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THE DEPARTMENT OF HUMAN PHYSIOLOGY AND BIOPHYSICS

MEDICAL BIOPHYSICS

Lectures

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Theme 1. PREFACE

THE SUBJECT OF BIOPHYSICS

The general development of contemporary sciences, technical progress stopped their firmly separation, having the aim of the studying of common problems or to use each other the methods and own methodology of investigation. Biophysics has more than other sciences the concept of interdisciplinary science.

Being a science that was developed by integration of two sciences, physics and biology that are so named classical, have the deep relation with physiology, physical chemistry, cytology, genetics, physiopathology, informatics, cybernetics, philosophy, etc.

The transmission of nervous influx, for example, is the problem of biophysics but also of neurophysiology, the mechanism of replication of double helix of the molecule of deoxyribonucleic acid is studied in the same time by biophysics, biochemistry, physical chemistry or genetics. The interdisciplinary character of biophysics sometimes is realized not by biological object but common technology. The modern technique, especially complicated one is solicited in the equal solicitation by a lot of sciences. For example can be: methods of diffraction by X rays, electronic microscopy, spectroscopy of magnetic resonance, variety of radio isotopic techniques, the technique of microelectrodes, genetic engineering etc.

A lot of definitions were given today for biophysics, but there is not yet a good consensus about complete definition.

The professor W. Beier from the University of Leipzig defined biophysics as the **"science that deals with physical analysis of functional structures and biological behavior".**

The professor V. Vasilescu from the University of Medicine and Pharmacy of Bukureshti – "Biophysics is the science that studies physical phenomena from biological systems by physical – mathematical techniques and theories".

The professor Tarassov from the University Lomonosov of Moscow – **"Biophysics consists in the studying of phenomena and physical – chemical molecular structures that stay on the base of primary mechanisms of biological processes"**. The analysis of suggested definitions allows us to confirm: **"Biophysics is the science that deals with the physical analysis of primary mechanisms that are referred to the composition, structure, the existence and the development of alive matter".**

It is difficult to establish with precision in time the apparition of biophysics, and though with approximation it is considered that biophysics as the object and the field with proper methodology has already existed fifty – sixty years. The study of physical phenomena from the alive organisms or effects of physical factors of all that is alive is much than before.

Hippocrates (460–373 b. o. m.) super named "parent of medicine" in his theoretical description titled "About air, water and fires" then he referred to the action of physical factors of the medium on the organism and the application of some factors and mechanisms of physical order in the prevention and the treatments of ills.

The well known personality of Renaissance, Leonardo da Vinci (1452–1519), studied hemodynamics; discovered the role of lens of the crystalline and explain the formation of images on the retina etc. Later, the Italian physicist and physiologist Luigi Galvani (1737–1798) took into consideration the relation between muscle contraction and electrical current. The English physician and physicist Thomas Young (1773–1829) elaborated the three chromatic theory of colored vision. The German physicist and physiologist Herman Helmholtz (1821–1894) measured in 1850 the speed of propagation of nervous influx, but in 1863 elaborated the theory of audition receiving on the base of resonance phenomenon.

These observations and random researches with biophysical evident character during one hundred years were absorbed by physiological patrimony, from which biophysics was separated half a century ago and in some circumstances it continues to be separated.

So, other interdisciplinary sciences were detached from physiology such as biochemistry, endocrinology and just biocybernetics; **the physiology remains to be defined as the science of integrated processes from the human healthy organism.**

It is necessary to mention the names of some scientists from different countries – laureates of Nobel Prizes related with the researches of biophysical character.

– **Willem Einthoven** (1860–1927) – Dutch physicist and physiologist, for the elaboration of theoretical bases of electrocardiography – 1924;

– **Alan Lloyd Hadgkin** (b. 1914) and **Andre Fielding Huxley** (b. 1917) – English physiologists, for the elaboration of some model of the dependence of ionic currents from the axonal membrane on the membrane potential – 1963;

– **John Carew Eccles** (b. 1903) – Australian physiologist and **Bernard Katz** (b. 1911) – English biophysicist, for the study of the mechanism of synaptic transmission – 1963 and 1970;

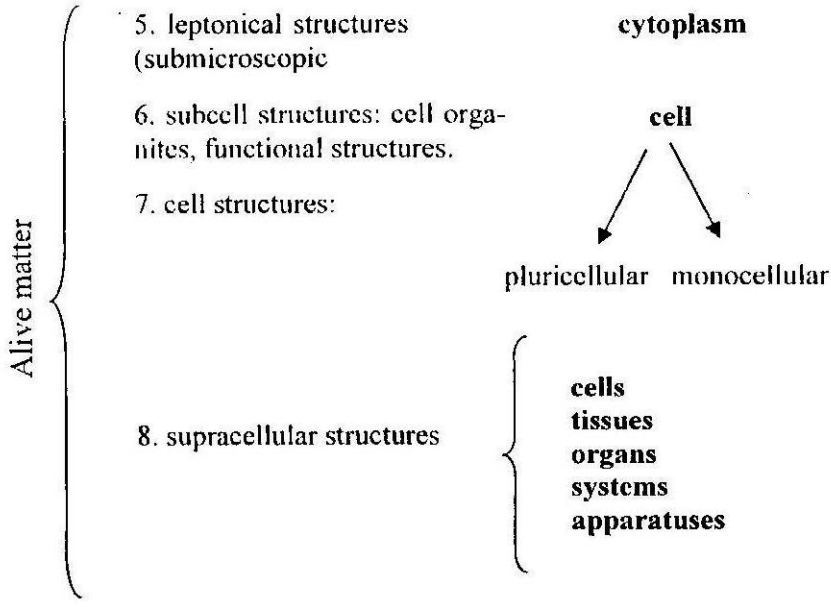
– **George Emil Palade** (b. 1912) – Rumanian scientist settled in the USA, for the multiple discovering, from which also the application of electronic microscope in the biological research in 1970.

We will have to know that the whole nature surrounding us from the galaxies and stars up to the grounds, plants, animals and peoples, everything consists of atoms and molecules. The last ones as the dependence of the arrangement and organization form the different bodies from which some are absent of life but others are alive.

The levels of organization of the matter from the fundamental particles up to the superior pluricellular organisms are presented in the table 1.1.

Table 1.1

1. Fundamental particles: proton, neutron, electron	
2. chemical elements	C, H, O, N, S, P, etc.
3. chemical structures	Organic molecules, ions and mineral molecules
4. complex chemical structures	Macromolecules complex colloidal system



The unity of the world is confirmed by the fact that there is not any chemical element proper for alive matter. On the other side we can not say about the chemical composition of alive matter, so that we can say about chemical composition of certain inorganic composition because the chemical elements are included in the alive organisms in the combinations not so very complicated but also extreme instable. The nature only for a reduced number of substances that belong to alive matter have been managed to be identified, due to of liability of these combinations.

According to the appreciation of well known American scientist Linus Pauling, "the human body would be constituted approximately of 100000 types of proteins from that today are known better only at least ten".

Till now from those 105 chemical elements identified in the Universe and included in the periodical system of elements in the alive matter above 60 elements were identified, that were named **biogenesis** or **bioelements**.

Only carbon, hydrogen, oxygen and nitrogen have an essential role in the realization of alive structures that with their abundance are named macro elements. Another category of elements such as: sulphum, ferrum, calcium, magnesium, potassium, phosphor and others are in small concentrations in the organization of alive matter but perform an important extreme role. Due to of small concentration they were named **microelements**.

Regarding the physical laws, there were not any evidence till now in the difference between the non alive objects and alive beings. Biophysics does not reduce the problems of biology and medicine at simple physical - chemical problems, the life differs qualitatively from physical – chemical phenomena and depends on the specific laws.

The technical scientific progress in the field of medicine can take place only taking into consideration the tight relations between biology and physical – chemical sciences, that the great physicist of XX century N. Bohr (1885–1962) wrote: "There are no results of biological researches that can be described univocally different that only in the base of physical and chemical notions".

The problems of biophysics can be systematized as the function of the field from physics on which application is based. Resulting from this fact biophysics uses nearly all classical fields and modern ones of physics:

Biomechanics studies different types of animal locomotion up to cellular motility and mechanical properties of cellular constituents;

Bioelectricity studies the ensemble of electrical phenomena from the alive matter to the cell level, tissue and organ one;

Biothermodynamics and **bioenerghetics** deals with both the generation, storage and the conversion of energy at cellular level and organism, and the energetic problems of great biological systems of super individual level.

Radiobiology is a chapter of biophysics that studies the interactions between the radiant energy and alive matter.

Another systematization can be realized as the function of organizational level of alive matter that is studied:

Molecular biophysics that studies the physical properties of the molecules and physical phenomena that are produced at molecular level and supermolecular one;

Cellular biophysics that studies the physical properties of the cells and physical phenomena that are taken place at the cellular level;

Biophysics of complex systems studies the biophysical aspects, beginning from the tissue level up to the level of supra individual biological systems.

More two modern chapters can be included in the fields of biophysics:

Bionics that studies the structures and mechanisms from alive systems for finding some solutions of technical problems;

Biocybernetics studies the principles and concrete mechanisms of commands, regulation, conservation, processing and transmission of information in the biological systems.

As the subject of study, the medical biophysics has the aim the formation of one scientific vision in the understanding of more detailed processes of life.

Both for theoretical preparation and the solving of complex problems of practices the future physicians must posses an clear image about the essentiality of vital processes.

Biophysics will help you to analyze the vital processes at the molecular and sub molecular level, to have a deeper insight in the dynamics and laws of alive matter.

From the extreme variety thematic of biophysics for the study in the universities of medicine it is necessary to select such problems that are in tight connection with major directions of development of medicine.

Theme 2. THE THEORY OF SYSTEMS

2.1. The sense and delimitation of the notion of system

A characteristic feature of material bodies is the fact that each of them is behavior as the whole, as the unit with respect with the surrounding bodies saving their individuality shorter or in time. This is due to the tights between the constituents parts of each body. From the physical point of view it is considered that these material bodies, alive or dead represent systems.

The well known German biophysicist Walter Beier defined the system as the ensemble of connected parts in some modality. Grodins in 1963 referred concretely to this notion:

"The system can be defined as the collection of components interconnected by different modalities. The components can be physical, chemical, biological or the combination of those three groups."

Essentially, the system can be defined as the ensemble of objects which interactions lead to the apparition of some proprieties which do not posses the constituent parts taken separately.

Molecules ⟶ **system**

A particle of sand ⟶ **summary conglomerate**

There are general principles, valid for systems not taking into consideration the nature of constituent elements and every relations or "forces" would be that connect them. These principles are: automatic regulation, feed back and others. So, **the theory of systems represents a new branch of science that deals with the expressing of general properties of the systems indifferently of their nature and their structure.**

The idea about the possible general theory of systems was emitted by American biologist of Austrian origin Ludwig van Bertalanffy in 1938, but the concept of "general theory of systems" was applied in the scientific publications at the ends of 1951.

For the study of alive matter, the theory of systems has a great role because in its conception, the organism, the species etc. are not considered as conglomerates of parts but as complex systems that possesses a certain morphofunctional organization, the organization that confers certain integrality. The theory of systems underlines the great role of interactions between the parts in the qualitative determination of the respective system.

Related with the fundamental ideas of cybernetics, the theory of systems opened the large possibilities of knowledge in biology and implicitly in biophysics.

2.2. The classification of systems

a. Physical systems

The classification of physical systems is performed by several criteria:

– as the function of internal structure there are two categories:

Homogeneous system that is formed of the same constituent, so that do not have separation surfaces inside.

Heterogeneous system that is formed of different constituents and present the separation internal surfaces.

– as the function of variation of properties on different ways from the system:

Isotropical system for which the properties do not vary on different directions.

Anisotropical system for which the properties vary on different directions.

– as the function of relations of the system with surrounding medium, three categories of systems are distinguished:

Isolated system which does not change energy and substance with the surrounding medium.

Closed system, that changes only energy with the external medium.

Opened system, that changes both the energy and substance with the external medium. For such a system both the quantity of energy and the quantity of substance are variable in time.

b. Biological systems

The opened systems, anisotropic and heterogeneous represented by alive organisms are named biological systems.

The metabolism, growing and the development and others phenomena that take place in the alive organisms are performed by structures based on heterogeneously and anisotropy. The essential condition for the maintaining of life consists in the permanent change of energy and substance between the organism and surrounding medium.

For example for people, the interruption of the connection with the external medium after 5 minutes determines the degradation of the system.

The biological systems possess a series of peculiarities:

1. A generic connection exists between the systems and each integrated systems with the sense that some ones originate from others or have a common origin. Also a certain graduation is evidenced with the sense that what is a part of system consists in its turn a system with respect to its constituent parts and so on. So that, the biological systems are the complexes made of units or subsystems. For example the cardiovascular system is made of heart, arterial system and venous system as the subsystems. The functioning and the organizations of constituent parts of one biological system are subordinated to the more general laws of the whole from which they belong.

2. Dynamical equilibrium

Comparing physical systems that resist a lot of time without changing the structure, the biological systems can not maintain the structure but consuming the substance and energy from external medium. This state of the biological system, characterized by a permanent flux of substance and energy in the system with the saving of the integrality of the system being in the continuously renewing was named **dynamical equilibrium**.

3. Auto regulation

Each organism is acted continuously by the action of external factors. In general, these stimuli tend to deregulate the dynamical

equilibrium of the given system. But the biological systems maintain their homeostasis due to of their capacity to receive, store and process the received information, to elaborate the "commands" and to transmit these commands to the organs of reactions.

The biological systems have the property of self control and self regulation the functions in each moment, due to of the fact that the alive organisms are the cybernetic systems.

4. Ever biological systems are characterized by: growing, development, reproduction and death.

Each type of cell and just each cell at the superior organisms have their individuality, the past, present and their future. The final of the future is real and in the same time tragic because all the alive organisms have the finite existence in time.

In the development of biological systems the **biogenetical law** is applied formulated by German biologist E. Haeckel in 1866: the ontogenesis (the development of the organism from the moment of fecundation up to the stage of individual) repeats the phylogeny (the development of the species regarded as the process of historical development as the result of the evolution of alive world).

2.3. The elements of biocybernetics

The theoretical fundament of some technical systems allowed the analogies with the systems that exist in the alive beings reaching the conclusions that the concrete realization of the systems of regulation or the processing of information is different in the technical devices with respect to the alive beings; the abstract scheme of the system is the same.

Taking these considerations the cybernetics was developed from 1948 with two fundamental papers: "the cybernetics is the science of commands or communication of beings and machines" – N, Wiener and "Mathematical theory of communication" – Cl. Shannon.

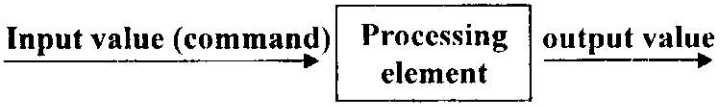
In order to emphasize the central role of the information A. Kolmogorov defined the cybernetics as "the science of modali-

ties of reception, save, transformation and the application of the information by machines, alive organisms and their reunion".

We will refer to the elements of the chapter more important for cybernetics: "The systems with automatic regulation".

a. The system of command

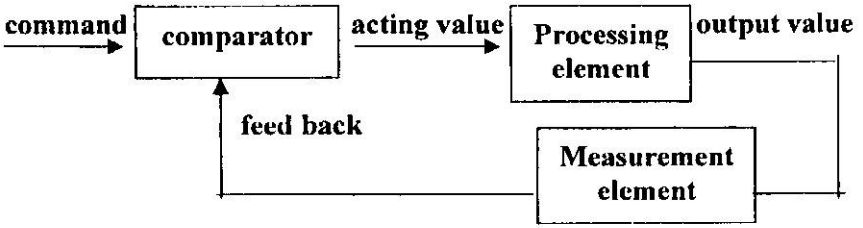
The system of command is made of one element or the ensemble of elements that for a certain value of entering generates a certain output value.



The relation between the command and the output value is depended by the structure of the processing element.

b. System of automatic regulation

For the control of processing of the command is necessary the measurement of the output value that due to of perturbations can have the deviations from the expected values. The received information for the measurement of output value is applied for the modification of the input value so that the output real value coincides with that expected. The obtained system is named **the system of automatic regulation.**



c. Feed back

In order to assure the function at the request parameters of one automatic system it is necessary that at the apparition of some deviations the acting value must be modified so that the deviation must be decreased. Such feed back is named **negative feed back** and assure the stability of the system.

In the case when the apparition of some deviation generates the modification so that the deviation will be increased we will have the **positive feed back**.

The effects of the positive feed back can be limited by the apparition of some negative feed back, or by the qualitative transformation of the system ("mutation").

2.4. Homeostasis and the mechanisms of control in the alive systems

The term of homeostasis is applied in medicine to indicate the maintaining of some static conditions or constant ones in the internal medium. Generally all organs and tissues of the body have functions that help the maintaining of these constant conditions. For example the lung provides the cells with the oxygen, kidneys maintain constantly the concentrations of electrolytes, and the intestine gives the nutritive substances.

A great part of the study of medicine is related to the possibility that each organ or tissue contributes to the homeostasis by some control systems. Some of them operate in the internal part of the cells and check the intracellular functions, others act at the level of organs and check only the functions of some individual parts of the organ but others check the interrelations between different organs.

The existence of numerous systems of automatic regulation offers for the alive systems the extraordinary possibilities to react adequately at the modifications of the medium (external or internal).

We will present schematically some examples here.

a. Pupil reflex

The pupil plays the role of the diaphragm of one device of photographing. The size of the pupil is checked by two smooth muscles, a ring shape sphincter and radial dilatator placed in the iris. Besides the control of light flux, the pupil contributes to the decreasing of the aberrations of sphericity.

The pupil reflex of myosis (the decreasing of the diameter of pupil) and mydriasis (the increasing of the diameter) is performed by a cybernetic system (*fig. 2.1*).

The contraction of circular fibers of the iris determines the myosis, but those radial produces mydriasis.

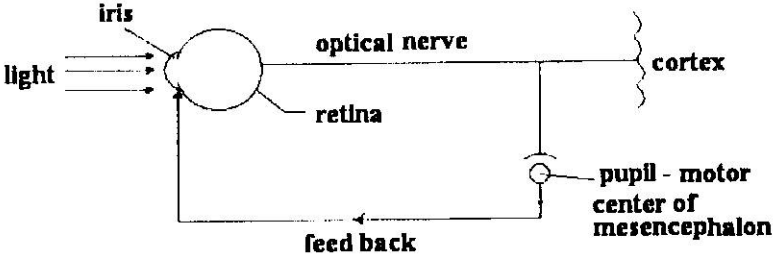


Fig. 2.1. **The pupil reflex – the system of command with automatic regulation**

A relative strong flux of light falls on the retina and determines a signal on the optical nerve that excites the sensitive zone from the cortical protection. The appeared influxes are transmitted to the neurons from the pupil motor center of mesencephalon and the formed command here is transmitted on the nervous efferent ways (of reaction) to the muscle fibers of iris, as the result the relaxation of radial muscles takes place and the contraction of ring shaped muscles takes place that produces myosis.

In the case when the flux of light that falls on the retina is relative weakly, the formed command in the pupil – motor center leads to the relaxation of ring shaped muscles and the contraction of radial muscles takes place that produces the mydriasis.

b. The regulation of arterial pressure

A series of nervous receptors named ***pressoceptors*** exist in the walls of big arteries that are stimulated by the tension of arterial wall. If the arterial pressure becomes greater, these pressoceptors are stimulated and impulses are transmitted in the ***medullar*** portion of the brain. Here the impulses inhibit the ***vasomotor*** cen-

ter that in its turn makes to be decreased the number of impulses transmitted to the heart and blood vases by the sympathetic nervous system. Due to of these impulses the pumping activity of the heart is decreased and provokes the decreasing of arterial pressure below the normal value.

As the pressure of the blood in the big vases is decreased this determines a relaxation of the walls of the vases acting on the receptors and produces an increasing of the activity of vasomotor center above the used level and therefore an increasing of arterial pressure takes place till the normal value.

c. The regulation of the concentration of CO_2 and O_2 in the extra cellular liquid

The oxygen is a substance necessary for the chemical reactions that are produced in the cells. It is evidently that the speeds of these reactions depend on the concentrations of the oxygen in the extra cellular liquid. The special system for the maintaining of the constant concentration of oxygen in the extra cellular liquid depends on the chemical characteristics of hemoglobin presented in all red blood corpuscles.

The hemoglobin is combined with the oxygen then when the blood passes at the level of lungs. In the capillaries from the tissues it does not release the oxygen if in the extra cellular liquid its concentration is great. Contrary the oxygen is released for the reestablishment of the concentration at the necessary level. This automatic regulation is named ***buffer function of the oxygen of hemoglobin***.

The dioxide of carbon is one of main final product of these oxidative reactions that are formed in the cells. In order that the speed of these reactions to be maintained constantly it is necessary to maintain the constant concentration of CO_2 in the extra cellular liquid.

An increased concentration of CO_2 excites the respiratory center leading to the acceleration and amplification of the breathing. This determines an increased elimination of dioxide of car-

bon from the blood to the alveolar air. So, the concentration of CO_2 is decreased from the extra cellular liquid.

Vice versa, a decreased concentration of CO_2 determines a slow respiratory rhythm with the consequent accumulation of dioxide of carbon in the extra cellular liquid. These processes continue up to the situation when the concentration of CO_2 in the extra cellular liquid becomes normally.

2.5. Integronics or the science of hyperintegrated systems

In order to maintain the stability, the cybernetic systems must contain at least a mechanism of feed - back for each parameter.

But for the regulation of some parameters such as the arterial pressure, the biological systems posses a lot of feed – back mechanisms that contribute to the increasing of the viability of system. If they are very numerous, they can not keep the stability of entire cybernetic system, because they can not check the regulated element. The center of commands, the processing organs, receptors and the communication ways remain "discovered". As this situation does not take place, they must be in its turn the regulated elements of some superior mechanisms. Therefore, the feed - back mechanisms from the biological systems are interposed and super ordered. In order to keep the stability in the diverse conditions, the system needs a mechanism of prevention of variations, so named "feed – before". The function of the organism is not regulated only retroactively but also anticipatory by the information received from the exterior.

Taking into consideration this fact, no one of the "feed back" mechanisms and those of "feed before" can not explain the modality in which the biological systems are able to be self -regulated, to be self – reproduced, to be self – generated and to be self – repaired. For this, there are others mechanisms of regulations such as: organization on a lot levels, coupling and chaining of phenomena; information and structural redundancy; functional redundancy; organization in the net; extra saturation with the internal bonds; combinatorial redundancy; specialization of some appara-

tuses and organs in the integration of elements; the completion of the written programs in the structure with those inherited.

Truly, the system can not be self regulated and self – repaired if it is not organized on a lot of levels, from molecular one which the life starts up to the level of entire organism at which its relations are regulated with the medium. In one very complex system such as the cell, for which hundreds or thousands chemical reactions takes place per second, hardly checked from the exterior due to of extreme big number of them, the biological systems appealed to the chaining of phenomena that are conditioned reciprocally. So, in order the enzymes can act on the substrate it is necessary the recognizing of the respective molecules. All molecules of biological systems have in the same time with the substance and with the energy from which are formed certain structural information, represented by their spatial conformation, able to be recognized by some molecules with a complementary configuration as in the case of enzymes, of antibodies and cellular receptors. Such, this structural information made possibly for enzymes to recognize the substrate and to accelerate the respective reactions that are chained and conditioned reciprocally, providing so for the system the energy and the necessary substances for the functioning and its regeneration.

Often, no acceleration, no coupling, no chaining of the phenomena is not sufficiently to keep the stability of the system, that many times the perturbation factors manages to destroy a lot of structures than to be regenerated. Therefore, the biological systems appealed to the structural redundancy.

They posses a lot of cells more than necessary and can lose per day several millions of hematies and leukocytes and several hundreds or thousands of neurons, without changing their stability.

