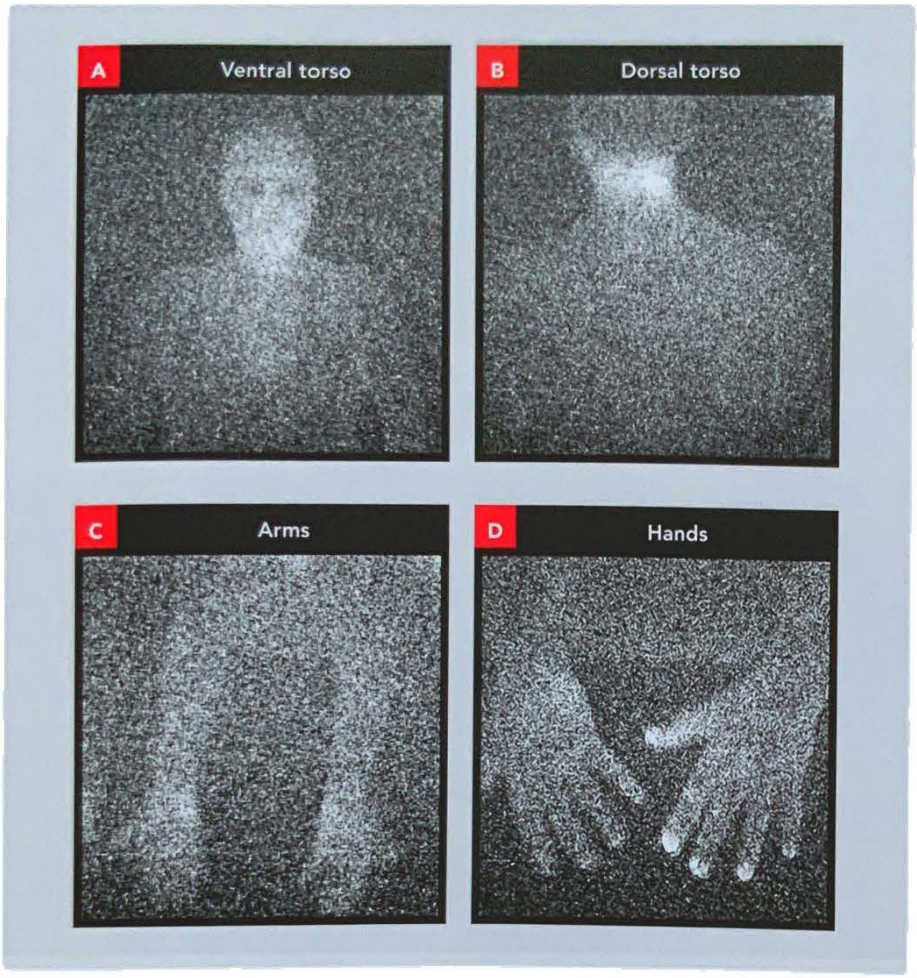

Figure 19.1
Ultra-weak photon emission images of a human subject. The upper panels represent images of the ventral torso, the dorsal torso, arms, and hands of the subject. The image of ultra-weak photon emission of the hands represents the dorsal of the left hand and the palm of the right hand. The dorsal hand side demonstrates more structure in the emission pattern than the palm side.

The capacity of such technology subsequently stimulated research addressing the stability of the emission pattern.

Another series of images made in the same laboratory focused on the hands and feet of a single subject. The images were captured throughout two consecutive days. Each experimental day, the subject was imaged twice: early morning and late afternoon. During the series, no special measurement protocol was utilized because, in this pilot study, data on the variation in hand and foot emissions were not available. The series of foot images is illustrated in Figure 19.2. Each image includes the left and right foot. The variability of emission intensity was observed between right and left foot on both days. Another difference of intensity was seen between the toe tips of the left and right feet on consecutive days.

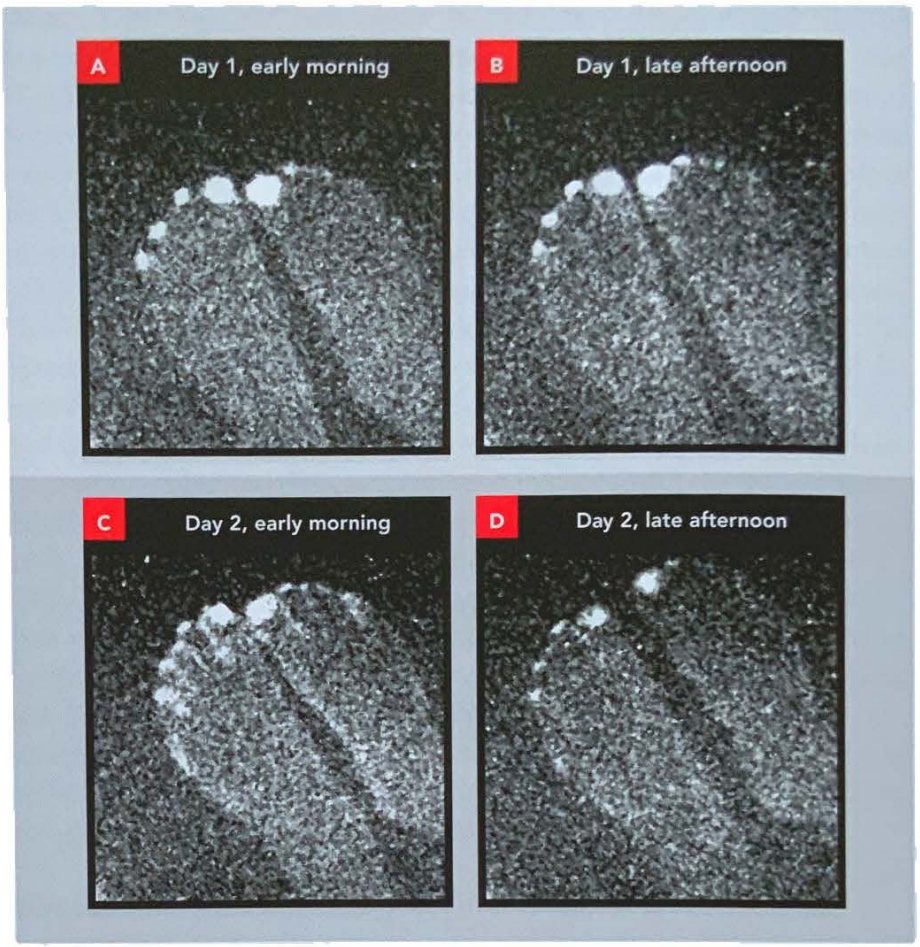

Figure 19.2
A series of ultra-weak photon emission images of feet of a single subject. Images were captured throughout two consecutive days.

A discussion of the intensity fluctuations of hands was based on two series of images. The first series included images of the palms of both hands. Such images were difficult to obtain due to the positioning of the subject. Exposing the palms in sitting position for 20 min in front of the camera was extremely difficult and resulted in a high degree of uncontrollable movement with subsequent loss of sharpness. Therefore, the Figure 19.3 images were made with the subject in a backward position with hands above the head and thumbs and index fingers pointing towards each other. In that position, the palms were relatively relaxed when exposed to the CCD camera. However, a small loss of sharpness cannot be totally avoided. Each image included both left and right palms. As documented with the feet, the variability of palm emission intensity was also observed within a single subject. An interesting aspect was discovered when the images were com-

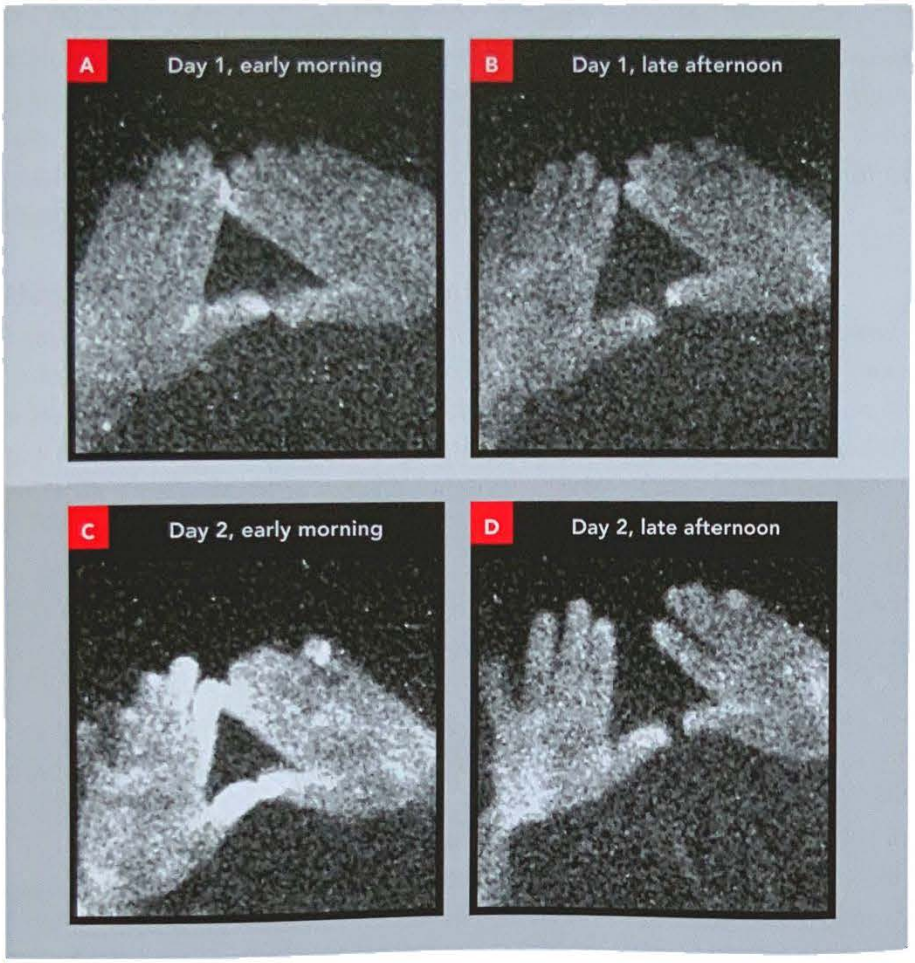

Figure 19.3
A series of images of the ultra-weak photon emission of palms of a single subject. Images were captured throughout two consecutive days.

pared in the order of the overall intensity (i.e., B, A, D, C). In particular, the thumb and index fingers suggested that the increase in intensity represents a pattern of preferential emission.

Images of the dorsal sides of the left and right hand are presented in Figure 19.4. Most intensity is observed at the tips of the fingers. However, the high emission is not limited to the nails. In some images, the intensity is seen to extend over larger areas (e.g., Figure 19.4.A). For the images of the palms (Figure 19.3) and dorsal (Figure 19.4), it was evident that left/right differences were relatively small.

In recent studies with the improved CCD camera and lens systems, Kobayashi and colleagues decided to systematically investigate the head

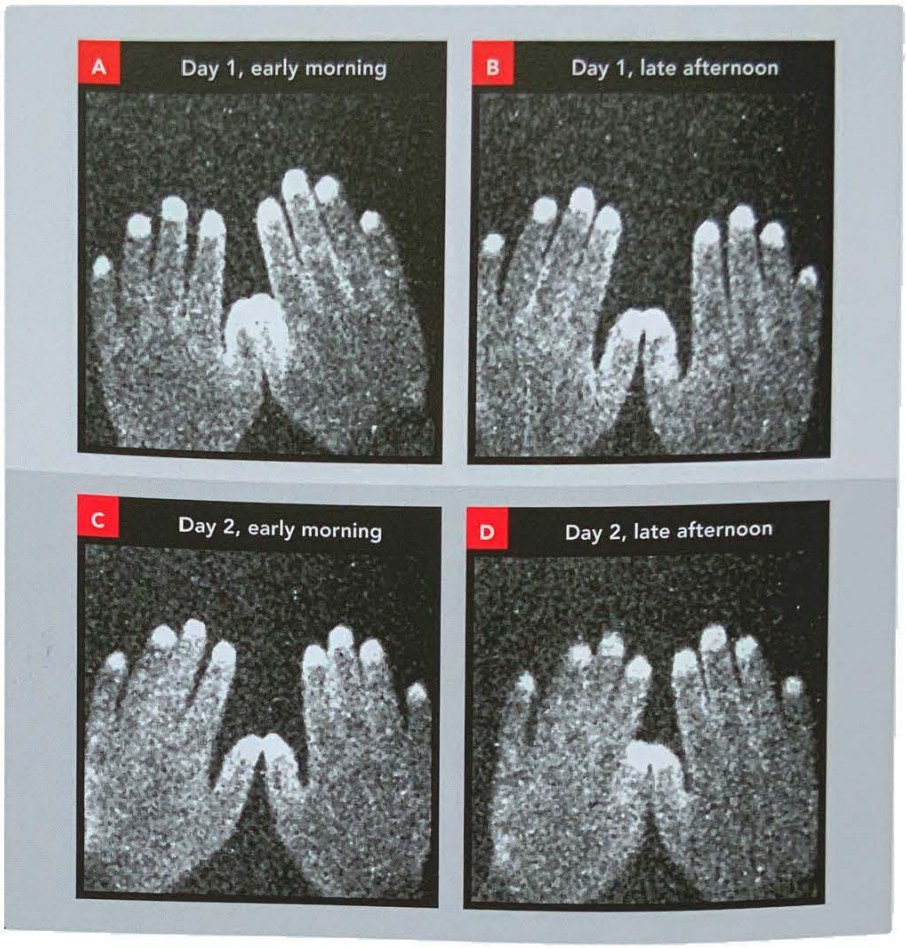

Figure 19.4
A series of images of the ultra-weak photon emission of both dorsal hands of a single subject. Images were captured throughout two consecutive days.

and upper torso of five males regarding the temporal variations of emission during the day. On the days of photon imaging, volunteers were kept in a dim illuminated room adjacent to the dark room. The subjects, one by one, were invited into the darkroom. The partially naked (face and frontal torso) subject remained for 15 minutes in the dark room in order to dark adapt. Subsequently, the subject in sitting position was exposed for 20 minutes to the CCD camera. Measurements were recorded every 3 hours from 10:00 A.M. to 22:00 P.M. Data clearly demonstrated the daily variation of photon emission. Averaging the daily variation across the 5 volunteers resulted in a typical daily fluctuation pattern. The photon emission intensity from the face and upper body was weak in the morning, increased in the afternoon, and peaked in the late afternoon. To further support this information, three volunteers were kept awake. Photon emission was recorded

at 1:00 A.M., 4:00 A.M., and 7:00 AM. Photon emission revealed a peak during late afternoon, then gradually decreased and remained low between 1:00 - 7:00 AM. In this experiment, the surface body and oral temperatures were taken utilizing thermography. The lack of correlation of the daily photon intensity and temperature suggested that the diurnal rhythm of photon emission was not a consequence of a change of temperature.

More on the dynamics in the emission pattern

The observed 1.) daily variation of photon emission from the face and frontal torso, 2.) the non-homogeneous distribution of the emission of these anatomical areas, and 3.) the distinct non-homogeneity in photon emission of the hands raised further questions about emission pattern variability. In the next paragraph we will focus on a series of new hand images.

Such was initiated in The Netherlands by Van der Kraats and the Van Wijk's team. They utilized a large dark imaging room painted with black graphite coater (which had no phosphorescence). The dark room was positioned inside a temperature stable dim light intensity room. The CCD camera system utilized was a iXon 888^{EM+} (back-illuminated), highly sensitive *electron-multiplying* CCD 201 (EMCCD) camera system from Andor Technology Ltd (Belfast, Northern Ireland). Spectral sensitivity of the CCD ranged between approximately 200-1000 nm with a peak (92%) in quantum efficiency at 575nm. A Xenon 0.95/25mm lens was also utilized. Xenon lenses were designed to cover the visible spectrum (400-700nm). Therefore, the overall spectral sensitivity of the camera and lens system was reduced in the UV and IR regions.

A series of images of a single subject were captured throughout the year. Each experimental day, the subject was imaged twice. The images included the dorsal side of the left and right hands. The time between those two imaging periods on the same day was variable. No special measurement protocol was followed with an intermediate time ranging between a few minutes and as long as 6 hours. The series of images consisted of 12 sets (Figure 19.5). Each set included two images of the left and right dorsal sides of each hand. The first imaging each day provided a baseline used for comparison with a second image made later on that same day. The baseline images are presented in Figure 19.5. It demonstrates the high variability of both emission intensity and pattern. In order to initiate a systematic evaluation and description of the images, three categories of images were distinguished based on overall photon emission intensity. Category 1 included the four images with the lowest emission intensity, four images of category 3 with the highest photon emission intensity, and category 2 with four images of intermediate overall intensity. Comparing the left and right hands, a high degree of similarity was observed. When a right hand emit-

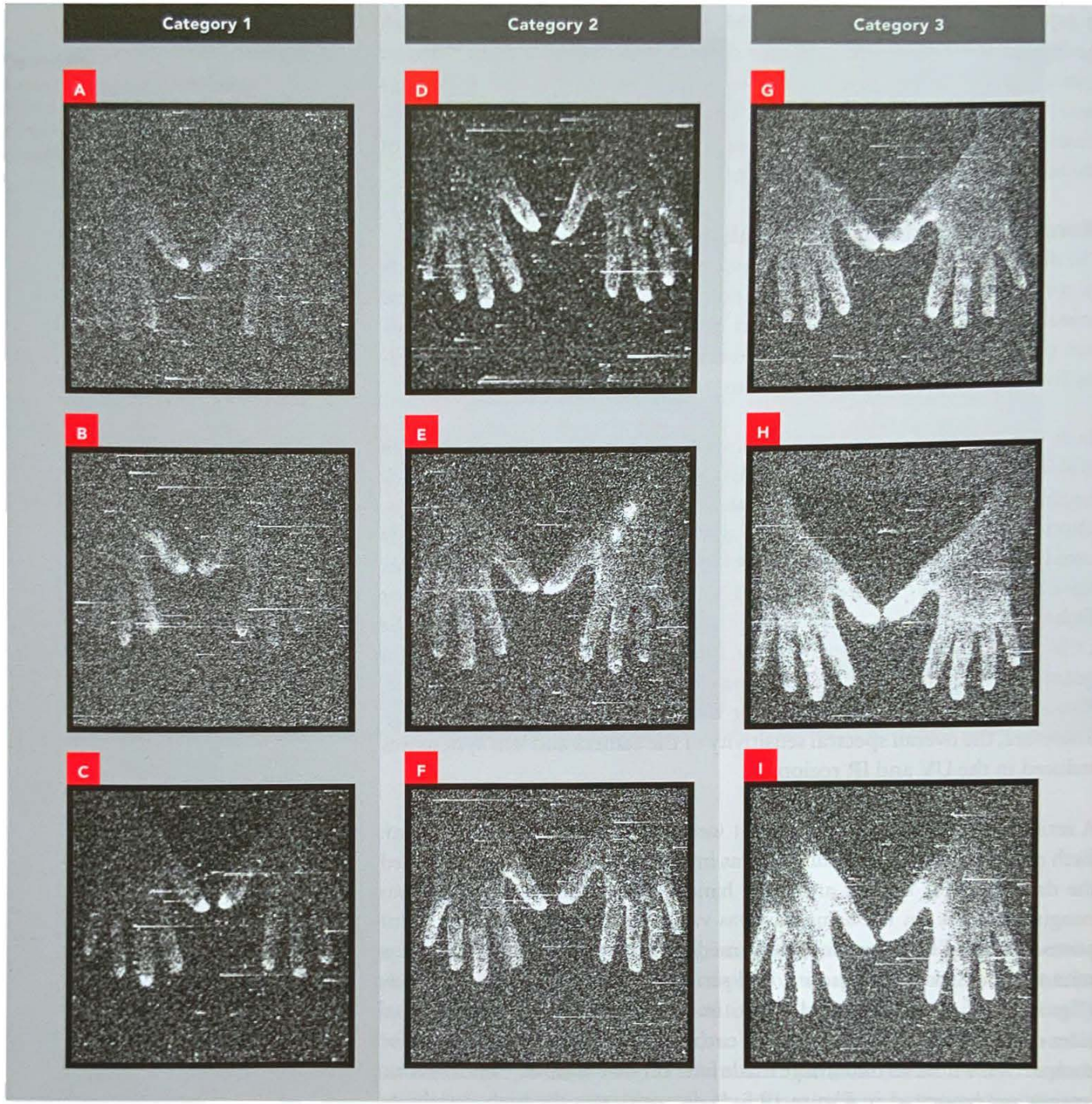

Figure 19.5
The variability in ultra-weak photon emission of the dorsal sides of the right and left hands of a single subject. Images are selected (from a larger range) and categorized by intensity. Category 1 represents images with low intensity. Category 2 represents images with intermediate intensity. Category 3 represents images with high intensity.

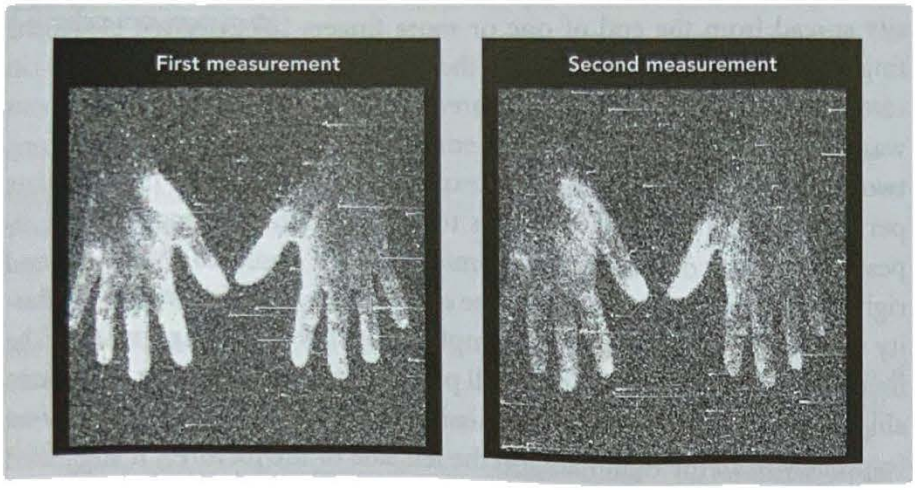

Figure 19.6
Illustrative examples of changes (2.5 h between images) in ultra-weak photon emission of the dorsal sides of the right and left hands of the same subject.

ted a weak intensity, so did the left. The same was observed for those pairs with the intermediate and high emissions. However, left/right symmetry was not perfect. The most intense images (category 3) included the greatest differences of emission patterns.

By zooming in on the specific features of the images with the weakest overall intensity, it was possible to document a pattern. In general, one or more finger end points emitted more light than the rest of the hand. In addition, a higher emission was detected on the medial side as well as the nail of the thumb, plus in some cases (i.e., image B), the lateral side of the index finger. The images with moderate intensity demonstrated that the category 1 spots with high intensity had become brighter and spread over a larger area of the fingers. Particularly, the medial side of the thumb (including the finger tip) emitted a relatively high intensity. At a higher overall intensity, more high emission areas were detected as, for example, on the little finger (image F). Another interesting observation was the bright spot area on image E. In the images with the highest overall intensity (third category), in general, the hand areas basically corresponded with category 2 but spread more in the direction of the central part of the hand. Most pronounced in intensity were the thumb and index fingers plus other fingers that began to become more pronounced (i.e., image I). Apparently, a higher emission spread over a larger area as if the bright intensity (on the thumb side, for instance) moved upwards on the radial side towards the wrist. This was more clearly illustrated on the right hand rather than on the left.

The most remarkable suggestion obtained from the images was (except for some highly local bright spots on the back of the hand) that the inten-

sity spread from the end of one or more fingers (in category 1) via the fingers (in category 2) and then to the dorsal central area of the hand (in category 3). In order to test such spreading of intensity in time, the focus was placed on the dynamics of the emission pattern. As explained before, two images were made on the same experimental day with an intermediate period ranging from a few minutes to up to 6 hours. If images were repeatedly measured, additional dynamics could be observed. When left and right hand were compared, it became evident that a high degree of similarity was present. An interesting example is highlighted in Figure 19.6. The image illustrates a decrease in overall photon emission intensity. A remarkable feature was the decrease in emission from the thumb up to the wrist (especially from the right hand on the left side of the picture). It suggested that fluctuations took place in the range of several hours.

Conclusion

It can be concluded that these novel data present an unique line of research in the area of human ultra-weak photon emission imaging. A major conclusion is that the body cannot be considered as a large, homogeneous emitter. Instead, emission is highly heterogeneous with high emitter anatomical locations such as the face and the hands. In the low emitting trunk and back parts of the body, no detailed light emission patterns were observed. In contrast, patterns with more details were detected in the high emitting face and hand regions. The detailed heterogeneity of the face demonstrated higher emission around the mouth and the cheeks, whereas, lower emission was found at the lateral area and the orbits. The detailed heterogeneity of the hand emission was primarily illustrated by the major emission at finger tips. Both high emitting anatomical areas revealed that the emission pattern was highly dynamic. Regarding the hands, under conditions of increased emission, the pattern spreads in size (i.e., from the tips of thumb and index fingers to a particular side of the back of the hand). Another observation was the remarkable bright spots that were sometimes observed. They may correspond with acupuncture points according to the thinking of Inaba and a few other researchers who focused attention on ultra-weak photon emission from hand and finger acupuncture points. Is such one of the perspectives involved in biophoton research?

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20

A Perspective: Biophotons as a Bridge between Western and Chinese Medicine?

The advent of new photon counting technologies in the early 1960's provided the tools to demonstrate the existence of a ubiquitous low level luminescence in organisms. This provided convincing evidence that light from living organisms was not restricted to life forms having special organs containing enzyme systems such as luciferase/luciferin. The early research was done in the USSR and therefore published in Russian (sometimes translated into English). The spontaneous ultra-weak photon emission by living systems is the result of normal biochemical reactions wherein electrons transition in and out of electronically excited states. Identification of such states was achieved in well-defined chemical systems. However, the task became more problematic in complex biological systems (e.g., in cells or intact organisms). Outside of the USSR, the existence of such radiation was either neglected or became a subject of dispute. However, finally in the 1970's, the existence of this photonic emission was confirmed by research teams in Japan, Australia, Poland, Germany, China, and the USA. Emissions were in the order of 10-10^4 photons/s.cm^2. It was demonstrated in all organisms studied and can provide a useful tool to examine oxygen dependent radical damage.