All cells, organs and apparatuses that born from the organization of redundancy structures of the organism are connected between them by some substantial connections and energetic ones, such as the anatomical connections and metabolic ones and through some informational connections. Due to of this a great combi-

natory redundancy is reached. So, by the connection of those 14 milliards of neurons from the brain a net of milliards possible circuits is obtained. This thing can not be characterized by others cells of the organism. So, a fantastic net of communications is obtained. The specific of communications in this net is given by the structural information of the molecules of chemical messages that will act only there where the capably receptors exist for the recognizing.

The biological systems are more than cybernetic systems. They exceed the organization in the closed circuit being located in the hyper net, located on a lot of levels and supersaturated with the internal connections in which all or almost all are connected. This hyper integration leads to a great dependence of elements. Therefore, the elements of biological system can not exist separately. Of course, the hyper integration leads to a great capacity of influence, of compensation and reciprocal replacing of different elements that leads to the increasing of possibilities of saving of stability of biological systems.

The principles of such organization for which all elements located on a lot levels are connected between them, leading to a hyper integration, for which all elements can be recognized, influenced and reciprocally be compensated, for which some systems and mechanisms such as the nervous system, endocrine system, the cardiovascular system, the metabolism, but also the immunity system were specialized only in the integration of others elements, exceed the principles of organization of cybernetic systems. As they are the result of the hyper integration, the science that could study them would be named integronics. Without any contradictions of the theory of systems and cybernetics, the integronics just would complete them as in the following table.

It is necessary to accentuate that the principle of integration, of organization and complexity in spite of the second principle of thermodynamics that postulates the increasing of the entropy, so of disordering, leads to the integration also of diversification of the systems – integration that was finished with the apparition of biophysical systems.

Therefore, in order to understand better the biological systems and to build the technical systems more performed it is necessary to make an appeal to the principles of integronics that are less known.

Table 2.1

The completion of the theory of systems and cybernetics by integronics

	Theory of systems	Connections among elements Integrity of the system Unitary reactivity Inputs, outputs
	cybernetics	The organization in the closed circuit Informational connections Programs, auto regulations Homeostasis, stabilization
integronics		Organization on the lot of levels Coupling of complementary elements or contradictory ones The chaining of phenomena The structural information of elements Structural redundancy Functional redundancy The organization in the net The super saturation with the internal connections The combinatory redundancy All is connected with all All depend on all All is helped by all The combination of the recorded programs in the structure with those inherited Self providing, self generation, self repairing, adaptation

Theme 3. MODEL SYSTEMS

3.1. The importance of modeling

Definition of model system: **The model is a theoretical system or material one by which the properties and the transformations of another more complex system can be studied indirectly and presents an analogy with the first system.**

In the contemporary sciences we meet different models, such as the atomical model of Bohr, models of economical increasing, technical models, etc. A large spreading of the models in medicine is directed by the following main goals.

– the facilitation of the access to the knowledge of different complex functions of the human body, in the normal and pathological conditions;

– realization of some models for the testing of medical substances, or with the aim of introduction of some methods and new techniques of diagnosis and treatment;

– the temporary substitution or definitive one of some organs or functions from the organism, especially in the case of artificial pulmonary-cord or the artificial kidney.

– Realization of some prosthesis, especially denture, articulation and for limbs.

Process of realization of one model is named the modeling.

The reproduction of behavior of one system (physical or biological) with some analogue one, constituted especially on the base of some rules is understood by modeling.

3.2. Principles of modeling

Some essential features consist the principles of modeling belong to all process of modeling.

The first principle comes from the observation of Newton. He pointed that studying the movement of one material point we can make the conclusion about the movement of other bodies of different masses. Consequently this principle of the similitude was

generalized leading to the bearing of concrete rules, by which the models can be elaborated.

The second principle is born from the first and is referred to the fact that at the practical solving of the model, the abstraction of qualitative properties of compared systems is made, passing from the concrete values to the abstract digits. Frequently, after the solution of the problem about model the possibility exists to return to the concrete values.

The third principle (used for example for the modeling of the functions of nervous central system) is the principle of "black box". The essentiality is the following: Only the input and output values are known, the modality for which the input data are processed in order to obtain the output data is unknown, or is known in the extreme quantity.

3.3. The steps of modeling

In the realization of some models, so that in the modeling, some steps are passed systematically, from which only three are the most important:

– A simplification and the essentiality is made only for the first step, by other words only the fundamental features are established for the modeled system. The processes are mentally for this step.

– The reproduction of original is the second step, using other materials, at other level of organization or other form of the movement of the matter.

– Finally, the third level consists in the utilization of the medium for the studying of some causal or functional relations.

Recently, an extreme role in the realization of the models is performed by computers. A computer can be programmed so that can be considered as a physical system, complex chemical system, a hoemodynamic system, or a series of different endocrine glands which interact between them. Generally, if the biologist has an idea or a hypothesis, then their validation can be tested by the

computer before to perform the biological experiences. This represents an important aspect of biological research.

3.4. Classification of the models

A great variety of models makes difficult a complete classification. The group of interest models for the field of medicine and biology can be classified as the function of three criteria:

– as the function of the aim;

– as the function of the matter form, which the model is concretized in;

– as the function of the relation between the model and the modeled system.

A. As the function of the aim the models can be:

1 – Demonstrative (didactical);

2 – Researching or heuristic;

3 – Practical;

4 – Mixed.

1. **The demonstrative or didactical models** are conceived for the facilitation of understanding of one structure or one phenomenon. For example modeling of one anatomical region from the organism, electronical model of one axon fragment, etc.

2. **The researching models (heuristic).** This group of models is more important, representing the basic support of experiments.

A great role in the development of molecular biology has a series of researching models which opened the new ways in the knowledge of live structures. For example the model of double spiral, named also the live spiral was elaborated by Watson and Crick (see *fig. 3.1*). Actually we can not imagine a valuable scientific researching without models.

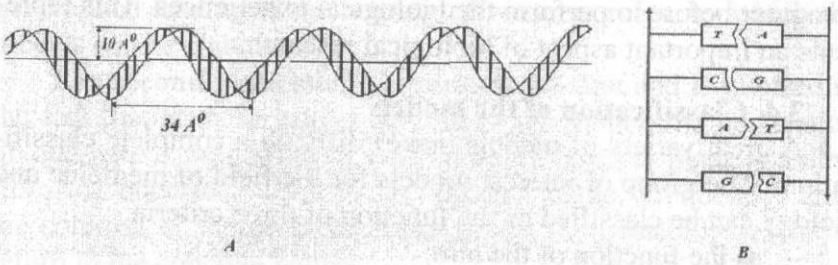


Fig. 3.1. **A – the original model of demonstration of double spiral of DNA, B – the same schematic model with the specification of nitric pairs.**

3. Practical models are realized with the aim of solving of some practical problems. For example before the finalization of one object or complex apparatus, the tries are performed using a model with the reduced sizes.

4. The mixed models are those which belong from two or all those three groups mentioned before.

B. As the function of the matter form for which the models are concretized they are divided in two big groups:

- Theoretical, abstracts or ideal models;
- Material or substantial models;

C. As the function of the relation between the model and the modeled system, physical models, chemical and physical – chemical can be:

- Similar, engineering;
- Homologue;
- Analogue;

If the analogy is performed respecting the structure of modeling object, then the modeling is named structural modeling or bionic one. When it is taken into consideration the obtaining of one result, without existing any structural similitude with the modeled object, then the analogue modeling will be of functional type or cybernetic.

3.5. Biological models

There are two big groups of biological models:

a) Natural;

b) artificial.

The form of movement of the matter is the same in both cases, both in the model and in the modeled system. This fact is possible by using the experienced animals.

This is the case of some animals which make the disease comparable with people or present a series of hereditary modifications which studies is of interest for human medicine not introducing any artificial element.

Artificial biological models are obtained by total or partial exclusion of some tissues or organs, following the effects, or by introduction in the organism, by adding of some factors, for example, a chemical substance or a medical substance.

3.6. Limits of modeling

The model and the object are in the relation not of analogy and not of identity, from this fact it is necessary to take into consideration that outside of the model the rest remains. This rest does not represent specific properties of live systems.

Non rational using of the modeling in biology can represent following risks:

– the defectively and exaggerated simplification leads to the removing from reality;

– the excessive mentioning of some common elements hiding which are specific;

– the mathematic schematization of the phenomenon, which can lead to the abstraction of the form by the content;

– The absolution of analogy value can leads to the erroneous conclusions.

The modeling opened and opens big perspectives for biology and medicine with all of these limits, having an extremely good cognitive value.

3.7. Possibilities of diagnosis by modeling The logical mathematics can be used for the calculation of some diagnoses more probably on the base of symptoms presented for patients. The application of logics in this issue has as the basic concept the symptom - disease complexes.

A symptom – disease complex is a list both of symptoms and diseases by which a patient would have or would not have to posses. For example in the table 3.1, only two symptoms S(1) and S(2) and two diseases D(1) and D(2) are taken into consideration.

Table 3.1

S(1)	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
S(2)	1	1	1	1	0	0	0	0	1	1	1	1	0	0	0	0
D(1)	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0
D(2)	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0

Each column represents a disease – symptom complex for which "1" shows that the patient has the respective symptom, or the disease, but "0" means that the patient should not have the respective symptom or disease. The columns from the table represent all symptoms – disease complexes which can be formed by two symptoms and two diseases.

If it is taken into consideration (p) symptoms and (q) diseases, then will be possible $N=2^{p+q}$ disease-symptom complexes.

If the patient has the symptom S(1) **and** S(2) then it will have the diseases D(1) **and** D(2).

So that, it means:

$$S1 \cdot S2 = D1 \cdot D2 \qquad (1)$$

But from all disease-symptom imaginable complexes not all are possible (a part of them are not met in the medical practice) in concordance with the formula (1). The sign · means the logical operation of multiplying or the operation **and**. In accordance with the formula (1), the values from grey backgrounds are not possible (the table 3.2).

Table 3.2

S(1)	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
S(2)	1	1	1	1	0	0	0	0	1	1	1	1	0	0	0	0
D(1)	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0
D(2)	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0

There is also the operation **or** besides the logical operation of **and.** The operation or is named also the logical sum.

$$S1+S2=D1+D2 \qquad (2)$$

In accordance with the formula (2), the values from grey backgrounds are not possible (the table 3.3).

Table 3.3

S(1)	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
S(2)	1	1	1	1	0	0	0	0	1	1	1	1	0	0	0	0
D(1)	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0
D(2)	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0

The results of calculations from grey backgrounds are false, but from those of non colored backgrounds are true respectively both for the table 2 and 3.

There is also the operation of logical negation —. For example: $\overline{1} = 0$; $\overline{0} = 1$; $\overline{\overline{0}} = 0$;

There are also operations: $\overline{S}_1 \cdot \overline{S}_2 = \overline{D}_1 \cdot \overline{D}_2$ and $\overline{S}_1 + \overline{S}_2 = \overline{D}_1 + \overline{D}_2$

The calculation is performed by computers when there is a lot of disease – symptom complexes which present a list of possible diagnoses.

Theme 4. THE STRUCTURE AND PROPERTIES OF WATER MOLECULE

4.1. Biological role and repartition in organisms

The physical properties of water are different with respect to other liquids. These properties facilitate maximally the apparition of live phenomena. All live beings such as animal and vegetal ones contain an important proportion of water. For example, medusa contains up to 96–97% of water of entire body. But contrary the spores and bacteria contain less than 50% of water of entire body. The mammals and the people are situated within these limits. Their content of water is about 65–70 % of the weight. The role of water in the organism is determined by the fact that it represents an important constituent of live matter and takes part at fundamental processes of the organism.

We mention the important roles of water which take place in the organism:

– the water is the essential element which regulates the osmotic pressure;

– It represents the medium in which the multiple chemical and biochemical reactions take place in the organism;

– Participates at the constant maintaining of the temperature of organism;

– Being a good solvent, the water forms solutions with a series of substances;

– Water participates at the elimination of residues from the organism by renal way;

– It has an important role in the mechanical protection of fetus.

Table 4.1

The content of water of main tissues	
Skin	71%
Striated muscle	70%
Nervous tissue	70% in the white substance
Nervous tissue	85% in the grey substance
Skeleton	33%
Heart	77%
Red blood corpuscles	65%
Liver	73%
Lungs	80%
Kidney	80%
The content of water of biological liquids	
Plasma	90-92%
Cephalo-rachidian liquid	98.5%
Lymph	96%
Saliva	99.5%
Gastric juice	99.4%

Only 70% of water represents the intercellular water, 23% the interstitial water and 7% the circulated water from entire quantity of water of the organism.

4.2. The structure of water molecule

The molecule of water is formed by an atom of oxygen and two atoms of hydrogen bound to the oxygen atom by covalent bounds.

$$H\text{-}O\text{-}H \quad (H_2O)$$

It was established that the water posses the triangle form by the diffraction of X rays (*fig. 4.1*).

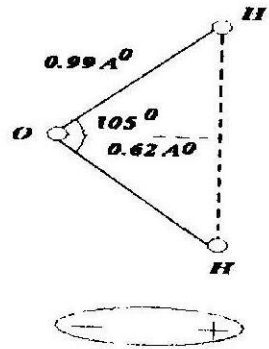


Fig. 4.1. **The structure of water molecule**

The water molecule can be considered as an association of one ion O^- and two ions H^+ due to of uncqual distribution of electrons. The nucleus of oxygen attracts strongly each electronical doublet removing them from the hydrogen, so that each OH binding has a polar character. Besides a lot of experimental arguments, the existence of dipole properties obliges us to state that the water molecule is triangle, declining so the hypotheses of linear structure.

Another characteristic of water molecule is the formation of hydrogen bounds. The typical example is the hydrogen binding that exists between two water molecules (*fig. 4.2*).

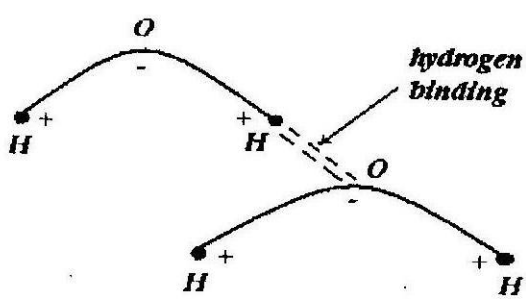


Fig. 4.2.

The dipolar character of water molecule and the ability to be arranged in the hydrogen bounds explains the properties of the water in those three states of aggregation. The usual liquid water is formed by a mixture of free monomer molecules H_2O, dimmers $(H_2O)_2$, tetramers $(H_2O)_4$, octamers $(H_2O)_8$, their percentage is a function of temperature. The penetration of the monomers as the interstitial molecules takes place in the free spaces of the crystalline structure at the temperatures between 0^0 and 4^0 C. This explains the increasing of water density from 0^0 till 4^0 when it becomes maximal (*fig. 4.3*).

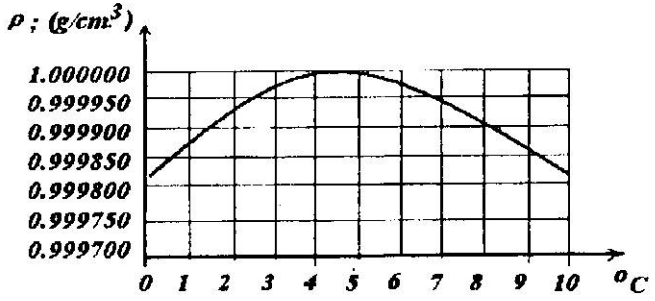


Fig. 4.3. **The variation of density of water with the temperature**

At the temperatures greater than 4^0 C these monomers go out from the nodes of crystalline structure due to of thermal agitation and in this way the density of water is decreased. The quantity of 50 % of hydrogen bounds is unbounded and the water becomes more fluid at the temperatures of 40^0C. Therefore, this temperature is considered as "the second melting temperature of water".

The fact that the homoeothermic animals fixed the normal temperature of the organism between 35–41 ^{0}C is related with the water properties in this interval of temperatures. The dimmers predominate in water in this interval of temperatures and have high chemical activity. In these conditions the cells can easily create the crystalline structures of water of different forms. Therefore the

water is considered as the "life matrix". The hydrogen bounds stay on the base of supramolecular structures of $(H_2O)_n$ both in the solid state and the liquid state (*fig. 4.4*).

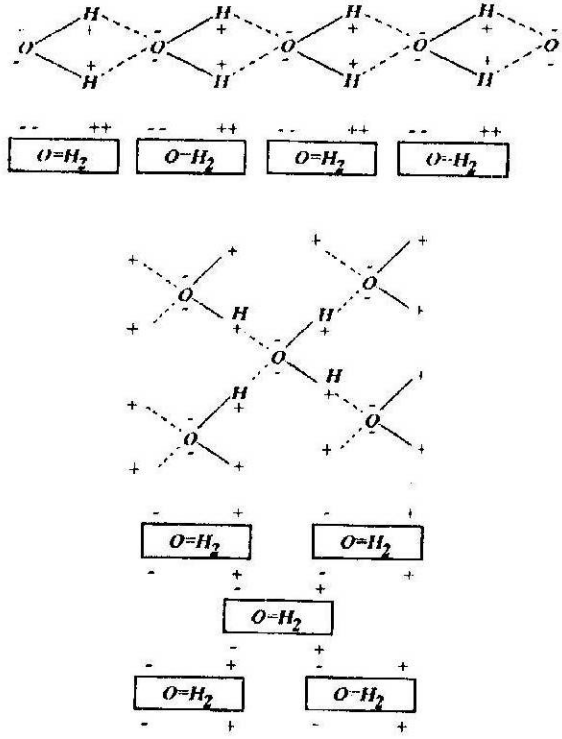


Fig. 4.4. **The association of water molecules of different spatial structures: A – the linear association; B- globular association**

4.3. Modification of the structure in presence of solutes

The structure of water is modified in the presence of solutes as the function of their characteristics. In the aqueous solutions the electrolytes are completed dissociated in ions. These attract the water molecules (dipoles) with the pole of opposite sign. So, the supramolecular structures are formed with the spherical symme-

try. The bound water has the name of ***hydration water*** and has the properties different of ***free water***. The process is named the hydration or solving of ions (*fig. 4.5*).

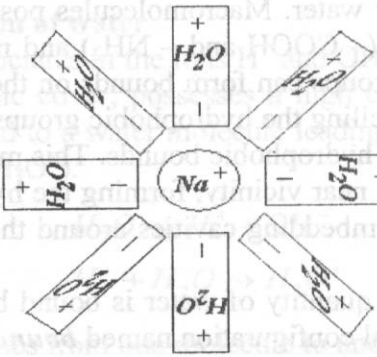


Fig. 4.5. **The hydration of one ion: The ion of Na^+**

The solutes which are hydrophobic molecules without dipolar moment can form with the water only weak Van – der - Waals bounds. In order to decrease the potential energy, the number of hydrogen bounds is increased between surrounding water molecules, leading to the formation of structures with pentagonal faces (polyhedron with 12 faces), and the hydrophobic molecule is situated in the center of this structure. These structures are named ***embedding cavities*** (*fig. 4.6*).

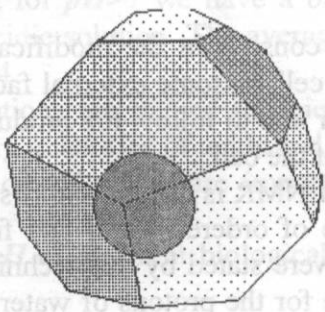


Fig. 4.6. **Embedding cavities**

There is a possibility to form bounds among hydrophobic molecules. The hydrophobic binding appears in the aqueous solution not as the result of attraction among hydrophobic molecules but of their repelling by water. Macromolecules posses groups of different types: polar (– COOH and – NH_2) and nonpolar (hydrophobic). The polar groups can form bounds on the base of electrostatic attraction, repelling the hydrophobic groups, which agglomerating can form the hydrophobic bounds. This modifies the structure of water from the near vicinity, forming the hydration water around the ions and embedding cavities around the hydrophobic radicals.

So, a certain quantity of water is bound by a macromolecule in a high structural configuration named ***bound water*** with properties and different behavior of ***free water***. There are diverse opinions about the state of water in the live cells. The single point of view which is accepted is the fact that there is a fraction of intracellular water with a high degree of ordination in the live cell and this fraction would play an important role in the development of biological phenomena which characterize the life of the cell. This fact can be researched by two ways:

– one of them consists to follow the modifications in the ordering state of cellular water during the development of some functional processes (the depolarization of membrane, the cellular excitation, the conduction of nervous influx, muscle contraction, anesthesia, etc.)

– another way consists in the modification of the ordering state of water in the cell by some external factors with the known effect upon the water and to follow the produced modifications in the functional state of the cell.

The technique of NMR is used in the first method. The modifications of the ratio of ordered water and free water during the muscle contraction were stated by this technique. The determination of NMR spectra for the protons of water of nerve shows that the depolarization by electrical factors or chemical ones of the membranes of nervous fibers is followed by pronounced changes

in the state of intracellular water. Anesthesia leads to the modification of organization degree of intracellular water which has the temporal blocking of the capacity of the cell to be excited.

4.4. Dissociation of water

The water dissociates in the ions H^+ and OH^-. The ion H^+, not having the electronic cover, possesses a high capacity of hydration. It can be bound to a water molecule leading to the formation of hydronium ion (H_3O^+).

$$H_2O \rightarrow H^+ + OH^-$$

$$H^+ + H_2O \rightarrow H_3O^+$$

The proton passes from one molecule to another molecule having a high mobility. The degree of dissociation of water is small in the pure water at 25^0C.

$$\left[H^+\right] = \left[OH^-\right] = 10^{-7}\ moles\ /\ l$$

The logarithm with the opposite sign of the concentration of hydrogen ions is named ***pH***:

$$pH = -\lg\left[H^+\right]$$

So, for the pure water, ***pH=7*** at ***25 ^{0}C***. For ***pH=7*** we have a neutral solution, but for ***pH>7*** we have a basic solution and for ***pH<7*** we have an acidic solution. The average value of ***pH*** of the organism is about 7.4.

The buffer solutions are named the biological solutions for which the relative big quantities of acids and bases are added without any changing of ***pH***. They are important for the constant maintaining of ***pH***.

The values of ***pH*** of different biological liquids are given in the table 4.2.

Table 4.2

The liquid	pH
Blood	*7.4*
Gastric juice	*1.8*
Pancreatic juice	*8.0*
Urine	*5.6*

4.5. The heavy water. Polywater

In the 1932 Urey remarked that the residues of electrolytic vases had the high density in comparison with the usual water. This leaded to discovering of the ***heavy water***. It is in small quantity in the usual water. The electrolysis of usual water at high voltages reaches the percentage of heavy water.

There are two heavy waters:

$$D_2O \quad and \quad DOH$$

D is the deuterium which is the isotope of hydrogen ${}_1^2H$. Physical properties of heavy water differ of that usual water. The main physical properties of the form D_2O are given in the next table 4.3.

Table 4.3

Physical property	H_2O	D_2O
Molecular mass	18	20
Density	1.0000 g/cm^3	1.1079 g/cm^3
The temperature of maximal density	4.0 ^{0}C	11.6 ^{0}C
The melting temperature	0.00 ^{0}C	3.802 ^{0}C
The boiling temperature	100,00 ^{0}C	101.42 ^{0}C
Surface tension (dyne/cm)	72.75	67.8
Viscosity, in poise, at 20^{0}C	$10.09 \cdot 10^{-3}$	$12.06 \cdot 10^{-3}$

The water introduced in the live organisms can be marked as the heavy water in biology which is one of the studying methods

of the metabolism of the water. The part of heavy water in the usual water can be determined by the measurement of the ***refractive index***.

Regarding the biological role of heavy water, it was observed that beginning at some concentration of heavy water, the metabolic processes are stopped. An inhibition of cell division is observed, the sterility of animals is provoked, the inhibition of active transportation and muscle contraction takes place, and the deep modifications take place in the function of myocardium emphasized clearly by electrocardiogram, the level of excitability is increased.

All these phenomena are explained if we suppose that in the processes of cell energetic, possibly in the synthesis of ATP, the proton plays an important role that can not be performed by deuteron.

The recent researches emphasized the existence of one special form of water named "polywater" which has a modified series of properties of the usual water. The boiling temperature is 400°C, the temperature of solidification is – 51°C, the density is 1.5g/cm^3, viscosity 15 times greater than in the usual water.