Since the early 1980's, it has been known that peroxidal lipid reactions are important components in the etiology of diabetes, liver and lung diseases, arteriosclerosis, aging, and cancer. This realization led to the possibility that ultra-weak photon emission evaluated in blood samples might be a general marker for health and could be utilized for diagnostic and clinical purposes. Early studies established the interdependence between various diseases and emission intensity by measuring the differences between the emission from blood samples of healthy as well as diseased human subjects. The development of a diagnostic assay based on ultra-weak photon emission remained limited due to difficulties and requirements of ultra-high sensitive photon counting systems that might be simultaneously applied with biomedical instruments. The utilization of chemiluminigenic probes (substances whose oxidation gives a high yield of electronically excited products) solved many of these issues by increasing the photon output by several orders of magnitude. For this purpose, luminol (and sporadically lucigenin) was employed as a chemiluminigenic enhancer.

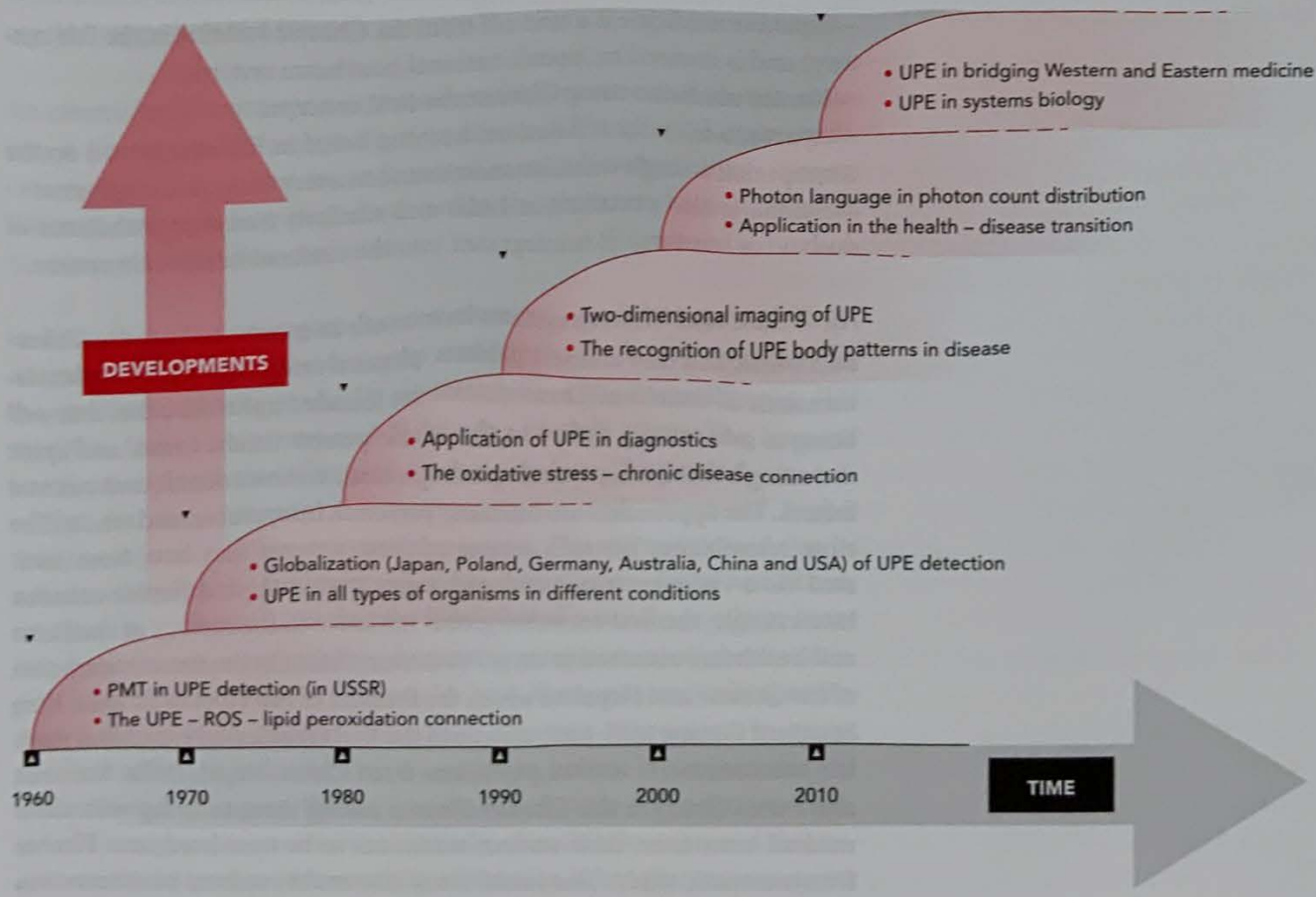
The next great challenge for the field of ultra-weak photon emission detection (in relationship to oxidative processes) was to provide images of very weak signals in addition to physiological information of diseased states. As indicated in the previous chapter, a two-dimensional photon counting imaging of a rat's brain was technically achieved in 1999 demonstrating the effects of local ischemic conditions of the brain. In that chapter, the application of two-dimensional imaging of photon emissions in experimentally induced cancer were described. In bladder cancer (transplanted into the feet of nude mice), the increase of photon emission in early developing cancer revealed active proliferating cancer before any detection of necrosis, hemorrhage, or leukocyte infiltration. Cancer imaging in mice transplanted with ovarian cancer cells utilizing a highly sensitive CCD camera system confirmed the increased photon emission of the tumors. In 2004, Chen and colleagues demonstrated the feasibility of imaging increased ROS levels in a rheumatoid arthritis mouse model utilizing, in their experimental protocol, luminol (injected intravenously) which acted as a luminigenic reporter of ROS. Also using a mouse model of early rheumatoid arthritis, a recent collaboration between the Van Wijk and the Kobayashi teams aimed to image ultra-weak photon emission both with and without the luminol enhancer. Their data suggested that utilizing a highly sensitive imaging system, a higher intensity in the joints of arthritis induced animals was detectable (even without luminol). Such data also offered interesting perspectives for human application since in humans the use of luminol is not preferred.

The major trends over the last 50 years are schematically shown in Figure 20.1. Since the original observation of ultra-weak photon emission, detection methodology has matured to be both uniquely positioned for not only providing insight into detailed biochemical processes but also to provide a non-invasive tool for observing and even understanding system organization. Therefore, it is possible (utilizing detection and quantification of spontaneous photon emission) to non-invasively assess a person's health and wellness (specifically, at the level of stress in both biochemical and metabolic systems via the production of reactive oxygen species). The 50 years of progress in this field has, thanks to advances in technologies for measuring ultra-weak photon emissions, led to the emerging discipline of "human photon emission".

A recent development has been the conduction of a systematic peer review of scientific literature on ultra-weak photon emissions from humans. It was a collaboration between the Samueli Institute of Information Biology and the Sino-Dutch Center for Preventive and Personalized Medicine. Can spontaneous photon emissions from humans be utilized as a non-invasive health assessment? Such an assessment was actually conducted across eight

relevant databases up to October, 2011. The inclusion criteria were met by 56 studies out of which one was a RCT (randomized controlled trial), 27 were CCT (controlled clinical trials), and 28 were observational and descriptive studies. The authors described the characteristics of all of the included studies, the outcomes assessed, and the effectiveness of photon emission as a potential health assessment tool. The report demonstrated that the peer reviewed literature regarding human photon emission measurement (in particular) was surprisingly large. Most of the human emission literature is good to high quality. The utilization of this approach indicates the value of both a medical and research tool.

Figure 20.1
Developments and present state of the art in ultra-weak photon emission (UPE) research. Abbreviations: PMT: photomultiplier tube; ROS: reactive oxygen species.



Fifty years after the first assessment of photon emission utilizing photomultiplier technology, the perspective about this research, more than ever, continues to be developed as a diagnostic tool. Such development is interesting. However, it needs to be considered not simply within the view of Western medicine, but also globally. The diagnostic perspective has been developed vis-à-vis research spread over the world within other cultural systems. Since the 1990's, developments have taken place both in Western (Europe and USA) as well as in Eastern cultures (China, Japan, Korea). To design future research, it is necessary to take cultural diversity into account. So far (illustrated in previous paragraphs), most attention has focused on the role of photon emission in relationship to oxidative processes, excited oxygen species and the damage resulting in well defined disease states according to the Western diagnostic system. However, there exist as many healing systems as there are cultures. Following are descriptions of a few traditional systems (which today are practiced together with Western medicine and researched for scientific validations):

—Chinese medicine is the oldest, established more than 2000 years ago. It encompasses many different practices including acupuncture, herbal remedies, and is rooted in the ancient philosophy of Daoism and life energy (known as Qi).

—Japanese medicine is a spin off from the Chinese system (in the 7th century) and is covered by Japan's national healthcare systems.

—Korean medicine using Chinese medical concepts.

—Ayurveda in India is based on a strong belief in life energy and on the concept that a single consciousness connects everything in the universe.

—Siddha is also practiced in India and similarly based on imbalance of doshas (or humors). It is integrated into the national health care system.

All of these non-Western systems have much in common. They are universal, holistic and they connect patients' physical symptoms with all other dimensions of their lives. These systems are founded upon the belief that well being is intrinsically linked to the whole person (body, mind, and spirit including behavioral, psychological, spiritual, environmental, and cultural factors. The approaches are intensely personal, inseparable, and whole. The close resemblance between several of these systems may have been initiated vis-à-vis interactions and exchanges among these different cultures. Interestingly, the first recorded global initiative in the history of medicine and healthcare occurred in early 7th century Asia. On the recommendation of his Chinese and Nepalese wives, the founder of the Tibetan Empire, King Songtsen Gampo (605-650) convened the first recorded international medical conference. He invited physicians from China, Nepal, India, Kashmir, and Persia (Iran) to the Tibetan Plateau asking them to bring with them medical texts from their various traditions to be translated into Tibetan for syncretistic study. This initial foray into multi-national healthcare was

so successful that in the subsequent 8th century King Trisong-Detson organized a second multi-cultural conference. Physicians were invited from China, Nepal, India, Kashmir, Persia and the Turkish regions of central Asia (including what is now Afganistan). The proceedings were synthesized in a single great book, the *Gyu-zhi* (*The Four Medical Tantras*) which is still studied today. Interesting to note, in 2008 and 2011, the first translation of the first 3 tantras of the *Gyu-zhi* were published.

Whereas Western medicine tends to focus on curing illness in various parts of the body (frequently relying upon costly technology), other traditions rely upon a continuum of preventive medicine, health maintenance, local plants as medicine, food as medicine, and long-term relationships with healers. An interesting phenomenon occurs when the rigors of Western reductionistic scientific methodology move toward increased specialization (i.e., knowing more and more about smaller and smaller phenomena). As the lens of investigation narrows its scope, so does the clinical and diagnostic value of the lessons learned. When there is only a single focus on a singular disease without evaluation of the effect of a therapeutic agent on the entire person, we lose the perspectives of a holistic approach to healing. TCM and Ayurveda intrinsically consider the entire person when developing their approach to a person's healing.

An interesting phenomenon occurs when the assessment of photon emission is situated in the framework of traditional Chinese and Korean medicine vis-à-vis biophysical research rooted in such Eastern cultures. Several different examples have been described in the previous chapters based on the concepts of Chinese medicine.

In 2000, Inaba and colleagues focused attention on photon emission from acupuncture points of the forearms and hands. The paper reported that the emission intensity gradually decreased along the Large Intestine (LI) meridian from Shang-yang and Ho-ku to Chuchih points. The intensity was compared with that of non-acupuncture points. The paper documented that emission intensity of acupuncture points was, in general, higher than the intensity of non-acupuncture points. The intensity could also be influenced by specific treatments of acupuncture points. Inaba had already reported in 1998 and 1999 that the insertion of a needle or a laser beam into an acupuncture point induced intensity enhancement of photon emission from other acupuncture points. In 2000, Yanagawa and colleagues reported the effect on photon emission via thermal stimulation by moxa, another method to influence skin acupuncture points. In another study by Slawinski and colleagues, the electrostimulation of the JG4 point was studied and compared to control. In this study, chemiluminescent and fluorescent molecular probes were overlaid on the skin in an effort to investigate

MORE ABOUT...

BOX 20.1

■ A FOCUS SPECIFICALLY ON CHINESE MEDICINE

The basics of the Chinese medical concept developed over a period of at least 3000 years and were firmly established during the Han Dynasty (206 B.C. - 220 A.D.). By the 4th century A.D., the medical foundations of Chinese medicine were written. Most important was the *Yellow Emperor's Inner Classic*. It belonged to the oldest extant books on Chinese medicine and is still frequently cited in modern days. In later history, Chinese medicine continued to evolve. However, the most prominent basics were maintained. Those are the concepts of Yin and Yang, Qi, and the channel theory.

In Chinese cosmology, all creations are derived from the unity of two opposite aspects, Yin and Yang. Yin and Yang are both opposite to each other, but cannot exist without each other. Gradually, this logic was developed into a system of thought that was applied to other areas. Chinese medicine is an example of one area where the Yin-Yang theory is utilized to understand complicated relationships in the body. The Yin and Yang properties of an object are not absolute, but rather relative. The state of the body is determined by the balance of Yin and Yang.

Qi and Blood are the important substances and are part of the body. They sustain the vital activities and nourish the body in order to maintain optimal functions of the tissues and organs. Qi is the animating force that provides the capacity to move, think, feel, and work. Blood is the foundational substance that creates bone, nerves, skin, muscle, and organs. The ancient Chinese philosophers believed that everything in the universe was generated by the motion and changes of Qi.

The Channels are an important and unique concept in Chinese medicine. They are regarded as three-dimensional passageways through which the Qi energy and Blood flow at different levels of the body. Because of this three-dimensionality, the term "channel" is preferred over the two-dimensional term "meridian" (that is common in English translations). According to traditional theory, the channels integrate all of the body's separate parts and functions into a unified organism. The channels form a web which crisscross the body vertically and horizontally. They join the internal Organs with the skin, flesh, ligaments, bones and all other tissues integrating each part with the whole. However, these channels have no definite anatomical channel structure.

There are two principal types of channels. The main trunks (jing) are generally distributed vertically over the body through relatively deep tissues including the twelve Primary, twelve Divergent and eight Miscellaneous channels. Among these, the twelve Primary channels are by far the most important for clinical purposes. Each has been named by combining traditional Yin and Yang polarity with one of the twelve Organs with which it is associated (e.g., the Absolute Yin Liver channel, the Greater Yang Small Intestine channel, etc). Peripherally, six of the Primary channels are associated with the leg and the remaining six with the arm. The three leg and three arm channels that traverse the lateral and posterior aspects of the limbs (and then join internally with Yang Organs) are referred to as Yang channels. They are commonly translated as the Lesser Yang, Yang Brightness, and greater Yang channels of the arms and legs. The remaining six channels follow the medial and anterior aspects of the limbs, join internally with the Yin Organs, and are called Yin channels. These include the Lesser Yin, Greater Yin, and Absolute Yin channels of the arms and legs. Each Yin channel and its related Yin Organ is paired with one of the Yang channels and its related Yang Organ.

As said earlier, the Channels not only reach into the body cavities to join with associated Viscera, but also circulate through superficial tissues on the surface of the body. Each of the twelve Primary channels is associated with the connective tissues (muscles, tendons, ligaments) and cutaneous areas along its path.

There are twelve Cutaneous Regions and twelve Muscle channels associated with the twelve Primary channels. This latter aspect has been included in discussions within the Channel theory since the Tang Dynasty (founded 618 A.D.).

A study of the channels might therefore provide an essential basis for understanding the reciprocal relationship and influences among the various physiological, pathological, diagnostic and therapeutic aspects of traditional Chinese medicine. The Qi and Blood circulate throughout the body via this network of channels. Thus, the intimate relationship between the internal Viscera and the periphery of the body is primarily maintained by the channels. This means that when the various entities and forces in the body are in harmonious balance, there is health. When this balance is disturbed, there is illness.

the intensity of ultra-weak photon emission using the sensitive "Night Owl" CCD camera. It appeared that the electrostimulation of the JG4 acupuncture point resulted in weaker biophoton emission than the stimulation of neighboring non-active areas. It is evident that further systematic studies are required to shed light on the relationship between photon events and acupuncture points in Traditional Chinese and Korean medicines.

With regard to the dynamics of the photon emission pattern, the question was asked whether it is analogous to the Qi energy flow in Traditional Chinese Medicine? Reading the hand data regarding ultra-weak photon emission, attention must be focused on the six channels (which, according to TCM, are bilaterally oriented in the body and traverse the hand). The name of each is derived from the extremity which it traverses, the particular aspect of the limb through which it passes, and the Organ with which it is associated (see also Box 20.1). The circulation of Qi essentially follows the specific paths of the channels. In general, the Qi energy in the three Arm Yin channels travels from the internal Organs to the hands. The Qi in the three Arm Yang channels travels from the hands to the head. More specifically, the flow in these hand channels is as follows:
—The "Arm Greater Yin Lung Channel" (L) ends in the *thumb*. It descends along the upper arm and elbow to the pulse and then extends to the tip of the thumb. A branch from the main channel splits at the wrist and ends at the tip of the *index finger*. This channel begins in the region of the stomach, crosses the diaphragm, and enters its associated organ, the Lung. It then emerges between the Lung and the throat, descends along the upper arm and elbow where it extends to the tip of the thumb and the index finger.

—The "Arm Yang Brightness Large Intestine Channel" (LI) begins at the tip of the *index finger* and proceeds upward to the wrist and upper arm. It continues along the upper arm to the shoulder joint. Behind the shoulder, it turns upward and enters into the supraclavicular fossa before descending across the diaphragm to the associated organ, Large Intestine. At the supraclavicular fossa, a branch separates from the main channel moving upward through the neck and terminates at the side of the nose.

—The "Arm Absolute Yin Pericardium Channel" (P) ends at the tip of the *middle finger*. It descends along the arm and separates in the palm proceeding along the *4th finger* to the tip. The channel begins in the chest at its associated organ, the Pericardium. It then descends into the abdomen. A branch of the main channel runs along the chest and ascends to the axilla and from there, it descends along the upper arm and reaches both the middle finger and the 4th finger.

—Another channel, the "Arm Lesser Yang Triple Burner Channel" (TB) originates on the *4th fingertip*, traverses the hand, and continues upward to the shoulder. Then, it crosses back over the shoulder and travels to the mid-chest region where it descends across the diaphragm to the abdomen linking with the upper, middle, and lower burners of the Triple Burner (to which this channel belongs). A branch of the main channel separates in the chest and ascends to the neck. It then proceeds upward behind the ear and across the cheek to below the eye. Another branch separates behind the auricle and then traverses to the cheek.

—The "Arm Lesser Yin Heart Channel" (H) ends at the tip of the *little finger*. It begins in its associated Organ, the Heart and travels downward across the diaphragm. In the heart, a branch of the main channel separates and ascends alongside the esophagus to the face and then joins the tissues surrounding the eye. Another branch goes directly from the heart to the lung and emerges below the axilla. From here, the channel descends along the upper arm where it continues downward and enters the palm following the medial side of the little finger to the finger tip.

—Finally, in the opposite direction, the "Arm Greater Yang Small Intestine Channel" (SI) originates at the tip of the *little finger* and ascends to the wrist. It passes the elbow and proceeds along the upper arm emerging behind the shoulder. At the top of the shoulder, the channel turns downward and descends along the esophagus. It then crosses the diaphragm before reaching its associated organ, the Small Intestine. Like the other two arm Yang channels, a branch of this channel travels upward from the supraclavicular fossa and crosses the neck and cheek to the eye and ear region. Another branch separates from the former branch on the cheek and ascends to the eye region.

A testable hypothesis regarding future research might be that the distribution of Qi over the specific channels is related to specific dynamics of hand

photon emission patterns. According to traditional theory, the channels integrate all of the body's separate parts and function as a unified organism. Pathological symptoms associated with the primary channels are supposed to appear as local manifestations in their related cutaneous regions. One research step to possibly follow regarding the hypothesis might be to image hand emission patterns of different subjects to detect personalized patterns in relationship to both disease development as well as the possibility to subtype subjects based on the principles of Chinese medicine. Another possibility to research the same hypothesis may be to focus on the photon emission patterns of hands in relationship to organ functioning and thereby characterize the metabolites representing organ functioning. Utilizing this line of thinking, it would be helpful to further compare the cutaneous representation of the channels by photon imaging with the patterns of metabolism by utilizing metabolomics and other "omics" research fields.

The biophoton research could lead to an enrichment of diagnostics based on the combination of Western and Eastern biomedical knowledge. Such opens a new order of thinking. The journey for the future, focusing on the diagnostic value of the internationally researched ultra-weak photon emission would have a significant global perspective. Globalization is not limited to the increasing transnational circulation of money, goods, and information world-wide. It is presently recognized as one of the most powerful forces shaping current diagnostics into future diagnostics. By collaboration both across cultures and respecting and conserving the richness and diversity of each type of medicine, it may lead to a universal integration, ensuring complementarities and mutually enriching hybridization. The burgeoning awareness of systems biology is encouraging more open minded and multi-disciplinary approaches to problem solving. Complex diseases have called for complex integrative approaches leading to a new acceptance of outside traditions. Biomedicine is now encouraging the Western medical culture to accept holistic and integrative medical practices as viable options. With the dissolution of multi-national boundaries and the universal free access to information, the notion of holistic and global based care is emerging as the future of medicine. In many respects, the ultra-weak emission (biophotons) of living organisms can be seen as a bridge between Western and Chinese medicine.

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Glossary

A

Acetylcholine. Neurotransmitter that functions at vertebrate neuromuscular junctions and at various neuron-neuron synapses in the brain and peripheral nervous system.

Acetyl CoA. Small, water-soluble metabolite comprising an acetyl group linked to coenzyme A (CoA), formed during oxidation of pyruvate, fatty acids, and amino acids. Its acetyl group is transferred to citrate in the citric acid cycle.

AC field. Alternating electrical current or magnetic field.

Achromatic lens (or achromat). Lens that is designed to limit the effects of chromatic and spherical aberration.

Actin. Abundant structural protein in eukaryotic cells that interacts with many other proteins. The monomeric globular form (G-actin) polymerizes to form actin filaments (F-actin). In muscle cells, F-actin interacts with myosin during contraction.

Action potential. Rapid, transient, all-or-none electrical activity propagated in the plasma membrane of excitable cells (e.g., neurons and muscle cells) as the result of the selective opening and closing of voltage-gated Na^+ and K^+ channels.

Activating energy. The input of energy to (overcome the barrier to) initiate a chemical reaction.

Active site. Specific region on the surface of an enzyme that binds a substrate molecule and promotes a chemical change in the bound substrate.

Active transport. Movement of an ion or small molecule across a membrane against its concentration gradient or electrochemical gradient driven by the coupled hydrolysis of ATP.

Acupuncture point. Point on the skin related to channel or meridian according to concept of Traditional Chinese Medicine. See also *Channel; Meridian; Traditional Chinese Medicine (TCM).*

Adaptation. Adjustment of sensitivity following repeated stimulation. This is the mechanism that allows a neuron or a bacterium to react to small changes in stimuli even against a high background level of stimulation.

Adenylyl cyclase. Membrane-bound enzyme that catalyzes formation of cyclic 3',5'-AMP (cAMP) from ATP; also called adenylate cyclase.

Adenosine 5'-triphosphate (ATP). A nucleoside triphosphate used in cells as a coenzyme; produced by enzymes from adenosine diphosphate (ADP) or adenosine monophosphate (AMP) and various phosphate group donors.

Adipocyte. A fat cell.

ADP (adenosine 5'-diphosphate). Nucleotide that is produced by hydrolysis of the terminal phosphate of ATP. It regenerates ATP when phosphorylated by an energy-generating process such as oxidative phosphorylation.

Adrenaline (epinephrine). Hormone released by chromaffin cells (in the adrenal gland) and by some neurons in response to stress. Produces "fight or flight" responses, including increased heart rate and blood sugar levels.

Aerobic. Referring to a cell, organism, or metabolic process that utilizes gaseous oxygen (O_2) or that can grow in the presence of O_2.

Aerobic oxidation. Oxygen-requiring metabolism of sugars and fatty acids to CO_2 and H_2O coupled to the synthesis of ATP.

Alga. Informal term used to describe a wide range of simple unicellular and multicellular eucaryotic photosynthetic organisms.

Allostery. Change in the tertiary and/or quaternary structure of a protein induced by binding of a small molecule to a specific regulatory site, causing a change in the protein's activity.

Alpha (α-) helix. Common protein secondary structure in which the linear sequence of amino acids is folded into a right-handed spiral stabilized by hydrogen bonds between carboxyl and amide groups in the backbone.

Alpha (α-) ketoacids. Product derived from amino acids after N removal; in metabolism used for gluconeogenesis or fatty acid synthesis.

Alpha (α-) particles. Consist of two protons and two neutrons bound together into a particle identical to a helium nucleus.

Alpha (α-) tocopherol. Form of vitamin E that is preferentially absorbed and accumulated in humans.

Amino acid. An organic compound containing at least one amino group and one carboxyl group.

Amino group. Weakly basic functional group derived from ammonia (NH_3) in which one or more hydrogen atoms are replaced by another atom. In aqueous solution it can accept a proton and carry a positive charge.

AMP (adenosine 5'-monophosphate). One of the four nucleotides in an RNA molecule. Two phosphates are added to AMP to form ATP.

Anabolism. System of biosynthetic reactions in a cell by which large molecules are made from smaller ones.

Anaerobic. Referring to a cell, organism, or metabolic process that functions in the absence of gaseous oxygen (O_2).

Anaphase. Mitotic stage during which the sister chromatids separate and move apart toward the spindle poles.

Aneurogenic (of embryonic parts). Developing without the normal neural component; a grafted limb bud is aneurogenic.

Angiogenesis. Growth of new blood vessels by sprouting from existing ones.

Angiogenesis inhibitor. Substance that inhibits the growth of new blood vessels. See also *Angiogenesis.*

Angstrom. Unit of length used to measure atoms and molecules. Equal to 10^{-10} meter or 0.1 nanometer (nm).