The polywater was obtained by the condensation of water steams in the capillaries of the diameter from 2 till 40 microns in the closed space with the relative humidity of 93–98 %. The polywater is divided in two no miscible phases at the temperatures small than – 12°C . It is supposed that the hydrogen bounds which establish the structure of polywater have a preponderantly covalent character. The biological implications of this form of the water are not clearly discussed till now.

Theme 5. MOLECULAR BIOPHYSICS

5.1. Forces and intermolecular bonds. Forces and ionical bonds

The structure of molecule is determined by attraction of intermolecular forces (among the constituent atoms). The molecule is

formed as the stable configuration when the attraction forces between atoms are approximately equal with the repelling forces and the potential energy of the system becomes minimal.

In order to understand the formation of biological structures from molecular components is necessary the knowledge of different types of interactions. Generally, the intermolecular forces are of electrical nature.

We will examine only such bonds which are met frequently in the structure of biopolymers or molecules of live matter.

An ionized substance (electrolyte) is formed of positive and negative ions.

The electrostatic forces exist among them given by the law of Coulomb:

$$F = \frac{q_1 q_2}{4\pi\varepsilon_0 \varepsilon r^2} \qquad (1)$$

These strong forces are decreased with the square distance $F \sim \frac{1}{r^2}$. The formation of ionic bond is created when two atoms are brought very closely. The electrons occupy different energetic levels, so that one electron can pass easily from one atom to another. This is the case when the atom of **Na** is brought very closely to **Cl** atom.

A single electron from the external orbital of **Na** atom passes to the external orbital of **Cl** filling the free space of it. As the result the Na atom is charged positively, but the **Cl** atom negatively. The atoms become ions, but the different electrical charging maintains the bond among them. In this case we can say about the apparition of ionic bond.

The ionic bond is broken easily in the solutions, so they two ions are in ionic free form. So, the separation of ions in solutions is the proving that the molecule in the non dissolved state contains an ionic bond.

The dipoles appear in the ionic bond, which attraction forces lead to the arrangement of molecules as the form of crystal. So, the crystal of **NaCl** is formed.

5.2. Van der Waals forces

The existence of these forces was predicted in 1878 by scientist Van der Waals and they represent the attraction force which exist for all types of atoms and molecules and are characterized by the factor a/V_0^2 from the equation of real gases:

$$\left(P + \frac{a}{V_0^2}\right)(V_0 - b) = RT$$

The neutral molecules can have a symmetrical spatial structure (for example methane: CH_4) or one asymmetrical which contrary charges sign are removed spatially (they have different mass center). Such molecule represents an electrical dipole.

The electrical forces named exist among dipoles. They are weaker than ionical forces and are decreased rapidly with the distance:

$$F \sim \frac{1}{r^2}$$

Van der Waals forces do not modify the structure and properties of molecule, staying at the fact of formation of molecular complexes.

Some molecules or atomical groups are permanent dipoles (water molecule, and the groups NH_2, COOH, CO, CHO in the organical molecules) (see the *fig. 5.1, a*).

The molecules of symmetrical structure can become dipoles near the electrical charge that attracts the charges of contrary sign, separating so the positive and negative charges: an induced dipole is obtained (*fig. 5.1, b*).

There are molecules which spatial asymmetrical repartition of electrical charges is given by the movement of electrons resulting

to the creation of one dipole that has a determined direction only in a very short interval of time: instantaneous dipole (*fig. 5.1, c*).

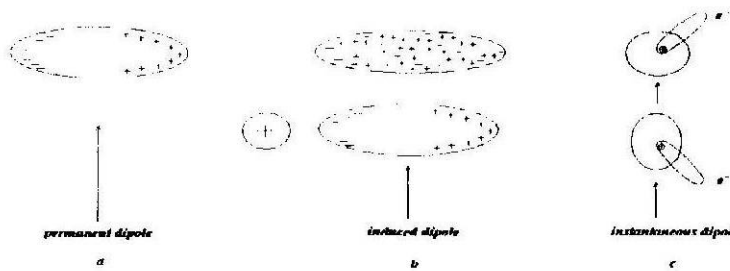


Fig. 5.1.

5.3. Coordinative bonds (Hydrogen bonds)

A great number of facts show that the groups which contain an atom of **H** are as the permanent dipoles. They are susceptible to contract with other molecules the hydrogen bonds.

The hydrogen bonds represent such particular case of Van der Waals forces of average distance (approximately $3\Lambda^0=3\cdot10^{-10}$m). A molecule that is formed of hydrogen atom and one electronegative atom leads to the formation of **covalent bond.**

The electron of the hydrogen is attracted to the electro-negative atom X leading to the formation of the dipole of negative charge at X atom and with the positive charge at H atom (*fig. 5.2, a*). As there is another atom Y in the near place of this dipole, then the bond named hydrogen bond appears between X-H and Y. (*fig. 5.2, b*).

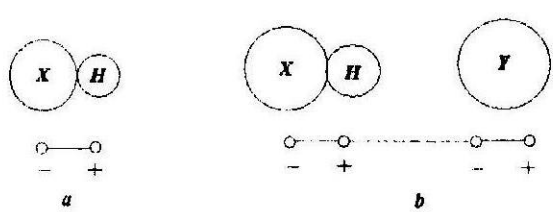


Fig. 5.2. **The formation of hydrogen bond with the dipole – dipole interaction**

The hydrogen bond has the next characteristics:

– as the proton is smaller than another atom, then the atoms X and Y are so closely that another electronegative atom can not be included. **So, the hydrogen bond can not be realized only between two atoms.**

– due to of electrostatic repelling between X and Y the hydrogen bond is always **in the straight line.**

The creation of hydrogen bonds explains the molecular aggregations which determine the non usual properties of water and plays an important role in the establishing of spatial structure of biopolymers.

At the small intermolecular distances, the repelling forces appear, especially due to of electrostatic repelling among nuclei. These are quickly decreased with the distances:

$$F \sim 1/r^{13}$$

The dependence of interaction of distance is shown in the next table 5.1.

Table 5.1

Type of interaction	Wp – proportional
Ion – ion	r^{-1}
Ion – dipole	r^{-2}
Dipole – dipole	r^{-3}
Ion-induced dipole	r^{-4}
Dipole – induced dipole	r^{-6}

Although the intermolecular forces are smaller than intramolecular, their effect becomes significantly due to of big number of interactions of this type. The small respective energy of these intermolecular forces allows the breaking and their formation easily enough, which are responsible of the biological functions of bio macromolecules.

5.4. Potential energy of intermolecular forces. The aggregation states

The variation with the distance of potential energy due to of intermolecular forces is represented in the *fig. 5.3.* That given by attraction force is negative, so it is represented in the inferior plane, but the energy determined by repelling forces which are positive is represented in the superior plane. The sign of potential energy is not taken by any fact, but results from the sense, that the variation of internal energy influences in which component is: the increasing of attraction forces (in absolute value) leads to the decreasing of internal energy (for example: the liquid have internal energy smaller than gases).

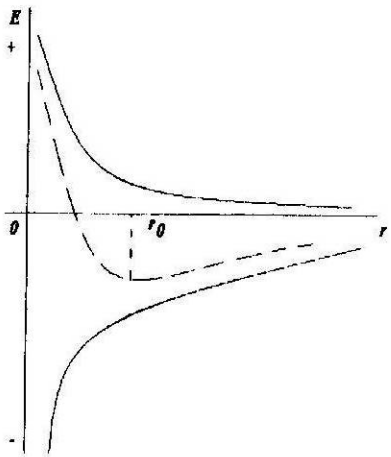


Fig. 5.3. **The potential energy due to of intermolecular forces**

The total potential energy is the resultant (algebraic sum) of those two energies. It is observed that as the result of more rapid decreasing of repelling forces with respect to those of attraction, the resultant presents a minimum at some distance among molecules.

Macroscopically the substances appear as three states of aggregation: gaseous, liquid, solid each with the characteristic properties. A delimitation can made among those three states begin-

ning from intermolecular distances, as the result from the intermolecular forces:

– **the gaseous state**: the intermolecular forces are neglected (it is exact as in the case of ideal gas)

– **liquid state**: the forces of attraction are important, those of repelling remain neglected, that determines a proper volume without the proper form;

– **solid state**: both the attraction forces and those of repelling are important, so that they have the volume and proper form. The structure of solid is arranged such that the intermolecular distance corresponds to the minimal potential energy (the resultant of attraction and repelling forces).

5.5. Phase transformations. Liquid crystals

The passage from one aggregation state to another one is named the phase transformation. It is realized in such conditions of temperature and pressure (*fig. 5.4*).

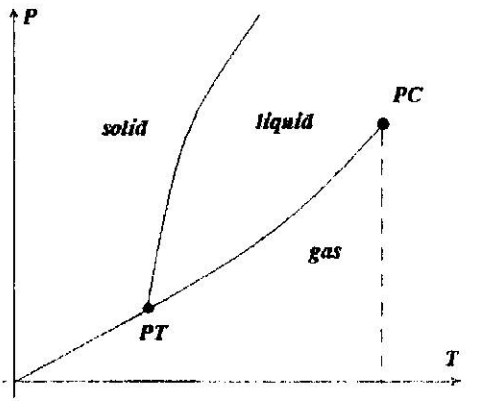


Fig. 5.4. **The phase transformations: PT – The treble point; PC – the critical point**

Treble point (TP) represents the point for which all three states of aggregation exist, in the dynamical equilibrium and it is characterized by a certain value of temperature and pressure specific to respective substance.

Critical point (CP) is the point over which the gas can not be transformed in the liquid indifferent if the pressure is increased.

The gases and liquids which have some common features are named generically **fluids.**

The isotropic solids, non crystallized, such as the glass, can be considered as super compressed liquids: there is not any discontinuity between the solid and vitrified state; the melting takes place slowly, by softening of the substance, not suddenly, at some temperature (the melting point) as in the case of crystals. The net differences appear only in the liquid state and crystalline one, the passage is always discontinuously.

The liquid crystals are the organic crystals for which the solid – liquid transitions not performed directly but passing through intermediary phases, the states for which the substance is anisotropic (similar to the solid crystal), but fluid.

The intermediary phases are: ***mesophases, mesomorphe phases or condensed fluids with the spontaneous anisotropy.***

The properties of liquid crystals are given by spatial orientation of the moleculesor the some molecular aggregates.

As the functions of formation conditions of mesophase, the liquid crystals can be classified in the following:

Thermotrope crystals, they are obtained in some interval of temperatures.

Lyotrope crystals, they are obtained in some interval of concentrations.

The thermotrope crystals are divided in its turn as the dependence of spatial orientation into:

Nematical: the long axis of the molecule is oriented under the preferential direction (*fig. 5.5, a*). They have a transparence of about 100% and do not posses optical activity.

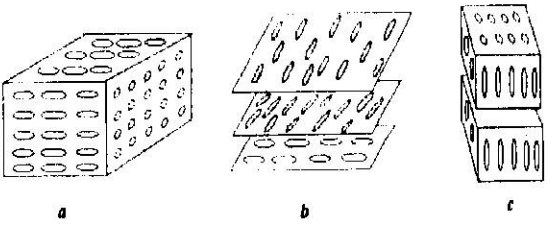


Fig. 5.5. **The thermotrope liquid crystals: a. nematical, b. cholesterical, c. smectical**

Cholesterical: they are formed of the structures of molecules with the long axis oriented parallel, but the orientation direction is rotated continuously with an angle from one layer to another making an helicoidally structure (*fig. 5.5, b*). They are optic active and the direction of rotation can be of the right or of the left. The liquid cholesterical crystals have the property to change the color in the whole visible range as the function of temperature. This characteristic allows their usage in the thermography to follow the development of vascular affections, the diagnosis of cancer etc.

Smectical: They have stratified structure (*fig. 5.5, c*). The long axis of the molecule is oriented normally or bent under the layer plane (for example: myelin)

An important property of liquid crystals is the changing the optical properties in the presence of weak electrical fields becoming the optical active substances, able to rotate the polarization plane of light. This property is explained by the orientation of molecules in the field which are considering as the electrical dipoles.

If such liquid crystals are situated behind optical active quartz plate then they absorb strongly the light when the electrical field is created on these crystals perpendicularly with respect to light polarization plane from the quartz and the visual field becomes darkened.

There are lyotrope liquid crystals with a certain field of concentration in the aqueous strong polar biological liquids. They form structures like spherical, cylindrical, prismatic, and la-

mellar with a maximal number of hydrophilic bonds and hydrophobic interactions (*fig. 5.6*). Such structure is found in the cell membranes, they have the characteristics of some lyotrope liquid crystals.

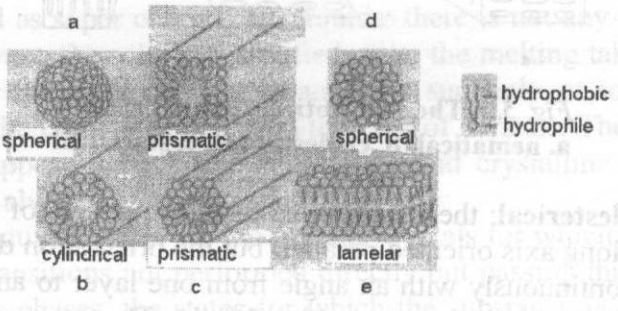


Fig. 5.6. **Lytrope liquid crystals**

5.6. General notions of biophysics of macromolecules. Definition and classification of macromolecules

The molecular mass of macromolecules is about 5000 daltons (D). The simple organic molecules and then biologic specific macromolecules are made of inorganic molecules in the organism.

The diversity of macromolecules from live matter is extremely large and the atomic molecular interactions and the types of chemical bonds from live matter do not possess any difference with respect to inorganic matter. One of principal argument for this affirmation represents the realization of artificial synthesis of biomolecules.

Biological macromolecules (named often biopolymers) synthesized artificially possess kinetic properties, electric, optic identical with synthesized macromolecules in the cells.

The fundamental types of biopolymers are:

1. Proteins that represent the macromolecules made of amino acids which are used in most important structures and processes from the organism.

2. Nucleic acids (made of nucleotides) which are of two types **DNA** and **RNA** and form the support of genetic information.

3. Polysaccharides: amylose which is a principal component of the vegetable cells and glycogen that plays a correspondent role in the animal cells. These macromolecules serve for the storage of glucose which is a basic "food" of the cell. Another macromolecule of this type is the cellulose which is an important constituent of the vegetable cells.

4. Lipids and especially phospholipids are the components of the cell membranes.

There are some criteria of classification of biological macromolecules:

1. the alternation of monomers: each biopolymer is made of a series of fundamental units named monomers. The polymers are named the macromolecular compounds made of groups of identical atoms which are repeated. Marking with A the grouping of atoms, a polymer can be written as follow:

$$A\text{-}A\text{-}A\text{-}A\text{-}A \quad or \ (A)_n$$

The copolymers are named the macromolecular compounds which monomers do not possess identical composition, for example:

$$A\text{-}C\text{-}B\text{-}A\text{-}C\text{-}D\text{-}A$$

Where A, B, C and D are different monomers. The most important type of copolymers is proteins which monomers are amino acids.

2. the geometrical form. From this point of view the macromolecular compounds are

- linear
- branched
- spatial

3. as the function of biological form in the organism. There are following types of macromolecules.

a) Bio colloids – the circulated proteins from the plasma and some soluble proteins from the cytoplasm (globulins, fibrinogen).

b) Macromolecules of the structure – organized in a series of structures such as: muscle proteins (actinic, myosin), collagen, coratin etc, as an extreme importance for the organism

4. As the function of informational level the macromolecules are classified in high carrying of information (nucleic acids and enzymes) and the macromolecules with the reduced information, or absent of information which are polysaccharides.

5.7. Composition, conformation, function and electrical properties of the proteins.

The proteins have the greatest part in the cells: 50% and more from the dried mass. The diversity of proteins from the live matter is $10^{10} - 10^{12}$. It was established by chemical and physical methods that the proteins which were examined consists of C, H, O, N, a lot of S, some have the elements as: P, Fe, Zn, Cu.

The molecular mass of them is 5000–220000 D some of them reach 1000 kD (1 D=$1.6 \cdot 10^{-27}$ kg).

The protein is unbounded by acidic hydrolysis in the simple organic compounds: α – amino acids, which contain an amino group – ($-NH_2$). The amino acids differ among them by the nature of the radical and the lateral chains (*fig. 5.7*).

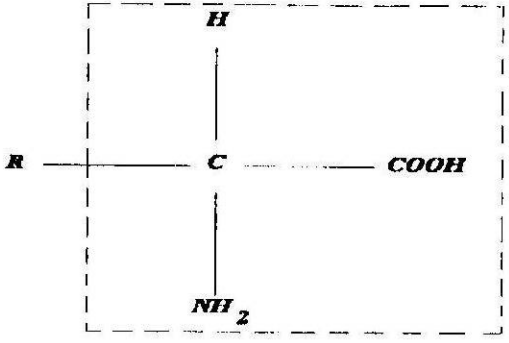


Fig. 5.7. **The general structure of aminoacids**

The proteins possess **the primary, secondary, tertiary and quaternary structure** (*fig. 5.8*).

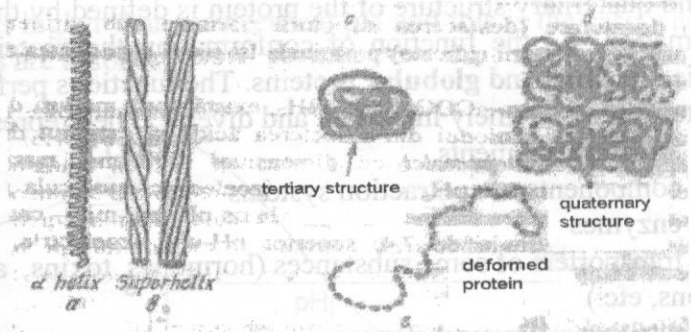


Fig. 5.8. **Conformation of the proteins**

The primary structure represents the covalent frame of the polypeptide chain with the specific segmentation of amino acidic components.

The secondary structure consists in the arrangement of polypeptide chains on a single spatial dimension, making the α helix structures. The spiraling is made in accordance with **Linus Pauling** rules as for: the diameter of one spiral is 10,1 A^0, the oxygen from – CO of one chain is situated in front of hydrogen from Nh of the next spiral. A hydrogen bond is formed between O and H which makes the spiral stable. The step of spiral corresponds to the minimal distance between two equivalent points and is 5.21 A^0.

The tertiary structure represents the three-dimensional spatial structure of one polypeptide chain. The intracathene bonds are specific to tertiary structure which assures the stability of coiled chain.

The quaternary structure is formed of the association of distinct polypeptide chains making the oligomer proteins. The component chains are named subunits or protomers. The stability and the functional characteristics are assured by the **intercathenary bond, ionic ones and hydrophobic ones**.

The spatial structures, tertiary and quaternary have an important role in the protein synthesis, the production of antibodies, enzymatic reactions, ionic channels, pumps, etc. The secondary, ter-

tiary and quaternary structure of the protein is defined by the term conformation. As the function of conformation the proteins are classified in **fibril** and **globular** proteins. The functions performed by proteins are extremely important and diverse. They represent:

- structural elements
- components of contraction systems
- enzymes
- transporters of some substances (hormones, toxins, antibodies, ions, etc.)
- nutritive reserve substances

The proteins lose the capacity of realization of biological functions by destruction (unbinding of normal structure under the influence of extreme pH or high temperature).

– There are two modalities of dissociation of the proteins (*fig. 5.9*) due to of the groups – COOH and $-NH_2$. The anion from acidic dissociation and the cathion from basic dissociation represent the protein ions with the dimensions greater than inorganic ions.

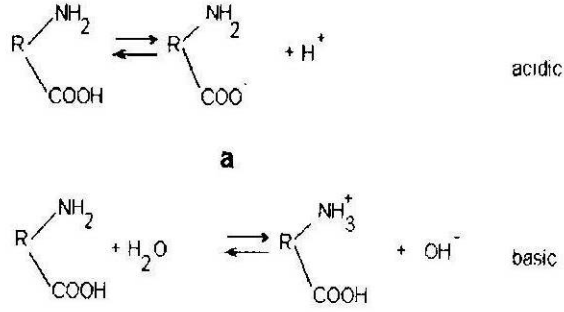


Fig. 5.9. **The dissociation of proteins: a. acidic dissociation; b. basic dissociation**

At some pH_i named isoelectrical pH , the protein molecule is neuter, at bigger value of pH it becomes anion, but at small pH it becomes cathion (*fig. 5.10*). The average pH in the organism is 7.4

which is higher than isoelectrical pH and this is the case when the proteins are anions.

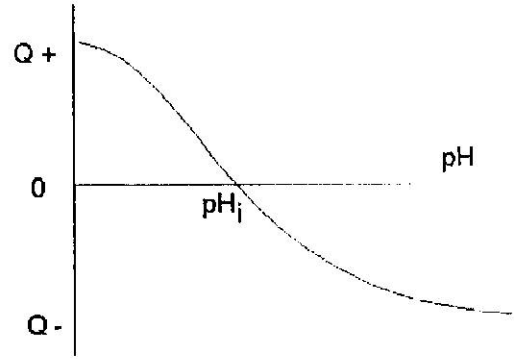


Fig. 5.10. **Modification of the electrical charge of protein as the function of pH of the medium: pH_i is the isoelectric pH**

Theme 6. PHYSICAL METHODS OF STUDY IN BIOPHYSICS

6.1. Planetary model of the atom. Theory of Bohr

Earlier theories about the construction of atom stated that the atom represented a sphere filled uniformly with the positive and negative charges, for which the total positive charge is equal to total negative charge of electrons and that is why the atom is electrical neutral. This was stated by Thomson. After that, the distribution of positive and negative charges in the atom was studied by Rutherfordt in the experiment of the scattering of α particles in the thin layers of substances. Rutherfordt observed from the experiment that some α particles scattered with the angle of 180^0 (they returned back). From the Thomson point of view, the α particles would not return back. They would penetrate the thin layer without any scattering back. Rutherfordt suggested that the electrons do not belong to the uniform sphere. They move on the orbits aro-

und the positive charge named nucleus. But from classical point of view if the electrons move on the circles with the acceleration they must all time irradiate the electromagnetical waves. The process of irradiation is followed by the losing of energy and finally the electron falls on the nucleus and the process of annihilation of positive and negative charges takes place and the atom disappears. This did not explain the stability of atom.

That time, the discrete spectra of some light sources were observed which was in contradiction with the theories of electrodynamics. This theory states, if the electrons move with the irradiation of energy, then the spectrum of the emitted light observed through prism must be continuous (the continuous passage from one color to another, as the colors of rainbow).

The problem was solved by the scientist Niels Bohr in 1913. He suggested two hypotheses:

1. Only discrete orbits satisfying some defined quantum conditions are realized from the infinite set of the electronical orbits possibly from classical mechanics. The electron belonging to one of these orbits does not irradiate the electromagnetical wave (light). These states are named stationary states. So, the stationary states are the states for which the atom does not absorb and does not emit the energy. The atom can be in this state long time if it is not acted by other interactions.

The values of energies of stationary states form a discrete series: $E_1, E_2, E_3, \ldots E_n$.

2. The atoms emit and absorb energy only if they pass from one stationary state to another stationary state. The emitted radiation of absorbed one in such transition has a frequency ν_{mn} determined by the relation:

$$h\nu_{mn} = E_m - E_n$$

where $h\nu_{mn}$ is the energy of emitted or absorbed photon, but E_m and E_n are the energies of stationary states, which the transitions takes place for.