Animal pole. In yolky eggs, that end free of yolk that cleaves more rapidly than the vegetal pole.

Anoxia. Total depletion of oxygen, an extreme form of hypoxia or "low oxygen". See also *Hypoxia.*

Anterior. Situated toward the head end of the body.

Anteroposterior. Described the axis running from the head to the tail of the animal body.

Antibody (immunoglobulin). Protein produced by B cells in response to a foreign molecule or invading microorganism. Binds to the foreign molecule or cell and facilitates its clearance by various mechanisms.

Antioxidant. Substance that balances production of free radicals.

Antiparallel. Describes the relative orientation of the two strands in a DNA double helix; the polarity of one strand is oriented in the opposite direction to that of the other.

Apatite. The mineral fraction of bone; microscopic calcium phosphate crystals deposited on the preexisting collagen structure of the bone, making it hard. See also *Collagen.*

Apical. Referring to the tip (apex) of a cell, an organ, or other body structure.

Aplysia. Genus of sea slugs, belonging to marine gastropod mollusks.

Apochromatic lens (also apochromat). Lens that has better correction of chromatic and spherical aberration than the achromat lenses. See also *Achromat.*

Apoptosis. Regulated process leading to cell death mediated by a caspase cascade and marked by a series of well-defined morphological changes; also called programmed cell death.

Aqueous. Pertaining to water, as for example, in an aqueous solution.

Ascites. Accumulation of fluid in the peritoneal cavity.

Asexual reproduction. Any type of reproduction (such as budding in *Hydra,* binary fission in bacteria, or mitotic division in eucaryotic microorganisms) that does not involve gamete formation and fusion.

Aster. Structure composed of microtubules (astral fibers) that radiate outward from a centrosome during mitosis.

ATP (adenosine 5'-triphosphate). A nucleotide that is the most important molecule for capturing and transferring free energy in cells.

ATP synthase. Protein complex bound to inner mitochondrial membranes and the bacterial plasma membrane that catalyzes synthesis of ATP during oxidative phosphorylation.

ATPase. One of a large group of enzymes that catalyze hydrolysis of ATP to yield ADP and inorganic phosphate with release of free energy.

Autoradiography. Technique for visualizing radioactive molecules in a sample by exposing a photographic film or emulsion to the sample.

Autotomy. Process in which an amputation towards the tip of the limb induces the animal to cast off the rest of the limb. It is caused by contraction of specialized muscles at the breaking point.

Axis (of the body). Imaginary longitudinal line through the vertical center of the body (head to feet). Development in vertebrates and most other animals occurs in a nearly symmetrical fashion around the body axis.

Axon. Long process extending from the cell body of a neuron that is capable of conducting an electric impulse (action potential) generated at the junction with the cell body towards its distal, branching end (the axon terminus).

Axonal transport. Directed transport of organelles and molecules along a nerve cell axon. It can be anterograde (outward from the cell body) or retrograde (back toward the cell body).

B

Bacteria. Members of the Bacteria, one of the two major divisions of procaryotes, the other being the Archea. Most exist as single cells and some cause disease.

Basal. Situated near the base. The basal surface of a cell is opposite the apical surface.

Basal lamina. A thin sheetlike network of extracellular matrix components that underlies most animal epithelia and other organized groups of cells separating them from connective tissue or other cells.

Base. Any compound that can accept a photon (H^+). Commonly used to denote the purines and pyrimidines in DNA and RNA.

Base pair. Association of two complementary nucleotides in a DNA or RNA molecule stabilized by hydrogen bond-

ing between their base components (for example, G pairs with C, and A with T or U).

Basolateral. Referring to the base and side of a cell, an organ or other body structure.

Benign. Referring to a tumor containing cells that closely resemble normal cells. Benign tumors stay in the tissue where they originate.

Beta (β-) particles. High-energy, electrons or positrons emitted by certain types of radioactive nuclei; a form of ionizing radiation also known as beta rays.

Beta (β-) sheet. A planar secondary structure of proteins that is created by hydrogen bonding between the backbone atoms in two different polypeptide chains or segments of a single folded chain.

Binding site. A region on the surface of one molecule (usually a protein or nucleic acid) that can interact with another molecule through noncovalent bonding.

Biological clock. Refers to circadian rhythm; a biological process that displays an endogenous entrainable oscillation of about 24 hours.

Biophores. Material particles that – in different combinations – are divided between blastomers and result in differentiation (Weismann).

Biophoton. A photon related to biological organization.

Bin (in photon count statistics). Measuring interval in a series of photon counts.

Bisoliton. A collective excitation provided by a specific molecular structure resulting in transfer of electron pairs. See also *Soliton*.

Blood plasma. Liquid component of blood that normally holds the blood cells; serves as the protein reserve and keeps electrolytes in balance.

Blastomers. Cells resulting from cleavage of the egg cytoplasm.

Blastopore. Opening into the archenteron during embryonic stages of an organism.

Blastema. The mass of primitive, unspecialized cells that appears at the site of an injury in animals that regenerate. Blastema cells specialize and form the replacement part.

Blastomere. One of the cells formed by the cleavage of a fertilized egg.

Blastula. Early stage of an animal embryo, usually consisting of a hollow ball of cells, before gastrulation begins.

Blood (in Traditional Chinese Medicine). Used as a broad term to describe the physical blood in the body that moistens the muscles, tissues, skin and hair, as well as nourishing the cells and organs.

BMI. See *Body Mass Index*.

Body Mass Index (BMI). A measure for human body shape based on an individual's weight and height.

Bond energy. Strength of the chemical linkage between two atoms, measured by the energy in kilocalories or kilojoules needed to break.

Brachial plexus. Network of nerve fibers, running from the spine and proceeding through the neck, the axilla (armpit region), and into the arm.

Budding yeast. A common name for a yeast which divides by budding off a smaller cell. Examples are the baker's yeast *Saccharomyces cerevisiae* and the brewer's yeast *Saccharomyces carlsbergensis*.

C

Calcium pump (Ca^{2+}-ATPase). Transport protein in the membrane of the sarcoplasmic reticulum of muscle cells (and elsewhere) that pumps Ca^{2+} out of the cytoplasm into the sarcoplasmic reticulum using the energy of ATP hydrolysis.

Calmodulin. A small cytosolic regulatory protein that binds four Ca^{2+} ions. The Ca^{2+}/calmodulin complex binds to many proteins, thereby activating or inhibiting them.

Calorie. A unit of heat (thermal energy). One calorie is the amount of heat needed to raise the temperature of 1 gram of water by 1°C. The kilocalorie (kcal) commonly is used to indicate the energy content of foods and the free energy of a system.

cAMP. See *Cyclic 3',5'-AMP*.

cAMP-dependent protein kinase. See *Protein kinase A*.

Cancer. General term denoting any of various malignant tumors, whose cells grow and divide more rapidly than normal, invade surrounding tissue, and generally spread (metastasize) to other sites.

Carbohydrate. General term for certain polyhydroxyaldehydes, polyhydroxyketones, or compounds derived from these usually having the formula $(CH_2O)_n$. Primary type of compound used for storing and supplying energy in animal cells.

Carbonyl group. Pair of atoms consisting of a carbon atom linked to an oxygen atom by a double bond (C=O).

Carcinogen. Any chemical or physical agent that can cause cancer when cells or organisms are exposed to it.

Carcinogenesis. Generation of a cancer.

Carcinoma. Cancer of epithelial cells. The most common form of human cancer.

Cardiac muscle. Specialized form of striated muscle found in the heart, consisting of individual heart muscle cells linked together by cell junctions.

Cardiomyocytes (or myocardiocytes). Cells that comprise cardiac muscle.

Cartilage. Flexible form of connective tissue composed of cells (chondrocytes); found in the joints between bones, the rib cage, ear, nose, bronchial tubes and intervertebral discs.

Cartilage cells. Cells that comprise cartilage; embedded in a matrix rich in type II collagen and chondroitin sulfate.

Caryokinesis. See *Karyokinesis.*

Catabolism. Cellular degradation of complex molecules to simpler ones usually accompanied by the release of energy. See also *Anabolism.*

Catalyst. A substance that increases the rate of a chemical reaction without undergoing a permanent change in its structure. Enzymes are protein catalysts that catalyze most biochemical reactions.

CCD camera. Abbreviation for Charged-Coupled Device camera.

Cell body. Main part of a nerve that contains the nucleus. The other parts are the axons and dendrites.

Cell cortex. Specialized layer of cytoplasm on the inner face of the plasma membrane. In animal cells it is an actin-rich layer responsible for movements of the cell surface.

Cell cycle. Ordered sequence of events in which a eukaryotic cell duplicates its chromosomes and divides into two; normally consists of four phases; G_1 before DNA synthesis occurs; S when DNA replication occurs; G_2 after DNA synthesis; and M when cell division occurs, yielding two daughter cells.

Cell cycle control system. Network of regulatory proteins that governs progression of a eukaryotic cell through the cell cycle.

Cell division. Separation of a cell into two daughter cells. In higher eukaryotes, it involves division of the nucleus (mitosis) and of the cytoplasm (cytokinesis).

Cell fate. In developmental biology, describes what a particular cell at a given stage of development will normally give rise to.

Cell-free system. Fractionated cell homogenate that retains a particular biological function of the intact cell, and in which biochemical reactions and cell processes can be more easily studied.

Cell line. A population of cultured cells that has undergone a change allowing the cells to grow indefinitely.

Cell strain. A population of cultured cells that has a finite life span and eventually dies.

Cellular pathology. Medicine dealing with the essential nature of disease, especially changes in body tissues and organs that cause or are caused by disease.

Central nervous system (CNS). Main information-processing organ of the nervous system. In vertebrates it consists of the brain and spinal cord.

Centriole. Either of two cylindrical structures within the centrosome of animal cells and containing nine sets of triplet microtubules.

Centromere. DNA sequence required for proper segregation of chromosomes during mitosis and meiosis; the region of mitotic chromosomes where the kinetochore forms.

Centrosome. Structure located near the nucleus of cells that is the primary microtubule-organizing center (MTOC). It divides during mitosis, forming the spindle poles.

Chi. See *Qi.*

Chain reaction. Series of events in which each induces or influences the next. In chemistry: A series of reactions in which one product of a reacting set is a reactant in the following set.

Channel (also called meridian). In Traditional Chines Medicine, a path through which the life-energy known as "qi" flows.

Channel protein. Membrane transport protein that forms an aqueous pore in the membrane through which a specific solute, usually an ion, can pass.

Chaperone. Collective term for two types of proteins that prevent misfolding of a target protein or facilitate its proper folding.

Checkpoint. Any of several points in the eukaryotic cell cycle at which progression of a cell to the next stage can be halted until conditions are suitable.

Chemiosmosis. Mechanism in which a gradient of hydrogen ions (a pH gradient) across a membrane is used to drive an energy-requiring process, such as ATP production.

Chemotaxis. Movement of a cell or organism toward or away from certain chemicals.

Cholesterol. A lipid containing the four-ring steroid structure with a hydroxyl group on one ring.

Chondrocyte (cartilage cell). Connective-tissue cell that secretes the matrix of cartilage.

Chromatid. One copy of a replicated chromosome, formed during the S phase of the cell cycle, that is joined at the centromere to the other copy; also called sister chromatid.

Chromatin. Complex of DNA, histones, and nonhistone proteins from which eukaryotic chromosomes are formed.

Chromosome. In eukaryotes, the structural unit of the genetic material consisting of a single, linear double-

stranded DNA molecule and associated proteins. In most prokaryotes, a single, circular double-stranded DNA molecule constitutes the bulk of the genetic material.

Chromosome condensation. Process by which a chromosome becomes packed up into a more compact structure prior to M phase of the cell cycle.

Cilium. Short, membrane-enclosed structure extending from the surface of eukaryotic cells and containing a core bundle of microtubules.

Circadian rhythm. The predominant biological cycle of all living organisms; from Latin "circa" ("approximately") and "dies" ("a day").

Citric acid cycle. A set of nine coupled reactions occurring in the matrix of the mitochondrion in which acetyl groups derived from food molecules are oxidized, generating CO_2 and reduced intermediates used to produce ATP; also called Krebs cycle and tricarboxylic acid (TCA) cycle.

Cleavage. (1) Physical splitting of a cell into two. (2) Specialized type of cell division seen in many early embryos whereby a large cell becomes subdivided into many smaller cells without growth. (3) Cleavage of a covalent bond with accompanying addition of water, –H being added to one product of the cleavage and –OH to the other.

Clone. A population of genetically identical cells or organisms descended from a common ancestor.

Codon. Sequence of three nucleotides in DNA or mRNA that are also called triplet.

Coenzyme A (CoA). See *Acetyl CoA.*

Coherence. Physics, an ideal property of waves that enables stationary (i.e. temporally and spatially) constant interference.

Collagen. A triple-helical glycoprotein rich in glycine and proline that is a major component of the extracellular matrix and connective tissues. The numerous subtypes differ in their tissue distribution. Tendons, ligaments, and scar tissue are composed almost entirely of collagen. It also forms the basic structure of bone. See also *Apatite.*

Collective excitation. In physics, collective excitations are emergent phenomena that occur when a microscopically complicated system behaves as if it contained different weakly interacting particles. For example, as an electron travels through a semiconductor, its motion is disturbed in a complex way by its interactions with all of the other electrons; however it behaves like an electron traveling unperturbed.

Collimator. In optics, curved mirror or lens with some type of light source and/or an image at its focus.

Computation. Any type of calculation or use of computing technology in information processing.

Condenser. Component of optical system of transmission light microscopes; Lens that serves to concentrate light from the illumination source that is in turn focused through the object and magnified by the objective lens.

Conductor (electrical). An object allowing the flow of electric charge.

Conformation. The precise shape of a protein or other macromolecule in three dimensions resulting from the spatial location of the atoms in the molecule.

Connective tissue. Any supporting tissue that lies between other tissues and consists of cells embedded in a relatively large amount of extracellular matrix. Includes bone, cartilage, and loose connective tissue.

Connective tissue cell. Any of the various cell types found in connective tissue, e.g. fibroblasts, cartilage cells (chondrocytes), bone cells (osteoblasts and osteocytes), fat cells (adipocytes) and smooth muscle cells.

Constellation (molecular). Association of molecules facilitating energy circulation.

Constitutive. Referring to the continuous production of a cellular molecule that is not regulated by internal or external signals.

Cooperativity. Property exhibited by some proteins with multiple ligand-binding sites whereby binding of one ligand molecule increases (positive cooperative) or decreases (negative cooperativity) the binding affinity for successive ligand molecules.

Contractile ring. Ring containing actin and myosin that forms under the surface of animal cells undergoing cell division and contracts to pinch the two daughter cells apart.

Coronary thromboses (acute). Obstruction of a coronary artery by a thrombus, often leading to destruction of heart muscle.

Covalent bond. Stable chemical force that holds the atoms in molecules together by sharing of one or more pairs of electrons.

Coupled reaction. Linked pair of chemical reactions in which the free energy released by one of the reactions serves to drive the other.

Coupled oscillators. More complicated system than is modeled by the harmonic oscillator which has a single degree of freedom. Coupled oscillator systems have more degrees of freedom. In such cases, the behavior of each variable influences that of the others. This leads to a *coupling* of the oscillations of the individual degrees of freedom. For example, two pendulum clocks (of identical frequency) mounted on a common wall will tend to synchronise.

Crayfish (also crawfish). Freshwater *Crustacea*, taxonomically, members of the superfamilies *Astacoidea* and *Parastacoidea.*

Crista. One of the folds of the inner mitochondrial membrane.

Crystal lattice. The precise, orderly arrangement of atoms in a crystal, forming a netlike structure.

Cyanide. Inhibitor of the enzyme cytochrome c oxidase in the fourth complex of the electron transport chain.

Cybernetics. Transdisciplinary approach for exploring regulatory systems.

Cyclic 3',5'-AMP (cAMP). A second messenger, produced in response to hormonal stimulation of certain receptors.

Cyclic 3',5'-GMP (cGMP). A second messenger that opens cation channels in rod cells and activates protein kinase S in vascular smooth muscle and other cells.

Cytochromes. A group of heme-containing proteins that function as electron carriers during cellular respiration.

Cytochrome b-c_1 complex. Second of the three electron-driven proton pumps in the respiratory chain. It accepts electrons from ubiquinone.

Cytochrome oxidase complex. Third of the three electron-driven proton pumps in the respiratory chain. It accepts electrons from cytochrome c and generates water using molecular oxygen as an electron acceptor.

Cytokinesis. The division of the cytoplasm following mitosis to generate two daughter cells.

Cytoplasm. Viscous content of a cell that is contained within the plasma membrane; outside the nucleus.

Cytoskeleton. Network of fibrous elements, consisting primarily of microtubules, actin microfilaments, and intermediate filaments, found in the cytoplasm of eukaryotic cells. The cytoskeleton permits directed movement of organelles, and the cell itself.

Cytosol. Unstructured aqueous phase of the cytoplasm excluding organelles, membranes, and insoluble cytoskeletal components.

Cytostatics. Inhibitor of cell growth; used to inhibit tumor cell growth.

D

Dalton. Unit of molecular mass.

Daoism (also Taoism). A philosophical and religious tradition that emphasizes living in harmony with the Dao (meaning "way" or "path"). In Daoism, Dao denotes both the source and the driving force behind everything that exists.

DC field. Any time current flow through a wire a magnetic field is generated. If that current is Direct Current, the resultant magnetic field has a constant orientation. If the current is AC or alternating current, the magnetic field will vary in direction and intensity, hence the term AC magnetic field. See also *AC field.*

Dedifferentiation. The process in which a mature, specialized cell returns to its original, embryonic, unspecialized state. During dedifferentiation the genes that code for other cell types are made available for use by derepressing them. See also *Differentiation, Redifferentiation, Undifferentiation.*

Degradational radiation. Radiation in living objects under unfavourable conditions; from unbalanced molecular constellations with high energy level for its maintenance.

Delayed fluorescence. See *Delayed luminescence.*

Delayed luminescence. Type of photostimulated luminescence. Fluorescence usually refers to light emission that rapidly fades within nanoseconds or picoseconds. Light that slowly fades in a period ranging from several seconds to several ten seconds is called delayed luminescence.

Delta wave. A high amplitude brain wave with a frequency of oscillation between 0-4 hertz.

Denaturation. Drastic alteration in the conformation of a protein or nucleic acid due to disruption of various noncovalent bonds caused by heating or exposure to certain chemicals; usually results in loss of biological function.

Dendrite. Process extending from the cell body of a neuron that is relatively short and typically branched, receives signals from axons of other neurons and carries it toward the cell body. See also *Axon, Neuron.*

Dense, heterogeneous medium. Medium which contains a considerable number of dispersed particles. In interaction with laser radiation the luminosity shows an optical breakdown which is characterized by a gradual change of the absorption coefficient.

Deoxyribonucleic acid. See *DNA.*

Depolarization. Change in the cytosolic-face negative electric potential that normally exists across the plasma membrane of a cell at rest, resulting in a less negative membrane potential.

Dermis. A layer of skin beneath the epidermis consisting of spongeous collagenous tissue.

Determination. In embryogenesis, a charge in a cell that commits the cell to a particular developmental pathway (cell fate).

Detergent. Type of small amphipathic molecule that tends to coalesce in water, with its hydrophobic tails buried and its hydrophilic heads exposed. It is widely used to solubilize membrane proteins.

Development. Overall process involving growth and differentiation by which a fertilized egg gives rise to an adult plant

or animal, including the formation of individual cell types, tissues, and organs.

Diabetes (or diabetes mellitus). A group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. See also *Prediabetes.*

Differentiation. The process in which a cell matures from a simple embryonic type to a mature, specialized type in the adult. Differentiation involves restricting, or repressing, all genes for other cell types. See also *Dedifferentiation, Gene, Redifferentiation.*

Diffusion. Net drift of molecules in the direction of lower concentration due to random thermal movement.

4-Dimethylaminoazobenzene. Carcinogenic substance.

Dipole (electric dipole). A pair of point charges with equal magnitude and opposite sign separated by a distance.

Disaccharide. A small carbohydrate (sugar) composed of two monosaccharides covalently joined by a glycosidic bond.

Dissipation (force of energy). Force causing energy to be lost or dissipated is called a dissipative force.

Diurnal (rhythm). Fluctuation in a 24h period.

Divergent channels. In Traditional Chinese Medicine: Channels belonging to the major channels.

DNA (deoxyribonucleic acid). Long linear polymer, composed of four kinds of deoxyribose nucleotides, that is the carrier of genetic information. In its native state, DNA is a double helix of two antiparallel strands held together by hydrogen bonds between complementary purine and pyrimidine bases.

DNA base modification. Modification in chemical structure of deoxyribonucleotides it may sometimes result in mutagenesis and cancerogenesis.

DNA repair. Collective name for those biochemical processes that correct accidental changes in the DNA.

DNA virus. Class of viruses having a DNA genome; replicating themselves commonly in the host-cell nucleus.

Domain. Region of a protein with a distinct tertiary structure and characteristic activity.

Doping. Tiny amounts of certain minerals mixed in semiconductor materials to change their characteristics.

Dorsal. Relating to the back of an animal or the upper surface of a structure.

Dorsoventral. Describes the axis running from the back to the belly of an animal or from the upper side to the underside of a structure.

Double helix, DNA. The most common three-dimensional structure for cellular DNA in which the two polynucleotide strands are antiparallel and wound around each other with complementary bases hydrogen-bonded.

Drosophila melanogaster. Species of small fly, commonly called a fruit fly, much used in genetic studies of development.

Delta (Δ) G^0. See *Standard free-energy change.*

Delta (Δ) G. See *Free-energy change.*

Dynode. Secondary electron emitter in photomultiplier tube.

Dysplasia. A change in cell growth and behavior in a tissue in which the structure becomes disordered.

E

Ectoderm. Outermost of the three primary cell layers of the animal embryo; gives rise to epidermal tissues, the nervous system and external sense organs.

Ectopic (limb). Additional limb formation in other parts of embryos, for instance by members of the fibroblast growth (FGF) family.

Egg. The mature female gamete in sexually reproducing organisms. It is usually a large and immobile cell.

Electrobiology (electrophysiology). Field of research dealing with the reactions of living organisms to both environmental electric stimuli and electrical phenomena within the organism.

Electrochemical gradient. The driving force that determines the energetically favorable direction of transport of a ion (or charged molecule) across a membrane.

Electrochemical proton gradient. The result of a combined pH gradient (proton gradient) and the membrane potential.

Electrode. A device, usually metal, that connects electronic equipment to a living organism for the purpose of measuring electrical currents or voltages in the organism, or delivering a measured electrical stimulus to the organism.

Electrodynamic theory (of life). Theory that explains the pattern of (living) organization by behavior and orientation of physicochemical components in reciprocal interaction.

Electrolyte. Any chemical compound that, when dissolved in water, separates into charged atoms that permit the passage of electrical current through the solution.

Electron. Negatively charged subatomic particle that generally occupies orbitals surrounding the nucleus in an atom.

Electron acceptor. Atom or molecule that takes up electrons readily, thereby gaining an electron and becoming reduced.

Electron carrier. Molecule such as cytochrome c, which transfers an electron

from a donor molecule to an acceptor molecule in coupled oxidation and reduction reactions.

Electron donor. Molecule that easily gives up an electron, becoming oxidized in the process.

Electron microscope. Type of microscope that uses a beam of electrons to create the image.

Electron-multiplying CCD system (EMCCD). A charge-coupled device in which a gain register is placed between the shift register and the output amplifier. The gain register is split up into a large number of stages. In each stage, the electrons are multiplied.

Electron spin resonance spectroscopy (ESR). Based on principle that electrons in the lower of two spin states in a magnetic field can absorb a photon of the right frequency and move to a higher state.

Electron transport chain. Series of electron carrier molecules along which electrons move from a higher to a lower energy level to a final acceptor molecule. The energy released during electron movement can be used to power various processes. Electron transport chains present in the inner mitochondrial membrane generate a proton gradient across the membrane that is used to drive ATP synthesis. See also *Respiratory chain.*

Electron volt (eV). A quantity (unit) of energy.

Electrostimulation (of acupuncture point). Type of stimulation of acupuncture point using a small electric current.

Embryogenesis. Early development of an individual from a fertilized egg (zygote). In animals, following cleavage of the zygote, the major axes are established during the blastula stage; in the subsequent gastrula stage, the early embryo invaginates and acquires three cell layers.

Embryonic stem cells (ES cells). Pluripotent stem cells derived from the inner cell mass of a blastocyst, an early-stage embryo.

Endocrine. Referring to signaling mechanism in which target cells bind and respond to a hormone released into the blood by distant specialized secretory cells usually present in a gland.

Endocrine cell. Specialized animal cell that secretes a hormone into the blood. Usually part of a gland, such as the thyroid or pituitary gland.

Endoderm. Innermost of the three primary cell layers of the animal embryo; gives rise to the gut and most of the respiratory tract.

Endoplasmic reticulum (ER). Network of interconnected membranous structures within the cytoplasm of eukaryotic cells contiguous with the outer nuclear envelope. The rough ER, which is associated with ribosomes, functions in the synthesis and processing of secreted and membrane proteins; the smooth ER lacks ribosomes.

Endothelial cell. Cell lining a blood vessel; divides in vascular growth.

Endothelium. Layer of highly flattened cells that forms the inner lining of all blood vessels and regulates exchange of materials between the bloodstream and surrounding tissues.

Enhancer. A regulatory sequence in eukaryotic DNA that may be located at a great distance from the gene it controls. Binding of specific proteins to an enhancer modulates the rate of transcription of the associated gene.

Enthalpy (H). Heat; in a chemical reaction, the enthalpy of the reactants or products is equal to their total bond energies.

Entropy (S). A measure of the degree of disorder or randomness in a system; the higher the entropy, the greater the disorder.

Enzyme. A protein that catalyzes a particular chemical reaction involving a specific substrate or small number of related substrates.

Epidemiology. Basic science of preventive and community medicine and its being applied to a variety of problems in health services.

Epidermal growth factors (EGF). A family of secreted signaling proteins that is used in the development of most tissues in most or all animals.

Epidermis. Epithelial layer covering the outer surface of the body having no blood vessels. It has different structures in different animal groups.