The mechanical model of the planetary atom is the model of one material point that moves on a circle orbit under the influence of one force which passes permanently through a fixed point as presented in the *fig. 6.1.*

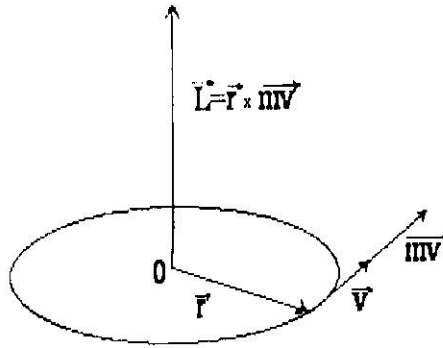


Fig. 6.1. **The kinetic moment of the material point with respect to the center**

The material point in the movement is characterized by the speed $\bar{v}$ and the impulse $m\bar{v}$ which are always tangent in the circular movement. The kinetical moment of the material point with respect to the origin of coordinates system is:

$$\bar{L} = \bar{r} \times \bar{p} = \bar{r} \times m\bar{v} \qquad (1)$$

$\bar{L}$ is perpendicular both on $\bar{r}$ and $\bar{v}$, so it is perpendicular each time on the plane of trajectory.

Only such orbits are realized from all orbits of electrons possibly from classical mechanics for which the impulse moment is equal to a whole number of Planck constant η (2).

$$L = n\eta \qquad (2)$$

$$mvr = n\eta \qquad n = 1,2,3,... \qquad (3)$$

Let us examine the electron moving in the field of atomical nucleus with the charge Ze. For Z=1 such a system corresponds to

the hydrogen atom, for other values Z to hydrogenous ions, which are the atoms with order number Z with one electron. The equation of movement of electron is:

$$m\frac{v^2}{r} = \frac{Ze^2}{4\pi\varepsilon_0 r^2} \qquad (4)$$

$$m^2v^2r^2 = n^2\eta^2 \qquad (5)$$

$$m^2\frac{v^2}{m} = \frac{Ze^2}{4\pi\varepsilon_0 r} \qquad (6)$$

$$m^2v^2 = \frac{Ze^2m}{4\pi\varepsilon_0 r} \qquad (7)$$

We write the relation m^2v^2 from (5) and substitute in (7)

$$m^2v^2 = \frac{n^2\eta^2}{r^2} \qquad (8)$$

The right parts of the relations (7) and (8) are equal, then:

$$\frac{Ze^2m}{4\pi\varepsilon_0 r} = \frac{n^2\eta^2}{r^2} \qquad (9)$$

The final relation about r is:

$$r = \frac{4\pi\varepsilon_0 n^2\eta^2}{Ze^2m}; \qquad n = 1,2,3,... \qquad (10)$$

We can see from this relation that the radiuses are quantified and are increased with the square law. The radius is changed with the square law (*fig. 6.2*).

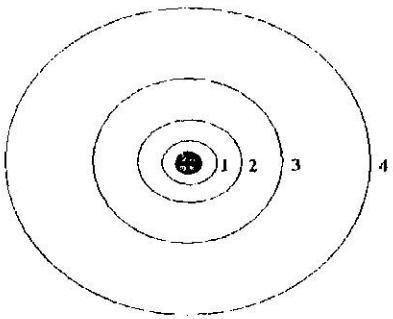


Fig. 6.2. **Planetary model of the atom**

The radius of the first orbit (n=1) of hydrogen atom (Z=1)is named the ***Bohr radius.*** Its value is:

$$r = \frac{4\pi\varepsilon_0 \eta^2}{e^2 m} = 0.529\,A^0 \qquad (11)$$

The internal energy of the atom consists of the kinetical energy of the electron and the energy of interaction of the electron with the nucleus.

$$E = E_{kin} + E_{pot} = \frac{mv^2}{2} - \frac{Ze^2}{4\pi\varepsilon_0 r} \qquad (12)$$

From (6) we have that $m\frac{v^2}{2} = \frac{Ze^2}{8\pi\varepsilon_0 r}$ and

$$E = \frac{Ze^2}{8\pi\varepsilon_0 r} - \frac{Ze^2}{4\pi\varepsilon_0 r} = -\frac{Ze^2}{8\pi\varepsilon_0 r} \qquad (13)$$

Substituting (10) in (13) we have:

$$E = -\frac{Ze^2}{8\pi\varepsilon_0 r} = -\frac{Ze^2 \cdot Ze^2 \cdot m}{8\pi\varepsilon_0 n^2 \eta^2 4\pi\varepsilon_0} = -\frac{mZ^2 e^4}{32\pi^2 \varepsilon_0^2 n^2 \eta^2} \qquad (14)$$

So:

$$E_n = -\frac{mZ^2e^4}{32\pi^2\varepsilon_0^2 n^2\eta^2} \qquad (15)$$

The energies of energetical levels are measured in eV in spectroscopy. 1eV=1.66·10^{-27} J

The scheme of energetical levels determined by the relation (15) is presented in the (*fig. 6.3*).

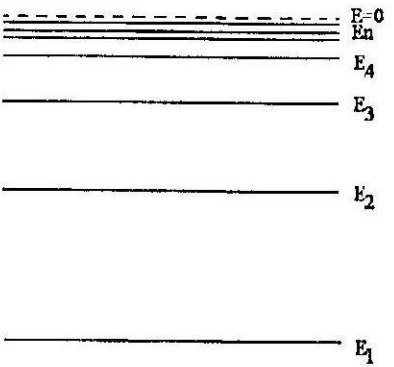


Fig. 6.3. **The scheme of energetical levels**

The energies of stationary states determined by the relation (15) are decreased with the quantum number ***n***. In the limit case for the infinite orbit ($r \to \infty, n \to \infty$), then $E=0$ (the interrupted line) (*fig. 6.3*). The fundamental state of the hydrogen atom is the state with the quantum number ***n=1.*** This state is named fundamental state. The other states with the energies E_2, E_3, ... E_n are named the excited states.

In order the electron to pass from the fundamental state (n=1) on the one excited state (E_m) is necessary to transmit the energy equal to $\Delta E = E_m - E_1$. This energy can be received by the absorption of one photon of energy $h\nu = \Delta E - E_m - E_1$ (*fig. 6.4*).

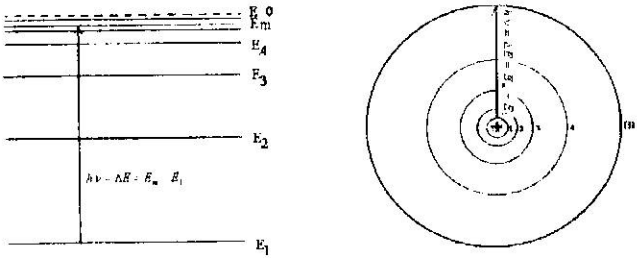


Fig. 6.4. **The energetical scheme of the excitation of atom**

The disexcitation of the atom, so that the returning back to the fundamental state is performed by the emission of one photon with the energy equal to the difference $h\nu = \Delta E = E_m - E_1$.

The model of Bohr was verified by the comparison of the calculated frequencies with the frequencies of spectral lines emitted by the atom of hydrogen. A good concordance was found. The atom of hydrogen passes from the fundamental state (n=1) into one excited state with the **quantum principal number m=2,3,4,....**by excitation. The radiations are emitted by disexcitation (*fig. 6.5*) which frequencies are given by the relation:

$$h\nu = Em - En = \frac{mZ^2e^4}{32\pi^2\varepsilon_0^2\eta^2}\left(\frac{1}{n^2} - \frac{1}{m^2}\right), \quad m=2,3,4,\ldots.; \; n<m \quad (16)$$

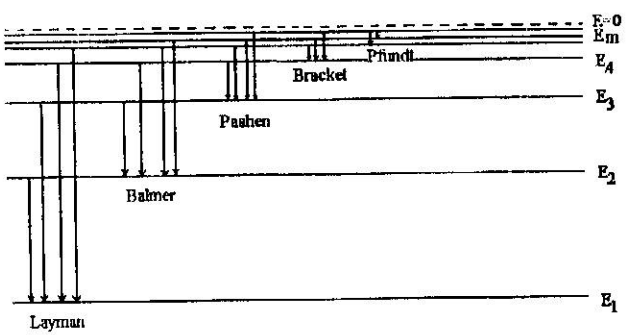


Fig. 6.5. **Spectral series**

$$\nu = \frac{c}{\lambda} = \frac{Em - En}{h} = \frac{mZ^2e^4}{32\pi^2\varepsilon_0^2\eta^2 \cdot h}\left(\frac{1}{n^2} - \frac{1}{m^2}\right) \qquad (17)$$

ν is the frequency of the emitted spectral line; λ is the wavelength of the spectral line; c the speed of light in vacuum or air ($c=3\cdot10^8$ m/s).

$$\tilde{\nu} = \frac{1}{\lambda} = \frac{Em - En}{c \cdot h} = \frac{mZ^2e^4}{32\pi^2\varepsilon_0^2\eta^2 \cdot h \cdot c}\left(\frac{1}{n^2} - \frac{1}{m^2}\right) = RchZ^2\left(\frac{1}{n^2} - \frac{1}{m^2}\right) \qquad (18)$$

$\tilde{\nu}$ is the wave number; the measure unit of wave number in spectroscopy is $[\tilde{\nu}]=cm^{-1}$

R is the constant of Rydberg $R=1.097373\ m^{-1}$; The Planck constants: $h=6.62\cdot10^{-34}$ J·s; $\hbar = 1.054$ J·s.

The series of spectral lines for which n=1 and m=2,3,4,... is named the Layman series.

The series of spectral lines for which n=2 and m=3,4,5,... is named the Balmer series.

The series of spectral lines for which n=3 and m=4,5,6,... is named the Pashen series.

The series of spectral lines for which n=4 and m=5,6,7,... is named the Bracket series.

The series of spectral lines for which n=5 and m=6,7,8,... is named the Pfundt series.

The Layman series is observed in the ultraviolet range of spectrum, Balmer in visible ranges, Pashen, Bracket and Pfundt in the infrared ranges.

So, the theory of Bohr explains the discrete spectrum of emission of hydrogen atom. The theoretical results are in good accordance with the experiment. For complex atoms the discrete spectra of emission are explained by the theory of ***quantum mechanics***. The theory of Bohr is a passage theory between the classical physics and quantum physics.

Each chemical atom has its own discrete spectrum of emission. This fact stays on the base of spectral analysis for the determination of chemical composition of the light source.

6.2. Luminescent analysis

All bodies which temperatures are over 0^0K radiate the electromagnetical waves named thermal radiation as the result of interatomical and intermolecular processes. The intensity and the spectral structure of thermal radiation depend on the temperature.

The supplementary radiation of the body with respect to thermal radiation at the given temperature is named luminescence.

The phenomenon of luminescence obtains different forms after the causes that they produce.

The body can become luminescent when it is touched by visible light radiation or invisible. This is the case of photoluminescence. The photoluminescence is classified in fluorescence (of short time) and phosphorescence (of long time).

The luminescence produced by the charged particles: by ions is named **ionoluminescence**, by electrons – **cathodoluminescence**; by nuclear radiation – **radioluminescence**. The luminescence produced by Roentgen radiation and γ is named **roentgenoluminescence. The triboluminescence** appears when the some special crystals are broken. The electrical field produces **electroluminescence**, a particular case of which is the luminescence of electrical discharge through gases. The luminescence produced by exothermal chemical reactions is named **chemiluminescence**.

The initial process of each luminescence is the excitation of the atom or a molecule with the external photon. In the simple case as usual this is realized in steams and monoatomical gases, the atom after a short time of 10^{-8} s returns to initial state irradiating a photon identical with that incident (*fig. 6.6*).

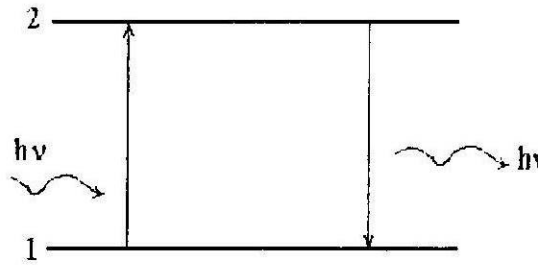


Fig. 6.6.

This phenomenon is named ***resonant luminescence***. More probably are the cases when the molecule from the excitation state **3** (*fig. 6.7*) without irradiation go to the level **2** and then spontaneously to the level **1**, irradiating one photon with the frequency smaller than that incident.

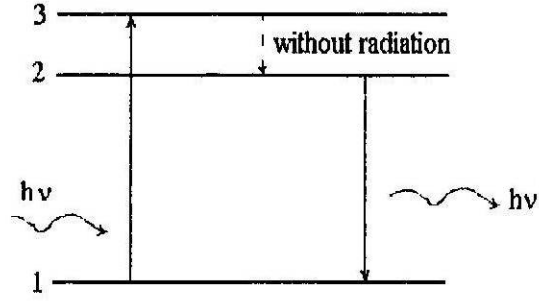


Fig. 6.7.

The transition can take place from the excitation state **4** to another **metastable intermediate state 3** in the complex organical molecules (*fig. 6.8*). The direct transition of the molecule from this state in the basic state is less probably.

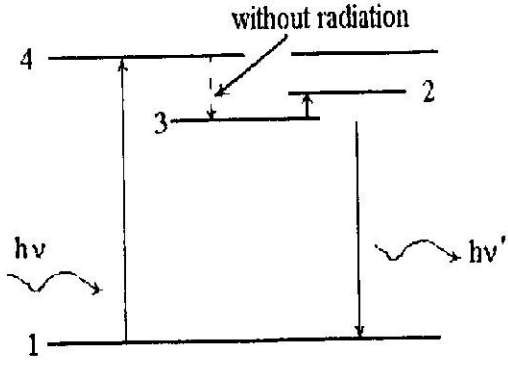


Fig. 6.8.

The transition of the molecule with the irradiation of the photon $h\nu'$ from the excitation level **2** to fundamental level **1** takes place as the result of thermal agitation or the external photon. This is one mechanism of the apparition of ***phosphorescence phenomenon.***

The ***Law of Stokes*** states that the luminescence spectrum is removed in the range of long wave lengths with respect to the spectrum that produces this luminescence (*fig. 6.9*).

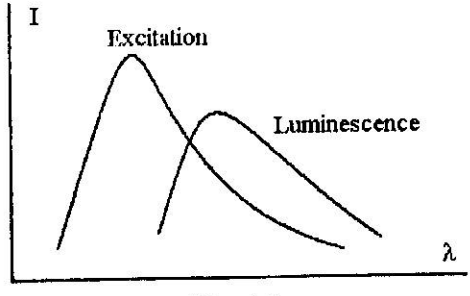


Fig. 6.9.

The luminescence could be of energies $h\nu' > h\nu$ or $\lambda' < \lambda$ Such a luminescence is named ***antistokes*** (*fig. 6.10*).

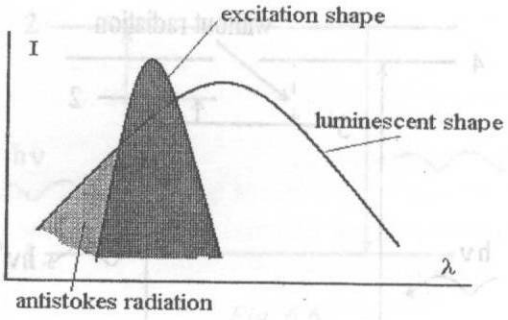


Fig. 6.10.

A series of biological molecules for example albumin molecules possesses fluorescence. The parameters of fluorescence are sensible to the structure of external medium surrounding the molecule, and the respective chemical transformations.

Last years the special fluorescent molecules which are stuck to the nonluminescent macromolecules are studied. Such molecules were named the **fluorescent probes** (in the case of noncovalent bond) or **fluorescent quotes** (in the case of chemical bonds).

The method named **fluorimetry** based on the excitation with the ultraviolet radiations of the researched structure is studied in biophysical researches (*fig. 6.11*).

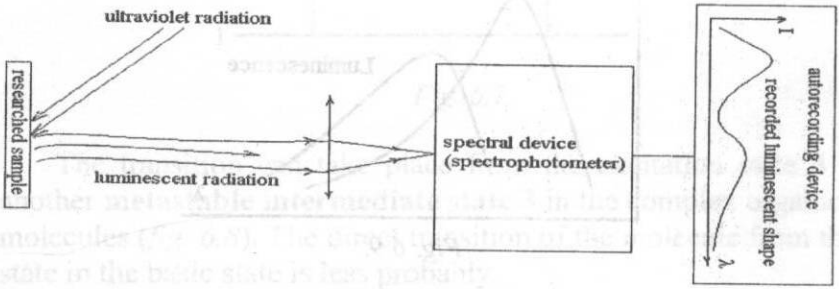


Fig. 6.11. **The principal scheme of the study of luminescence**

After the excitation of the researched substance with the ultraviolet radiation then the sample illuminates. The luminescent radiation is directed in the spectral device named spectrophotometer with the photoelectrical recording. The luminescent shape is recorded on the paper band moving with the same speed as the speed of movement of electrical motor changing gradually the position of dispersive element (prism or diffraction grating) in the spectral device. Each of luminescent maximum are related to one chemical element. The chemical elements are determined with the positions λ_1, λ_2 of luminescent maxima by special tables of spectral lines (*fig. 6.12*).

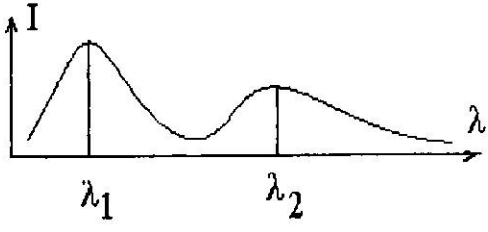


Fig. 6.12. **The luminescent shape**

If the height of luminescent maximum is small then the respective chemical element related to this is in small quantity. The determination of chemical element by luminescent method is named the **qualitative luminescent analysis.** The determination of percent content of chemical element with the heights of luminescence maxima is named **quantitative luminescent analysis.**

The fluorescent microscope is applied for the researching of micro objects. The last one is different of optical microscope, using an ultraviolet radiation. Producing the fluorescence of the researched structures the supplementary information are obtained.

6.3. Roentgen radiation. Methods of diffraction by Roentgen rays.

In 1985 the German scientist Roentgen discovered experimentally an extreme penetrable radiation. In order to emphasize the unknown nature of these radiations, Roentgen called them X rays.

Recently it is known that these radiations are of electromagnetical nature with the wavelengths of 0.01 A^0 ÷ 800 A^0.

X radiation can be obtained by the device represented in the *fig. 6.13.*

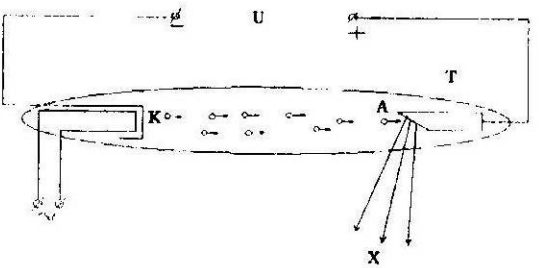


Fig. 6.13. **The electrical discharge Roentgen tube**

Where T – the vacuumed tube, A – anode; K – cathode, o – electron beam

The X rays appear during the break of the rapid electronic beam at the anode. The kinetic energy of these electrons is transformed into the energy of X radiation.

This X radiation named as radiation of break or white radiation has a continuous spectrum with the maximal frequency given by the relation:

$$eU = h\nu_{max} = h\frac{c}{\lambda_{min}}$$

The linear spectrum appears besides the X white radiation when the voltage U≥31800 V. These rays characterize the material of anode and that is why they are named **characteristic**.

The accelerated electrons can pull out the electrons from the deep levels of the atoms from anode. The free places are occupied by other electrons from high electronic covers (*fig. 6.14*).

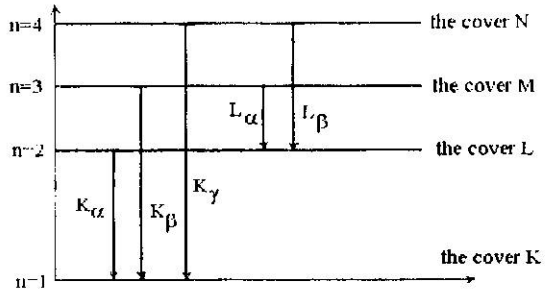


Fig. 6.14.

As the result of these transitions the X characteristic radiation appears. So the radiation spectrum of the Roentgen tube includes two components: the continuous spectrum (breaking radiation) and maxima superposed on it (characteristic radiation). The emitted spectrum of radiation by Roentgen tube with the anode made of molybdenum is represented in the *fig. 6.15.*

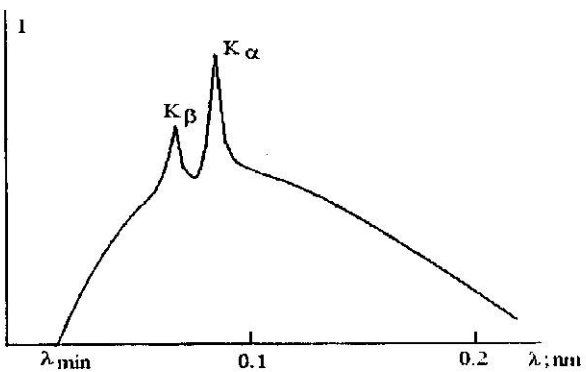


Fig. 6.15.

The frequency of the emitted line of characteristic spectrum can be calculated by the formula of Moseley:

$$\tilde{\nu} = R(Z-\sigma)^2\left(\frac{1}{n_1^2} - \frac{1}{n_2^2}\right)$$

R is the constant of Rydberg; Z – order number of element (anode); σ – screening constant; n_1 and n_2 – quantum numbers of transition.

After the discovering of Roentgen radiation the diffraction of these radiations through usual optical gratings was tried. The results were negatively. The English physicist L. Bragg (1890–1971) together with his father G. Bragg had the idea to use the natural crystalline gratings for the study of X rays. In such a grating the atoms of the substance are distributed in ordinary modality after those three directions of the space forming the **reticular** planes. The distance between two successive planes is the same and has the name of the constant of grating **d**.

The diffraction of X rays on the ordered structure (crystal, biopolymer) is phenomenon of diffusion. The scientists Bragg considered for the simplification that the diffraction of X rays as the phenomenon of reflection. The reflected waves from each parallel plane can be amplified or attenuated. These phenomena depend of the angle of slipping of the ray named also the angle of Bragg. The condition of maximum interference is realized for the case when the difference of ways of those two rays (*fig. 6.16*) is a whole multiple of wavelength, so that in the drawing **BC+BD=nλ**.

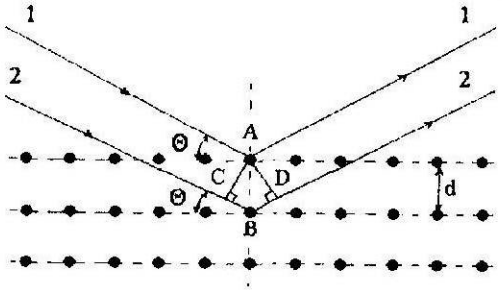


Fig. 6.16.

We can observe from the drawing that **BC=BD=d·sinΘ.** Finally the condition of interference maximum can be written as **2·d·sinΘ= nλ.** This is the equation of Bragg. The value of d can be

determined on the base of this equation if the wavelength of X rays is known. The spatial structures of different biological macromolecules as nucleic acids, proteins can be observed from the exact evaluation of the sufficient number of values **d**.

There are a lot of experimental methods, in general grouped in two cathegories of techniques.

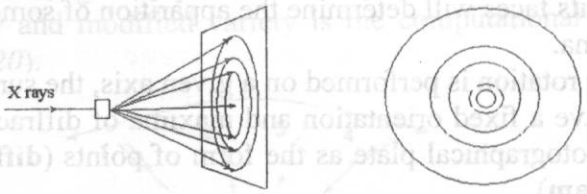


Fig. 6.17. **Method of powders**

1 – method of powders 2 – method of the block crystal.

The crystallized substance in the method of powders (*fig. 6.18*) is placed as the form of powders in front of X rays.

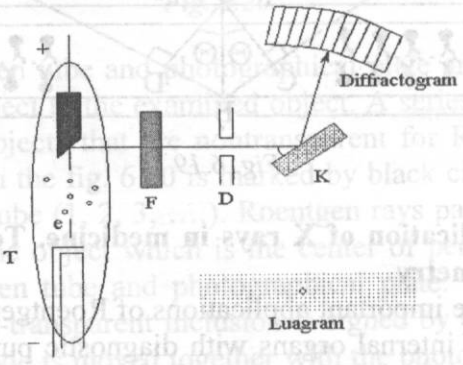


Fig. 6.18.