Epigenesis. One of two ways to explain the development of individual organic form: The development of a complex organism from a simple, undifferentiated unit, such as the egg cell. The organism gradually acquired form. The material was there but the formal cause only gradually plays out. It is the opposite of preformation, in which a complex organism was thought to develop from a smaller, but similarly complex, antecedent, such as the homunculus that some early biologists thought resided in the sperm or egg cell.

Epimorphosis. Type of "add-on" regeneration, for example, the old stump provides cells for blastema formation without drastic rearrangement of the remaining tissues. See also *Morphallaxis.*

Epinephrine. A catecholamine secreted by the adrenal gland and some neurons in response to stress; also called adrenaline.

Epiphyseal plate. Growth center in bone.

Epithelium. Sheetlike-covering; composed of one or more layers of tightly adhered cells, on external and internal body surface; general term for skin and for the lining of the digestive tract.

Epstein-Barr virus (EBV). A human gamma herpes virus.

Equifinality. Final result of the development (formation of a specific mature organism) may be reached by different pathways.

Equilibrium. State where there is no net change in a system. For example, equilibrium is reached in a chemical reaction when the forward and reverse rates are equal.

Equipotentially. All the parts (cells) of a whole (embryo) are equal in their potential morphogenetic capacity.

ER lumen. The space enclosed by the membrane of the endoplasmic reticulum (ER).

ER. See *Endoplasmic reticulum.*

Erythrocyte (red blood cell). Small, hemoglobin-containing blood cell of vertebrates that transports oxygen and carbon dioxide to and from tissues.

ES cell. See *Embryonic stem cell.*

***Escherichia coli* (*E. coli*).** Rodlike bacterium normally found in the colon of humans and other mammals and widely used in biomedical research.

Ester. Molecule formed by the condensation reaction of an alcohol group with an acidic group. Phosphate groups usually form esters when linked to a second molecule.

Estrous cycle. Period of identifiable changes in appearance of corpora lutea.

Etiology. Science of the causes of disease.

Eucaryote (also Eukaryote). Organism composed of one or more cells with a distinct nucleus and cytoplasm.

eV. See *Electron Volt.*

Excision-repair system. One of several mechanisms for repairing DNA damaged due to exposure to carcinogens.

Exciton. A collective excitation provided by specific molecular structures (for instance alpha helical structure in protein). Can be excited by light of corresponding frequency; it can also emit photons. See also *Soliton.*

Excited carboxyl compounds. Formed by dismutation of peroxyradicals; plays role in photon generation.

Extracellular matrix. A usually insoluble network consisting of polysaccharides, fibrous proteins, and adhesive proteins that are secreted by animal cells. It provides structural support in tissues and can affect the development and biochemical functions of cells.

Exudate. Liquid, sometimes containing cells, that diffuses out from a wound or surface structure of a living organism. Examples are a wound exudate and the slime exudate from the skin of fish.

F

FAD (flavin adenine dinucleotide). A diffusible electron carrier that accepts two electrons from a donor molecule and two H^+ from the solution. In mitochondria, the reduced form, $FADH_2$ transfers electrons to carriers that function in oxidative phosphorylation.

Fano factor. Named after Fano; ratio of variance and mean. Used in photon count distribution research.

Fano factor curve. The curve around which Fano factor fluctuates with bin size.

Fasting glucose blood values. Mean normal blood glucose level in humans is about 5.5 mM (5.5 mmol/L); the level fluctuates throughout the day and is usually lowest in the morning, before the first meal of the day: "the fasting level".

Fat. Energy-storage lipid in cells. It is composed of triglycerides – fatty acids esterified with glycerol.

Fatty acid. Any long hydrocarbon chain that has a carboxyl group at one end. Used as a major source of energy during metabolism and as a starting point for the synthesis of phospholipids.

Fat cell. Connective-tissue cell that produces and stores fat in animals.

Feedback inhibition. Type of regulation of metabolism in which an enzyme acting early in a reaction pathway is inhibited by a late product of that pathway.

Fermentation. Anaerobic energy-yielding metabolic pathway in which pyruvate produced by glycolysis is converted, for example into lactate or ethanol, with the conversion of NADH to NAD.

Fertilization. Fusion of a male and a female gamete (both haploid) to form a diploid zygote, which develops into a new individual.

Fibroblast. A common type of connective tissue cell that secrets collagen and other components of the extracellular matrix. It migrates and proliferates during wound healing.

Fibroblast growth factors (FGF). A family of secreted signaling proteins that are used in the development of most tissues in most or all animals.

Field of organization. Primary embryonic field.

Fission yeast. Common name often given to the yeast *Schizosaccharomyces pombe*, a common experimental organism. It divides to give two equal-sized cells.

Fluorochrome. Molecule that absorbs light at one wavelength and emits light at another (longer) wavelength.

Fourier spectrum. In physics and engineering, the Fourier transform is a mathematical transform that transforms a mathematical function of time into a new function of frequency with units of cycles/sec (hertz).

Free energy (G). A measure of the potential energy of a system which is a function of the enthalpy (H) and entropy (S).

Free-energy change (ΔG). The difference in the total free energy of the product molecules and of the starting molecules (reactants) in a chemical reaction. A large negative value of ΔG indicates that a reaction has a strong tendency to occur.

Free ribosome. Ribosome that is free in the cytosol, unattached to any membrane. It is the site of synthesis of all proteins encoded by the nuclear genome other than those destined to enter the endoplasmic reticulum.

Fungus. Kingdom of eucaryotic organisms that includes the yeasts, molds, and mushrooms. Many plant diseases and a relatively small number of animal diseases are caused by fungi.

G

G. See *Free energy.*

G_0 (G-"zero") phase. State of withdrawal from the eucaryotic cell division cycle by entry into a quiescent G_1 phase. A common state for differentiated cells.

G_1 phase. Gap 1 phase of the eucaryotic cell division cycle, between the end of cytokinesis and the start of DNA synthesis.

G_2 phase. Gap 2 phase of the eucaryotic cell division cycle, between the end of DNA synthesis and the beginning of mitosis.

Galleria. Moth of the family *Pyralidae.*

Galvanotaxis. The movement of a living organism toward or away from a source of electric current.

Gamete. Specialized haploid cell, either a sperm or an egg, serving for sexual reproduction.

Gamma (γ-) radiation. Refers to electromagnetic radiation of high frequency and high-energy per photon.

Ganglion. Cluster of nerve cells and associated glial cells located outside the central nervous system.

Gastrulation. The stage in animal embryogenesis during which the embryo is transferred from a ball of cells to a structure with a gut (a gastrula).

Geiger-Müller counter. Type of particle detector that measures ionizing radiation. It detects the emission of nuclear radiation by the ionization produced in a low-pressure gas in a Geiger-Müller tube; mostly used for nuclear radiation – alpha (α-) particles, beta (β-) particles, or gamma (γ-) rays.

Gene. Physical and functional unit of heredity, which carries information from one generation to the next.

Gene control. All of the mechanisms involved in regulating gene expression.

Gene expression. Overall process by which the information in a gene is converted into an observable phenotype.

Gene repressor protein. A gene regulatory protein that prevents the initiation of transcription.

Genome. Total genetic information carried by a cell or organism.

Genomic DNA. All the DNA sequences composing the genome by a cell or organism.

Genomics. Comparative analyses of the complete genomic sequences to predict the types of proteins produced by an organism.

Genotype. Entire genetic constitution of an individual cell or organism.

Germ cell. Any precursor cell that can give rise to gametes.

Germ line. Lineage of germ cells, which give rise to gametes and thus participate in formation of the next generation of organisms.

Giga. Prefix denoting 10^9 (from Greek "gigas", giant).

Glia. A tissue composed of a variety of cells, mostly glial cells, that makes up most of the nervous system. Glial cells have electrical properties that, while not the same as nerve impulse transmission, enable them to play a role in communication in the body.

Globular protein. Any protein with an approximately rounded shape. Such proteins are contrasted with highly elongated, fibrous proteins such as collagen.

Glucagon. A peptide hormone produced in the alpha cells of pancreatic islets that triggers the conversion of glycogen to glucose by the liver.

Glucose. Six-carbon monosaccharide (sugar) that is the primary metabolic fuel in most cells.

Glucogenic amino acids. Amino acids that are degraded to pyruvate, alpha-ketoglutarate, succinyl CoA, fumarate and oxaloacetate.

Glycogen. A very long, branched polysaccharide, composed exclusively of glucose units, that is the primary storage carbohydrate in animals. It is found primarily in liver and muscle cells.

Glycolysis. Metabolic pathway in which sugars are degraded anaerobically to lactate or pyruvate in the cytosol with the production of ATP.

Gradient. Amount of slope. In embryology related to *morphogen*, a signaling molecule that diffuses through an embryo during early development setting up concentration gradients.

Graft. In surgery: a portion of living tissue used in *otoplasty* for correcting deformities and defects.

Granulocyte. Category of white blood cell distinguished by conspicuous cyto-

plasmic granules. Includes neutrophils, basophils, and eosinophils.

Ground (and excited) state. After absorbing energy, an electron may jump from the ground state to a higher energy excited state.

Growth factor. An extracellular polypeptide molecule that binds to a cell-surface receptor, triggering an intracellular signaling pathway generally leading to cell proliferation.

H

Heat shock protein (stress-response protein). Protein synthesized in increased amounts in response to an elevated temperature or other stressful treatment, and which usually helps the cell to survive the stress. Prominent examples are hsp60 and hsp70.

HeLa cells. Line of human epithelial cells that grows vigorously in culture. Derived from a human cervical carcinoma.

Heme. Cyclic organic molecule containing an iron atom that carries oxygen in hemoglobin and carries an electron in cytochrome.

Hemiparesis. Weakness on one side of the body. It is less severe than hemiplegia – the total paralysis of the arm, leg, and trunk on one side of the body.

Hemisphere (or half sphere). The brain is composed of two nearly mirror-image lobes called the *left* and *right hemispheres.*

Hepatitis B. Infectious inflammatory illness of the liver caused by the hepatitis B virus (HBV).

Hepatocarcinogen. Substance that can cause tumor of the liver.

Hepatocyte. Liver cell.

Hertz. Cycles per second, the unit for measuring the vibratory rate of electromagnetic radiation. Named for Heinrich Hertz who made the first experimental discovery of radio waves in 1888.

Hexose. A six-carbon monosaccharide.

H^+. See *Proton.*

High-energy bond. Covalent bond that releases a large amount of energy when hydrolyzed under the usual intracellular conditions.

High-performance liquid chromatography (also referred to as high-pressure liquid chromatography), HPLC. Chromatographic technique used to separate a mixture of compounds with the purpose of identifying and quantifying the components of the mixture.

Histone. One of several small, highly conserved basic proteins, found in the chromatin of all eukaryotic cells, that associate with DNA.

Homeostasis. The ability of living organisms to maintain a constant "internal environment". For example, the human body maintains a constant amount of dissolved oxygen in the blood at all times by means of various mechanisms that sense the oxygen level and increase or decrease the breathing rate.

Homology. Similarity in characteristics (e.g., protein and nucleic acid sequences or the structure of an organ) that reflects a common evolutionary origin. Molecules or sequences that exhibit homology are referred to as homologs. In contrast, analogy is a dissimilarity in structure of function that does not reflect a common evolutionary origin.

Hormone. General term for any extracellular substance that induces specific responses in target cells; specifically, those signaling molecules that circulate in the blood and mediate endocrine signaling.

Homogenization (in cell biology). Process that involves breaking apart cells to release their cytoplasm.

Homoplastic implantation. Implantation of embryos in an adult.

Hydrocarbon. Compound that has only carbon and hydrogen atoms.

Hydrogen bond. A noncovalent interaction between an atom (commonly oxygen or nitrogen) carrying a partial negative charge and a hydrogen atom carrying a partial positive charge. Particularly important in stabilizing the three-dimensional structure of proteins and in formation of base pairs between nucleic acid strands.

Hydrogen peroxide (H_2O_2). The simplest peroxide (a compound with an oxygen-oxygen single bond). Compound generated spontaneously from superoxide anion O_2^-.

Hydrophilic. Interacting effectively with water. See also *Polar.*

Hydrophobic. Not interacting effectively with water; in general poorly soluble or insoluble in water. See also *Nonpolar.*

Hydroxyl (-OH). Chemical group consisting of a hydrogen atom linked to an oxygen, as in an alcohol.

Hydroxyl radical ($OH^•$). Reactive oxygen species (formed via reaction between O_2^- and H_2O_2; Haber-Weiss reaction); key radical in damaging molecules.

Hypertonic. Referring to an external solution whose solute concentration is high enough to cause water to move out of cells due to osmosis.

Hypocotyl. The stem of a germinating seedling found below the cotyledons (seed leaves) and above the radicle (root).

Hypoperfusion. Decreased blood flow through an organ; it may result in permanent cellular dysfunction and death.

Hypothyroidism. Lower state of metabolic activity.

Hypotonic. Referring to an external solution whose solute concentration is low enough to cause water to move into cells due to osmosis.

Hypoxia. Inadequate oxygen supply to cells and tissues.

I

Image processing. Computer treatment of images that reveal information not immediately visible to the eye.

Immune response. Response made by the immune system when a foreign substance or microorganism enters the body.

Induction. (1) In embryogenesis, a change in the developmental fate of one cell or tissue caused by direct interaction with another cell or tissue or with an extracellular signaling molecule. (2) In metabolism, an increase in the synthesis of an enzyme or series of enzymes mediated by a specific molecule (inducer).

Inflammatory response. Local response of a tissue to injury or infection – characterized by tissue redness, swelling, heat, and pain. Caused by invasion of white blood cells, which release various local mediators such as histamine.

Information theory. Branch of applied mathematics, bioinformatics, and computer science involving quantification of information.

Infusoria. Minute aquatic creatures such as ciliates, euglenoids, protozoa, and unicellular algae. In modern classifications, the microorganisms are mostly assigned to the kingdom *Protista.*

Inner membrane. The innermost of two membranes surrounding an organelle. In the mitochondrion, it encloses the matrix and contains the respiratory electron transport chains.

Insulin. A protein hormone produced in the beta cells of the pancreatic islets that stimulates uptake of glucose into muscle and fat cells; acts with glucagon to help regulate blood glucose levels. Insulin also functions as a growth factor for many cells.

Intermembrane space. The subcompartment formed between the inner and outer mitochondrial membrane.

Internal membrane. Eucaryotic cell membrane other than the plasma membrane. Examples are the membranes of the endoplasmic reticulum and the Golgi apparatus.

Integral membrane protein. Any protein containing one or more hydrophobic segments embedded within the core of the phospholipid bilayer; also called intrinsic membrane protein.

Intermediate filament. Cytoskeletal fiber (10 nm in diameter) formed by polymerization of several types of subunit proteins including keratins, lamins, and vimentin.

Industrial Medicine (also Occupational Medicine). Branch of medicine concerned with maintenance of health in working populations in the workplace.

Interphase. Long period of the cell cycle, including the G_1, S, and G_2 phase, between one M (mitotic) phase and the next.

In vitro. Term used by biochemists to describe a process taking place in an isolated cell-free extract. Also used by cell biologists to refer to cells growing in culture, as opposed to in an organism. (Latin for "in glass").

In vivo. In an intact cell or organism (Latin for "in life").

Iodoacetic acid. Toxic alkylating compound; reacts with cysteine in proteins.

Ion. An atom that has either gained or lost electrons in acquire a charge.

Ion channel. Transmembrane protein complex that forms a water-filled channel across the lipid bilayer through which specific inorganic ions can diffuse down their electrochemical gradient.

Ionic bond. Cohesion between two atoms, one with a positive charge, the other with a negative charge. One type of nonvalent bond.

Ionic conduction. Mode of conduction in solutions by the movement of ions.

Ionization energy. Amount of energy required to remove an electron from atom or molecule in gaseous state.

Iron-sulfur center. Electron transporting group consisting of either two or four iron atoms bound to an equal number of sulfur atoms, found in a class of electron transport proteins.

J

Joule. Standard unit of energy in the meter-kilogram system. One joule is the energy delivered in one second by a one-watt power source. Approximately equal to 0.24 calories.

K

Karyokinesis. Process of partition of a cell's nucleus into the daughter cells during cell division.

Keratins. A group of intermediate filament proteins found in epithelial cells that assemble into heteropolymeric filaments.

Ketogenic amino acids. Amino acids that are degraded to acetyl CoA or acetoacetyl CoA.

Key enzymes (of metabolic pathway). Enzyme controlling rate and direction of flux.

Kilo-. Prefix denoting 10^3

Kilocalorie (kcal). Unit of heat energy equal to 1000 calories. Often used to express the energy content of food or molecules: bond strengths, for example, are measured in kcal/mole.

Kilojoule. Standard unit of energy equal to 1000 joules, or 0.24 kilocalories.

Kinase. An enzyme that transfers the terminal (gamma) phosphate group from ATP to a substrate.

L

Laser. Device that emits light through a process of optical amplification based on the stimulated emission of electromagnetic radiation. Acronym for "Light Amplification by Stimulated Emission of Radiation".

LED. Light emitting diode.

Leucocyte. See *White blood cell.*

Leukemia. Cancer of white blood cells.

L-field. Field of life (which held the shape of an organism).

Ligand. Any molecule, other than an enzyme substrate, that binds tightly and specifically to a macromolecule, usually a protein, forming a macromolecule-ligand complex.

Light-independent and dark reactions. These reactions take the light energy from light-dependent reactions and perform further chemical processes on them.

Lipid. Any organic molecule that is poorly soluble or virtually insoluble in water but is soluble in nonpolar solvents. Major classes include fatty acids, phospholipids, steroids, polyisoprenoids, and triglycerides.

Lipid bilayer. Thin bimolecular sheet of mainly phospholipid molecules that forms the core structure of all cell membranes. The two layers of lipid molecules are packed with their hydrophobic tails pointed inward and their hydrophilic heads outward, exposed to water.

Lipid peroxidation. Oxidative degradation of lipids. This process proceeds by a free radical chain reaction mechanism.

Lipolysis. Degradation of lipid providing glycerol for gluconeogenesis.

Lipophilic. See *Hydrophobic.*

Lumen. The space within a tubular structure (e.g., a vessel or the intestine) or the interior volume of a membrane-bounded compartment within a cell.

Luminescent bacteria. Emit light as the result of a enzyme-catalyzed chemoluminescence reaction, wherein the pigment luciferin is oxidized by the enzyme luciferase. Luminescent bacteria exist as symbiotic organisms carried within a larger organism.

Lymphocytes. Two classes of white blood cells that can recognize foreign molecules (antigens) and mediate immune responses. B-lymphocytes are responsible for production of antibodies. T-lymphocytes are responsible for destroying virus- and bacteria-infected cells, foreign cells, and cancer cells.

Lysis. Destruction of a cell by rupture of the plasma membrane and release of the contents.

M

M phase. Period of the eucaryotic cell cycle during which the nucleus and cytoplasm divide.

Macromere. Larger cell in unequal cleavage during the first two cell divisions.

Macromolecule. Any large, usually polymeric molecule (e.g., a protein, nucleic acid, polysaccharide) with a molecular mass greater than a few thousand daltons.

Macrophage. Phagocytic cell derived from blood monocytes, typically resident in most tissues. It has both scavenger and antigen-presenting functions in immune responses.

Magnetosphere. The area around the earth in which the planet's magnetic field exerts a stronger influence than the solar or interplanetary magnetic field. It extends some 30000 to 50000 miles from the earth's surface. A prominent feature of the magnetosphere is the Van Allen belts, areas of charged particles trapped by the earth's magnetic field.

Magnetotactic. Active movement toward a magnetic pole.

Malignant. Referring to a tumor cell that can invade surrounding normal tissue and/or undergo metastasis. See also *Benign.*

Malondialdehyde (MDA). Highly reactive three-carbon dialdehyde produced as byproduct of polyunsaturated fatty acid peroxidation; combines with molecules including proteins, lipoproteins, and DNA.

Mediolateral. From the midline outwards to the left or to the right.

Medulla oblongata. Lower half of the brainstem.

Mega. Prefix denoting 10^6. (From Greek "megas", huge, powerful).

Melanocyte. Cell that produces the dark pigment melanin. Responsible for the pigmentation of skin and hair.

Membrane-bound ribosome. Ribosome attached to the cytosolic face of the endoplasmic reticulum. The site of synthesis of proteins that enter the endoplasmic reticulum.

Membrane channel. Transmembrane protein complex that allows inorganic ions or other small molecules to diffuse passively across the lipid bilayer.

Membrane potential. Voltage difference across a membrane due to the slight excess of positive ions (cations) on one side and negative ions (anions) on the other.

Membrane protein. Protein that is normally closely associated with a cell membrane.

Membrane transport. Movement of molecules across a membrane mediated by a membrane transport protein.

Membrane transport protein. Collective term for any integral membrane protein that mediates movement of one or more specific ions or small molecules across a cellular membrane regardless of the transport mechanism.

Meridians (Traditional Chinese Medicine). Major 12 pathways through which Qi (Chi) flows, supplying energy and nourishment to the body. Acupuncture needles are placed in points along these pathways to assist in correcting imbalances. See also *Acupuncture points; Channel.*

Meristem. Tissue in plants consisting of undifferentiated cells found in zones where growth can take place.

Messenger RNA (mRNA). RNA molecule that specifies the amino acid sequence of a protein. It is translated into protein in a process catalyzed by ribosomes.

Mesenchyme. Immature embryonic connective tissue derived from either the mesoderm or ectoderm in animals.

Mesoderm. The middle of the three primary cell layers of the animal embryo, lying between the ectoderm and endoderm; gives rise to the notochord, connective tissue, muscle, blood, and other tissues.

Metabolon. Multienzyme complex facilitating substrate channels between active sites.

Metallic conduction. Mode of conduction in a metal by a cloud of electrons.

Metaphase. Mitotic stage at which chromosomes are fully condensed and attached to the mitotic spindle but have not yet started to segregate toward the opposite spindle poles.

Metaplasia. A change in the pattern of cell differentiation in a tissue.

Metastability. Behavior of certain physical systems that can exist in long lived states that are less stable than the most stable metastable states; these states have finite lifetimes.

Metastasis. Spread of cancer cells from their site of origin and establishment of areas of secondary growth.

Miasma theory (also miasmatic theory). Refers to diseases such as cholera, chlamydia or the Black Death which were caused by a miasma, a noxious form of "bad air". The theory was displaced in the 19th century by the discovery of germs.

Micro-. Prefix denoting 10^{-6}.

Microfilament. Cytoskeletal fiber (=7nm in diameter) that is formed by polymerization of monomeric globular (G) actin; also called actin filament. Microfilaments play an important role in muscle contraction, cytokinesis, cell movement, and other cellular functions and structures.

Micromere. In unequal cleavage in the first two cell divisions, the smaller cells are specified as the micromeres.

Microsome. A small particle in the cytoplasm of a cell, typically consisting of fragmented endoplasmic reticulum to which ribosomes are attached.

Microtubule. Cytoskeletal fiber (=24 nm in diameter) that is formed by polymerization of alpha, beta-tubulin monomers and exhibits structural and functional polarity. Microtubules are important components of cilia, flagella, the mitotic spindle, and other cellular structures.

Milli-. Prefix denoting 10^{-3}.

Mindfulness. A mode of meditation (a spiritual or psychological faculty) that, according to the teaching of the Buddha, is considered to be of importance in the path to enlightenment.

Miscellaneous channels (Traditional Chinese Medicine). A number of channels (or meridians) throughout the body.

Mitochondrial DNA (mtDNA or mDNA). DNA located in mitochodria.

Mitochondrion. Large organelle that is surrounded by two phospholopid bilayer membranes, contains DNA, and carries out oxidative phosphorylation, thereby producing most of the ATP in eukaryotic cells.

Mitogen. Any extracellular molecule, such as a growth factor, that encourages a cell to commence cell division; usually a protein.

Mitogenesis. Induction (triggering) of mitosis.

Mitogenetic radiation. Light radiation that allowed the morphogenetic field to control embryonic development (according to Gurwitsch).

Mitosis. In eukaryotic cells, the process whereby the nucleus is divided to produce two generically equivalent daughter nuclei with the diploid number of chromosomes. See also *Cytokinesis.*

Mitotic apparatus. A specialized temporary structure, present in eukaryotic cells during mitosis, that captures the chromosomes and then pushes and pulls them to opposite sides of the dividing cell.

Mitotic spindle. Array of microtubules and associated molecules that forms between the opposite poles of a eucaryotic cell during mitosis and serves to move the duplicated chromosomes apart. See *Mitotic apparatus.*

Model organism. A species, such as *Drosophila melanogaster* or *Escherichia coli*, that has been studied intensively over a long period and thus serves as a "model" of the biology of a particular type of organism.

Molecular Correlation Concept (of cancer). Link of enzymic and metabolic imbalance with transformation and progression of tumors.

Monochromatic colors. Colors (tints, tones, and shades) of a single hue. Hue is defined as the degree to which a stimulus can be described as similar to or different from stimuli that are described as red, green, blue, and yellow.

Mononucleosis. Disease most commonly caused by the Epstein-Barr virus (EBV).

Monosaccharide. Any simple sugar with the formula $(CH_2O)_n$ where n=3-7.

Morphogen. A molecule that specifies cell identity during development, often as a function of its concentration.

Morphogenesis. Biological process that causes an organism to develop its shape.

Morphogenetic field. A group of cells whose position and fate are specified with respect to the same set of boundaries; it will give rise to its particular organ even when transplanted to a different part of the embryo.

Morphollaxis. Type of regeneration of tissue; a large majority of regenerated tissue comes from already-present tissue in the organism.

Motor protein. Any member of a special class of mechanochemical enzymes that use energy from ATP hydrolysis to generate either linear or rotary motion.

Moxa. Made from dried mugwort; it plays a role in the traditional medical systems of China, Japan, and Korea. Practitioners burn the fluff and use it indirectly, with acupuncture needles, or burn it on the patient's skin.

Mutagen. A chemical or physical agent that induces mutations.

Mutation. In genetics, a permanent, heritable change in the nucleotide sequence of a chromosome, commonly causes an alteration in the function of the gene-product.

Myeloid cell. Any white blood cell other than lymphocytes.

Myoblast. Mononucleated, undifferentiated muscle precursor cell. A skeletal muscle cell is formed by the fusion of multiple myoblasts.

Myofibril. Long, highly organized bundle of actin, myosin, and other proteins in the cytoplasm of muscle cells that contracts by a sliding filament mechanism.

Myosins. A class of motor proteins that have actin-stimulated ATPase activity. Myosins move along actin microfilaments during muscle contraction and cytokinesis and also mediate vesicle translocation.

N

NAD^+ (nicotinamide adenine dinucleotide). A widely used diffusible electron carrier that accepts two electrons from a donor molecule and one H^+ from the solution. In mitochondria, the reduced form, NADH, transfers electrons to carriers that function in oxidative phosphorylation.

NADH dehydrogenase complex. First of the three electron-driven proton pumps in the mitochondrial respiratory chain. It accepts electrons from NADH.

$NADP^+$ (nicotinamide adenine dinucleotide phosphate). Phosphorylated form of NAD^+; used extensively as an electron carrier in biosynthetic pathways.

Na^+/K^+ ATPase. An ion pump present in the plasma membrane of all animal cells that couples hydrolysis of one ATP molecule to export three Na^+ ions and import two K^+ ions. This pump is largely responsible for maintaining the normal intracellular concentrations of Na^+ (low) and K^+ (high) in animal cells.

Nano-. Prefix denoting 10^{-9}.

Nanometer (nm). Unit of length commonly used to measure molecules and cell organelles, 1 nm= 10^{-3} micrometer (μm) =10^{-9} meter.

Necrosis. Cell death resulting from tissue damage or other pathology; usually marked by swelling and bursting of cells with release of their contents. Contrast with apoptosis.

Negative control. Type of control of gene expression in which the active DNA-binding form of the regulatory protein turns the gene off.

Neoblast. An unspecialized embryonic cell retained in the adult bodies of certain primitive animals and called to the site of an injury and take part in regenerative healing.

Nephron. Basic structural and functional unit of the kidney.

Neural tube. Tube of ectoderm that will form the brain and spinal cord in a vertebrate embryo.

Neurite. Long process growing from a nerve cell in culture. A generic term that does not specify whether the process is an axon or a dendrite.

Neuroepidermal junction. A structure formed from the union of skin and nerve fibers at the site of tissue loss in animals capable of regeneration. It produces the specific electrical currents that bring about the subsequent regeneration.

Neurohormone. A chemical produced by nerve cells that has effects on other nerve cells or other parts of the body.

Neuropeptide. Peptide secreted by neurons as a signaling molecule either at synapses or elsewhere.

Neoplasm. Abnormal mass of tissue as a result of neoplasia (i.e., abnormal growth or division of cells).

Neuron (nerve cell). Any of the impulse conducting cells of the nervous system.

Neurotransmitter. Extracellular signaling molecule that is released by the presynaptic neuron at a chemical synapse and relays the signal to the postsynaptic cell.

Neutrophil. White blood cell that is specialized for the uptake of particulate material by phagocytosis and which enters tissues that become infected or inflamed.

Newt. An aquatic amphibian of the family *Salamandridae.*

Nicotine adenine dinucleotide. See NAD^+.

Nicotine adenine dinucleotide phosphate. See $NADP^+$.

Noncovalent interaction. Any relatively weak chemical interaction that does not involve an intimate sharing of electrons. Multiple noncovalent interactions often stabilize the conformation of macromolecules and mediate highly specific binding between proteins.

Nonpolar. Referring to a molecule or structure that lacks any net electric charge or asymmetric distribution of positive and negative charges. Nonpolar molecules generally are insoluble in water.

N-type semiconduction. A negative current by movement of excess electrons.

Nuclear envelope. Double-membrane structure surrounding the nucleus; the outer membrane is continuous with the endoplasmic reticulum and the two membranes are perforated by nuclear pore complexes.

Nucleic acid. A polymer of nucleotides linked by phosphodiester bonds. DNA and RNA are the primary nucleic acids in cells.

Nucleoside. A small molecule composed of a purine or pyrimidine base linked to a pentose (either ribose or deoxyribose).

Nucleotide. A nucleoside with one or more phosphate groups linked via an ester bond to the sugar moiety, DNA and RNA are polymers of nucleotides containing deoxyribose and ribose, respectively.

Nucleus. Large membrane-bounded organelle in eukaryotic cells that contains DNA organized into chromosomes; synthesis and processing of RNA and ribosome assembly occur in the nucleus.

O

O_2^- or Superoxide (also hyperoxide). Compound that contains the superoxide anion with the chemical formula O_2^-; product of the one-electron reduction of dioygen O_2.

$^1O_2$ or Singlet oxygen. Electronically excited state of molecular oxygen (O_2): less stable than the normal triplet oxygen; highly reactive.

Oil immersion lens. In light microscopy, a lens used to increase the resolution of a microscope. This is achieved by immersing both the objective lens and the specimen in a transparent oil of high refractive index, thereby increasing the numerical aperture of the objective lens.

Oncogene. A gene whose product is involved either in transforming cells in culture or in inducing cancer in animals. Most oncogenes are mutant forms of normal genes (proto-oncogenes) that encode proteins involved in the control of cell growth or division.

Oncology. Branch of medicine that deals with cancer.

Oocyte. Immature egg cell (ovum).

Oogenesis. Formation and maturation of oocytes in the ovary.

Optic nerve. Transmits visual information from the retina to the brain.

Organelle. Any membrane-limited subcellular structure found in eukaryotic cells.

Ortho-localization (of energies). The energy to localize a pair of pi (π-) electrons between adjacent carbon atoms unconjugated with the rest of the molecule.

Oscillator (electronic). An electronic circuit that produces a repetitive, oscillating electronic signal, often a sine wave or a square wave. Convert direct current (DC) to an alternating current (AC) signal.

Osteoblast. Cell that secretes matrix of bone.

Osteoclast. Macrophage-like cell that erodes bone, enable it to be remodeled during growth and in response to stresses throughout life.

Osteocytes. Bone cells; produce collagen.

Osteogenesis. The formation of new bone, whether in embryogenesis, postnatal development, or fracture healing.

Osteon. Unit of bone with a small canal with a blood vessel and a nerve.

Outer membrane. Outermost of the two membranes surrounding an organelle: the membrane adjacent to the cytosol.

Oxidation. Loss of electrons from an atom or molecule as occurs when a hydrogen atom is removed from a molecule or oxygen is added. The opposite of reduction.

Oxidation potential. The voltage change when an atom or molecule loses an electron, a measure of the tendency of a molecule to loose an electron.

Oxidative phosphorylation. The phosphorylation of ADP to form ATP driven by the transfer of electrons to oxygen

(O_2) in bacteria and mitochondria. This process involves generation of a proton motive force during electron transport and its subsequent use to power ATP synthesis.

Oxygen electrode. Electrode that measures oxygen on a catalytic platinum surface.

P

Pathology. The study and diagnosis of disease. Pathologies is synonymous with diseases.

Pentose. A five-carbon monosaccharide. The pentoses ribose and deoxyribose are present in RNA and DNA, respectively.

Peptide. A small polymer usually containing fewer than 30 amino acids connected by peptide bonds.

Peptide bond. The covalent amide linkage between amino acids formed by a dehydration reaction between the amino group of one amino acid and the carboxyl group of another with release of a water molecule.

Perhydroxyl radical (also hydroperoxyl radical). Protonated form of superoxide with the chemical formula $HO_2^{\bullet}$ Hydroperoxyl is formed through the transfer of a proton to an oxygen atom.

Periosteum. A layer of tough, fibrous collagen that surrounds each bone. It contains cells that turn into osteoblasts during fracture healing.

Peroxide. Compound containing an oxygen-oxygen single bond or the peroxide anion ($[O–O]^{2-}$). Oxygen atoms in the peroxide ion have an oxidation state of −1, simplest peroxide is hydrogen peroxide. Generated via a transfer of two electrons (instead of 4) to O_2.

Peroxisome. Small organelle that contains enzymes for degrading fatty acids and amino acids by reactions that generate hydrogen peroxide, which is converted to water and oxygen by catalase.

pH. A measure of the acidity or alkalinity of a solution defined as the negative logarithm of the hydrogen ion concentration in moles per liter: $pH= -\log[H^+]$. Neutrality is equivalent to a pH of 7; values below this are acidic and those above are alkaline.

Phagocyte. General term for a macrophage or neutrophil that is specialized to take up particles and microorganisms by phagocytosis.

Phagocytosis. Process by which relatively large particles (e.g., bacterial cells) are specifically internalized by certain eukaryotic cells; distinct from receptor-mediated endocytosis.

Phosphatase. An enzyme that removes a phosphate group from a substrate by hydrolysis.

Phosphorylation. Reaction in which a phosphate group becomes covalently coupled to another molecule.

Photocathode. A negatively charged electrode in a light detection device such as a photomultiplier or phototube that is coated with a photosensitive compound.

Photoelectric effect. Refers to the process of emission of electrons from solids, liquids or gases when they absorb energy from light. Electrons emitted are called photoelectrons.

Photoelectric material. A substance that changes light into electrical energy, producing an electric current when light shines on it.

Photomultiplier tube (photomultiplier or PMT). Class of sensitive detectors of light in the ultraviolet, visible and near-infrared ranges of the electromagnetic spectrum. These detectors multiply the current produced by incident light by as much as 100 million times, in multiple dynode stages.

Photon. Elementary particle of light and other electromagnetic radiation. They are massless particles of definite energy, definite momentum, and definite spin.

Photon field. In order to explain the photoelectric effect, Einstein assumed that the electromagnetic field consists of discrete energy parcels, photons.

Photon language. A popular term referring to specific information in the photon signal.

Photon sucking. Active absorption of photon energy by one part of the living system from another part (by an embryo from the shell or membrane).

Photoreceptor. Cell or molecule that is sensitive to light.

Pi bond. Result from overlap of atomic orbitals that are in contact through two areas of overlap. Electrons in pi bonds are referred to as *pi electrons.*

Pi (π-) electrons. Electrons in pi bonds are sometimes referred to as pi electrons.

Piezoelectricity. Ability of a material to transform mechanical stress into electrical energy.

Piezoelectric material. A substance that changes mechanical stress into electrical energy, producing an electrical current when deformed by pressure or bending.

Plasma membrane. The membrane surrounding a cell that separates the cell from its external environment.

Pluripotent (cell). A cell that generates almost every cell.

Polar. Referring to a molecule or structure with a net electric charge or asymmetric distribution of positive and negative charges.

Polarity. In cell biology, presence of functional and/or structural differences in distinct regions of a cell.

Polycyclic aromatic hydrocarbons (PAHs). Potent pollutants that consist of fused aromatic rings.

Polymer. Any large molecule composed of multiple identical or similar units (monomers) linked by covalent bonds.

Polypeptide. Linear polymer of amino acids connected by peptide bonds.

Polyp (*Hydra*). Small, simple, fresh-water animals that possess radial symmetry belonging to the phylum *Cnidaria* and the class *Hydrozoa.*

Polyribosome. A complex containing several ribosomes, all translating a single messenger RNA; also called polysome.

Polysaccharide. Linear or branched polymer of monosaccharides, linked by glycoside bonds. An examples is glycogen.

Positional information. Information supplied to or possessed by cells according to their position in a multicellular organism. A cell's internal record of its positional information is called its positional value.

Posterior. Situated toward the tail end of the body.

Potential. Another term for voltage, which may at times be limited to a voltage that exists without a current but is potentially able to cause a current to flow if a circuit is completed.

Prediabetes. State in which some but not all of the diagnostic criteria for diabetes are met. Often described as the "gray area" between normal blood sugar and diabetic levels.

Preformation. One of two ways to explain the development of individual organic form: the specific contention that all organisms were created at the same time and that succeeding generations grow from miniature versions (homunculi, animalcule) that have existed since the beginning of creation. See also *Epigenesis.*

Primary channels (in Traditional Chinese Medicine). Twelve regular meridians that correspond to each of the six Yin and six Yang Organs.

Progenitor cell. Cell that has a tendency to differentiate into a specific type of cell, but is already more specific than a stem cell. Important difference is that stem cells can replicate indefinitely, and progenitor cells only a limited number of times.

Programmed cell death. See *Apoptosis.*

Prokaryotes. Class of organisms that lack a true membrane-limited nucleus and other organelles. See also *Eukaryotes.*

Prometaphase. Phase of mitosis preceding metaphase in which the nuclear envelope breaks down and chromosomes first attach to the spindle.

Prophase. Early stage in mitosis, during which the chromosomes condense, the centrioles begin moving toward the spindle poles, and the nuclear envelope breaks down.

Protein. A linear polymer usually containing 50 or more amino acids linked together by peptide bonds and folded into a characteristic three-dimensional shape (conformation).

Protein domain. Portion of a protein that has a tertiary structure of its own. Larger proteins are generally composed of several domains each connected to the next by short flexible regions of polypeptide chain.

Proteome. The entire complement of proteins produced by a cell.

Proteomics. Comprehensive analysis of the identity, interactions, and locations of proteins within a cell.

Proton. Positively charged subatomic particle that forms part of an atomic nucleus. Hydrogen has a nucleus composed of a single proton (H^+).

Proton motive force. The energy equivalent of the proton (H^+) concentration gradient and electric potential gradient across a membrane; used to drive ATP synthesis by ATP synthase.

Proton pump. Integral membrane protein that moves protons across a biological membrane.

Proto-oncogene. A normal cellular gene that encodes a protein usually involved in regulation of cell growth or differentiation.

Protozoa. Free-living or parasitic, non-photosynthetic, single-celled, motile eucaryotic organisms, such as *Paramecium* and *Amoeba.*

Proximodistal. From the center to the outer; used in medicine especially in relation to newborns and infants.

P-type semiconduction. A positive current by movement of holes in their electron clouds (which can be thought of as positive charges).

Pump. Any transmembrane protein that has ATPase activity and couples hydrolysis of ATP to the active transport of an ion or small molecule across a biomembrane against its electrochemical gradient.

Purine. A class of nitrogenous compounds containing two fused heterocyclic rings. Two purines, adenine (A) and guanine (G), are the base components of nucleotides found in DNA and RNA. See also *Base pairs.*

Pyrimidines. A class of nitrogenous compounds containing one heterocyclic ring. Two pyrimidines, cytosine (C) and thymine (T), are the base components of nucleotides found in DNA; in RNA, uracil (U) replaces thymine. See also *Base pairs.*

Q

Qi or Chi (Traditional Chinese Medicine). Vital energy or life force which flows through the meridians (or channels) and is used to protect, transform and warm the body.

Qigong (Traditional Chinese Medicine). Set of exercises including meditative and physical movements. Used to move qi, thereby maintaining and regaining physical, emotional and spiritual health.

Quantum efficiency. Ratio of number of photoelectrons produced per number of incoming photons.

Quaternary structure. The number and relative positions of the polypeptide chains in multisubunit proteins.

Quiescent. Referring to a cell that has excited the cell cycle and is in the G_0 state.

Quinone (Q). Small, lipid soluble, mobile electron carrier molecular found in the respiratory electron transport chain.

R

Radicle. The first part of a seedling (a growing plant embryo) to emerge from the seed during the process of germination.

Rate limiting enzyme. Enzyme that has lowest activity in a sequence of metabolic steps in a pathway.

Receptor. Any protein that specifically binds another molecule to mediate cell signaling. Most commonly denotes a protein located in the plasma membrane or cytoplasm that is activated by binding a specific extracellular signaling molecule (ligand), thereby initiating a cellular response.

Redifferentiation. The process in which a previously mature cell that has dedifferentiated becomes a mature, specialized cell again. See also *Dedifferentiation, Differentiation.*

Redox pair. Pair of molecules in which one acts as an electron donor and one as an electron acceptor in an oxidation/reduction reaction; for example, NADH (electron donor) and NAD^+ (electron acceptor).

Redox potential. The affinity of a redox pair for electrons, generally measured as the voltage difference between an equimolar mixture of the pair and a standard reference. $NADH/NAD^+$ has a low redox potential and O_2/H_2 has a high redox potential (high affinity for electrons).

Redox reaction. A reaction in which one component becomes oxidized and the other reduced; an oxidation/reduction reaction.

Reduction. Gain of electrons by an atom or molecule as occurs when a hydrogen atom is added to a molecule or oxygen is removed. The opposite of oxidation.

Reduction potential. The voltage change when an atom or molecule gains an electron.

Regeneration. Reactivation of development in postembryonic life to restore missing tissues.

Relay. Transmission of excited states, both dark and light.

Resonance. In physics, the tendency of a system to oscillate with greater amplitude at some frequencies than at others; relative maximum is known as resonant frequencies, or resonance frequencies.

Respiration, cellular. General term for any cellular process involving the uptake of oxygen (O_2) coupled to production of carbon dioxide (CO_2).

Respiratory chain. Set of four large multiprotein complexes in the inner mitochondrial membrane plus diffusible cytochrome c in the intermembranous space and coenzyme Q through which electrons flow from reduced electron donors (e.g., NADH) to O_2. Each member of the chain contains one or more bound electron carriers that sequentially accept and donate electrons.

Respiratory enzyme complex. Any of the major protein complexes of the mitochondrial respiratory chain that act as electron-driven protein pumps to generate the proton gradient across the inner membrane.

Ribosome. A large complex comprising several different RNA molecules and more than 50 proteins, organized into a large subunit and small subunit; the site of protein synthesis.

RNA (ribonucleic acid). Linear, single-stranded polymer, composed of ribose nucleotides. Three types of cellular RNA – mRNA, rRNA, and tRNA play different roles in protein synthesis.

RNA virus. Virus that has RNA (ribonucleic acid) as its genetic material; usually single-stranded RNA, but may be double-stranded RNA.

Rough endoplasmic reticulum (rough ER). Endoplasmic reticulum with ribosomes on its cytosolic surface. Involved in the synthesis of secreted and membrane-bound proteins.

Reactive oxygen species (ROS). Chemically reactive molecules containing oxygen. ROS form as a natural byproduct of the normal metabolism of oxygen. Examples include oxygen ions and peroxides.

S

Saccharomyces. Genus of yeast that reproduce asexually by budding or sexually by conjugation. Widely used as simple model organisms in the study of eucaryotic cell biology.

Salamander. A group of amphibians related to frogs but retaining a tail throughout their lives. Since salamanders are vertebrates, with an anatomy similar to ours, and since they regenerate many parts of their bodies, they are commonly used in regeneration research.

Sarcoma. Cancer of connective tissue.

Sarcoplasmic reticulum. Network of internal membranes in the cytoplasm

of a muscle cell that contains high concentrations of sequestered Ca^{2+} which is released into the cytosol during muscle excitation.

Sciatic nerve. The main nerve in the leg. It includes both motor nerve fibers carrying impulses to the leg muscles and sensory nerve fibers carrying impulses to the brain.

Second messenger. A small intracellular molecule whose concentration increases (or decreases) in response to binding of an extracellular signal and that functions in signal transduction.

Secondary structure. In proteins, local folding of a polypeptide chain into regular structures including the alpha helix, beta sheet, and U-shaped turns and loops.

Semiconduction. The conduction of electrical current through orderly molecular structures (e.g. crystals) in which electrons or "holes" (the absence of electrons) move from electron cloud around one nucleus to the cloud around another. They are the basic materials of the transistors and integrated circuits used in most electronic devices.

Senescence. Process of accumulative changes to molecular and cellular structure with the passage of time, resulting in deterioration and death.

Signal transduction. Conversion of a signal from one physical or chemical form into another. In cell biology commonly refers to the sequential process initiated by binding of an extracellular signal to a receptor and culminating in one or more specific cellular responses.

Signaling molecule. General term for any extracellular or intracellular molecule involved in mediating the response of a cell to its external environment or to other cells.

Singlet oxygen (or $^1O_2$). Electronically excited state of molecular oxygen (O_2), which is less stable than the normal triplet oxygen.

Smooth endoplasmic reticulum (smooth ER). Region of the endoplasmic reticulum not associated with ribosomes. It is involved in lipid synthesis.

Smooth muscle cell. Type of long, spindle-shaped mononucleate muscle cell making up the muscular tissue found in the walls of arteries and of the intestine and other viscera. Called "smooth" because it lacks the striated myofibrils of skeletal and cardiac muscle cells.

Soliton. A collective of excitations provided by specific molecular structures (for instance alpha helix of proteins). A soliton is not excited by light and its probability for light emission is small. See also *Exciton.*

Somatic cell. Any cell of a plant or animal other than a germ cell or germ-cell precursor (From Greek "soma", body).

Somite. One of a series of paired blocks of mesoderm that form during early development and lie on either side of the notochord in a vertebrate embryo. They give rise to the vertebral column, muscles and associated connective tissue. Each somite produces the musculature of one vertebral segment, plus associated connective tissue.

Spark (electric). Type of electrostatic discharge that occurs when an electric field creates an ionized electrically conductive channel in air producing a brief emission of light and sound.

Spectrograph. Instrument that separates an incoming wave into a frequency spectrum.

Spemann's Organizer. Specialized tissue at the dorsal lip of the blastopore in an amphibian embryo; a source of signals that helps to orchestrate formation of the embryonic body axis.

S phase. Period of a eucaryotic cell cycle in which DNA is synthesized.

Squeezed state. *In biophoton research:* a characteristic of the holistic property of the emitter.

Squeezed state index. *In biophoton research:* establishes quantum value of photon signal.

Stem cell. A self-renewing cell that can divide symmetrically to give rise to two daughter cells with a developmental potential identical to the parental stem cell or asymmetrically to generate one daughter stem cell and one with a more restricted developmental potential than the parental cell.

Steroids. A group of four-ring hydrocarbons including cholesterol and related compounds. Many important hormone (e.g., estrogen and progesterone) are steroids.

Striated muscle. Muscle composed of transversely striped (striatedly) myofibrils.

Stress. An organism's response to a stressor.

Subcutis also hypodermis. Lowermost layer of the integumentary system in vertebrates.

Substrate. Molecule that undergoes a change in a reaction catalyzed by an enzyme.

Subunit. Component of a multicomponent complex – for example, one protein component of a protein complex or one polypeptide chain of a multichain protein.

Sucrose. Disaccharide composed of one glucose unit and one fructose unit.

Sugar. Small carbohydrate with a monomer unit of general formula $(CH_2O)_n$. Examples are the monosaccharides glucose, fructose and mannose, and the disaccharide sucrose (composed of a molecule of glucose and one of fructose linked together).

Superconduction. The conduction of an electrical current by a specific mate-

rial that under certain circumstances (generally very low temperatures) offers no resistance to the flow. Such a current will continue undiminished as long as the necessary circumstances are maintained.

Superoxide anion (O_2^-). Compound generated via the transfer of a single electron (instead of 4) to O_2.

Superoxide dismutases (SOD). Enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide; important antioxidant defense.

Synapse. Specialized region between an axon terminal of a neuron and an adjacent neuron or other excitable cell (e.g. muscle cell) across which impulses are transmitted.

Syncytium. A multinucleated mass of cytoplasm enclosed by a single plasma membrane. Example: *Physarum polycephalium.*

Synergy. Interaction of multiple elements in a system to produce an effect greater than the sum of their individual effects.

T

Tadpole. Larval stage in life cycle of amphibian, particularly that of a frog or toad.

Telophase. Final mitotic stage during which the nuclear envelope re-forms around the two sets of separated chromosomes, the chromosomes decondense, and division of the cytoplasm (cytokinesis) is completed.

Temperature hysteresis. Dependence of a system not only on its current temperature but also on its past environment; this arises because the system can be in more than one internal state.

Teratogen. Chemicals and environmental factors that are known to cause birth defects in humans and animals.

Teratoma. An encapsulated tumor with tissue or organ components resembling normal derivatives of all three germ layers.

Tertiary structure. In proteins, overall three-dimensional form of a polypeptide chain, which is stabilized by multiple noncovalent interactions between side chains.

Thalidomide. Drug that was introduced in the late 1950s to be used as sleeping pill, and later to help pregnant women with the effects of morning sickness. It was withdrawn after being found to causes birth defects.

Thiobarbituric acid reactive substances (TBARS). Byproduct of lipid peroxidation which can be detected by the TBARS assay using thiobarbituric acid as a reagent.

Thyroid. Largest endocrine glands found in the neck.

Thermography. Infrared thermography is an example of infrared imaging; thermographic cameras detect radiation in the infrared range of the electromagnetic spectrum (roughly 9,000-14,000 nanometer or 9-14 μm).

Totipotent. A cell that generates every cell in an organism.

Traditional Chinese Medicine (TCM). A broad range of medicine practices sharing common theoretical concepts which have been developed in China and are based on a long tradition, including various forms of acupuncture, exercise, and herbal medicine.

Transcendental meditation (TM). Refers to a specific form of mantra meditation called the TM technique. The TM technique and TM movement were introduced in the mid-1950s by Maharishi Mahesh Yogi (1918-2008).

Transformation. Conversion of a "normal" mammalian cell into a cell with cancer-like properties usually induced by treatment with a cancer-causing agent.

Transforming growth factor beta (TGF-β). A family of second signaling proteins that are used in the development of most tissues in most or all animals. Mutations in TGF-β signal transduction components are implicated in human cancer, including breast cancer.

Triacylglycerol. A source in fat cells yielding glycerol and fatty acids.

Tricarboxylic acid cycle. See *Citric acid cycle.*

Tubulin. The protein subunit of microtubules.

Tumor. A mass of cells, generally derived from a single cell, that arises due to loss of the normal regulators of cell growth, may be benign or malignant.

Tumor progression. The process by which an initial mildly disordered cell behavior gradually evolves into a full-blow cancer.

U

Undifferentiated. Unspecialized, a term applied to cells that are in a primitive or embryonic state. See also *Dedifferentiation, Differentiation, Redifferentiation.*

Unsaturated. Describes a molecule that contains one or more double or triple carbon-carbon bonds, such as isoprene or benzene.

Urodela. Group of species of amphibians.

V

Van der Waals interaction. A weak noncovalent interaction due to small, transient asymmetric electron distributions around atoms (dipoles).

Vascularization. Process of formation of blood vessels and capillaries in living tissue.

Vegetal pole. The end at which most of the yolk is located is an animal egg. The end opposite the animal pole.

Ventral. Relating to the front of an animal or lower surface of a structure.