The entire sample is rotated so that the crystals contained in the powders take all possible orientations with respect to the incident beam, inclusively the position for which the diffraction maxima appear. The dispersed beam will form different cones which

axis is parallel to the direction of incident beam. If a photographical plate is placed perpendicularly on the direction of incident beam, the diffraction maxima will have the aspect of concentric circles.

In the case of methods of block crystals (with the sizes of about 1 mm), the crystal is placed so that one of its principal axes will be perpendicular to the direction of incident beam of X rays. Rotating the crystal around this axis, the rays that fall consequently on all its faces will determine the apparition of some diffractional maxima.

If the rotation is performed on a given axis, the surfaces of the crystal have a fixed orientation and maxima of diffraction appear on the photographical plate as the form of points (**diffractogram** or **lauegram**).

The scheme of the obtaining of one diffractogram with X rays at the level of membrane bilipid layer is obtained in the *fig. 6.19.*

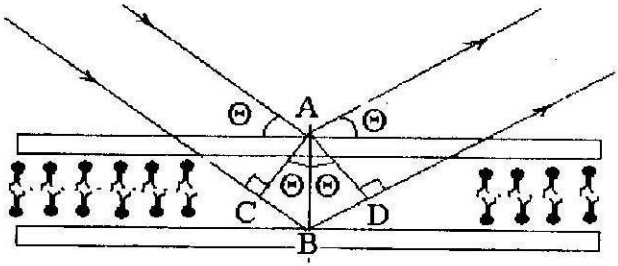


Fig. 6.19.

6.4. Application of X rays in medicine. Tomography and tomodensitometry

One of the important applications of Roentgen radiation is the radioscopy of internal organs with diagnostic purpose. This method of researching is named **Roentgen diagnosis**. Roentgen diagnosis is used in two varieties: **radioscopy** – the image is examined on the radioluminescent screen, **radiography** – the image is obtained on the photographical plate. If the radiation is used with the diagnostic purpose, then the intensity must not be high in order do not cause unexpected biological results. Therefore, a series of de-

vices exist that give good quality of images for small intensities. They are **electron – optical transducers**. Another example is **fluorography** for which the image from radioluminescent screen is obtained on sensible photoplate. The X radiation is also used with therapeutical purposes for the destruction of the tumors. This therapy is named **Roentgen therapy**.

An interesting variety of **radiography** is the method of radio tomography and modified variety is the computational tomography (*fig. 6.20*).

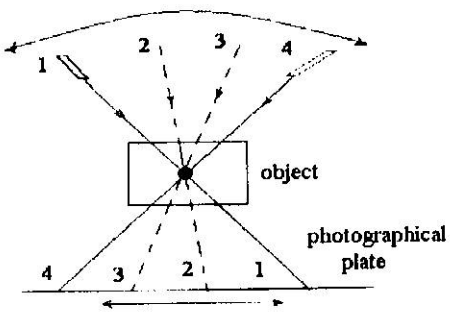


Fig. 6.20.

The Roentgen tube and photographical plate are moved constantly with respect to the examined object. A series of inclusions exist into the objects that are nontransparent for Roentgen rays. This inclusion in the fig. 6.20 is marked by black circle. For each position of the tube (1, 2, 3, ...). Roentgen rays pass through the same point of the object which is the center of periodical movement of Roentgen tube and photographical plate. This point, so that a small non-transparent inclusion is signed by dark circle. Its image of the shade is moved together with the photographical plate taking consequently positions 1, 2, 3 and so on.

Changing the position of the "oscillation center", the image in layers of the object could be obtained. The notion of **tomography** is introduced in connection with it. Using a beam of Roentgen radiation, the obtained image on the screen in tomography can be analyzed (instead of the photographical plate) which consists of

semiconductor detectors for the ionized radiation and the computer. This modern variety of tomography allows us to obtain the image in layers (lamellar) of the body on the screen of tube with the electronic beam or on the paper with the small details smaller than 2 mm at the different absorption of Roentgen radiation up to 0.1%. This allows for example to differentiate the gray substance and white from the brain and to observe very small tumors. The first Nobel Prize was awarded to C. Roengen (1901), but in 1979 this Prize was awarded to scientists G. Haymsfild and Mak – Kormac for the elaboration of Roentgen computational tomography.

6.5. Eletronical microscopy

The most important quality of one microscope is the **separation power** or the **resolution power**. If this is signed by **P**, then **P=1/d**, **d** is the minimal separable distance and represents the minimal distance between two distinctive points which are observed by microscope. The distance d is given by the relation:

$$d = 0.61\frac{\lambda}{n\sin\varphi/2} \qquad (1)$$

λ is the wavelength of the light source for the observation of microscopical sample; **n** is the refractive index of the medium that separates the sample by objective system; φ/2 is the half angle of light cone that touches the objective lens (*fig. 6.21*).

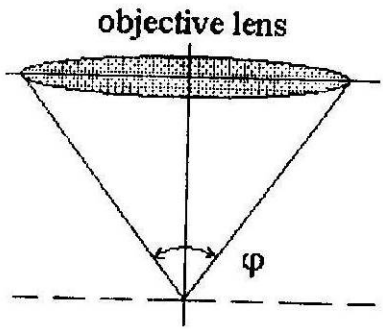


Fig. 6.21.

The product $n \sin \varphi / 2$ is named the **numerical aperture.** We can see from the relation (1) that the resolution power can be improved depending on **λ. The microobjects smaller than the wavelength of the source can not be observed. For optical microscopes d≥250 nm.**

The accelerated electronic beam possesses the associated wavelength according to the hypothesis of **Louis de Broglie.**

$$\lambda = \frac{h}{mv} \qquad (2)$$

v is the speed of electrons; m is the mass of electrons; h is the Planck constant. We can see from the relation (2) that the wavelength of electrons is smaller when the speed is greater. The speed in its turn depends on the acceleration voltage U.

$$\lambda = \frac{h}{mv} = \frac{h}{\sqrt{2emU}} \qquad (3)$$

So, the wavelength is equal to 1.3 nm when the voltage is 10 kV, and 0.3 nm when the voltage is 100 kV. The electronical microscope was elaborated with this fact. The convergence of electronic beam can be performed by magnetical "lenses" that represent the magnetical coils. The action of focusing of magnetical lens on electronic beam is presented in the *fig. 6.22.*

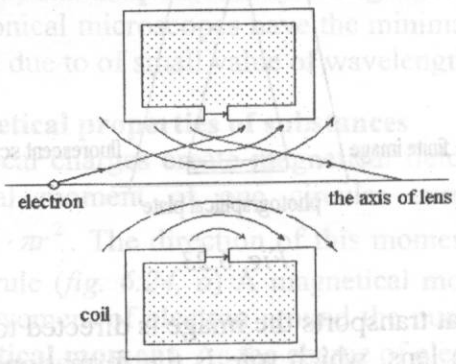


Fig. 6.22.

The electronical microscope is made of electronical canon, the system of concentration of electronical beam (magnetical lenses) and the devices of image formation (*fig. 6.23*). The emitted electrons from the canon are accelerated potential difference of anode. The electronical beam is directed by first lens with the big focal distance, which role is the concentration of the electronical beam on the object. While passing the object, the electrons are diffused under different angles. After passing the object, the beam is directed to the second lens named objective with small focal distance, which role is the formation of the first image of the sample.

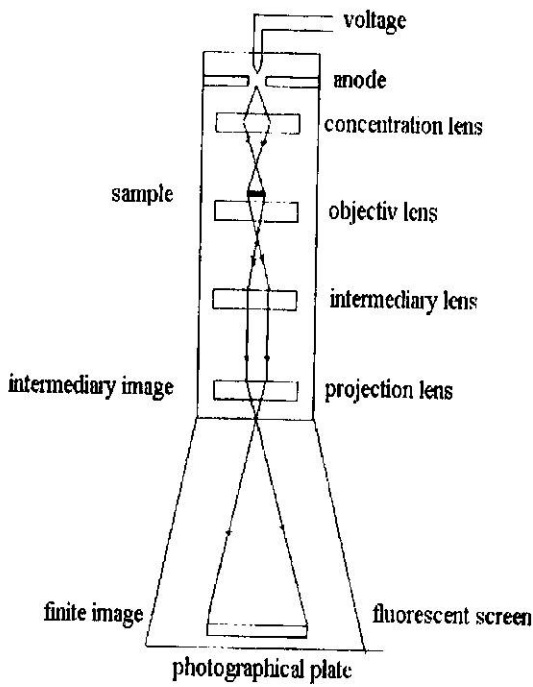


Fig. 6.23.

The beam that transports the image is directed to the third lens named projection lens, which role is the formation of real image on the fluorescent screen or on the photographical plate sensible to

rapid electrons. The above described microscope is named **conventional electronical microscope.**

There are two types of electronical microscopes in the field of biophysics.

a) **Scanning microscope**. Very thin electronic beam in this microscope is focused on the surface of sample that scans each line a limited surface, similar as television screen. The material emits the signals of different nature (especially secondary electrons) that together with the diffused electrons consist the useful elements in the formation of image on the screen of oscilloscope. This type of microscope offers an average resolution and is extremely useful in the study of three-dimensional forms of biological structures and the surfaces of these structures. The principle of this analysis is the following: We know that the irradiated body by the electronic beam emits X radiation as the continuous spectrum and a characteristic spectrum of each element of irradiated body in accordance with the law of Moseley. The inclusions are observed from the cell by this method.

b) **Microanalyzer with electronical probe.** This one is obtained by the connection of conventional electronical microscope with a spectrograph of X radiation. This device allows the realization of high chemical sensitivity analysis on the extremely reduced quantity of matter in the well defined points (in some internal points of the cell) and the **point analysis** is given by this method.

The electronical microscopes have the minimal separable distance d=0.2 nm due to of small value of wavelength.

6.6. Magnetical properties of substances

The electrical charges create magnetical field while moving. The magnetical moment of one circular current is defined as $\mu = I \cdot S = I \cdot \pi r^2$. The direction of this moment is determined by the gimlet rule (*fig. 6.24, a*) A magnetical moment is created also by the movement of electron around the nucleus, named the **orbital magnetical moment**. As the charge of electron is negative

then the kinetic and magnetic moments have opposite directions (*fig. 6.24, b*).

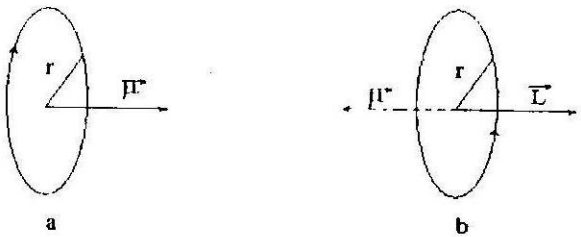


Fig. 6.24.

$$\mu = IS = \frac{eV}{2\pi r} \cdot \pi r^2 = \frac{eVr}{2} \qquad (1)$$

V=ωr and the relation is:

$$\mu = \frac{e\omega r^2}{2} \qquad (2)$$

The mechanical orbital moment L is quantified and is given by the relation:

$$L = \frac{h}{2\pi}\sqrt{l(l+1)} \qquad (3)$$

l is the quantum orbital number **(0, 1, 2, 3, 4..., n-1)**

$$L = mVr = m\omega r^2 = \frac{h}{2\pi}\sqrt{l(l+1)} \qquad (4)$$

$$\omega = \frac{h\sqrt{l(l+1)}}{2\pi m r^2} \qquad (5)$$

Substituting this value of ω in (2) we have:

$$\mu = \frac{eh}{4\pi m}\sqrt{l(l+1)} \qquad (6)$$

The value $\frac{eh}{4\pi m} = \mu_B$ is named magneton of Bohr.

It was established that the electron possesses also the rotation movement around own axis, named **spin** movement.

The spin mechanical moment L_s is also quantified:

$$L_s = \frac{h}{2\pi}\sqrt{S(S+1)} \qquad (7)$$

S is the quantum spin number and for electron is S=1/2. This movement represents an electrical charge and an elementary current is created and magnetical moment appears μ_s, and this is also quantified by the following formula:

$$\mu_s = 2\mu_B\sqrt{S(S+1)} \qquad (8)$$

All substances are divided in two classes by magnetical properties: **paramagnetic and diamagnetic.**

The paramagnetic substances are such substances which atoms posses permanent magnetical moment. They amplify the external magnetical field. Such substances are Pb; O_2; Al.

The diamagnetical substances are made of atoms without permanent magnetical moment. They are H_2; Cu; N etc. They decrease the intensity of external magnetical field. All electrons of these diamagnetic substances are coupled and spin magnetical moments are compensated (*fig. 6.25, a*).

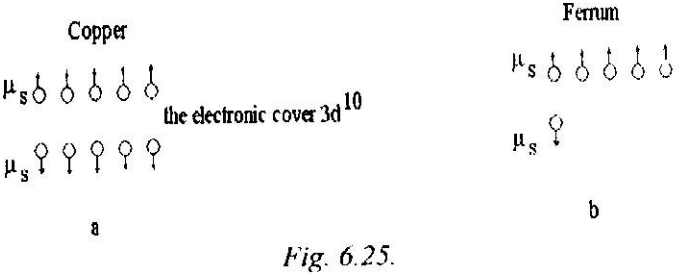


Fig. 6.25.

Both paramagnetism and diamagnetism are weak manifestations. Only a small group of paramagnetic substances such as Fe, Co, Ni posses pronounced magnetical properties because of non compensated spin magnetical moments of the electrons (*fig. 6.25, b*). These substances are named **ferromagnetic** substances and are large used in practice.

The theory of diamagnetism is based on the law of **Faraday**. The speed of rotation of electrons is decreased under the influence of external magnetical field which moments are directed in the direction of field and the speed is increased for those which orientation is opposite with respect to the field.

Magnetical properties of substances are characterized by the following macroscopic physical values such as the degree for which molecular magnetic moments of one material are directed in one particular direction and are described by a vector value named **magnetization** $\overline{M}$, and represents the magnetical moment of unit volume:

$$\overline{M} = \frac{\sum_{i=1}^{n} \overline{m}_i}{V} \qquad (9)$$

$\overline{m}_i$ is the molecular magnetic moment.

$\overline{M}$ depends on the nature of substance and the intensity of external magnetical field: $\overline{M} = \chi \overline{H}$. The material constant χ is named the **magnetical susceptibility**. χ=0 for vacuum; χ>0 for paramagnetic substances and χ<0 for diamagnetic substances.

The relation between the induction of magnetical field **B** and its intensity **H** is: $\overline{B} = \mu_0 \overline{H}$ and for a certain medium is $\overline{B} = \mu\mu_0 \overline{H}$, where μ_0 is the magnetic constant but μ is **magnetical permeability**. For vacuum μ=1.

$\mu > 1$ for paramagnetic materials; $\mu < 1$ for diamagnetic materials; and $\mu >>> 1$ for ferromagnetic materials.

The magnetical susceptibility χ depends on temperature and is given by the equation of **Curie**

$$\chi = \frac{C}{T} \qquad (10)$$

where **C** is the constant of Curie; **T** is the absolute temperature.

The ferromagnetic properties disappear at a certain temperature named Curie temperature of the ferromagnetic. For example Fe, the Curie point is 768^0C; for Ni is 358^0C.

6.7. Magnetical moment of the atom

If the electron possesses the orbital moment $\bar{L}_l$ and spin moment $\bar{L}_s$, then the resultant of these moments gives the total mechanical moment $\bar{L}_j$. The vector sum of them is:

$$\bar{\mu}_j = \bar{\mu}_s + \bar{\mu}_l \quad (3)$$

Analogically the total magnetical moment $\bar{\mu}_j$ is related to total mechanical moment by the relation:

$$\mu_j = g\mu_B\sqrt{j(j+1)} \quad (4)$$

j – quantum number of total moment.

g – gyromagnetic factor (Lande factor).

The respective projections of orbital and spin mechanical moments on a certain axis **z** are:

$$\begin{aligned} L_{lz} &= m_l\eta \\ L_{sz} &= m_s\eta \\ L_{jz} &= m_j\eta \end{aligned} \qquad (5)$$

where m_l, m_s and m_j are the quantum projection numbers of orbital, spin and total magnetical moments respectively.

The numbers m_l, m_s and m_j take respectively the values:

$$m_l = -l, -l+1, -l+2, \ldots\ldots l-1, l \qquad (6)$$

$$m_s = -s, -s+1, -s+2, \ldots\ldots s-1, s$$

$$m_j = -j, -j+1, -j+2, \ldots\ldots j-1, j$$

so that m_l obtains $2l+1$ values and m_s obtains $2s+1$ values.

When the atom contains a single electron (or one unpaired electron) then quantum spin number of this electron $s=1/2$ *and* $m_s=\pm 1/2$

The relations (2) can be written as:

$$\mu_l = \mu_B \sqrt{l(l+1)} = \mu_B \frac{L_l}{\eta}$$

$$\mu_s = 2\mu_B \sqrt{s(s+1)} = 2\mu_B \frac{L_s}{\eta} \qquad (7)$$

The projections of magnetical moments μ_l and μ_s on a certain axis z are:

$$\mu_{lz} = \mu_B \frac{L_{lz}}{\eta} = \mu_B \frac{m_l \eta}{\eta} = \mu_B m_l$$

$$\mu_{sz} = 2\mu_B \frac{L_{sz}}{\eta} = 2\mu_B \frac{m_s \eta}{\eta} = 2\mu_B m_s \qquad (8)$$

$$\mu_{jz} = g\mu_B \frac{L_{jz}}{\eta} = \mu_B g \frac{m_j \eta}{\eta} = \mu_B g m_j$$

When the atom contains a single electron (or one unpaired electron) then quantum spin number of this electron $s=1/2$ and $m_s=\pm 1/2$, then:

$$\mu_{sz} = \pm \mu_B \qquad (9)$$

The vector μ_j is not collinear with the vector L_j because of the double magnetism of the spin. The vectors $\bar{L}_l$ and $\bar{L}_s$ rotate around the direction L_j including in this precession the resultant vector of magnetical moment μ_j (*fig. 6.26*). After enough time of observation the average value of the vector μ_j will be registered and this is represented in the fig. 6.26 as $\langle \bar{\mu}_j \rangle$.

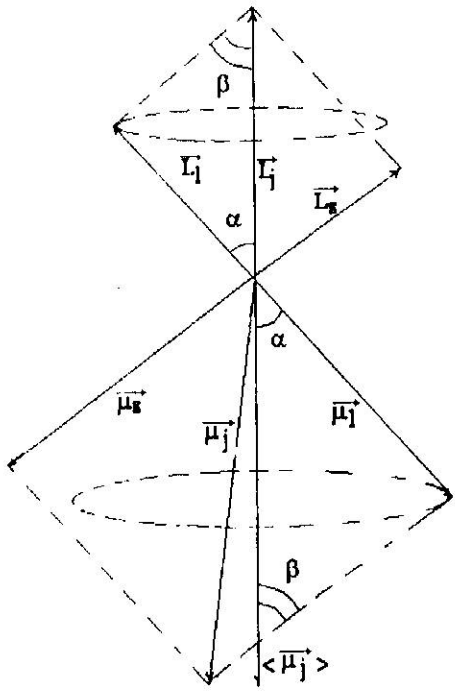


Fig. 6.26.

The gyromagnetic factor **g** is determined by the expression:

$$g = 1 + \frac{j(j+1) + s(s+1) - l(l+1)}{2j(j+1)} \qquad (10)$$

In the case when the total spin moment of the atom is equal to zero (***s=0, the case when all electrons are paired***), then total moment coincides with the orbital (***j=l***), and in this case ***g=1***. In the case when the total orbital moment of the atom is zero (***l=0***), then total moment coincides with the spin moment (***j=s***). The substitution of these values in (10) gives the result ***g=2.***

The states of the electron in the atom are described by quantum numbers *n, l, m_s and m_j*

For the principal quantum number *n, l* takes the values: *0, 1, 2, 3, ... n-1.*

The symbols of the states for the values l are given in the table below.

l=0	*1*	*2*	*3*	*4*	*5*	...
s	*p*	*d*	*f*	*g*	*H*	...

6.8. Zeeman effect

The Zeeman effect is named the splitting of energetical levels while the magnetical fields is acting on the atom. The splitting of the levels leads to the splitting of spectral lines in several components. The splitting of spectral lines while the magnetic field is acting on irradiating atoms also is named the Zeeman effect.

The Zeeman splitting of the levels is explained by the fact that the atom possessing the magnetical moment μ_j, **then the atom will posses the supplementary energy**

$$\Delta E = \mu_{jz} B \qquad (1)$$

where B is the induction of magnetical field. The projection of magnetical moment is:

$$\mu_{jz} = -\mu_B g m_j; \quad m_j = -j, -j+1, \ldots\ldots, j-1, j \qquad (2)$$

The energetical level is splitting in $2j+1$ equidistant sublevels, but the value of the splitting depends on the Lande factor (gyromagnetic factor).

At first we examine the Zeeman splitting of the levels for the spin quantum number S=0 and thus g=1. Then the formula (1) is:

$$\Delta E = \mu_B B m_j \qquad (3)$$

The splitting of the levels and spectral lines for the states with **L=1** and **L=0** (transition P→S) is presented in the *fig. 6.27*.

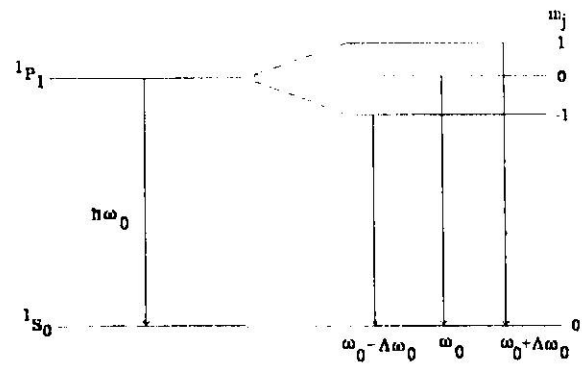


Fig. 6.27.

When the magnetical field is absent only one spectral line is observed with the frequency ω_0. There are lines with $\omega_0 + \Lambda\omega_0$ and $\omega_0 - \Delta\omega_0$ besides the line with the frequency ω_0 when the field is included. The analogical scheme for more complicated case is represented in the *fig. 6.28* for the transition D →P. At first we can see that the initial line is splitted in seven components, but in reality only three components are: the line with the frequency ω_0 and two symmetrical lines with the frequencies $\omega_0 + \Lambda\omega_0$ and $\omega_0 - \Delta\omega_0$. This is explained by the fact that there is a selection

rule for quantum magnetic number $\mathbf{m_j}$ according to this the transitions are possibly for which $\mathbf{m_j}$ remains constant or is changed by **1**: $\Delta m_j = 0;\pm 1$.

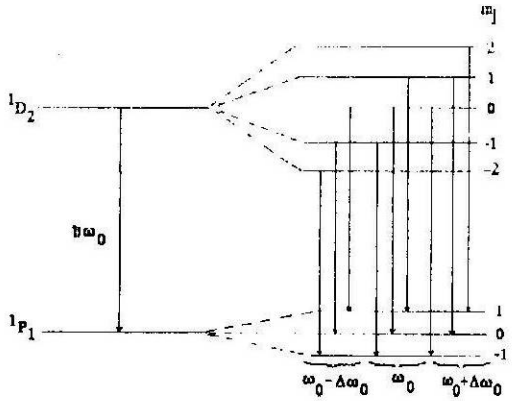


Fig. 6.28.

The transitions are possible shown in the fig. 6.28 in accordance with this rule. Three components with the same frequencies are obtained similar with those shown in the fig. 6.27. **The deviation of the components $\Delta\omega_0$ obtained in the above cases is named normal or Lorentz deviation.**

Taking into consideration formula (3) this deviation is:

$$\Delta\omega_0 = \frac{\mu_B B}{\eta} \qquad (4)$$

The researched splitting in three components two of them are deviated from initial line with normal deviation $\Delta\omega_0$ is named simple Zeeman effect.

The simple Zeeman effect is obtained for that case when the total spin S=0. In the case when S≠0 the number of splitting components is greater than three. Such effect is named **complex Zeeman effect.**

For example the splitting of spectral lines of **Na** atoms we examine in *fig. 6.29* and *fig. 6.30*.

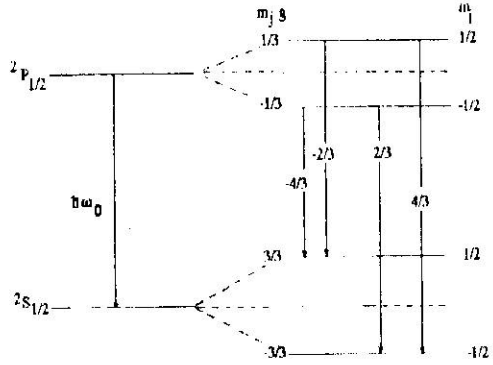


Fig. 6.29.

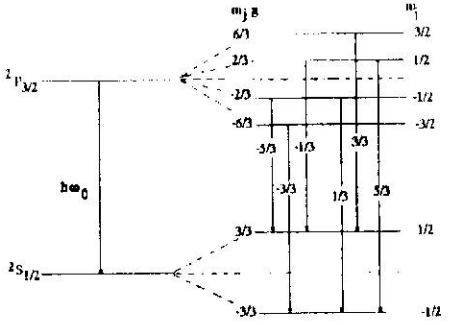


Fig. 6.30.

The transitions for Na atoms are: $3^2P_{1/2} \rightarrow 3^2S_{1/2}$ (fig. 6.29) and $3^2P_{3/2} \rightarrow 3^2S_{1/2}$ (*fig. 6.30*).

The **Lande factor** has the values:

a) $3^2S_{1/2}$ ***(l=0, s=1/2, j=1/2)***

$$g = 1 + \frac{1/2 \cdot 3/2 + 1/2 \cdot 3/2 - 0 \cdot 1}{2 \cdot 1/2 \cdot 3/2} = 1 + 1 = 2$$

b) $3^2P_{1/2}$ *(l=1, s=1/2, j=1/2)*

$$g = 1 + \frac{1/2 \cdot 3/2 + 1/2 \cdot 3/2 - 1 \cdot 2}{2 \cdot 1/2 \cdot 3/2} = 1 - 1/3 = 2/3$$

b) $3^2P_{3/2}$ *(l=1, s=1/2, j=3/2)*

$$g = 1 + \frac{3/2 \cdot 5/2 + 1/2 \cdot 3/2 - 1 \cdot 2}{2 \cdot 3/2 \cdot 5/2} = 1 + 1/3 = 4/3$$

We can write the supplementary energy for the energetical level $3^2S_{1/2}$ **as:**

$$\Delta E' = \mu_B B g' m'_j$$

g'=2=6/3

For the level $3^2P_{1/2}$ **:** $\Delta E'' = \mu_B B g'' m''_j$

g''=2/3

We can write the deviation of the line with respect to initial line the following expression:

$$\Delta\omega = \frac{\Delta E'' - \Delta E'}{\eta} = \frac{\mu_B B}{\eta}\left(g'' m''_j - g' m'_j\right) = \Delta\omega_0\left(g'' m''_j - g' m'_j\right)$$

The numbers represented on spectral lines in the Fig. 6.29 and Fig. 6.30 represent the relation $\left(g'' m''_j - g' m'_j\right)$.

There is not any initial nondeviated spectral line in this case in comparison with the simple Zeeman effect. Four lines appear (fig. 6.29) instead of initial line which deviations are: **-4/3; -2/3; 2/3 and 4/3** and can be written as:

$$\Delta\omega = \Delta\omega_0\left[\pm 2/3; \pm 4/3\right]$$

and for the Fig. 6.30: $\Delta\omega = \Delta\omega_0\left[\pm 1/3; \pm 2/3; \pm 5/3\right]$

6.9. Electronic paramagnetic resonance (Spin electronic resonance)

The transition between two neighbor sublevels could take place if we use external electromagnetic field. The necessary condition is that the the frequency of electromagnetic field must be coincident with the frequency of photon which energy corresponds to the energy between the sublevels. In this case the absorption of energy of electromagnetical field could be observed and this effect is named **electronic paramagnetic resonance or spin electronic resonance.**

Theoretically the condition of resonance could be performed by two ways:

1) **ν=const and B is changed**
2) **B=const and ν is changed**

The condition of resonance is:

$$h\nu = \mu_B B g$$

The wavelengths of electromagnetical field belong to the range of microwaves **(λ=1cm);** $\nu = \frac{c}{\lambda}$; **ν=3·10^{10}Hz**

The first modality could be performed because **B** is modified easily.

The installation for the research of SER applied for the researching substances is named **radiospectrometer**. It consists of following components (*fig. 6.31*).

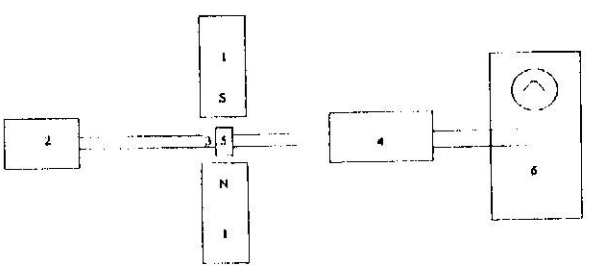


Fig. 6.31.

1 – electromagnet that creates strong and homogeneous magnetical field and the induction of magnetical field is changed slowly.

2 – generator of electromagnetic waves.

3 – resonator in which the researched sample is introduced.

4 – the electronical scheme that allows the observation of registration of SER.

5 – the researched sample

6 – oscillograph

One of the applications of SER consists in the researching of free radicals. A free radical possesses paramagnetic properties.

For example, the spectra of SER of irradiated proteins allow explaining the mechanism of formation of free radicals and modification in the primary products and secondary ones after the irradiation.

The SER of the irradiated proteins allow explaining the particular mechanism at photosynthesis. The cancer activity of some substances is researched. The SER is applied at the determination of the concentration of the radicals in the air medium with sanitary hygienic purpose.

For the study of SER of biological materials that do not possess paramagnetical properties the method of **marked spin** was proposed. The essentiality of this method consists in the fact that a paramagnetical formation of one well known structure is connected to the molecule of the researched object. The position of marked spin in the molecule is determined by the spectrum of SER. Introducing markers in different parts of the molecule, the distribution of different groups of atoms, their interaction can be determined and the nature and orientation of chemical bonds, molecular movement are determined. The reunion of some marked spins to the molecule, for example two spins gives possibility to obtain the information about the distances of marked groups and their reciprocal orientation.

The **spin probes** also are used that are paramagnetical particles which are connected noncovalently with the molecules. The

variation of the spectra of SER of spin probes gives the information about the states of surrounding molecules. The spectrum of SER of nytroxilen radical which is as the spin probe is introduced in the glycerin is shown in the *fig. 6.32*. The viscosity of glycerin is decreased when the temperature is increased that leads to the changing of SER spectrum.

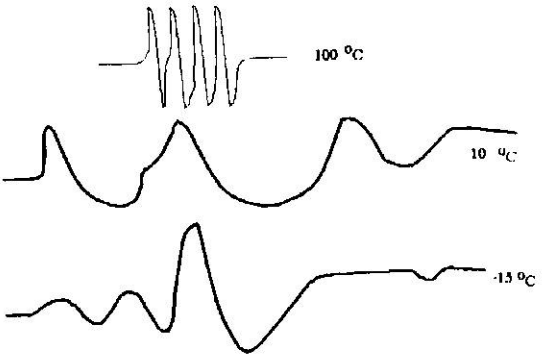


Fig. 6.32.

The microviscosity can be determined by the form of SER spectrum which is the viscosity from the near vicinity of spin probe. Thus in particular, the microviscosity of lipid layer of the membrane can be determined. So, there are a lot of great researches of biological objects by SER.

6.10. Magnetic nuclear resonance

MNR is a similar phenomenon to SER. In this case magnetical spin moments are characteristic to proton and neutrons of nucleus. Vectorial sum of them gives the spin magnetical moment of the nucleus.

Magnetical moment of the nucleus situated in magnetical field can take only discrete orientation. It means that the energy of nucleus will correspond to the sublevels which distance depends on magnetical induction. If in this condition the electromagnetical field will act on the nucleus then the transitions between the suble-

vels will take place. In order to obtain these transitions and the absorption of energy of electromagnetical wave it is necessary to be respected the following condition:

$$h\nu = \mu_n B g_n$$

μ_n – nuclear magneton

g_n – gyromagnetic factor of nucleons

$$\nu = 60 \div 100 \quad MHz$$

λ receives the values of meters referring to the range of **radio waves.**

The resonant frequency ν is named also Larmor frequency. Magnetical field used in NMR are typically in the range 1 to 4 T. The corresponding Larmor frequencies are about $\nu = 60 \div 100 MHz$. These frequencies are in the radio frequency range, which are much lower than the X rays and do not disrupt living tissue.

The driving pulse (radio wave) and the emitted NMR signal are shown schematically in the *fig. 6.33.*

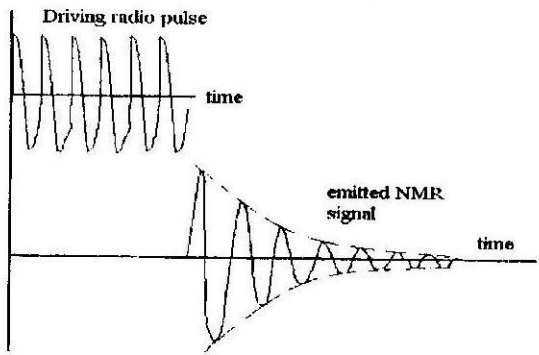


Fig. 6.33.

For a given initial driving pulse, the magnitude of the emitted NMR signal is a function of the number of hydrogen nuclei in the material. Bone, for example, which contains relatively few water

or other hydrogen – containing molecules, produces relatively low NMR signal. The post pulse radiation emitted by fatty tissue is much higher.

The time – constants T_1 and T_2 characterizing the rate of decay of the emitted NMR signal provide information about the nature of the material within which the precessing (rotating) nuclei are located. For example, with an external magnetical field of 1 T, for **fat** T_1=240 msec and T_2=80 msec; for **heart tissue** T_1=570 msec and T_2=57 msec.

6.11. Imaging with magnetic nuclear resonance

In order to obtain a three dimensional image using nuclear magnetic resonance, we must isolate and identify the location of signals from small sections of the body and then build the image from these individual signals. In Computational Tomography scanning, such tomographic spatial images are obtained by extracting the information from intersection points of narrowly focused X – ray beams. This can not be done with NMR because the wavelengths of the radio frequency driving signals are long, in the range of meters, which cannot be collimated into the narrow beams required to examine small regions of interest.

In the 1970s, several new techniques were developed to utilize NMR signals for the construction of two – dimensional tomographic images similar to those provided by CT scans. One of the first of these was described by P. C. Lauterbur in 1973. He demonstrated the principle using two tubes of water, A and b as shown in the *fig. 6.34, a.* In a uniform magnetic field (B_0) the Larmor frequency of the two tubes is the same. Therefore, the post pulse NMR signals from tubes 1 and 2 cannot be distinguished. The NMR signals from the two tubes can be made distinguishable by superimposing on the uniform field B_0 a magnetic field gradient B(x) as shown in the *fig. 6.34, b.* The total magnetic field now changes with position along the x – axis, and the associated Larmor frequencies at the locations of tubes **1** and **2** are now different.

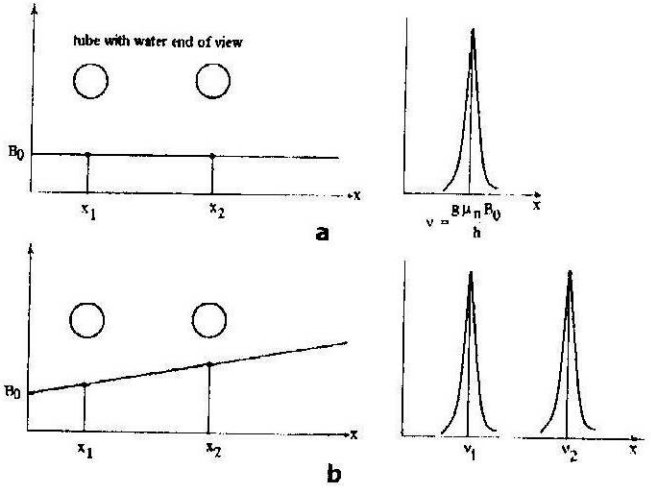


Fig. 6.34. **a – in a uniform magnetic field (B_0) the Larmor frequency of two locations in space x_1 and x_2 is the same. b – When a magnetic field gradient is superimposed on the uniform field, the Larmor frequency at the locations 1 and 2 are different**

As is evident, each point (actually small region Δx) on the x – axis is now characterized by its unique Larmor frequency. Therefore, the NMR signal observed after excitation with a pulse of a given frequency can be uniquely associated with a specific region in the **x** – space. A field gradient in one direction yields projection of the object onto that axis. To obtain a tomographic image in the **x- y** plane, a fields gradient in both the **x** and **y** directions must be introduced. A magnetic field gradient is also applied in the z direction to select within the body the slice to be examined. A very large number of NMR signals have to be collected and synthesized to construct an MRI image. For this purposed, the intensity as well as the time constants T_1 and T_2 of the NMR are needed. The process is more complex than for CT scans and requires highly sophisticated computer programs.

An image of different organs of human body can be obtained also if the gradient of the magnetic fields in the **x, y, z** directions is applied. The magnetic field is created by liquid helium superconducting magnets to produce the high magnetic fields required for the production of high resolution images (*fig. 6.35*).

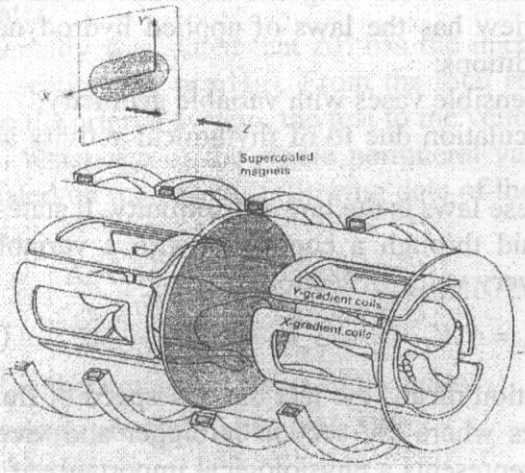


Fig. 6.35.

Theme 7. BIOPHYSICS OF SANGUINE CIRCULATION

7.1. Vascular resistance of blood flowing

The cardio vascular apparatus assures the transport of substances between the parts of the body: the oxygen necessary for the metabolic reactions, nutritive elements, electrolytes, hormones, water, etc. An important role plays the heat changes in the thermoregulation.

The main components of the cardio vascular system are:

– The heart as the role of pump in the rhythmical regime.

– The big pipes as the role of transportation net.

– Capillaries, representing the zones where the permanent change takes place with the medium of the cells.

– The blood as the transport agent.

A series of manifestations take place in the functioning of the cardio vascular system that conventionally can be divided in following: electric manifestations; mechanic manifestations related with the construction of the heart; oscillation mechanics. All these manifestations are studied thoroughly in the course of physiology.

The discussion about sanguine circulation from the biophysical point of view has the laws of applied hydrodynamics in the particular conditions:

– the extensible vases with variable geometry;

– the circulation due to of rhythmical activity and pulsation of the cord

One of these laws is the law of continuity. It states that the debit of one fluid through a conductor with a variable section is constant for every value of section:

$$S_1V_1 = S_2V_2 = ... = S_nV_n = const \qquad (1)$$

This equation means the fact that the speed of fluid is smaller in those places where the section is bigger and vice versa. The phenomenon represents a physiological importance if we take into consideration the sanguine capillaries where the section is bigger and the speed of circulation is small, facilitating so the metabolic changes tissue – blood.

For generalization we represent some data regarding the circulation of the blood for people:

– the speed of the blood in the aorta is **30 – 40** cm/s but in capillaries **0.05 – 0.08** cm/s;

– the transversal section of all capillaries has a total area of **700** times bigger than of aorta;

– the speed of the blood in veins increases again reaching the value of **6-14** cm/s in the superior cave vein;

An analogical relation of the law of Ohm from the electricity allows to calculate the resistance R that appears at the blood flowing: $R = \frac{\Delta P}{Q}$.

Where ΔP is the efficient pressure that assures the circulation; Q – the sanguine debit.

As the consequence of the fact that along of vascular pipes Q=const (according to the continuity law), results that the resistance at the blood circulation will be bigger there where the pressure falling is greater.

Experimentally was stated that ΔP has the maximal value in the arteriolar section (**60 mmHg**). From the total resistance 93% are referred to the arterial system, the rest to the venous system.

The total resistance of the whole peripheral vascular system can be calculated on the base of following data of the healthy people in the rest:

$$R = \frac{\Delta P}{Q} = \frac{\bar{P}_a - P_v}{Q} = \frac{95 mmHg}{85 ml/s} = 1.1 UPR$$

where $\bar{P}_a$ is the average pressure in the aorta;

P_V the average pressure in the cave vein;

$1 UPR = 1 \frac{mmHg}{ml/s}$ – a unit of peripheral resistance.

During the physical effort the peripheral resistance is decreased facilitating so the cardiac activity.

The relation of Poiseuille says:

$$Q = \frac{\pi r^4 \Delta P}{8 \eta l}; \qquad \frac{\Delta P}{Q} = \frac{8 \eta l}{\pi r^4} \quad (2)$$

So, for the peripheral resistance we obtain the expression:

$$R = \frac{8 \eta l}{\pi r^4} \qquad (3)$$

The last relation takes into the evidence the pronounced dependence of the local resistance of dimensional parameters of the sanguine pipe.

The resistance R given by the relation (2) is named the hydraulic resistance and is similar to the electrical resistance. The laws of consecutive and parallel connections in the electricity are similar also to hydraulic pipes. **If we remember the law of Ohm then ΔP is similar to the potential difference but Q to the intensity of current.**

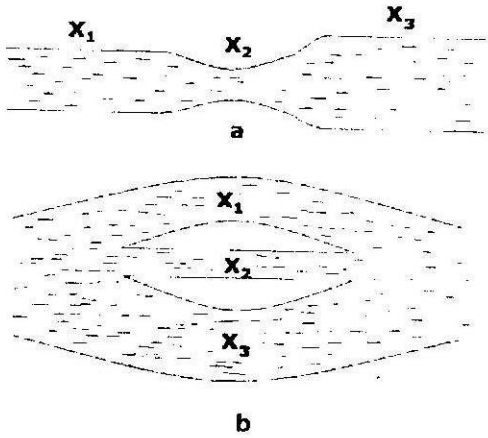


Fig. 7.1.

The total resistance of the pipes connected consecutively is: (*fig. 7.1, a*)

$$X = X_1 + X_2 + X_3 \quad (4)$$

The total resistance of the pipes connected in parallel is: (*fig. 7.1, b*)

$$X = \left(\frac{1}{X_1} + \frac{1}{X_2} + \frac{1}{X_3} \right)^{-1} \quad (5)$$

In order to generalize the law of Pouseuille available for the pipes with variable sections we substitute $(p_1-p_2)/l$ by gradient of pressure dp/dl and then:

$$Q - \frac{\pi R^4}{8\eta} \cdot \frac{dp}{dl} \quad (6)$$

We put the manometric tubes in different places of one cylindrical pipe of different sections through which a viscous liquid circulate. (*fig. 7.2, a*). The static pressure indicated by these tubes is decreased along the pipe with different sections proportionally with *l*; *dp/dl=const*. If *Q* is the same, then the gradient of pressure is greater in the pipes with small radius.

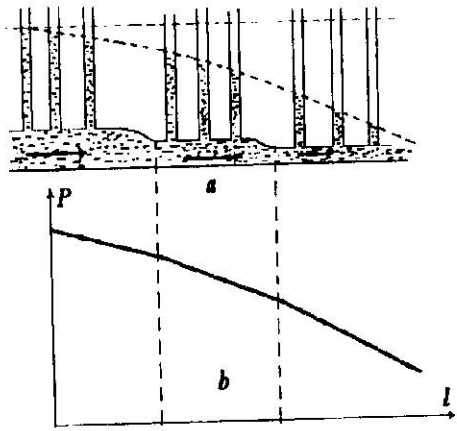


Fig. 7.2.

The graphic of approximate dependence of the pressure with the distance along the pipes is represented in the *fig. 7.2, b*.

7.2. The laminar and turbulent flowing. The number of Reynolds

The flowing of the liquid studied before is stratified or laminar. The increasing of the speed of flowing of one viscous liquid as the result of no homogeneity of the pressure along the transversal section of the pipe leads to the apparition of whirlpools and the movement becomes turbulent. The speed of particles in each point varies continuously and chaotic for the turbulent flowing.

The character of flowing of one liquid through a pipe depends on the properties of the liquid, the speed of flowing **V**, the size of the pipe **D** and is determined by the number of Reynolds:

$$\mathrm{Re} = \frac{\rho_l V D}{\eta} \qquad (1)$$

ρ_l is the density of liquid; **D** – diameter of the pipe.

If the number of Reynolds is greater than critical value ($\mathbf{Re > Re_{cr}}$), then the movement of the liquid becomes turbulent. For example, for cylindrical pipes with smooth walls $\mathbf{Re_{cr}=2300}$.

As the number of Reynolds depends on the viscosity and the density of liquid, it is more convenient to introduce the notion of **cinematic viscosity** equal with the ratio between them:

$$\nu = \frac{\eta}{\rho_l} \qquad (2)$$

Using this notion, we can express the number of Reynolds as follow:

$$\mathrm{Re} = \frac{VD}{\nu} \qquad (3)$$

The measurement unit of the cinematic viscosity is the square meter on second (m^2/s) in the **IS**, in the **CGS system** – Stokes; the relation between them is following:

$$\mathbf{1\ St = 10^{-4}\ m^2/s.}$$

The character of flowing of the liquid depends greatly on the size of pipe. For example, one tube with the diameter of 2 mm, the flowing of water will become turbulent for a speed over 127 cm/s, but for a tube with the diameter of 2 cm – at 12 cm/s (the temperature of 16 ^{0}C). The flowing of the blood through such pipe will become turbulent at the speed of 50 cm/s, but in the sanguine vases with the diameter of 2 cm the turbulent flowing can practically appears at small values of the speed.

The normal flowing of the blood through arteries is laminar; a small turbulence appears only in the vicinity of the valves. During pathology, when the viscosity is smaller than the normal value, the number of Reynolds can exceeds the critical value and the movement can become turbulent.

A supplementary energy is consumed at the turbulent flowing, so for the blood leads to the supplementary work of the heart. The noise that appears at the turbulent flowing of the blood can be used at the diagnosis of diseases. This noise is heard during the measurement of the blood pressure at the humeral artery.

7.3. Peculiarities of the viscosity of circulated blood

The blood is a biological liquid product and no homogeneous that represents a system of complex dispersion, knowing all form of this: molecular solutions, colloids, suspensions, etc. So, it contains the crystalloids (solution), macromolecules (colloidal dispersion) and figurative elements (suspension).

The values of the relative viscosity coefficient of the normal blood measured with respect to the viscosity of water (37 ^{0}C) are **3.8÷5.5**. The viscosity of the blood essentially differs with the value of the speed of circulation, with the radius of tube and with the value of hematocryte (the percentage of the total volume of the blood occupied by figurative elements).

The normal value of hematocryte is 45 – 50 %.

Increasing the hematocryte over 58% the relative viscosity of the blood increases exponentially (*fig. 7.3*).

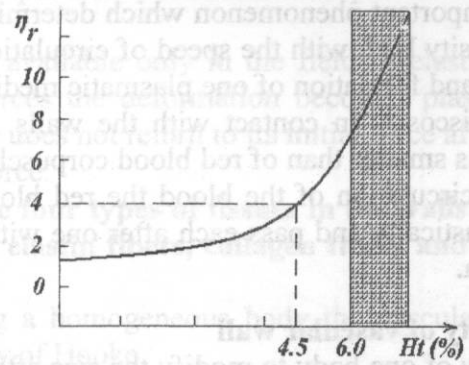


Fig. 7.3.