Ventricular fibrillation. Uncoordinated contraction of the cardiac muscle of the ventricles in the heart.

Vertebrate. Any of the animals that have backbones, including all fish, amphibians, reptiles, birds, and mammals. All vertebrates share the same basic anatomical arrangement, with a backbone, four extremities, and similar construction of the muscular, nervous, and circulatory systems.

Virus. A small intracellular parasite consisting of nucleic acid (RNA or DNA) enclosed in a protein coat that can replicate only in a susceptible host cell; widely used in cell biology research.

Voltage-gated cation channel. Type of ion channel found in the membranes of excitable cells (such as nerve cells and muscle) which opens in response to a shift in membrane potential past a threshold value.

W

Wheatstone bridge. Electrical circuit to measure an unknown electrical resistance by balancing two legs of a bridge circuit, one leg of which includes the unknown component.

White blood cell (leucocyte). General name for all the nucleated blood cells lacking hemoglobin. Includes lymphocytes, neutrophils, eosinophils, basophils, and monocytes.

X

***Xenopus laevis* (South African clawed toad).** Species of frog (not toad) frequently used in studies of early vertebrate development.

Y

Yang (Traditional Chinese Medicine). Represents heat and the ability to generate and maintain warmth and circulation. See also *Yin.*

Yeast. Common term for several families of unicellular fungi. Includes species used for brewing beer and making bread, as well as pathogenic species.

Yin (Traditional Chinese Medicine). Represents cool and the substances of the body, including blood and bodily fluids that nurture and moisten the organs and tissues. See also *Yang.*

Yoga. A generic term for the physical, mental, and spiritual disciplines which originated in ancient India with a view to attain a state of permanent peace. It is one of the six schools of Hindu philosophy.

Yolk. Nutritional reserves rich in lipids, proteins and polysaccharides, present in the eggs of many animals.

Z

Zen meditation. Zen is a school of Mahayana Buddhism that developed in China during the 6th century, and then spread to Vietnam, Korea and Japan. The word Zen is derived from the Sanskrit word dhyāna which can be translated as "absorption" or "meditative state".

Zygote. A fertilized egg; diploid cell resulting from fusion of a male and female gamete.

Author Index

Abbe, 37
Aladzhalova, 259, 269, 292
Allen, 142, 240
Amato, 68, 71
Ampère, 228
Andres, 61, 70, 75
Arnold, 117, 289
Arvanataki, 283, 288
Audubert, 93, 105-106, 108, 110-111
Ausprunk, 71
Austin, 113, 241, 270
Bachman, 250, 253, 256, 270
Bang, 67, 70, 373
Barnard, 208, 275
Barton, 233, 240-241
Bassett, 251, 253, 256-257, 271
Bateman, 101, 108, 110-111
Becker, 244-245, 248-249, 251, 253-254, 256-257, 270-271, 286, 288, 291
Belogolowy, 61, 70, 75
Benolken, 283, 288
Berenblum, 65, 70
Bieske, 343, 359
Blokha, 119, 127
Boling, 233, 241
Boltzmann, 104
Bonnet, 18-19
Borodin, 101, 109
Boveri, 45-46, 51, 73
Boveris, 142-144, 149-150, 214-215
Brizhik, VII, 332, 373
Brown, A. M., 283, 288
Brown, H. M., 283, 288
Buchner, E., 162, 176
Buchner, H. E. A., 162
Bütschli, 37
Burk, 167-169, 177
Burr, 148, 215, 225, 230-244, 291
Buu-Hoï, 277-287
Cadenas, 142-144, 149-150, 287
Cameron, 169, 177
Chalazonitis, 283, 288
Chance, 142-144, 149-150
Child, 46-47, 50-51, 73, 227
Choi, 355, 360
Chwirot, VII, 285, 289
Cilento, 139, 150, 205, 215, 281, 285, 287-289, 297, 301, 319
Claus, 101, 105-106, 110, 113
Cohen, 70, 343, 359
Cohnheim, 59-60, 69
Cori, C., 165, 182-183
Cori, G., 165, 182-183
Danforth, 208, 215
Davydov, 329-330, 332, 340
Denham, 202
Dinsmore, 15, 32-33
Dredge, 68, 71
Driesch, 41-42, 44-46, 50-51, 73, 79, 81, 85, 153, 227, 234, 323
Dunaway, 193, 196
Dürken, 61, 70, 75
Einstein, 79, 103-105, 110, 113, 153, 401
Ellermann, 67, 70
Embden, 165
Endo, 195, 216
Eremko, 332
Faraday, 104, 319
Fischer, 148, 283, 288
Flanigan, 169, 177
Folkman, 68, 71, 212
Frank, 91, 93, 108, 111
Franke, 263, 269
Frank-Kamenetskii, 264
Fridovitch, 202
Friedewald, 65, 70
Fröhlich, 288, 332
Fukada, 252, 257
Galtsoff, 61, 70, 75
Galvani, 266
Gauss, 228
Gerschman, 202, 214
Goldenberg, 93
Goldblatt, 169, 177
Goldfarb, 193
Goodsir, 37
Gorlich, 115
Gott, 233, 241
Granger, 208
Gray, 113
Greengard, 193
Grennel, 236
Gurwitsch, A. A., 99-100, 284, 289
Gurwitsch, A. G., 50, 79, 85-94, 96-101, 106, 108-109, 111, 113, 118, 121, 123, 138, 153, 227, 234, 283-285, 288-289, 292-293, 325, 398
Gutteridge, 151, 204, 215
Halliwell, 151, 204, 214-215
Harary, 263, 269, 292
Harden, 162, 165, 176
Harvey, 20, 236-237, 241
Hatzfeld, 193, 196
Hauser, 59, 69
Hay, 26-27, 32
Hearse, 208, 215
Henkart, 283, 288
Hertz, 101-102, 105, 110, 300, 395
Hill, VI, 94, 149, 240
Ho, 325, 337, 340, 373
Hollaender, 101, 105-106, 109-110, 113
Hooke, 35
Hörstadius, 48, 52, 73
Hunter, 56
Iams, 115
Ichikawa, 65
Inaba, 129, 131-132, 134, 146-147, 285, 289-290, 342, 358, 361-363, 372-373, 379
Jenning, 208, 215-216
Jung, 321, 355, 360
Kalendaroff, 94
Katz, 208, 215
Kennedy, 197
Kikuchi, 283, 288
Klimenko, 119
Kobayashi, VII, 146-147, 290, 344, 359, 363-364, 367, 373-374, 376
Komarov, 119
Konev, 79, 118-121, 127, 136, 138
Kratochwill, 62, 70, 75
Kubetsky, 114, 116
Lane, 232, 240
Laville, 230, 240-241, 291
Lehninger, 197
Lenard, 103-104, 110
Lenhoff, 18, 32
Levi, 60, 70, 106
Levy, 93, 105, 110
Lilius, 286, 290
Lister, 35
Locatelli, 24
Loffe, 89
Lorenz, 110, 113

Lubischev, 89
Lubsandorzhiev, 116, 126
Lund, 230-231, 241, 243
L'vova, 123, 127
Lysenko, 98
Malmgren, 169, 177
Marinesco, 93-94
Marnilla, 286
Mauro, 235, 241
Maxia, 98
Maxwell, 102-104, 110, 260, 310
McCord, 202, 214-216
Metcalf, 123, 126
Meyerhof, 165, 176
Mitchell, 201, 257, 270
Mittal, 213
Morgagni, 56
Morgan, 19, 22, 32, 83
Morris, 179, 186, 193, 195-196, 319-320
Müller, 37, 58-59, 69, 106-111, 394
Murard, 213
Musselman, 233, 240-241
Nakamura, 342, 358
Neuberg, 165
Newport, 37
Nims, 232-233, 240
Northrop, 234, 240
Obruchev, 89
Oesterheld, 283, 288
Ostrowski, 123, 127, 215
Palade, 197
Parnas, 165
Perelygin, 119, 127
Pitot, 193, 195, 320
Pizzarello, 65, 70, 75
Planck, 104, 110, 171
Popp, VII, 100, 141-142, 148-149, 273-274, 277, 280-281, 285-290, 292, 308, 310, 312, 316, 319, 321-322, 340, 343-344, 359
Pott, 56, 58, 65, 275
Potter, 193-195, 320
Presman, 266-270, 273, 292, 301
Pullman, 215, 275, 280, 286, 292
Que Hee, 134, 147
Quellet, 113
Quickenden, 121, 123, 126, 134, 136-137, 147
Rahn, 101, 110, 113
Ramazzini, 56
Ras, 101, 109
Raspail, 35
Réaumur, 13, 15, 18
Reske, 111, 119, 127
Ribbert, 59, 70
Richards, 101, 109
Richardson, 320
Rose, 24, 26, 32, 62, 68, 70-71, 75, 215
Rous, 65, 67, 70
Roux, 41-42, 51, 70, 340
Ruth, 141-142, 148-149, 287-288, 303, 319, 321
Rutherford, 106, 110
Salzberg, 115
Samuel, 59, 70
Sato, 146-147, 193, 195, 216, 373
Sauermann, 344, 359
Schamhart, 285, 289, 306, 320-321
Schleiden, 37
Schmidt, 275, 286, 292, 321
Schoeffel, 101, 109
Schwann, 37
Seilern-Aspang, 62, 70, 75
Sel'kov, 264
Seyfert, 105-106, 110
Shnol', 264, 269
Shtrankfel'd, 119, 127
Shubik, 65, 70
Sies, 144, 149-150, 214-215, 287
Singer, 27-28, 33
Sinyukhin, 243-244
Sjostrand, 197
Slawinska, 138, 148-149, 215, 289-290
Slawinski, VII, 128, 138-139, 147-149, 205, 215, 284, 286, 289-290, 321, 379
Slepian, 113
Soh, VII, 344, 355, 359-360, 384
Spallanzani, 15, 19, 32, 44, 172
Spemann, 47-48, 51-52, 73
Starke, 113
Stauf, 119
Stauff, 111, 123, 127, 151, 215
Stoletov, 102-103, 110
Strehler, 117
Strumpf, 73
Stumpf, 48, 50, 52
Summer, 116, 126
Sung, 277, 280, 286, 288, 292
Szent-Gyorgyi, 263-264, 269
Tada, 208, 215
Taffel, 236-237, 241
Tamm, 89
Tarusov, 79, 118-119, 127, 136, 140, 215
Taylor, 101, 109, 208, 215, 358
Tennant, 208, 215
Thiersch, 58, 69
Thompson, 261, 320
Thornton, 26, 29, 32
Tilbury, 121, 134-138, 147-148, 285, 290
Tillmanns, 58
Todd, 24, 32
Trabka, 263, 269
Trembley, 15, 17-18, 32, 44
Tsong, 335, 339
Turing, 323, 340
Usa, 134, 147, 290, 373
Van Allen, 397
Van Aken, 286, 290, 321
Van Wijk, IV, 175, 178, 285-286, 289-290, 303, 306, 308, 312, 320-321, 344, 348, 351-352, 355, 359-360, 373-374, 376, 384
Vassiliev, 93
Virchow, 35, 37, 58-59, 69-70, 74, 85
Vladimirov, 79, 118, 123, 127, 136
Von Helmsbach-Meckel, 58, 69
Wang, 121, 151, 321
Warburg, 165-166, 169-172, 176-177, 197, 219
Weber, 61, 70, 75, 186, 193-196, 320
Weinberg, 171, 176-177
Weinhouse, 193, 195-196, 320
Weismann, 37, 40-42, 51, 83, 387
Weiss, 48, 52, 61, 70, 75, 227, 265, 269
Westinghouse, 113
Wicks, 306, 320
Wiener, 263, 269
Wiggers, 208, 215
Williams, 71, 124, 144, 150, 215-216
Wolff, 101, 109, 251, 358
Wolpert, 48, 52, 73
Wolsky, 48, 52, 65, 70, 75
Wooldridge, 261, 292
Yamagiwa, 65
Yanagawa, 342, 358, 379
Yasuda, 252
Young, 162, 165, 176
Zavadovsky, 89
Zhabotinskii, 264
Zhirmunskii, 244
Zhuravlev, 79, 118, 123, 127-128, 205, 215, 290
Zworykin, 115-116

Subject Index

Acetoacetyl CoA, 302, 396
Acetyl CoA, 172, 180, 184, 197, 199-200, 302, 385, 389, 396
Acetylcholine, 28, 385
Actin, 269, 330, 332, 385, 389-390, 398-399
Action potential, 300, 385-386
Acupuncture, 144-145, 150-151, 342, 355, 358-360, 363, 372, 378-379, 381, 384-385, 392, 398-399, 405
  meridian, 144-145, 150-151, 355, 360, 379-380, 384-385, 388, 398, 402
  point, 144-145, 342, 363, 372, 379, 381, 385, 392, 398
Adenosine diphosphate (ADP), 165, 190, 201, 331, 385-386, 400
  ADP reductase, 190
Adenosine triphosphate, 177, 215, 382, 387, *see also* ATP
Adenylosuccinase, 190
Adenylosuccinate synthetase, 190
Aerobic, 136, 162-163, 165-166, 169-170, 172-173, 175-178, 182, 197, 201, 220, 314, 385-386, 393-394
  aerobiosis, 165-166, 169-170, 172-173, 175, 177, 290
  alternation cycle between anaerobiosis and aerobiosis, 169-170, 172, 176
  and anaerobic, 136, 162-163, 166, 169-170, 172-173, 175, 177-178, 197, 220, 314, 386, 393-394
  and glycolysis, 136, 162-163, 165-166, 169-170, 172-173, 175-178, 182, 197, 220, 393-394
  metabolism, 170, 176, 177
Aging, 144, 220, 214-216
Alcoholic fermentation, 111, 162, 165-166, 176
Aldolase, 163, 176-177, 189, 306-307
  aldolase B, 306
Allium cepa, 105, 110
Alpha cells, 184-185, 394
Alpha helical structure, 250, 326, 329-330, 332, 335, 385, 393, 404
Alpha ketoacids, 302, 385
Alpha ketoglutarate, 199, 302, 320, 394
Alpha particles, 106, 110, 385, 394
Alpha rays, 106, 110
Alpha tocopherol, 132-133, 147, 204, 207, 385
Alternating electric field, 339, *see also* Electric field
Alternation cycle, 169-170, 172, 176
  between anaerobiosis and aerobiosis, 169-170, 172, 176
Amino acid, 170, 182-184, 186, 194, 203, 302-303, 307, 327, 385, 394, 396, 398, 401-402
  degradation, 185, 302, 303
  metabolism, 183, 184, 185, 186, 302, 303
  and protein structure, 327
Amphibians, 22, 244, 256-257, 270-271, 325, 403, 405-406
Amplitude, 141, 268, 335, 337, 390, 403
  and frequency, 268, 335, 403
  window, 337
Amputation, 15, 19, 21-22, 24, 26, 28-29, 32, 59, 62, 65, 73-74, 245-246, 251, 386
Anaerobes, 166, 169-170, 172, 176
  facultative anaerobes, 167, 170, 172, 176
Anaerobic, 136, 162-163, 166, 169-170, 172-173, 175, 177-178, 197, 220, 314, 386, 393-394
  and aerobiosis, 165-166, 169-170, 172-173, 175, 177, 290
  alternation cycle between anaerobiosis and aerobiosis, 169-170, 172, 176
  anaerobiosis, 165-166, 169-170, 172, 175, 177, 290
  bacteria, 136, 162
  and glycolysis, 136, 162-163, 165-167, 171-173, 197, 219, 393-394
Anaplasia, 60
Aneurogenic limb, 28-29, 74, 386
Angiogenesis, 28, 68, 71, 212, 220, 386
  factor, 28, 68, 220
  inhibitors, 68, 71, 212, 220, 386
Anisotropy, 335
Anoxic, 206, 211-212, 215
  cell pocket, 210-211
  and ischemia, 151, 160, 203, 206-210, 212-216, 220, 263, 376
Antero-posterior dominance, 38, 46, 51
Antioxidant, 131, 204, 207, 209, 214-216, 359, 386, 405
  catalase, 204, 205, 209
  superoxide dismutase, 202, 204-205, 209, 214, 216, 405
  system, 204, 209
Apatite, 250-251, 253-254, 256, 291, 386, 389
Apical cap, 22, 26-27, 74
Ascites cancer cells, 142, 150, 169-170, 270, 386
  Ehrlich ascites cells, 142, 150, 170, 270
Atherosclerosis, 127, 214-215
ATP, 125, 163, 165-166, 172-173, 182-183, 189-190, 197, 199-202, 206, 208, 301, 330, 332, 335, 339, 385-389, 392, 394, 397-402
  formation, 163, 165, 166, 172, 189, 197, 201, 208, 332
  hydrolysis, 165, 183, 330, 332, 335
  synthase, 200, 201
Autotomy, 15, 386
Axon, 28, 74, 124, 283, 386, 388, 390, 399, 405
Bacterium, 1, 105-106, 118, 121, 123-124, 126, 136, 142, 147, 162, 172-174, 177-178, 199, 203-204, 231-232, 283, 285, 288, 290, 385-386, 393, 397, 401
  anaerobic, 136, 162
Base, 209, 297, 315-316, 318, 386-387, 395, 402
  base modification, 209, 391
  DNA, 209, 297, 315-316, 318, 386-387, 395, 402
  metabolism, 189, 190, 191, 192
Benzo[a]pyrene, 66
Beta cells, 183, 185, 396
Beta particles, 106, 387, 394
Beta rays, 387
Beta-carotene, 204
Biochemical imbalance, 186, 188-190, 193-194, 219
  in carbohydrate metabolism, 188, 194, 219
  in colon tumor, 193
  in muscle sarcomas, 193
Biocommunication, 290, 339-340

and biological organization, 339
Bioelectrodynamic process, 340
Bioelectromagnetism, 5, 8, 133, 225, 227, 230, 235, 259-260, 293, 338, 339, 340
bioelectromagnetic field, 8, 230, 339
Biological clock, 264, 387
rhythm, 261, 283, 343, 359, 387, 389, 391
rhythmic body function, 261, 387, 389, 391
Biological organization, 82, 98, 339, 387
and biocommunication, 339
Biological semiconduction, 225, 246, 256, 291
Bioluminescence, 1, 123-124, 126-127, 146-148, 151, 153, 215, 285, 289, 358
Biophores, 40, 387
Biophotochemical reaction, 280, 301
Biophoton, V, VI, 3, 5, 7, 75, 123, 129, 131, 133, 145, 147-149, 226, 262, 280-283, 285, 287, 289-290, 297, 321, 339-340, 343-344, 358-361, 372-374, 381, 383, 387, 404
biophoton emission, V, 123, 129, 131, 133, 147-148, 226, 262, 282, 285, 289-290, 321, 340, 359-360, 373, 381
biophoton language, 8, 293, 297-298, 339, 341, 356, 401
Biosurface, 134
electric potential of, 134, 234, 243, 391
Blastema, 22, 24-32, 58-59, 62, 70, 74-75, 161, 225, 246, 251, 291, 387, 392
in regeneration, 14, 26, 28, 29, 31, 32, 42, 62, 74, 160, 161, 387, 391
Blastomer, 38, 41-42, 45-46, 387
Blastophore, 47
Blastula, 38, 41-42, 46, 48, 73, 336, 387, 392
Blood, 22, 28, 31, 39, 53-55, 61, 68, 71, 83, 108, 127-128, 131-132, 134, 146-148, 154, 162, 169, 179-180, 182-185, 203, 210-212, 220, 236, 245, 250-251, 259, 289, 302, 351, 356, 360, 375, 380-381, 385-387, 391-400, 402, 406
supply, 28, 68, 179, 210-212, 220
in Traditional Chinese Medicine, 380, 387, 406
vessel growth, 68, 210-212, 220, 386, 392, 406
Body pattern, 8, 225, 298, 323, 337, 340
Bone, 22, 31, 52, 84, 225, 250-257, 271, 291, 380, 386, 388-389, 392, 400-401, 406
cells, 250-251, 389, 400
and copper, 254
formation, 251
fracture, 225, 245, 250-251, 254, 256-257, 270-271, 291, 400-401
as a light emitting diode (LED), 253-255
responds to stress, 250-254, 256, 291
Brachial nerve, 28-29, 244, 387
ganglia, 244
Brain, 83, 93-94, 146, 149, 179-180, 184, 206, 210, 225, 240, 244, 259, 261-263, 268-269, 283, 289-290, 292, 364, 373, 376, 385, 388, 390, 395, 397, 399-400, 404
brain research, 259, 283
Breath, 144, 150, 316, 352, 395
photon emission in breath, 144, 150
Cancer, 2, 7-8, 13-14, 53-56, 58-59, 61-63, 65-71, 74-75, 155, 159, 166-172, 176-177, 179, 186-190, 193-197, 206, 209-210, 212-214, 216, 219-220, 225, 230, 238-241, 264, 273, 275, 277-278, 286, 291-292, 299, 319-320, 360, 363, 373, 375-376, 387, 391, 397-400, 403, 405
as electromagnetic disturbance, 225, 230, 291
epidemiology, 56
research, 62, 65, 69, 169, 171, 176, 186, 193, 292, 320
thermodynamic state of cancer cells, 171
Candida, 24, 123, 136, 147
and photon emission, 123, 136, 147
Capacity, 8, 20, 22, 28, 62, 66, 73, 84-85, 106, 117, 140, 168, 170, 172, 182, 190, 250-251, 274, 281, 297, 306, 308, 311, 314, 329, 364, 380, 393
Capillary, 31, 210-211, 406
endothelium, 211
in tumor growth, 210
Carbamoylphosphate synthetase II, 189
Carbohydrate metabolism, 162, 175, 177-178, 186, 188-189, 195, 320
in cancer, 186, 188-189, 195
and Molecular Correlation Concept, 176-187, 320
Carbonyl groups, 136, 206, 387
in photon emission, 136, 206
Carcinogen, 55-56, 58, 60, 62-63, 65-66, 70-71, 74, 166, 275, 277-278, 280, 286-287, 292,320-321, 387, 391, 393, 395
and carcinogenesis, 55, 56, 60, 62, 63, 65-66, 70-71, 166, 275, 278, 280, 286-287, 292, 301, 320, 387
and electronic excitation energies, 275, 277, 278, 280, 292
resonance theory for carcinogenesis, 8, 281, 286, 292
Carcinoma, 14, 55, 59-60, 69, 108, 177, 193, 195, 209, 238, 387, 395
Caryokinesis, 86, 388
Catalase, 204-205, 209, 216, 401
antioxidant system, 204-205, 209, 216, 401
CCD, V, 134, 298, 359, 363-364, 366-369, 373-374, 376, 381, 388, 392
Cell cycle, 31, 120-121, 126, 304-305, 388-389, 396-397, 403-404
phases, 120-121, 274, 304-305, 336, 386, 388-389, 396-397, 402-405
and photon emission, 120-121, 126, 141
regulation, 304-305, 388
Cell death, 38, 53, 68, 141, 206-207, 210, 299, 386, 399, 402
and photon emission, 141-142
Cell differentiation, 39, 60, 68, 159, 237, 398
Cell directional motion, 265
Cell division, 13, 18, 38, 41, 53, 68, 74, 79, 87-88, 91, 109, 121, 138, 147, 155, 167, 212, 226, 264, 292, 299, 304-305, 361, 388-389, 394, 396-398
Cell lines, 60, 289, 303, 306-309, 312, 320-321
and degree of differentiation, 60, 303, 307-308, 320-321
and photon emission, 297, 299, 303, 308, 312, 314
Cell maturation, 86, 400
Cell membranes, 27, 62, 84, 87, 143, 147, 150, 197, 199-201, 203-205, 210, 217, 220, 248, 262, 274, 283, 287-288, 314-315, 325, 327, 330, 332-333, 335-339, 385-394, 396-403, 405-406
Cell proliferation, 22, 33, 55-56, 60, 159, 161, 211-212, 219, 221, 237, 395
Cell reproduction, 86
Cell specification, 48, 50
Cell theory, 21, 37, 69, 73

Cellular ecosystem, 48
Central Nervous System, 94, 216, 259, 327, 364, 388, 394
and mitogenetic radiation, 94
and photon emission, 94, 364
Cerebral cortex, 261
and mitogenetic radiation, 261
and photon emission, 261
Cerebral visual tract, 94
and mitogenetic radiation, 94
and photon emission, 94
Chain reactions, 96, 98-99, 153, 207, 209
Charge density, 275, 277-278, 292
distribution within molecule, 275, 277, 278
Chemical induction of carcinogenesis, 273
Chemiosmotic theory, 200-201
Chromosomes, 40, 45, 265, 270, 274, 288, 304, 388, 398, 400, 402, 405
Chronic, 144, 151, 341, 352, 355-356
disease, 144, 355-356
psychosocial stress, 352
Citric acid cycle, 165-166, 172, 174-175, 180, 197, 199-201, 205, 263, 302, 385, 389, 405
Cleavage, 38, 41-42, 46, 163, 205-206, 327, 336, 387, 389, 392, 397-398
Coal tar-induced cancer, 56, 65-66, 275
Coherence, 262-263, 281, 288-290, 321, 325, 360, 389
coherent excitations, 288-289, 332
Collagen, 237, 250-251, 253-254, 256-257, 291, 360, 386, 388-390, 393-394, 400-401
and apatite, 250-251, 253-254, 291, 386, 389
and bone, 250, 251, 253
formation, 237, 250, 251
and piezoelectricity, 253-254, 257, 291
Collective excitations, 297, 389, 327, 329, 404
Color filters, 117, 342, 348
and spectral analysis, 117, 342, 348
Communication, VI, 1, 124, 126, 129, 265, 273, 281, 283-285, 288, 290, 319, 339-340, 394
biocommunication, 290, 339-340
Compensatory regeneration, 31
Competence, 47
Conduction, 94, 108, 225, 246-248, 250, 256, 262, 283, 291, 328, 376, 396, 398, 400, 402, 404
ionic conduction, 247, 396
metallic conduction, 246, 398
Conformational state, 264, 280-282, 286, 327, 330-333, 339, 389-390, 400, 402
of DNA, 280, 286, 327, 389-390, 400, 402
of protein, 264, 281-282, 330-333, 339, 389-390, 400, 402
Conjugated dienes, 208
Contraction, 15, 93, 125, 226, 263-264, 266, 292, 330, 340, 385-386, 398-399, 406
and myofibrils, 330
of striated muscles, 93, 125, 226, 263, 264, 266, 292, 330
Copper, 201, 204, 254
in bone, 254
Coronary thrombosis, 207
Cosmic rays, 108, 230, 268
Coupling of electric energy to chemical potential energy, 337
Cristae, 26, 199, 307
in mitochondria, 199, 307
CTP synthetase, 189
Current of injury, 225, 236-238, 241, 243, 246, 248, 252-257, 271, 291
Cyclic 3'5'-AMP, 175, 184, 303-304, 306, 320, 385, 387, 390
in catabolite repression, 175
in DNA duplication, 306
in growth inhibition, 304, 306
in liver regulation, 183, 184
as secondary messenger, 183, 303, 304
Cytochrome c oxidase, 201-202, 390
Cytoskeleton, 38, 289, 314, 327, 331-332, 390, 396, 398
and actin, 269, 330, 332, 385, 389-390, 398-399
and microfilaments, 331-332, 336, 390, 398-399
Darkroom, 341-342, 344-345, 368
Decay, 207, 280-281, 308-309, 312, 315, 317, 325
Dedifferentiation, 26, 29, 31-32, 60-61, 70, 74-75, 171, 213, 256-257, 271, 390-391, 403, 405
and growth, 26, 31, 70, 74-75, 213
Delayed luminescence, 117, 139, 142, 148-149, 290, 308-309, 313-314, 317, 319, 321, 360-361, 390
delayed photon emission, 303, 308-309, 312-315, 317, 319, 321
Delayed photon emission, 303, 308-309, 312-315, 317, 319, 321
delayed luminescence, 117, 139, 142, 148-149, 290, 308-309, 313-314, 317, 319, 321, 360-361, 390
Dendrites, 261, 388, 390, 399
and ganglion cells, 244, 261, 394
Dense heterogeneous medium, 284, 390
Dermis, 22, 26-27, 32, 38, 47, 390, 392, 404
Diabetes mellitus, 214, 391
Dibenz-anthracene, 65
Differentiation, 3, 7-8, 13, 26-27, 29, 31-33, 39-41, 45-46, 50, 53, 56, 60-62, 68, 70, 73-75, 82, 88, 98, 155, 159, 161-162, 167, 169-172, 176-177, 179, 186, 190, 193-195, 197, 206, 210, 212-213, 219-220, 234-235, 237, 256-257, 271, 297, 299, 303, 307-308, 319-321, 323, 327, 387, 390-391, 398, 402-403, 405
dedifferentiation, 26, 29, 31-32, 60-61, 70, 74-75, 171, 213, 256-257, 271, 390-391, 403, 405
degree of, 8, 167, 186, 190, 195, 289, 297, 303, 307-308, 321
and photon storage, 297, 303, 308
Differentiation/proliferation duality, 32, 60, 159-160, 176, 212, 216, 219-220, 237, 299
Dihydrothymidine dehydrogenase, 189
Dihydroxyacetone phosphate, 182-183
Dipole, 228, 260, 262, 264, 297, 391, 405
dipole/dipole interactions, 264
Dissipation of energy, 139, 285, 301, 333, 391
DNA, 8, 29, 37-38, 50, 66, 82, 120, 138, 155, 175, 180, 187, 189-190, 192, 194-195, 202, 209-210, 219, 269, 280, 286-287, 292, 297, 304-306, 315-316, 318-322, 329, 386, 388-395, 397-402, 404, 406
damage, 66, 209, 220, 280, 392
polymerase, 189
repair, 210, 391, 393
Doping, 254, 391
Drosophila, 19, 288, 325, 336-337, 340, 391, 398
development, 336-337, 340
Dying and photon emission, 141-142
Dynode, 114-115, 391, 401, *see also*

Secondary electron emitters
Earth electric field, 267-268
Earth magnetic field, 267-268, 397
Ectodermis, 38, 50, 336-337, 391, 398-399
Ectopic limb, 29, 74, 391
Ehrlich ascites cells, 142, 150, 170, 270
Electric, 29, 102-104, 115, 119, 125-126, 134, 215, 228, 234, 236-237, 241, 244, 249, 254, 256-257, 260-262, 266-271, 288-289, 303-304, 310, 328, 333-334, 337, 338-339, 386, 389, 391-392, 394, 400-402, 404
charge, 228, 254, 260, 328, 389, 400-401
field, 29, 115, 228, 234, 241, 245, 249, 254, 256-257, 260, 262, 267, 268, 270-271, 288, 303-304, 310, 338-339, 404
potential, 134, 228, 234, 240, 243, 256, 390, 402
Electrical, 29, 84, 93, 102, 104, 106, 110, 114, 125, 133, 147, 201, 208, 225, 227-228, 230-234, 236, 240-241, 243, 246, 252-254, 256-257, 259-263, 266, 268, 270-271, 274, 288, 290-291, 323, 335, 337, 339, 385, 389, 391, 394, 399, 401, 404, 406
current, 29, 102, 103, 107-108, 114-115, 147, 201, 208, 225, 228, 231-233, 243-244, 246, 248-249, 252-257, 260, 270-271, 290-291, 385, 390, 391, 392, 394, 399, 400-402, 404
resistance, 84, 102, 106, 232-233, 236, 405-406
voltage, 102, 107, 109, 114-115, 225, 231-233, 235-236, 238-239, 241, 244, 246, 248-249, 253, 255, 257, 270-271, 291, 334, 385, 391, 397, 400, 402-403, 406
Electrical stimulation, 257, 266, 270-271
Electrodynamic, 225, 230-231, 234-236, 239-240, 291, 323, 337, 340, 391
field, 230-231, 234-236, 240
theory of life, 225, 231, 234, 240, 291, 391
Electrode, 102, 107-108, 114-115, 125, 134, 170, 230-233, 235, 244, 250, 257, 271, 391, 401
Electromagnetic, 2, 7-8, 91, 102-104, 149, 225-228, 230, 239-240, 243, 259-260, 262-270, 273-274, 281, 283-286, 288, 291-293, 297, 301, 310-311, 315, 319, 321, 323, 332, 335, 337-340, 394-395, 397, 401, 405
bio-information, 226, 271, 286, 288, 319
energy, 230, 266, 274
radiation, 260, 262, 264, 268, 281, 284-285, 289, 301, 332, 392, 394, 397, 401,
spectrum, 91, 266, 401, 405
Electromagnetic field, 7-8, 104, 225-227, 230, 239-240, 243, 259-260, 263-269, 273, 283-285, 291-292, 297, 301, 310, 319, 323, 337-338
environmental field, 236, 265-269, 391
external field, 260, 267-268, 276, 335, 337, 339
interrelationship between macromolecules, 226, 259, 263-265, 267, 269, 289, 292
in living organisms, 7-8, 225-227, 230, 239-240, 243, 259-261, 263-271, 273, 283-285, 288-289, 297, 301, 310, 319, 323, 337-338
oscillatory system, 225, 261-262, 292
Electrometric, 225, 232-234, 236-239, 241, 291
correlates, 225, 232, 234-239, 241, 291
studies and wound healing, 225, 238-239, 241, 291
Electromotive force, 228
Electron, IV, V, 4, 26-27, 29, 102-105, 107, 110, 113-116, 129, 138, 146, 149-150, 165-166, 197, 199-203, 205-210, 219, 225, 227-228, 230, 238, 246-250, 252-253, 256, 261, 264, 270, 274-277, 280, 282, 284, 286-287, 289, 292, 300-302, 306, 314, 319, 322, 328-329, 332, 340, 363-364, 369, 373, 375, 387, 389-393, 395-396, 398-405
electron spin resonance spectroscopy, 208-209, 392
motive force, 200-201
pairing, 332
Electron transport chain, 165-166, 199-201, 390, 392, 396, 403
Electrostatic fields, 235, 239, 249, 268, 270
Embden-Meyerhof pathway, 165
Embryo, 2, 5, 13-15, 18, 20-21, 24, 28-29, 35-48, 50-52, 59-62, 68-71, 73, 75, 84, 86, 88, 98-99, 159, 161, 169-170, 193-194, 220, 234-235, 251, 257, 271, 323, 325, 327, 336-338, 340, 386-387, 389-396, 398-401, 403-405
embryonic development, 21, 44, 47, 86, 193-194, 220, 398
embryonic field, 35, 323, 393
embryonic rest hypothesis, 59
Endoplasmic reticulum, 26, 306-307, 392-394, 396-398, 400, 403-404
Endothelial cells, 31, 68, 210-213, 312, 392
Energy, 1, 5, 7, 24, 50, 74-75, 95-100, 102-105, 109-110, 117, 126, 139, 142, 145, 153, 155, 159-162, 165-166, 170, 172-175, 179-180, 195, 197, 199-202, 205-207, 210, 213, 219-220, 230, 235, 243, 252, 254-255, 266, 274, 277, 280-281, 285-286, 288, 292, 297, 300-301, 311-312, 319, 321, 325, 328-330, 332, 335, 337, 339-340, 358, 361, 373, 378, 380-381, 385-402
from ATP hydrolysis, 330, 332, 387, 399
of hydrogen bond, 327, 329, 385-387, 389
transfer, 280, 297, 328-329, 340
Entelechy, 44-45, 81, 83, 85, 234
principle, 85
Enzyme, 1, 31, 125, 159, 162-163, 165-167, 170, 172-178, 180, 183, 186-190, 193-197, 199-205, 207-209, 215-216, 219, 264-265, 269, 283, 299, 302-303, 306-307, 319-320, 332, 335, 337, 339, 375, 385-386, 388-390, 392-393, 396-399, 401, 403-405
fetal enzymes, 193, 196
isozym, 186, 188-189, 193, 195-196, 306-307
key enzymes, 159, 173, 186-190, 193, 219, 302-303, 307, 396
Epidermis, 22, 27, 32, 38, 47, 390, 392
Epigenesis, 13, 18-21, 32, 35, 40, 44, 73, 392, 402
Epimorphosis, 22, 31, 392
Epiphysis, 250
epiphyseal plates, 250
Epithelium, 26-27, 30-31, 33, 41, 55, 58-59, 62, 69-70, 238, 386-387, 392, 395-396
Equifinality, 42, 73, 393
Equipotentiality, 42, 44, 50, 73, 393
Estrous cycle, 233-234, 241, 393

and voltage, 233-234, 241
Excited, 18, 93-97, 119, 133, 136, 138-139, 143, 150, 160, 202, 205-207, 209, 213, 215, 275, 280-284, 287, 292, 300-301, 315, 319, 321-322, 328-329, 332, 375, 378, 393, 395, 400, 403-404
carbonyl compounds, 132-133, 136, 138, 205-207
oxygen dimers, 136, 138, 206-207
Exciton, 297, 326-329, 332, 340, 393, 404
metastable exciton, 332
and soliton, 297, 326-329, 332, 340, 393, 404
Exponential phase of growth, 136-138, 147
and photon emission, 136-138, 147
Exposure to light, 94, 132, 268, 281, 334
Facultative anaerobes, 166, 170, 172, 176
FAD, 199-201, 205, 390, 393
in respiration, 199-201, 205
FADH, 199-201, 205, 393
in respiration, 199-201, 205
Fasting, 183-184, 194, 356, 393
early fasting state, 183-184
Fatty acids, 177, 180, 183, 207, 210, 302, 385, 393, 397, 401, 405
Fermentation, 7, 111, 159, 162, 165-167, 169-173, 176-177, 219, 393, *see also* Alcoholic fermentation; Glycolysis
Fertilization, 37, 39, 46, 125, 166, 193, 265, 327, 336, 393
Fetal liver, 193
Fibroblast, 28-31, 33, 74, 108, 169, 236-237, 312-313, 327, 389, 391, 393
growth factor, 28, 31, 74, 327, 393
proliferation, 236-237
Field, V, 1-3, 5, 7-9, 13, 22, 29, 32, 35, 44-45, 47-48, 56, 68, 73, 75, 79, 85-86, 88-89, 97-100, 104, 113-116, 119, 127, 131, 136, 145, 153-154, 161, 176, 202, 213-214, 216, 219-220, 225-228, 230-232, 234-236, 239-241, 243-245, 248-249, 256-257, 259-260, 262-271, 273-275, 280, 283-286, 291-293, 297, 301, 310, 319, 323, 325, 329, 333-340, 358, 376, 383, 385, 390-393, 397-399, 401, 404
biophoton field, 5, 7, 75, 102
field of organization, 35, 323, 393
Fracture, 225, 245, 250-251, 254, 256-257, 270-271, 291, 400-401, *see also* Bone fracture
Free radical, V, 95, 131-133, 143-144, 150, 154, 159-160, 202-206, 208-210, 213-216, 219-220, 256, 264, 285, 352, 360, 386, 397
in blood plasma, 130, 132
process of lipid peroxidation, 131, 132, 143, 144, 154, 203, 352
scavenging system, 133, 204-205, 207, 209, 215-216
Frequency, 67-68, 87, 89, 103-105, 116, 254, 257, 259-266, 268-271, 291, 311, 329, 332, 334-335, 337, 339, 389-390, 392-394, 404
spectrum, 259, 291, 404
window, 297, 337, 339
Frog, 18-19, 24, 26, 38, 41, 47, 60-63, 94-95, 105-106, 108, 110, 119, 140, 235, 241, 244-246, 254, 261, 266, 283, 403, 405-406
in development, 38, 41, 47, 60, 62
in regeneration, 24, 26, 62, 63, 245, 246, 254
Fumarate, 302, 394
Fungi, 121, 124, 140, 394, 406
photon emission of, 121, 124, 140
Galleria mellonella, 48
Gamma rays, 106, 108, 230, 267, 394
Gastrulation, 38, 46, 336, 387, 394
Geiger-Müller counter, 79, 95, 106-111,153, 394
Gene, 159, 175 187-190, 211, 216, 219, 320, 337, 391-392, 394, 399, 400, 402
regulation, 29, 175, 189, 193-194, 211, 304, 306, 337, 394
Germ plasm, 37, 40-41
theory, 37, 40
Germination, 117-118, 126, 134, 139, 148, 169, 361, 363, 373, 395, 403
germinating soybean, 134, 146-150, 361-363, 373,
Glucagon, 183-185, 303-304, 306, 394, 396
Glucogenic amino acids, 302-303, 394
Gluconeogenesis, 180, 182-186, 189, 194, 302-303, 385, 397
Gluconeogenic enzymes, 159, 188, 219, 303
Glucose, 31, 83, 100, 150, 159, 162-163, 165-166, 172-175, 177-180, 182-186, 189, 194-195, 197, 201, 203, 219, 302, 304, 306, 320, 356, 393-394, 396, 404
Glucose 6-phosphate, 180, 183-184, 189
glucose 6-phosphatase, 189
glucose 6-phosphate dehydrogenase, 189
Glutamine PRPP amidotransferase, 189
Glutathione peroxidase, 204
and antioxidants, 204
Glycogen, 180, 183-185, 195, 394, 402
Glycolysis, 7, 136, 159, 162-163, 165-167, 171-173, 176-177, 180, 182, 184-185, 189, 194, 197, 206, 219, 306, 320, 393-394
glycolytic enzymes, 159, 163, 173, 180, 188, 219, 306, 307, 309
GMP synthetase, 190
Gradient, 13, 46-48, 50-52, 73, 200-201, 225, 227, 233, 235, 237-241, 256, 265, 291, 335, 339, 385, 388, 391-392, 394, 396, 402-403
Growth, 3, 7, 19, 22, 26, 28-29, 31-33, 39, 35, 53, 56, 58-61, 65, 68-71, 74-75, 86, 88, 91, 98, 120-121, 129, 136-138, 144, 147, 153-155, 161-162, 167, 169-170, 172-175, 177-179, 186, 190, 192, 194-195, 210-213, 219-220, 225, 227, 234, 239-241, 243, 250-251, 253, 256-257, 270-271, 273, 275, 291, 299, 303-304, 306-307, 319-320, 327, 361, 386, 389-393, 395-396, 398-400, 402, 405
growth rate, 91, 161-162, 167, 169-170, 175, 179, 186, 190, 192, 194-195, 219, 299, 303, 306-307, 320
H35, 303, 306-309, 320
Haber-Weiss reaction, 203, 215, 395
Hall effect, 248-249, 291
Harden-Young ester, 165, 176
Healing, 26, 29, 225, 236-238, 241, 243-245, 248, 251, 254, 256-257, 270-271, 291, 362, 378-379, 393, 399-401
wound healing, 26, 29, 225, 236-238, 241, 243-245, 248, 257, 271, 291, 362, 393
Heart muscle cells, 31, 33, 327, 388
Heme, 201, 390, 395
Hemiparesis, 355, 360, 395
Hepatocarcinogenesis, 66, 179, 186, 193, 195, 301, 395
Hepatocytes, 31, 144, 149, 206, 306-309, 312, 320-321, 395
Hepatoma, 159, 176, 186, 188, 190, 192,

194-196, 216, 219, 297, 299, 303-304, 306-309, 312, 314, 319-321
Morris hepatoma, 179, 186, 193, 195-196, 319-320
Novikoff hepatoma, 179, 186, 194
Hepatoma cell lines, 193, 297, 303, 306-309, 312-314, 320
Hertz effect, 101
Hexokinase, 163, 180, 189, 195, 306-307
High energy potentials, 96
High-energy, 96, 98, 102, 105, 117, 153, 155, 197, 199-200, 202, 205-206, 213, 219-220, 277, 297, 387, 394-395
excited states, 202, 205, 213, 220, 277
excited states in photon emission, 96, 98, 105, 117, 153, 155
Homeostasis, 83, 140-141, 148-149, 154, 183, 259, 285, 290, 357, 395
perturbation of, 140-141, 148-149, 154, 285, 290
Homogenate, 123, 131-132, 143, 146, 149, 195, 315, 388, 395
of internal organs, 132, 143, 146, 149, 195
Homoplastic, 14, 60-61, 70, 75, 395
HTC, 303, 306-309, 313-314, 320
Hydra, 15, 17-19, 21, 31-32, 51, 230, 386-387, 402
in regeneration, 15, 17-19, 21, 31-32, 51
Hydroperoxide, 143, 149, 203, 206, 208, 214-215
Hydroxyl radical, 144, 151, 202-204, 208, 213, 395, 401
stimulates guanylate cyclase, 213
Hypoxia, 169, 206-207, 210-211, 215-216, 351, 386, 396
hypoxia-inducible factor I (HIF-I), 211-212
hypoxic injury, 206
IMP dehydrogenase, 189-190
Implantation, 14, 51, 60-61, 65, 70, 75, 257, 270, 363, 395
Inaba Biophton Project, 129-134, 146-147, 361-363, 373, 379
Inflammation, 58-59, 151, 203, 395-396, 400
Information, V, 3, 5, 7, 24, 29, 42, 48, 50, 52, 62, 73-75, 80, 82, 85, 87, 119, 129, 131, 138, 142, 145, 150-151, 154-155, 178, 221, 226, 232, 259, 263-264, 267, 273, 280-284, 286, 288, 291-293, 297, 319, 323, 332, 335, 339, 348, 361-362, 364, 368, 373, 376, 383, 388-389, 391, 394, 396, 400-402
positional information, 5, 42, 48, 50, 52, 73-75, 402
Initiation in carcinogenesis, 14, 62, 66-67, 91, 211
Injury, 22, 30-32, 53, 73, 92, 206-209, 213-216, 220, 225, 236-237, 241, 243-246, 291, 361-363, 387, 396, 399
current and regeneration, 246, 291
injured limbs, 225, 244, 362
Inosine phosphorylase, 189
Insulin, 183-185, 304, 306, 391, 396
Intracellular morphology, 306, 320
Iodoacetate, 206
Ionic current, 246-248, 396
Iron, 2, 84, 204, 214, 225, 228, 250, 291, 395-396
iron-sulfer clusters, 201, 396
Irritation, 58
Ischemia, 151, 160, 203, 206-210, 212-216, 220, 263, 376
ischemia/reperfusion injury, 160, 203, 208-208, 213-214, 216
ischemic myocardium tissue, 151, 208, 214, 216
ischemic reperfused heart, 208, 216
ischemic reperfusion and pathogenesis of cancer, 206, 214
Isozyme, 186, 188-189, 193, 195-196, 306-307
isozyme shift, 189, 195-196, 306
JEDEC (Joint Electron Devices Engineering Council), 116
K^+ influx, 334-335, 385, 399
potassium pump, 224-335, 337, 339, 399
Ketogenic amino acid, 302, 396
L-field, 230-231, 397
Lactate dehydrogenase, 183
Language, 8, 145, 261, 293, 297-298, 335, 339, 341, 356, 401
cell language, 8, 293, 298, 335, 339, 401
Laterality, 29, 38, 52, 89, 195, 234-236, 274, 288, 364, 371-372, 380-381, 387, 397
Leukocytes, 142-143, 146, 149, 287, 376
Life, IV, V, VI, 2-3, 7-8, 13, 15, 20-22, 35, 37, 45, 56, 81, 83, 100, 121-123, 125, 131-132, 134, 144, 146, 150, 153, 159, 161, 169-170, 172, 202, 208, 210, 214, 216, 225-226, 230-232, 234, 240-241, 243, 261, 266-267, 273, 282-283, 285, 291, 301, 311, 316, 319-320, 335, 341, 352, 356, 358-360, 373, 375, 378, 388, 391, 396, 398, 400, 402-403, 405
definition of, 84
life style, 2, 8, 56, 134, 341, 356
Ligand binding, 335, 337, 389, 397, 403
and frequency, 337
Light emission, V, VII, 1-2, 5, 7-8, 79-80, 91, 103, 113, 117-123, 126, 129, 131-134, 136-150, 153-155, 159-160, 194, 197, 205-207, 209, 214-215, 220, 225-226, 253-255, 262, 273, 282, 284-286, 289-290, 293, 297, 301, 303, 308-309, 312-315, 317, 319, 325, 321-322, 327-329, 339, 340-346, 348-355, 357-369, 370-379, 381, 383, 384, 390, 397, 404, *see also* Photon emission
in muscle stimulation, 119, 122
in nerve excitation, 119, 122
Light-induced photon emission, 142, 301, 308-309, 312-313, 315-316, 317, 319, 321
Limb, 13, 15, 22, 25-33, 62, 70-71, 74, 225, 243-246, 248-251, 256-257, 270, 291, 380-381, 386, 391
aneurogenic limb, 28-29, 74, 386
ectopic limb, 29, 74, 391
regeneration, 13, 15, 19, 22, 24, 25, 26, 27, 28, 29, 30, 31, 62, 74, 245, 246, 251
Lines of force, 2, 103-104, 110, 225, 228, 230, 244, 248, 291
Lipid peroxidation, 131-133, 143-144, 146, 149, 154, 203, 208, 214-216, 219, 397, 405
lipid endoperoxides, 208
lipid hydroperoxides, 143, 203, 208
Liver, 29, 31-33, 38, 51-53, 83-84, 100, 123, 126-127, 131-133, 143-144, 146, 149, 167-168, 170, 172, 177, 179-180, 182-187, 189, 192-196, 209-210, 214, 219, 227, 283, 299, 302-304, 306-309, 312, 319-320, 375, 380, 394-395
in carcinogenesis, 66, 159, 179, 186, 193, 219, 301
liver-specific glucokinase, 180, 189, 195
Living Systems Theory, 75, 84, 153