The phenomenon is available only when the diameter of the capillary of viscosimeter or sanguine pipe exceeds **1 mm** and is

conditioned by the accumulation of red blood corpuscles that are deformed due to of reciprocal pressure that hinders their free movement.

When the diameter of the capillary is smaller than 0.5 mm (the case of the sanguine capillary pipes) an accentuated decreasing of viscosity is stated that improves the circulation in the small pipes (*fig. 7.4*).

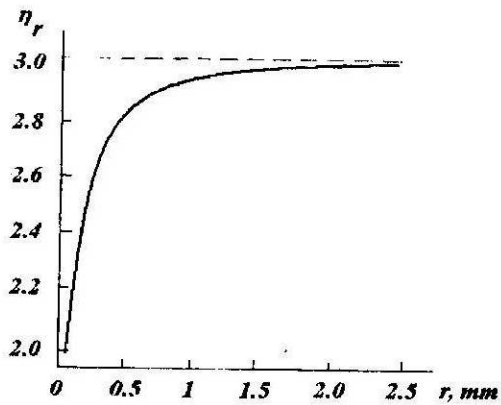


Fig. 7.4.

The most important phenomenon which determines the variation of the viscosity both with the speed of circulation and the radius of the pipe and formation of one plasmatic medium takes place with small viscosity in contact with the walls. In capillaries which diameter is smaller than of red blood corpuscles, in order to be possible the circulation of the blood the red blood corpuscles are deformed elastically and pass each after one with small speed rallied by plasma.

7.4. Elasticity of vascular wall

The property of one body to modify the size after the application of one force and to return to the initial state after the finishing of the force is the elasticity of the body. An homogenous elastic body respects the law of Hooke according to this the relative stret-

ching $\Delta l/l$ is proportional with the applied force on the unit surface (*fig. 7.5*).

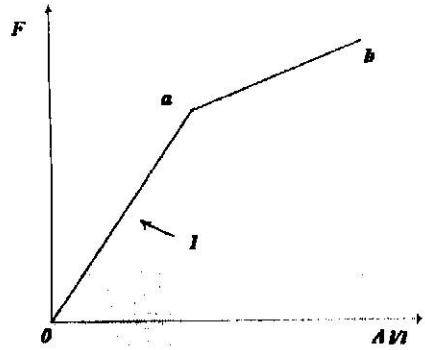


Fig. 7.5. **The diagram force – elongation: Oa – elastical deformations ab – plastic deformations b – the point of breaking**

$$\Delta l / l = \frac{1}{E} \cdot \frac{F}{S}$$

E – the elastic module of Young or the elasticity and represents the force per one unit surface that produces an stretching of 100% ($\Delta l = l$).

Hooke is available only in the field of elastic deformations. For bigger forces the deformation becomes plastic (the straight AB), the body does not return to its initial force after the disappearance of the force.

There are four types of tissues in the walls of blood pipes: endothelium, elastin fibers, collagen fibers and smooth muscle fibers.

Not being a homogeneous body the vascular wall does not respect the law of Hooke.

The elastin and collagen fibers have the elastic modules more different: $3 \cdot 10^5$ N/m^2 for the elastin and 10^8 N/m^2 for the collagen. The collagen fibers have the complex structure and they are accentuated after some stretching, when the curls are opened (*fig. 7.6*).

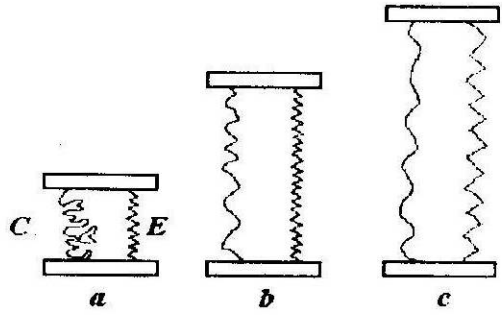


Fig. 7.6. **The consequent changing of elastin fibers (E) and collagen (C) in the vascular wall under the stretching: a: the elastin begins stretching while it acts b: the collagen begins its action after the curls were unbounded c: the elastic characteristics are determined by both types of fibers**

Then, the shape extension – tension of the arterial wall is non linear: the big extension at small forces (the portion OC) and small extension at big forces (the portion CD). It means that the vascular wall resists at bigger tensions when it is more stretched.

The diagrams tension – relative stretching of one arterial pipe are represented in the *fig. 7.7.*

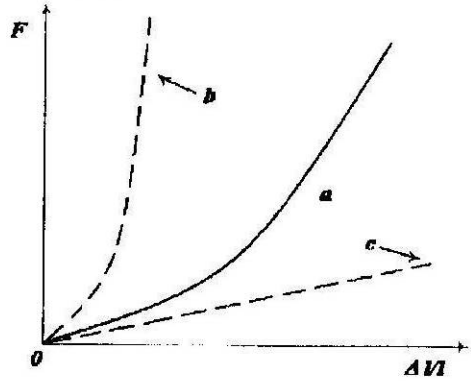


Fig. 7.7. **The diagram elongation – force of one vascular wall (a) resulting from the composition of the characteristics of elastin (c) and collagen (b).**

a – the normal state

b – the destroyed elastin fibers

c – the destroyed collagen fibers.

The sanguine pipes can be stretched not only longitudinally but also transversally possessing an elastic module three times bigger.

In this case, the force corresponds to the parietal tension but the elongation to the variation of the radius (*fig. 7.8*).

The smooth muscle fibers perform an active mechanical tension physiologically controlled. Their contraction modifies the radius of the pipes and indirectly the debit through it. This control is very effective at the arterioles level that possesses a considerable smoothed muscle (the capillaries are absent of this layer).

The parietal rigidity increases by the contraction of smooth muscles and the shape vascular radius – parietal tension is removed to the left (*fig. 7.8*).

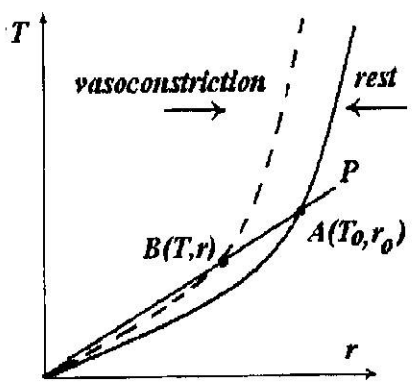


Fig. 7.8. **The relation parietal tension-vascular radius in the state of rest and vasoconstriction**

7.5. The dynamic pressure and the average pressure of the circulated blood

The law of Bernoulli from hydrodynamics shows that the sum of static pressures (lateral), dynamic and hydrostatic is constant in

every points of one conductor of variable section and orientation in the space (*fig. 7.9*).

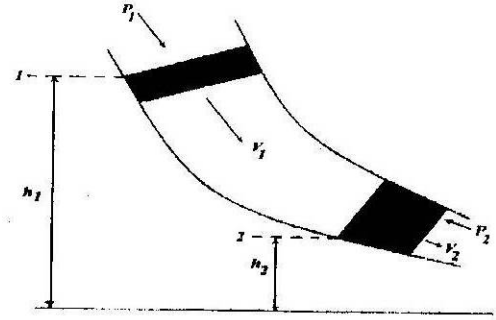


Fig. 7.9. **The parameters of Bernoulli law: P_1, P_2 the pressure; V_1, V_2 the speed of the liquid; h_1, h_2 the height of the liquid column (in the points 1 and 2 respectively)**

$$P_1 + \rho g h_1 + \frac{1}{2}\rho v_1^2 = P_2 + \rho g h_2 + \frac{1}{2}\rho v_2^2 = \ldots = P_n + \rho g h_n + \frac{1}{2}\rho v_n^2 = const$$

where: P – the static pressure that realizes the distension of the vase at the level of some section;

$\frac{1}{2}\rho v^2$ – dynamical pressure at the level of the some section;

$\rho g h$ – hydrostatic pressure of the respective level of the section;

ρ – the density of the liquid;

v – the average speed at the level of respective section; g – gravitational acceleration.

The law of Bernoulli has the conclusion the fact that the narrowed portions of the circulatory blood net where eventually the speed of circulation is big, the lateral pressure is minimal. Therefore, in the case of narrowing by atherosclerosis the phenomenon leads to the accentuation of the closing of vascular lumen in the place of obstruction.

Measuring the average systolic pressure (P_s=**16 kPa**) and the average diastolic pressure (P_d=**11 kPa**) the average pressure of the blood (P_m) can be determined from the relation:

$$P \approx \frac{P_s + 2P_d}{3}$$

The average pressure has in different zones the values:

100 mm Hg – in the aorta;

35 mm Hg – at the end of arterioles;

25 mm Hg – in the capillaries;

15 mm Hg at the end of veins;

Below than 10 mm Hg – in the cave vein;

The necessity appears often when the measured pressures in mmHg, cmH_2O must be transformed in the units of pressure from the international system named **Pascal** (1 Pa=1 N/m^2). We present the connection relations between different units of pressures mostly used:

1 cmH_2O = 0,098 kPa;

1 mmHg = 0,133 kPa

1 mbar = 0,1 kPa

760 mmHg = 101,3 kPa

7.6. The pulsation wave

The physical model of the cardiovascular system represents a system of tubes with elastic walls that are branched gradually and filled with the real liquid which flowing is sustained by the work of one pump in the rhythmical regime.

A tube with the elastic wall filled with real liquid is presented in the *fig. 7.10* at the end of which a piston is leaded by the action of periodical force *($F=F_0\cos\omega t$)*.

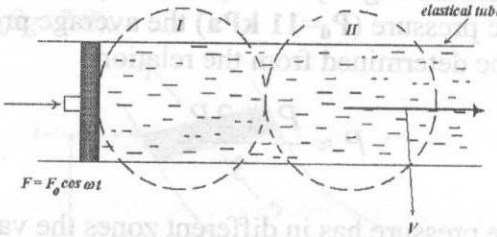


Fig. 7.10. **The mechanism of formation of pulsation wave**

The front of pressure created in the vicinity of the piston will be propagated along the tube as the form of wave due to of elasticity of the wall. The speed of propagation of the wave depends on a lot of factors like the density of the liquid ρ and the elastic module E of the walls of the tube.

A similar phenomenon takes place in the arteries due to of rhythmic contraction of the left ventricle and pulsate throwing of the blood portions in the aorta. The so named **pulsation wave** is propagated along arterial pipes. The speed of propagation of the pulsation wave through arterial vases is determined by the relation of **Moens**:

$$V = F\sqrt{\frac{Ed}{2r\rho}}$$

ρ – the density of blood

E – the module of elasticity of arterial wall;

d – the thickness of the wall;

r – the internal radius of the vase;

F – constant factor

The variation of the speed of the pulsation wave with the age is represented in the *fig. 7.11*. The aortic wall plays a role of accumulator of energy that is charged in systole and discharged in diastole. Both the flowing of the blood and propagation of the pulsation wave takes place with this energy.

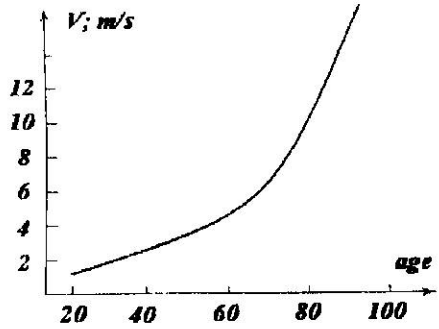


Fig. 7.11. **The relation age – speed of the pulsation wave**

We will have to mention that the speed of propagation of the pulsation wave and the speed of circulation of the blood differs essentially: for the blood is 0.3 – 0.5 m/s; for the pulsation wave is 5–10 m/s. The determination of the pulsation wave is useful in the diagnosis of some cardio vascular maladies.

7.7. The physical bases of clinic method of the measurement of blood pressure. (Method of Korotkov of the measurement of blood pressure).

The arterial blood pressure is an important indicator of the health of an individual. Both abnormally high and abnormally low blood pressures indicate some disorders in the body that require medical attention.

High blood pressure, which may be caused by constrictions in the circulatory system, certainly implies that the heart is working harder than usual and that it may be endangered by the excess load. Blood pressure can be measured most directly by inserting a vertical glass tube into artery and observing the height to which the blood rises.

This was, in fact, the way blood pressure was first measured in 1733 by Reverend Stephen Hales, who connected a long vertical glass tube to an artery of a horse. Although sophisticated modifications of this technique are still used in special cases, this

method is obviously not satisfactory for routine clinical examinations. In the medicine a most large used method is the method of Korotkov N. that is non blood flowing. Although this method is not as accurate as direct measurements, it is simple and in most cases adequate. In this technique, a cuff containing an inflatable balloon is placed tightly around the upper arm.

The balloon is inflated with a bulb, and the pressure in the balloon is monitored by a pressure gauge. The initial pressure in the balloon is greater than the systolic pressure, and the flow of blood through the artery is therefore cut – off. The observer than allows the pressure in the balloon to fall slowly by releasing some of the air. As the pressure drops, she listens with a stethoscope placed over the artery downstream from the cuff. No sound is heard until the pressure in the balloon decreases to the systolic pressure. Just below this point the blood begins to flow through the artery; however, since the artery is still partially constricted, the flow is turbulent and is accompanied by a characteristic sound. The pressure recorded at the onset of the sound is the systolic blood pressure. As the pressure in the balloon drops further, the artery expands to its normal size, the flow becomes laminar, and the noise disappears.

The pressure at which the sound begins to fade is taken as the diastolic pressure.

In clinical measurements, the variation of blood pressure along the body must be considered. The cut – off blood pressure measurement is taken with the cuff placed on the arm approximately at the heart level.

7.8. The determination of the speed of the circulation of blood

There are some methods of determination of the speed of circulation blood. The most important is **the ultrasound method**. This method is based on the Doppler effect. The signal from the generator **1.** of electrical oscillations of ultrasound (*fig. 7.12*) is transmitted to the emitter of US.

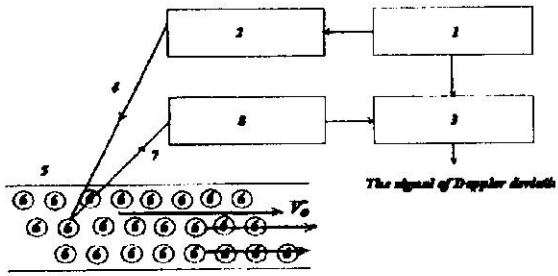


Fig. 7.12.

2. and to the device of the comparing of frequencies **3**. The wave **4.** penetrates into the sanguine pipe **5.** and is reflected from the movement erythrocytes **6.** The reflected ultrasound wave **7.** is transmitted to the receiver **8**. where is transformed into the electrical oscillation and is amplified. The amplified electrical oscillation goes to the device **3**. The oscillations that correspond to the incident and reflected waves are compared in this device and the variation of frequency with Doppler effect is separated as the form of electrical oscillation:

$$U = U_0 \cos 2\pi \nu_D t \qquad (1)$$

The essentiality of Doppler effect consists in the following. An object moves with the speed V_0 (*fig. 7.13*).

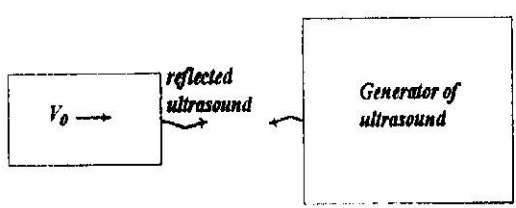


Fig. 7.13.

The generator emits an ultrasound with the frequency ν_g. The moving object as the receiver observes the frequency ν_1 that can be determined by the formula:

$$\nu_1 = \frac{V + V_0}{\lambda} = \frac{V + V_0}{V} \nu_g \qquad (2)$$

where V is the speed of propagation of mechanical wave (ultrasound).

The ultrasound wave with the frequency ν_1 is reflected from the object in the state of movement in the direction of generator of ultrasound. The receiver will receipt another frequency (Doppler effect) with which we can determine by the formula:

$$\nu_{rec} = \frac{V}{V - V_0} \nu_1 \qquad (3)$$

Taking into consideration the relation (2) then (3) can be written as:

$$\nu_{rec} = \frac{V + V_0}{V - V_0} \nu_g \qquad (4)$$

So, the difference of frequencies (Doppler deviation) is:

$$\nu_D = \nu_{rec} - \nu_g = \frac{V + V_0 - V + V_0}{V - V_0} \nu_g = \frac{2V_0}{V - V_0} \nu_g \qquad (5)$$

This frequency ν_D is named Doppler deviation.

In the applications of medicine the speed of ultrasound is greater than the speed of movement of object ($V >> V_0$).

$$\nu_D = \frac{2V_0}{V} \nu_g \qquad (6)$$

The speed of erythrocytes (Fig. 7.12) can be determined from the relation (6) by the following relation:

$$V_0 = \frac{V}{2} \frac{\nu_D}{\nu_g}$$

where V is the speed of propagation of mechanical wave (ultrasound).

Theme 8. BIOLOGICAL MEMBRANES. TRANSMEMBRANE TRANSPORTATION. BIOELECTROGENESIS. THE REGISTRATION OF BIOPOTENTIALS

8.1. Fundamental roles of biomembranes

The live elementary system able to exist by itself, to be developed and to be reproduced is the cell – open thermodynamic system.

The main conditions of the existence of the cells are the autonomy with respect to the external medium and the continuous regulated changing of substance and energy with the external medium. So, the main condition of the existence of the cell represents the biological membrane.

The fact that the cell membranes represent the discussed subject in the biomedical scientific research is motivated by fundamental roles of these structures.

– **the role of border**, of delimitation of intracell medium with respect to that extra cellular, assuring so in the same time the passive changing of substance and active with the external medium;

– the biomembranes create the conditions to the **mechanisms of asymmetrical distributions** of the ions at the cell level with respect to the extra cellular medium;

The ionic asymmetry is of great importance, constituting the base of following phenomena:

– The creation of membrane electric potentials;
– Synaptic transmission;
– Processes of secretion and digestive absorption;
– Hydroelectric equilibrium;

The **transportation of intercellular information** takes place by the specific receptors located at the membrane level;

– The cellular membranes has the important role in the assuring of adhesive - the intercellular coupling that determines the formations of tissues;

– The regulation or the limitation (by the inhibition of contact) of the growing of one organ;

– Some biomembranes have the **particularly roles;**

– the mitochondrion membrane takes place at the energetical processes (the conversion of chemical energy from ATP);

– the mielinic axonic membrane has the role of electrical isolant;

– the membranes of endoplasmatic reticulum together with the membranes belonging to the **Golgi** apparatus concur at the leading of extra cellular secretion, etc.

The diversity of the extreme important role of membranes in the vital processes is in good concordance wit the relative big value membrane surfaces sum from the organism. For example, the total surface of the membranes reaches ten square meters.

8.2. Physical methods of the study of structural organization of biomembranes

The realization of one image about the structure and the molecular composition of biomembranes was possible more before the apparition of electronical microscope, by the application of physical methods, relative simple for study.

The first model of biological membrane was proposed by Overton in 1902. He observed that the substances dissolved in the lipids easily penetrate the biomembranes. This fact allowed him to elaborate the supposing that the biological membranes represent a **monolayer of phospholipids.** The numerous series of lipids appeared while science was developed in which molecules an extremity soluble in water is observed named **hydrophilic head** and the second extremity is the trend formed of carbon atoms and hydrogen atoms insoluble in water and named **hydrophobic tails** (*fig. 8.1*).

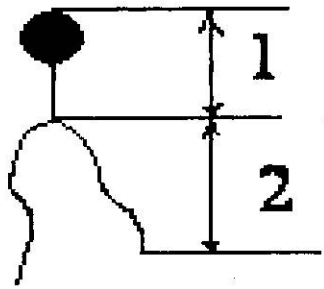


Fig. 8.1. **The phospholipid molecule 1 – polar part (hydrophilic) 2 – nonpolar part (hydrophobic)**

The phospholipidic molecule has the hydrophilic head formed of phosphate group (PO_4). The hydrophobic tails consist of the trend of fatty acids (made of acidic function of COOH and a trend of hydrogen atoms and carbon).

The model suggested by Overton was sustained by the fact that the phospholipids form a monomolecular layer at the surface of separation of polar and nonpolar media (for example, water and air) (*fig. 8.2*).

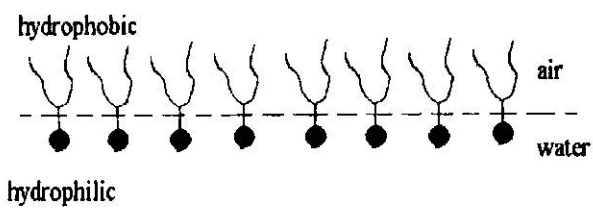


Fig. 8.2. **The arrangement of phospholipidic molecules in the monolayer**

The scientists **E. Gorter** and **F. Grendel** using acetone extracted the lipids from membranes from a known quantity of erythrocytes in 1925. They stated that the area of the monolayer surface is **two times greater** than the area of sum surfaces of erythro-

cytes membranes. **Gorter and Grendel** suggested then a new model according to this the lipids are situated as the form of double layer (*fig. 8.3*).

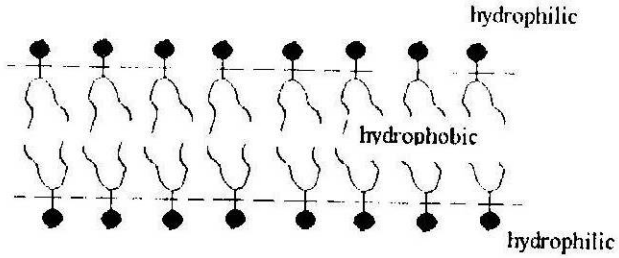


Fig. 8.3. **The double phospholipidic layer**

This supposing was confirmed also by researching of electrical parameters of biological membranes by **Coul and Chertis** (1935). The electrical resistance R_s and electrical capacitance C_s of one unit surface of the membrane have relative big values:

$$R_S \approx 10^7 \Omega / m^2 \qquad C_S \approx 0.5 \cdot 10^{-2} F / m^2$$

ting from this results, the biological membrane can be considered as the electrical capacitor (*fig. 8.4*), the conducting plates of which are formed by electrolitical solutions (from the exterior and the interior of the cell), separated by a double layer of lipids.

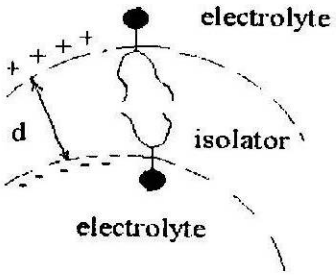


Fig. 8.4. **The membrane – electric capacitor**

The lipids are dielectric with the permeability ε=2. The capacity of one capacitor is determined by the equation:

$$C = \frac{\varepsilon\varepsilon_0 S}{d} \qquad (1)$$

ε_0 – the electrical constant (ε_0=8.85·10^{-12} F/m).

d – the distance between the plates of the capacitor. The capacity per one unit surface is:

$$C_s = \frac{\varepsilon\varepsilon_0}{d} \qquad (2)$$

The distance between the plates can be determined from the equation (2).

$$d = \frac{\varepsilon\varepsilon_0}{C_s} \approx \frac{8.85 \cdot 10^{-12} \cdot 2}{0.5 \cdot 10^{-2}} = 3.6nm$$

The obtained value corresponds to the order of thickness of nonpolar part of double bilipidic layer.

We mention that the hypothesis of double lipid layer was maintained in all ulterior membrane models.

The coefficient of surface tension at the interface water – membrane was measured by **Danielli and Davson** and found the value of 0.2 dynes/sm, but the value corresponding to the interface lipid – water is greater – 36 dynes/cm.

These results and the fact that the proteins are tensioactive substances made **Danielli and Davson** to conclude that there are proteins on the surface of membrane. So, they elaborated the "sandwich" model, according to which the bilipidic layer is included between two layers of proteins (*fig. 8.5*). One of drawbacks of this method consists in the fact that the compact structure made of the layers of lipids and proteins was not compatible with the rapid diffusion of water at the membrane level, and also for ions.