Long-range interactions, 139, 259, 289, 311, 321
of electromagnetic fields, 259
Luminol, 142-143, 375-376
luminol-mediated luminescence, 142-143, 375-376
Macrophages, 31, 39, 142-143, 149, 397, 400-401
Magnet, 2, 44, 114, 225, 228, 291
magnetic field, 2, 7-8, 104, 225-228, 230, 239-240, 243, 248-249, 259-260, 263-270, 273, 283-285, 291-292, 297, 301, 310, 319, 323, 336-338, 340, 385, 390, 392, 397, 401
magnetism, 5, 103-104, 110, 225, 228, 257, 259-261, 271, 293
Malonaldehyde, 208
Marrow cells, 31, 251
in bone regeneration, 251
Mature cartilage cells, 26, 32, 62, 251, 388-389
Maxwell equation, 102-104, 260, 310
Maxwell-Boltzmann kinetic theory of gases, 104
Maxwell's thermodynamic relations, 104
Mechanical, IV, 5, 20-21, 58, 75, 79, 81, 85, 89, 93, 98, 104, 114, 139, 149, 153, 250, 252, 256, 278, 285, 289, 297, 332, 337, 401
load in bone, 251-257, 291
loads, 139, 251-257, 289, 330
stress and electrical energy, 251-254, 256-257, 291, 401
Meditation, 352-354, 359-360, 398, 403, 405-407
photon emission in, 352-354, 359-360
Membrane, 27, 62, 84, 87, 143, 147, 150, 197, 199-201, 203-205, 210, 217, 220, 248, 262, 274, 283, 287-288, 314-315, 325, 327, 330, 332-333, 335-339, 385-394, 396-403, 405-406
membrane potential, 201, 262, 283, 390-391, 397, 402, 406
and photon emission, 134, 142, 315, 325
Meristem, 86, 89, 398
and photon emission, 86, 89
Mesenchyme, 46, 398
Mesoderm, 38, 47, 52, 312, 336-337, 398, 404
Metabolism, 5, 8, 50, 75, 83, 127, 145, 155, 159-160, 162, 165, 166, 168, 170, 173-180, 183-186, 188-190, 193-197, 199, 203, 209, 214-216, 219-220, 226-227, 264, 280, 286, 292-293, 306, 320, 364, 373, 383, 385, 393, 396, 403
metabolic alterations, 159, 186, 196, 219
metabolic lesion, 179
Metallic conduction, 246, 398
Metastability, 97, 332, 398
Metastasis, 14, 53, 54, 56, 59, 61-62, 69, 74-75, 170, 216, 299, 397-398
Methylcholanthrene, 65
MH_1C_1, 303, 306-307, 320
Miasmic organic influences, 58, 398
Microfilaments, 331-332, 336, 390, 398-399
Microtumors, 68, 210-212
Microwaves, 230, 260, 262, 268, 270, 288, 311
Minimal deviation tumor, 186, 195
Mitochondria, 7, 26-27, 84, 123, 127, 143-144, 149, 165, 171, 174, 183, 197, 199, 201-203, 205-206, 209-210, 215-217, 220, 306-307, 315, 320, 332, 386, 389-390, 392-393, 396, 398-399, 401, 403
mitochondrial DNA, 210, 398
mitochondrial matrix, 26, 199, 200, 201
mitochondrial membrane, 199, 205, 386, 390, 392, 396, 403
and ROS (free radicals), 143, 203, 210
Mitochondrial functioning, 7, 144, 171, 210, 220, 306
defective mitochondria, 210, 306
mitochondrial dysfunction, 7, 171, 220
Mitogenetic radiation, 5, 8, 91-94, 96-101, 109-111, 118, 121, 123, 126, 136, 138, 147, 206, 288, 398
Mitogenic growth factor, 28, 33, 398
Mitosis, 7, 18, 28, 31, 33, 35, 37, 38, 41, 53, 68, 73, 79, 86-87, 89, 91, 120, 138, 147, 153, 155, 190, 210, 237, 274, 304, 310, 320, 336, 386, 388, 390, 394, 396, 398, 402, 405
mitotic divisions, 33, 41, 73, 86, 336
Molecular complexes, 97, 200-201, 315, 327, 386-388, 390, 396-399, 402-403, 405
molecular constellations, 97
molecular orderliness, 96-97, 248, 404
multienzyme complexes, 200, 398, 403
Molecular Correlation Concept, 7, 29, 31, 159, 186-187, 194, 219, 301, 303, 306-307, 319-320, 399
Morphallaxis, 31, 392
Morphogenesis, 5, 13, 42, 48, 50-52, 73, 75, 79, 85-86, 88, 98, 101, 153-154, 234, 323, 340, 393, 398-399
morphogenetic field, 5, 48, 75, 79, 81, 88, 101, 153-154, 234, 323, 398-399
Morphogenetic Field Theory, 88, 98, 153, 227, 231
morphogens, 8, 48, 73, 225, 323
Morris hepatoma, 186, 195
Mosaic development, 35, 40-42
Moxa, 145, 342, 379, 399
Multialkali photocathode, 117
Multienzyme complexes, 200, 398, 403
Muscle, 15, 26, 29, 31-32, 35, 38, 41, 53, 55, 59, 62, 83-84, 93, 108, 119, 127, 165, 170, 172, 179-180, 182-184, 193, 196, 203, 210, 215, 226, 230, 240, 244, 261, 263-264, 266, 270, 292, 330, 332, 340, 380-381, 385-390, 394, 396, 398-399, 404-406
gastrocnemis muscle, 119
photon emission of, 119, 127
sartorius muscles, 119
Mutation, 66, 203, 205-206, 210, 337, 393, 399, 405
and patterning of abnormalities, 338, 340
Myofibrils, 26, 330, 399, 404
Myosin, 269, 330, 332, 385, 389, 399
Na^+ efflux, 334-335
NAD^+, 165, 199-200, 399-400, 403
NADH, 199, 200-201, 205, 393, 399, 403
NADH-Q oxidoreductase, 201
$NADP^+$, 199, 399-400
NADPH, 181, 203, 215
Neo-vitalism, 41, 44, 50-51, 81, 243
Neoplasm, 54, 56, 58, 60, 65-67, 71, 186-187, 190, 193-195, 209, 220, 299, 399
Nerve, 21, 24, 26-30, 32-33, 35, 41, 67-68, 71, 74, 84, 92-95, 100, 105-106, 108, 110, 119, 127, 153, 235-236, 241, 244-245, 248, 250, 254, 256, 259, 262, 266, 270, 283, 288, 291, 380, 386-388, 394, 399-400, 404, 406
bulbs, 27, 283

fibers, 26-27, 33, 94, 248, 387, 399, 404
Nervous system, 29, 33, 38, 47, 55, 93-94, 99, 183, 216, 225, 234, 236, 241, 244-245, 259, 261, 291, 327, 364, 385, 388, 391, 394, 400, *see also* Parasympathetic nervous system; Peripheral nervous system
Neuronal membrane, 283, 288
Neurodegeneration, 214, 216
neurodegenerative disease, 214, 216
Neurotransmitter, 28, 385, 400
Neuroepidermal junction, 22, 26-27, 399
Novikoff hepatoma, 186, 194
Nucleotide, 146, 180, 186, 189-190, 193-194, 199-200, 203, 306, 316, 320, 330, 385-386, 389, 391, 393, 399-400, 402-403
O_2, 132-133, 143, 165-166, 169, 175, 184, 192, 199-203, 206-207, 209, 302, 306, 320, 362-363, 385-386, 389, 395, 400-401, 403-405, *see also* Oxygen
and differentiation, 159, 161-162, 167, 169-172, 197, 206, 210, 212-213
excited O_2 species, 133, 136, 138, 143, 159-160, 202, 205-207, 209, 213, 220, 378, 393, 400, 404
tension, 143
Oncology, 53, 58, 65, 74, 128, 159, 171, 176, 186, 216, 400
Onion experiment, 87, 89-91, 105-106, 108, 110
Optically coupled, 138
Orderliness, 96-97, 311
Organ transplantation, 22, 24-25, 28-29, 47-48, 51, 60-63, 70, 75, 208, 216,257, 271, 399
Organization, 3, 5, 8, 13, 20, 24-26, 47-48, 53, 55, 73, 75, 79, 82, 84, 96-98, 100, 153-154, 227, 230, 234, 236, 241, 267, 269, 293, 297, 299, 301, 310, 318, 327, 338-340, 376, 387, 391, 393
organiser, 47-48, 73
organizational field, 8, 24-25, 154
Oscillation, 3, 226, 259, 262-263, 269, 292-293, 339, 343, 387, 389-390
oscillating field, 104, 289, 333, 339
Osteoblast, 251, 389, 400-401
Osteogenesis, 251, 257, 271, 400
Osteon, 250
Oxidation, 51, 79, 119, 131-133, 143-144, 146, 148-149, 151, 154, 165, 197, 199, 201, 203-204, 208-209, 214-216, 219, 263-264, 284, 352, 375, 385, 392, 397, 400-401, 403, 405
oxidation reactions, 79, 119, 143, 151, 284
oxidation/reduction center, 201
oxidative deterioration, 131, 146, 396
Oxidative phosphorylation, 127, 199-201, 385-386, 393, 398-400
Oxidative processes, 47, 376, 378
oxidative radical reactions, 80, 143, 149, 154
oxidative stress, 143-144, 209, 213-215, 352, 364, 373
Oxygen, 5, 7, 56, 68, 79, 96, 118, 125-126, 132-133, 136-138, 142-144, 146-149, 151, 153-154, 159, 163, 165-166, 168-172, 176-177, 182, 197, 199-212, 214-216, 219-220, 286-287, 302, 313-314, 342, 351, 360, 374-378, 385-387, 390, 393, 395-396, 400-401, 403-405, *see also* O_2
consumption, 164-166
deficiency, 165-166, 168-170, 176, 211
deprivation in carcinogenesis, 159, 166, 168-169
oxygen pressure in the tumor, 169, 170
oxygen-dependent radical damage, 202
oxygenation, 144, 351
pressure, 159, 166, 169-170, 219
reoxygenation, 7, 212
Pancreas, 83, 183-185, 194, 209, 391, 394, 396, *see also* Alpha cells; Beta cells
Parasympathetic nervous system, 183
Patterning abnormalities, 338
Pentose phosphate pathway, 180, 187, 203
PEP carboxykinase, 183, 303, 306, 320, *see also* Phosphoenolpyruvate carboxykinase
Periosteum, 251, 254, 291, 401
Peripheral nervous system, 236, 385
Peroxide, 131, 136, 143-144, 146, 148-151, 201-206, 208-209, 213-216, 287, 352, 395, 400-401, 403, 405
Perturbed homeostasis, 140-141, 148-149, 154, 285, 290
Phagocyte, 203, 290, 401
Phagocytosis, 39, 143, 146, 154, 265, 400-401
Phosphoenolpyruvate carboxykinase, 183, 303, 306, 320, *see also* PEP carboxykinase
Phosphofructokinase, 163, 173, 189, 196
Phosphoribosylpyrophosphate (PRPP), 189
Photo-effect, 102-103
first law of photo-effect (Stoletov's law), 102-103
photoelectric effect, 101-102, 104-105, 110, 113, 153, 254, 256, 401
Photobiochemistry, 139, 280-281, 283, 285, 287-289, 301
without light, 139, 280-281, 285, 287-289, 301
Photocathode, 95, 114-117, 123, 361, 363, 401
Ag-O-Cs, 114-115
multialkali, 117
Photoelectrons, 102, 105, 114, 116, 129, 138, 363, 401, 403
Photomultiplier, V, 79, 109, 113-117, 119, 121, 123, 126, 129, 134, 138, 144-145, 153-154, 209, 281, 283, 297, 303, 308, 341-342, 344-345, 348-349, 355, 358-359, 361-362, 364, 374, 377-378, 391, 401
photomultiplier tube, V, 79, 113-117, 119, 121, 129, 145, 153, 281, 303, 308, 341-342, 344-345, 349, 355, 361, 377, 391, 401
Photon, V, VI, VII, 1-3, 5, 7-9, 75, 79-80, 91, 93, 96-99, 102, 104-105, 108, 110, 113, 117-121, 123, 126, 129, 131-134, 136-151, 153-155, 159-160, 194, 197, 205-207, 209, 213-215, 219-220, 225-226, 255, 262, 268, 273-275, 280-290, 292-293, 297-298, 300-301, 303, 308-315, 317, 319, 321-323, 325, 327-328, 339, 340-346, 348-361, 363-374, 375-379, 381, 383, 384, 386-387, 392-394, 401, 403-404
Photon count distribution, 348, 359-360, 362, 369, 393
Photon emission, V, VII, 1-2, 5, 7-8, 79-80, 91, 103, 113, 117-123, 126, 129, 131-134, 136-150, 153-155, 159-160, 194, 197, 205-207, 209, 214-215, 220, 225-226, 253-255, 262, 273, 282, 284-286, 289-290, 293, 297, 301, 303, 308-309, 312-315, 317, 319, 325, 321-322, 327-329, 339, 340-346,

348-355, 357-369, 370-379, 381, 383, 384, 390, 397, 404, *see also* Light emission
from algae, 117, 121-122
from animals, 119, 121-124, 131-133, 140, 144, 362-364
from bacteria, 105-106, 118, 121-124, 126, 136, 143, 285, 290, 397
from fungi, 121-122, 124, 140
from human, VII, 297, 341, 343, 346, 351, 353, 355, 358-359, 364, 376-377
from insects, 121, 124-125
from muscles, 108, 119, 122
from yeast, 91-93, 108, 119-124, 126, 134, 136-138, 147-149, 154, 290
Photon imaging, V, 1, 131, 297-298, 344, 359, 361-364, 368-369, 372-374, 376, 383
Photon storage, 275, 281, 292, 297, 301, 303, 308, 310-312, 319, 344
coherent modes of, 274-275, 280-281, 285, 310
model, 274-275, 292, 297, 308, 312, 315
Physarum, 236, 405
Piezoelectricity, 251-254, 256-257, 291, 401
Planaria, 22, 31, 46, 51
Plasma membrane, 27, 203, 336, 385-386, 388, 390, 396-397, 399, 401, 403, 405
Pluripotent, 42, 44, 73, 392, 401
Polarity, 13, 42, 44-47, 51, 73, 238, 243, 245, 249, 288, 333, 337, 380, 386, 398, 401
Polycyclic aromatic hydrocarbons, 66, 275-280, 286, 292, 401
Polyp, 15, 17-18, 32, 148, 250, 289, 326-327, 329, 387, 395, 402-405
Polyunsaturated fatty acids, 210, 397
Positional information, 5, 42, 48, 50, 52, 73-75, 402
Potassium pump, 224-335, 337, 339, 399
K^+ influx, 334-335, 385, 399
Preconditioning, 213
Preformation, 13, 18-21, 32, 35, 40-42, 44, 73, 392, 402
Proliferation, 22, 26-33, 35, 54-56, 59-62, 74, 159, 161, 176, 186, 190, 193, 210-212, 219-220, 236-237, 240, 273, 299, 320, 363, 376, 393, 395
Promotion, 66-67, 216
Protein, 2, 8, 26-27, 29, 38, 50, 124, 127, 149, 170, 172, 175-176, 178, 183-184, 193, 199-202, 209, 211, 215, 217, 220, 227, 250, 262, 264, 274, 281, 288, 297, 302, 304, 315-320, 326-327, 329-335, 337, 339-340, 385-400, 402-406
breakdown of, 183
synthesis, 27, 29, 50, 178, 183-184, 302, 320, 395, 397, 403
Proton, 199-201, 227, 248, 264, 283, 385, 390-392, 395, 399, 401-403
motive force, 200-201, 401-402
pump, 200-201, 283, 390, 399, 402
Protoplasmic streaming, 236
Purine metabolism, 159, 189-190, 219
Pyrimidine metabolism, 159, 189-190, 219
Pyruvate, 163, 165-166, 172, 174, 180, 182-184, 189, 197, 199, 219, 302, 306-307, 320, 385, 393-394
pyruvate carboxylase, 183
pyruvate kinase, 163, 189, 306-307
Qigong, 342-343, 358-359, 403
Quantum, 3, 105, 108, 115, 128-129, 146, 148, 205, 264, 268, 273, 278, 280, 286, 290, 300, 316, 319, 321-322, 328, 340, 349, 356-357, 359-360, 369, 403-404
efficiency, 115, 349, 369, 403
of light, 104, 125, 205, 153
revolution, 105
Radial symmetry, 89, 402
Radiation, 3, 5, 7-8, 13, 56, 75, 79-80, 85, 88-89, 91-111, 113, 117-119, 121, 123-127, 132, 134, 136, 138, 141-142, 147-148, 150, 153-155, 206, 214, 220-221, 230, 260, 264, 267-270, 273, 280-281, 283-286, 301, 309-310, 321-323, 325, 332, 337, 341, 375, 387, 390, 394-395, 397-398, 401, 405
degradative radiation, 96, 140-142, 325, 390
field, 8, 13, 75, 79, 81, 85, 89, 153-154
spectrum, 91-92, 94, 101, 109, 117, 121, 123-124, 127, 131, 136-137, 141, 150, 153, 207, 230, 259, 263, 266, 268-269, 291, 299, 315, 343, 348-349, 369, 401, 404-405
Radio, 33, 93, 106-108, 110, 114, 124, 126, 230, 260, 263, 265-266, 269-270, 311, 359, 386-387, 395
communication, 265
waves, 230, 395
Rate limiting enzymes, 187-190, 403
Reaction diffusion model, 323
Reactive oxygen species, 7, 79, 126, 142, 202-204, 206, 215, 219, 313, 374, 376-378, 395, 403
Realization factor, 86-87
Receptor, 87-88, 281, 288, 390, 395, 401, 403-404
Redox regulation, 160, 164, 213-214
Regeneration, 2, 5, 13-19, 21-22, 24, 26-33, 42, 46-47, 51, 60, 62-63, 65, 67, 70-71, 73-75, 92, 161, 165, 168, 186, 194, 200, 204, 220, 243, 245-246, 251, 256-257, 270-271, 360, 385, 387, 392, 399, 403
compensatory, 31-32
in humans, 29, 33
of kidney, 31
regenerative process, 24, 28-29, 65, 74, 243, 246
Relay, 28, 96, 400, 403
Reperfusion, 7, 160, 203, 206-210, 212-216, 220
and arrhythmias, 208
injury, 208-209, 213-214, 216, 220
of ischemic tissue, 220
and ventricular fibrillation, 208
Resonance, 3, 8, 87-88, 208-209, 256, 264, 273, 276-277, 280, 287, 292, 297, 310, 328, 332, 339, 392, 403
principle of resonance, 88
resonating contour, 88
theory for carcinogenesis, 280, 287, 292
Respiration, 7, 125, 142, 147, 159, 166-167, 169-172, 174-178, 182, 197, 200, 203, 205-206, 213, 216, 219, 306-307, 320, 390, 392, 396, 399, 403
and differentiation, 7, 159, 167, 169-172, 179, 197, 206, 213, 219, 307, 320
and photon emission, 7, 142, 159, 197, 206, 207
and reactive oxygen, 7, 203, 206
Respiration/glycolysis duality, 176
Respiratory burst, 142, 203
Rhythmic bodily functions, 261, 263, 288, 343, 359, 369, 374, 387, 389, 391
Ribonucleotide reductase, 189
Ribose 5-phosphate, 180, 189
Right/left symmetry, 39, 234-235, 348,

351, 354-356, 360, 371
RLC, 303, 306-307, 320
ROS, 7, 202, 208-210, 214, 216, 302, 336, 341, 355, 376-377, 403
Rough endoplasmic reticulum, 306, 403
S-numbers (Spectral numbers), 117
Saccharomyces, 123, 136, 138, 147-148, 175, 177-178, 290, 387, 393, 403
Salamander, 15, 19, 22, 24, 26-27, 32, 62-63, 70, 234-235, 243-246, 248, 251, 256, 403
  egg, 235
Schizosaccharomyces, 123, 136, 147, 193
Secondary electron emitters, 114-115, 391, 401, *see also* Dynodes
Secondary radiation, 94, 96
Seedling, 117-119, 126, 141, 147-148, 361-363, 373, 395, 403
  and photon emission, 117-119, 126, 141, 147-148, 361-363, 373
  and photon imaging, 361, 362, 363
Selenium, 204, 289
Semiconduction, 225, 246-248, 250, 253-254, 256, 291, 328, 389, 391, 400, 402, 404
  in living tissues, 225, 246, 250, 256, 291
Single photon counting systems, 138, 151, 373
Singlet, 132-133, 136, 138, 143-144, 146, 148, 203, 205-207, 214, 400, 404
  molecular oxygen, 133, 138, 143-144, 146, 205-207, 404
Skin, 22, 24, 26, 29-30, 32, 56, 58, 62, 65-66, 70-71, 74, 125, 129, 144, 151, 236-237, 244-246, 265, 270, 275, 341-346, 348-349, 351-353, 355, 358-359, 374, 378-380, 385, 387, 390, 392-393, 397, 399
  grafts, 24-25, 71, 75
  and photon emission, 151, 342-346, 348-350, 352-353, 359, 374
  surface temperature, 151, 270, 341, 343, 351-352, 359, 369
Slime mould, 235-236, 241
SOD, 202, 204-205, 209, 214, 216, 405, *see also* Superoxide dismutase
Sodium pump, 334-335, 337, 339, 399
Soliton, 297, 327-332, 335, 340, 387, 393, 404
  and metastable excitons, 332
Spark gap, 102
Spectral, 8, 91-94, 101, 104, 109-110, 116-117, 121, 123-127, 131, 136-138, 141, 143, 146-151, 153-154, 186, 192-193, 206-209, 219, 230, 256, 259, 263, 265-266, 268-269, 273, 283, 288-291, 298-300, 315, 325, 327-329, 342, 348-349, 359-360, 364, 369, 392-393, 401, 404-405
  distribution, 117, 121, 138, 141, 147-148, 154, 206, 348-349
  of mitogenetic radiation, 91, 121, 206
  response of photo-cathodes, 95, 115-117
Starvation, 183-184
Starved-fed cycle, 183-184
Stationary phase of growth, 136-137, 147
  and photon emission, 136-137, 147
Stem cells, 31, 44, 69, 392, 402
Storage, 8, 180, 183-184, 274-275, 281, 292, 297, 301, 303, 308, 310-312, 319, 321, 327, 344, 393-394
  capacity, 281, 297, 308, 311
  of photons, 275, 281, 292, 297, 301, 303, 308, 310-312, 319, 344
Stress, 2, 7, 119, 132, 140-141, 143-144, 147-149, 154, 172, 206, 209, 213-216, 226, 250-254, 256, 283, 285-286, 290-291, 352, 364, 373, 376, 385, 392, 395, 401, 404
  and photon emission, 2, 119, 124, 132, 140-142, 144, 146-149, 154, 226, 280, 283, 285, 287, 290, 337, 376
  stress-perturbed systems, 140-141, 280, 284, 286
Superoxide, 143, 146, 149, 151, 201-205, 209, 213-216, 395, 400-401, 405
  superoxide anion, 143, 149, 201-204, 212
  superoxide dismutase, 202, 204-205, 209, 214, 216, 405
Surface potentials, 134, 236, 241, 244
  in peripheral nerve injury, 236, 241
Symmetry, 38, 47, 51, 234-235, 283, 348, 351, 354-356, 360, 371, 402
  dorsal/ventral symmetry, 234, 348
  radial symmetry, 89, 402
  right/left symmetry, 39, 234-235, 348, 351, 354-356, 360, 371
Symplast, 86
Synapse, 27-28, 385, 399-400, 405
Syncytium, 86, 236, 336-337, 405
Synergy, 65, 281, 289, 321, 405
Temperature, 83-84, 100, 104, 118, 127, 138-139, 148, 150-151, 270, 285, 289, 319, 341-343, 351-352, 358-359, 369, 387, 395, 405
  hysteresis, 139, 148, 404
  and photon emission, 118, 127, 138-139, 151, 289, 319, 341-343, 351-352, 358-359, 369
Teratocarcinoma, 68
Teratomas, 59, 62, 70
Tetratogen, 68, 404
Thalidomide, 68, 71, 405
Thiobarbituric acid assay, 131, 143, 405
Thymidine, 33, 189-190, 192
  degradation, 190, 192
  synthesis, 192
Thyroid, 39, 68, 144, 362, 392, 395, 405
Tissue damage, 399
Tobacco hypothesis, 65
Tolerance, 213
  tolerance to ischemia, 213
Torula, 120
Totipotent, 22, 42, 44, 73, 405
Toxin, 31, 58, 119, 141, 151, 204, 209, 214, 216, 396
Transcendental meditation, 352, 405
Transmembrane complexes, 200-201, 333, 335, 337, 396-397, 402
Transplantation, 22, 24-25, 28-29, 47-48, 51, 60-63, 70, 75, 159, 168-169, 176, 179, 186, 193-195, 208, 216, 219, 241, 257, 271, 299, 303, 319-320, 363, 376, 399
  of eggs and embryos, 48, 51, 60, 63, 70, 75
  transplanted hepatomas, 159, 176, 186, 193-195, 299, 303, 319-320
  of tumor, 62-63, 159, 169, 177, 179, 186, 193-195, 219, 241, 299, 303, 319-320, 363, 376
Triturus, 32-33, 51-52, 65, 70
Tumor, 2, 5, 7-8, 13, 38, 53-56, 58-59, 61-63, 65-71, 73-75, 92, 105-106, 131, 142, 144, 155, 160-161, 166-170, 173, 176-177, 179, 186, 190, 192-195, 197, 206, 209-213, 216, 219-220, 270, 286-287, 289-290, 292, 297, 299, 301, 303, 306, 308, 312-313, 320-321, 363, 376, 387, 390, 395, 397, 399, 405
  biology, 2, 5, 13, 57, 281, 290, 299, 301, 321

growth, 56, 58-61, 65, 68-69, 75, 144, 155, 161, 167, 169-170, 179, 186, 190, 192, 194-195, 210-212, 219-220, 275, 299, 303, 306, 319-320
initiator, 62, 66-67
progression, 7, 56, 64, 66, 68, 186, 189-190, 209, 213, 220, 306, 399, 405
promoters, 66-67
tumor angiogenesis factors, 68, 71, 212
viri, 66-67
Two-dimensional, 134, 297-298, 333, 361-364, 373-374, 376, 380
photon recording, 134, 297, 299, 361, 364, 373, 375-376
Tyrosine transaminase, 302-303, 306, 320
Ultra-weak photon emission, V, 1-2, 79-80, 118-119, 121, 123, 126, 129, 131-134, 136-140, 142-145, 154, 160, 197, 205-206, 209, 214, 220, 273, 284-285, 297, 313, 327, 353, 355, 359-361, 363-368, 370-377, 381, 383-384
and lipid peroxidation, 131-133, 143-144, 146, 149, 154, 219
Ultraviolet, 56, 79, 81, 91, 93-97, 102-103, 105-106, 108-111, 119, 123, 127, 131-132, 136-138, 147-148, 153-154, 206, 213, 219, 230, 254, 273, 287, 290, 301, 319, 321, 348, 369, 401
light and cell division, 91, 138
Vacuum-tube voltmeter, 231, 236, 240
Vascularization, 68, 71, 213, 220, 406
Vascular endothelial growth factor (VEGF), 211-212
VEGF gene, 211-212
Vibrational, 226, 264, 269, 292, 300-301, 327
Virus, 58, 66-67, 171, 391, 393, 395, 397, 399, 403, 406
viral oncogenes, 66-67
Vision, IV, 7, 13, 18, 28, 38-39, 41-42, 45, 53, 60, 68, 73-74, 79, 81, 84, 86-89, 91, 97-98, 109, 121, 138, 145, 147, 153, 155, 167, 210, 212, 226, 264, 281, 292, 299, 304-305, 307, 336, 356, 361, 386, 388-390, 394, 396-400, 405
Vitalism, 20, 41, 44, 50-51, 81, 243
Vitamin C, 204, 263-264
Vitamin E, 204, 214, 385
Voltage gradients, 225, 233, 235, 238-239, 241, 291
Voltmeter, 231, 236, 240
vacuum-tube voltmeter, 231, 236, 240
Weak magnetic fields, 338, 340
Wheatstone Bridge, 232, 406
Wolff's Law, 251, 253
Wound, 22, 26, 29-30, 32, 66, 74, 210, 225, 236-238, 241, 243, 245, 250, 257, 271, 291, 362, 391, 393
surface, 22, 26, 393
Wound healing, 26, 29, 225, 236-238, 241, 243-245, 248, 257, 271, 291, 362, 393
human wound healing, 236-238, 241, 291, 362
and photon emission, 362
X-rays, 108
Yang, 150, 355, 379-382, 384, 402, 406
Yeasts, 91-93, 108-109, 113, 119-121, 123, 126, 134, 136-138, 147-149, 154, 162, 165, 172-178, 288, 290, 387, 393-394, 403, 406
Yin, 150, 355, 360, 380-382, 384, 402, 406
Yoga, 352-353, 406
Zinc, 204, 231