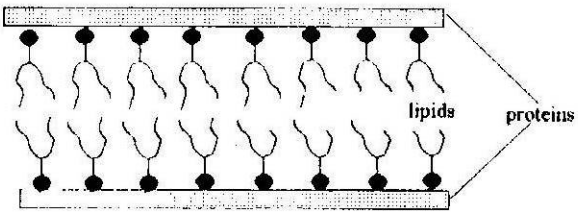


Fig. 8.5. **The "sandwich" model**

In order to cancel this drawback the authors of this model admitted the existence of some membrane pores in 1956. Obtaining new experimental data then the sandwich model was omitted.

An important role in the study of the structure of biomembranes belongs to physical methods of researching performed by technical devices of high precision. We can mention such device: **electronical microscopy and cryofracture.**

The ordered arrangement of the lipid molecules in the double layer was confirmed by the method of X rays and the linear dimensions from the structure of biomembranes were determined with the different precisions.

The electronical microscopy was applied in the study of biomembranes for first time by Robertson (1957). The thre layers were observed in the electronogram (*fig. 8.6*). The total thickness of the treble layer for the membranes of different cells varies from 7 till 15 nm.

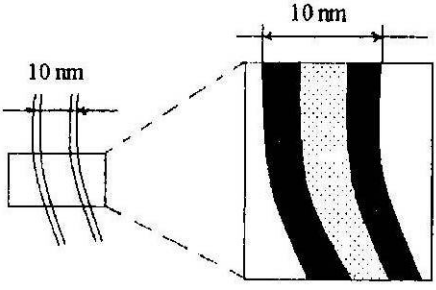


Fig. 8.6. **The electronogram**

8.3. The contemporary vision of the structure of biomembranes

The experimental data obtained by the methods described above and by other physical and chemical methods made possibly that in 1960-1970 a lot of trying of new membrane models was realized. In 1972 the mosaic model of lipoprotein fluid was elaborated by **Singer** and **Nicolson.** This model maintains the basic structure of double lipid layer penetrated by membrane proteins like integrals and peripheral proteins with different modalities of connections of membrane proteins (*fig. 8.7*).

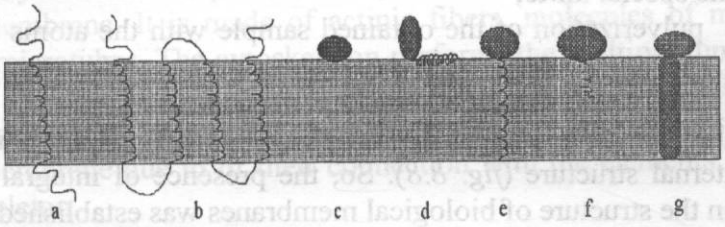


Fig. 8.7. **Modalities of connections of membrane proteins a – single transmembrane segment; b – multiple ransmembrane segment; c – electrostatic bonds; d – hydrophobic bonds; e – hydrophobic bonds by anchoring to a terminal segment that penetrates the membrane; f – anchoring by lipid molecule bound covalently; g – connection to another membrane protein**

The peripheral proteins are attached to the surface of membrane or penetrate to it through a certain thickness; they are implied frequently in the transmission of the information to the internal side of the cell. These proteins can interact with the surface of bilipidic layer:

– by electrostatic bonds;

– by hydrophobic bonds; without deep penetration in the lipid layer or are anchored by a terminal segment that penetrates the membrane;

– by covalent bonds with the lipid anchor;

– by attaching to other membrane proteins. The integral proteins penetrate the membrane whole thickness and are implied in

general in the transportation processes. The penetration of membrane can be performed one time or several times.

The fluid mosaic model was confirmed by numerous biophysical techniques, the most productive is the **cryofracture** of the cell structures and then examination at electronical microscope.

This method has several consecutive steps:

- the accelerated cooling of the cell in the nitrogen liquid;
- the separation of cell membrane;
- introduction into the vacuumed chamber;
- fracture of the membrane along the interface of lipid layers with the special knife;
- pulverization of the obtained sample with the atoms of Pt and C;
- Studying at the electronical microscope.

The membrane can be divided in two layers, emphasizing so the internal structure (*fig. 8.8*). So, the presence of integral proteins in the structure of biological membranes was established.

The lipid and protein compositions of different membranes are very variable; the ratio of masses of lipids -proteins varies from **4** for the myelin up to **0.3** for the internal membrane of mitochondrion, with all intermediary possible values. This ratio depends on the performed functions of the respective cell.

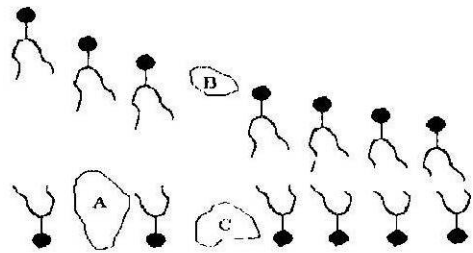


Fig. 8.8. **The illustration of the modality for which the membrane protein (A) can be seen by cryofracture**

The important phenomena with the structure and functions of cell membrane present those two structures from the vicinity of membrane: glycocalix (the cell cover) and cytoskeleton.

The glycocalix is situated in the external side of membrane and represents a layer made of polyozides, to which proteins and lipids can be connected. Both the structure and the composition of this layer are variable. It is rigid at the bone cells; at erythrocytes it represents a layer of ozides (glycolipids and glycoproteins).

Glycocalix plays an important role in the reciprocal action among the cells and the functions of receivers.

Cytoskeleton represents a layer situated on the internal side of membrane. It is made of actinic fibers, molecules of myosin and microtubes. The cytoskeleton performs the multiple functions both dynamic and static and refers to the membrane particles. Particularly, it was stated that some proteins are fixed in the structure of membrane due to of their connection with the elements of cytoskeleton.

8.4. Possibilities of movement in the bilipidic layer

The molecules both lipid and protein posses the characteristic to correspond to the fluid aggregation state. They are in the translation movement and rotation one due to of proper thermal agitation and the collision of the molecules which are in contact.

The movements of lipid molecules from the cell membrane (*fig. 8.9*).

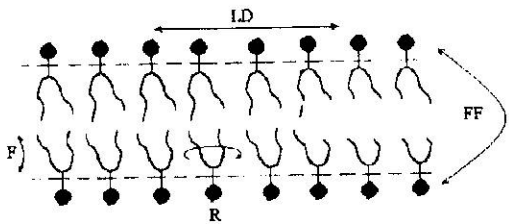


Fig. 8.9.

LD – translation in the layer in which it is (lateral diffusion)
R – rotation around its own axis;

F – flexion;

B – Flip – flop transition

The average time referring to the lateral transition of the phospholipidic molecule is $\tau_{lat} \approx 10^{-7} - 10^{-8} s$, but for flip – flop transition is $\tau_{ff} \approx 1 hour$.

The movements of proteins molecules from the cell membrane (*fig. 8.10*).

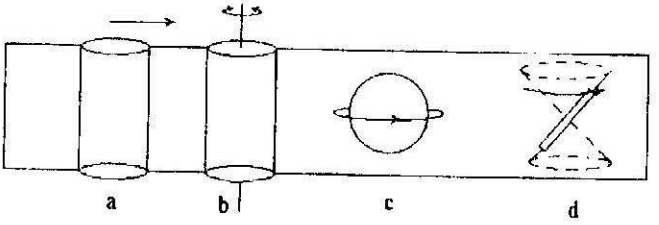


Fig. 8.10.

a – translation (lateral diffusion) through bilayer;

b – Rotation around the perpendicular axis on the bilipidic layer.

c – Isotropic rotation, without preferential axis (small molecules, spherical and hydrophobic ones)

d – Rotation describing the conical surfaces (hydrophobic molecules, as the rigid stick).

We have to mention that these movements are influenced by the dynamical viscosity of the membrane lipid phase. The modification of viscosity of the membrane influences essentially on the activity of the cells and can be the reason of a lot of maladies. The scheme of appreciation of viscosity of the cell membrane after the level of polarization of fluorescent radiation is represented in the *fig. 8.11.*

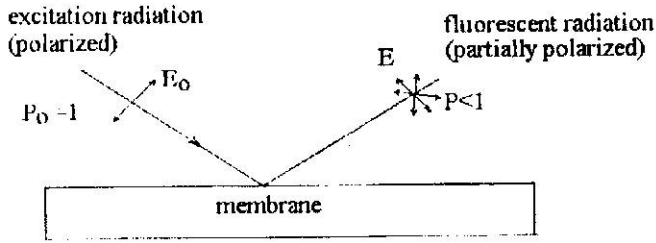


Fig. 8.11.

8.5. Transportation phenomenon. Notion of chemical and electro – chemical potential

The transportation of substances created by the gradient of concentration, potential and pressure takes place in the dispersed systems in the sense of increasing of entropy so to the thermodynamic equilibrium. This is performed in two general modalities:

– diffusion, representing the transportation of solute under the action of electro – chemical gradient;

– Osmosis, representing the transportation of solvent under the action of pressure gradient.

– Diffusion, from the thermodynamic point of view is explained as follow: the solute possesses a certain energy $\mathbf{W_c}$ named chemical potential $\boldsymbol{\mu_c}$

The chemical potential has the following equation at the constant pressure and volume:

$$W_c = \mu_c = \mu_0 + RT \lg a$$

where **a** is the activity of the respective component of the dispersed system;

a=f·c; $\boldsymbol{\mu_0}$ – the chemical potential for **a=1, c** – the concentration; **f** – the activity coefficient;

When **f=1**, **a=c** and the equation become:

$$W_c = \mu_c = \mu_0 + RT \lg c$$

In the case of electrolytic solutions the value of electric potential energy of one mole of substance is calculated by the formula:

$$W_e = VnF$$

Where: V – the electric potential from the respective compartment;

F – the number of Faraday;

N – the valence of ion; is considered as the positive charge (+) for the positive ions and (-) for the negative ions.

So, for each compartment and for each ion, the total potential energy which also is named **electro – chemical potential ($\mu_{e\text{-}c}$) is:**

$$W_c = \mu_c = \mu_0 + RT \lg c + VnF$$

The chemical gradient, the concentration gradient in two zones of different concentrations appears on the direction X: $\frac{dc}{dx} = X_c$ it represents a thermodynamic force under which action the transportation of substance from the big concentration to small concentration takes place: **diffusion.**

The diffusion is described quantitatively by the laws of **Fick:**

First law of Fick is referred to the speed of transportation:

The quantity of substance that diffuses normally through a unit surface in the unit time is proportional to the concentration gradient: The mathematical equation is:

$$J_c = \frac{dy}{dt} = -D\frac{dc}{dx}$$

where: **y** – the quantity of transported moles; D – the diffusion coefficient and is measured in $\mathbf{m^2/s}$.

If we consider the total flux through the surface of area S:

$$J_c S = \frac{dy}{dt} = -DS\frac{dc}{dx}$$

For the spherical particles of radius **r**, the diffusion coefficient is given by the equation:

$$D = \frac{kT}{6\pi\eta r}$$

η is the viscosity coefficient of the medium.

Second law of Fick is referred to the speed of variation of the concentration: the speed of variation of the concentration in each point of the system is proportional to the variation in space of the concentration gradient:

$$\frac{dc}{dt} = -D\frac{d}{dx}\left(\frac{dc}{dx}\right) = -D\frac{d^2c}{dx^2}$$

This is the second derivative of the concentration on the X direction.

8.6. Origin of bioelectrical potentials. Concentration element of Nernst

The live organisms contain a great quantity of water that represents the solvent for the dispersed polyphasic systems, including the crystalloids in the ionized form (Na^+, K^+, Cl^-, Ca^+, Mg^{++}, etc.), small molecules and macromolecules (in general anions at physiologic pH equal to 7.4).

The simplest model of the source of electrical energy of biological tissues represents the **concentration element of Nernst** (*fig. 8.12, a*).

Solutions of different concentrations (C_1 and C_2) in it of one salt are separated by a membrane that possesses different permeability for cations and anions in which the salt is dissociated. As the result a double layer of electrical charges is formed on the membrane (*fig. 8.12, b*).

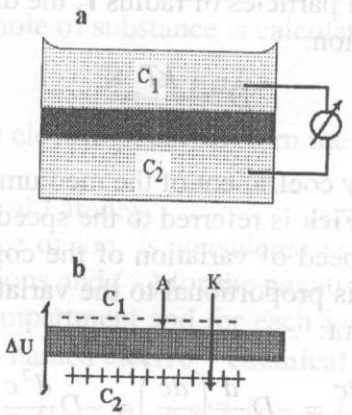


Fig. 8.12.

The electro motion voltage of the element formed by the solution of one single salt is determined by the relation of Nernst:

$$\varepsilon = \frac{RT}{nF} \ln \frac{[k_1]P_k + [A_2]P_A}{[k_2]P_k + [A_1]P_A}$$

For the same difference of concentrations in the case of monovalent ions (**n=1**) the electro motion voltage of the element is two times greater with respect to its value for the bivalent ions (**n=2**).

It is important to mention that the electromotion voltage in the Nernst element in great measure depends on the properties of membrane.

In order to clarify the mechanisms of bio electro genesis it is necessary to take into consideration the following moments: the difference of concentrations of the Na ions and K ions in the intra and extra cellular media; the cations have a great permeability with respect to anions through cellular membrane; a great role in the maintaining of ionic asymmetry plays the active transportation (the Na^+-K^+ pump); the presence of ionic channels; the cell mem-

brane is impermeable for the protein anions that leads to a unequal repartition of the diffusible ions K^+ and Cl^-.

So, the source of electrical energy in each cell serves the concentration element made of ionic solutions separated by plasmatic membrane with different permeability for cations and anions.

8.7. Rest membrane potential. Equation of Goldman – Hodgkin-Katz

The difference of potential between two faces of membrane can be proved from the condition of annulations of the net transportation for all species of ions as the function of concentration and permeability.

The equation Goldman – Hodgkin – Katz is obtained that establishes the polarization of membrane in the rest state: the rest membrane potential.

$$V_R = \frac{RT}{nF} \ln \frac{\sum_j P_{cj}[C_j^+]_e + \sum_j P_{Aj}[A_j^-]_i}{\sum_j P_{cj}[C_j^+]_i + \sum_j P_{Aj}[A_j^-]_e}$$

here: $[C_j^+]$-the concentration of cations;

$[A_j^-]$ - the concentration of anions;

P_{cj} - Permeability of membrane for cations;

P_{Aj} - Permeability of membrane for anions;

J=1, 2, 3, ... The sum is performed for all diffusible ions;

e and **i** means the external face and internal respectively of the membrane.

The equation was written for the ions with the same valence – **n**.

The main ions that lead to the membrane equilibrium are the monovalent ions Na^+, K^+ and Cl^- and the above equation becomes:

$$V_R = \frac{RT}{F} \ln \frac{P_K[K^+]_e + P_{Na}[Na^+]_e + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_e}$$

The name of membrane potential is correct and represents the potential of internal face of the membrane because the potential of interstitial liquid is considered null by convection and is taken as the refference potential.

The membrane potential can be measured by the technique of microelectrode. One electrode is situated in the external side of membrane (the frequently used is the membrane of gigantic axon of sepia). A very thin edge of one microelectrode of glass (d<1 μm) is introduced in the cell (*fig. 8.13*). The microelectrode is filled with a saturated solution of KCl in contact with the electrode of Ag over which AgCl was formed.

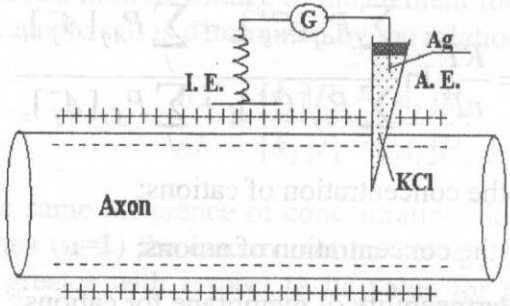


Fig. 8.13. **The scheme of measurement of membrane potential at the gigantic axon of sepia: A. E. the active electrode; I. E – indifferent electrode**

The experimental data of the concentrations and permeabilities of main ions (Na^+, K^+, Cl^-) are substituted in the equation of Goldman – Hodgkin – Katz and are in good accordance with those obtained values. For different cells and different species the membrane potential is 40–90 mV. The nonessential differences are created by other ions of small concentrations (Mg^{++}, Ca^{++}, etc.)

and have an important role in the realization of the function of some cells and tissues (example: the striated muscle, the cardiac muscle).

8.8. Physical bases of electrocardiography

Both the internal and the external faces of the membrane are the equipotential surfaces in the rest conditions. This state is modified at the excitation. The excited zone of the internal side becomes positive with respect with the neighbor zones being in the state of rest. The excited zone of the external surface possesses negative potential with respect with nonexcited zones of the same surface. This allows the registration of bioelectrical activity not only by microelectrode method but also the placement of the electrodes on the surface of one axon muscle fiber, tissue or organ.

The electrical activity of one organ results from the adding of constituent cells activity, the cells that are excited asincronically, due to of time propagation of the signal from the point of apparition up to different points of the organ. In the general case, the cells can have the excitation thresholds, speeds of propagation and forms of AP different. From this fact, the electrical signal of the organ has a different form of the cell action potential.

The AP is propagated as the form of **excitation wave**.

The wave front corresponds to the separation line between the active zone and those nonactive. The wavelength represents the length of depolarized zone at the given moment and depends on the difference between the speeds of propagation in different constituent cells (beside the duration of action potential).

The registration of electric activity of one organ is performed with the extra cellular electrodes and has the name of electrocardiogram. The potential difference before the excitation between electrodes is null, indifferent of their position on the surface of organ (the extra cellular medium is equipotential).

When the organ is excited, the measurement electrodes register the variation of potential difference between the points where they are placed. By convection, the modification of the potential

with respect to the rest potential at the apparition of wave front is represented by a positive deflection although the zone becomes more electronegative. The registration could be **bipolar and unipolar.**

Bipolar registration. Both electrodes are active, so that they are situated on the surface of the organ in the active zones. The distance between the electrodes is small in comparison with the wavelength of excitation (emphasized rectangle in the *fig. 8.14*).

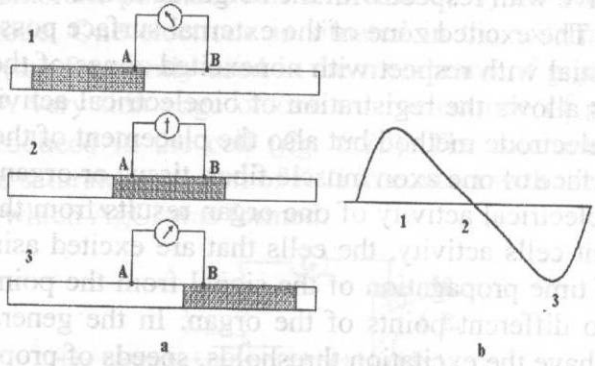


Fig. 8.14. **The bipolar registration a – the position of excitation wave with respect to the electrodes A and B; b – the presentation of potential difference between electrdodes; the emphasized rectangle represents the wavelength**

The following steps are distinguished:

1. **A activated, B inactive.** While approaching the wave front the point B, the potential difference is increased, reaches the maximum and then is decreased (the positive deflection);

2. **Both A and B are activated**. Thee is a moment for which both are situated at the same potential, so that the potential differrence between them is null (the curve passes through zero);

3. **A inactive, B active.** The deflection is negative. After passing of excitation wave, the potential difference between the points **A** and **B** returns to zero. Such a registration is named **biphasic.**

Unipolar registration.

The electrode B taken as the reference electrode is situated in a zone maintained permanently depolarized by sectioning, cauterization, so that the constant potential difference appears in the state of rest between those two electrodes and this potential is named **lesion potential** (*fig. 8.15*).

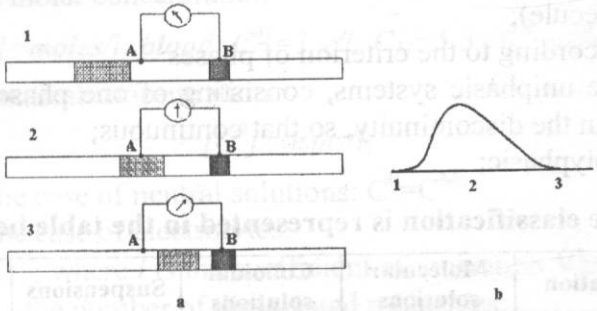


Fig. 8.15. **The unipolar registration: a – the position of excitation wave with respect to electrodes; b – the presentation of potential difference between electrodes with respect to the lesion potential; the black emphasizing corresponds to the lesion region.**

When a stimulus is applied, the modification of the potential appears at the electrode **A**. After the passing of excitation wave, the potential difference returns to its initial value equal to the lesion potential. So, the monophasic registration is obtained.

Theme 9. AQUEOUS SOLUTIONS. CLASSIFICATION OF DISPERSION SYSTEMS

9.1. Aqueous solutions. Dispersed systems

The solution is the mixture that consists of several types of dispersed molecular substances, one of them is the solvent another are soluble substances. The homogeneous mixture forms only one phase. Nonhomogeneous mixture form several phases. The biological solutions are aqueous solutions.

Classification of aqueous solutions:

After the degree of the dispersion of the particles:

- $\Delta = \frac{1}{d}$; d is the diameter of the particle; we can suppose that the particle has the spherical form;
- after the quantity of atoms containing in the dispersed particle (molecule);
- according to the criterion of phases:

a. the uniphasic systems, consisting of one phase, that does not contain the discontinuity, so that continuous;

b. polyphasic;

The classification is represented in the table below.

Classification	Molecular solutions	Colloidal solutions	Suspensions	The author
Dispersional degree	$\Delta > 10^9$	$10^7 < \Delta < 10^9$	$\Delta < 10^7$	Ostwald
As the dependence of the quantity of ions in the particle	$n < 10^3$	$10^3 < n < 10^9$	$n > 10^9$	Staudinger
After the criterion of phases	monophasic	polyphasic		

The researching of particles under the microscope

The researching of particles under the microscope can be studied by the scheme represented in the *fig. 9.1.*

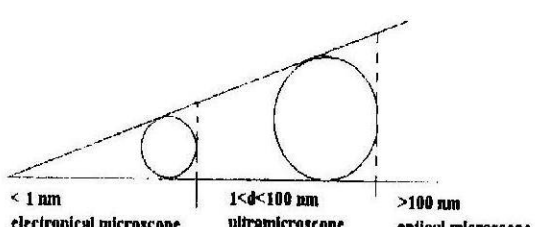


Fig. 9.1. **The scheme of researching of particles under the microscope**

The measurement units of solutions can be represented by different modalities:

1. The concentration related to the unit volume of solution

a. The mass concentration:

$$[C^M]=g/l;$$

b. The molar concentration

$[C_M]=moles/l$; **blood:** $C^M=1\ g/l$; $C_M=5.5\cdot10^{-8}\ moles/l$

c. Osmotical concentration

$$[C^0]=osms/l;$$

- in the case of neutral solutions: $C^0=C^M$;
- in the case of electrolytes:

$\mathbf{C^0=i\cdot C^M}$; where i is the coefficient of ionization Vant – Hoff

$\boldsymbol{\alpha}\cdot C^M$ – the number of dissociated molecules;

$C^M-\boldsymbol{\alpha}\cdot C^M=C^M(1-\boldsymbol{\alpha})$ – the number of non dissociated molecules;

$\mathbf{C^0=C^M(1-\alpha)+\alpha\cdot I\cdot C^M}$; I – the number of ions;

$\mathbf{C^0=C^M\cdot[1+\alpha\cdot(I-1)]}$; $\boldsymbol{i=[1+\alpha\cdot(I-1)]}$- the coefficient of Vant – Hoff;

For example: Suppose that there are two solutions of $CaCl_2$ with different concentrations.

1) $C^M{}_1=0.01$ *moles/l*; $\alpha=100\%\equiv1$

We obtain at dissociation $\mathbf{Ca^{2+}}$ and $\mathbf{2Cl^-}$

I=3; $\mathbf{C^0=i\cdot C^M=3\cdot0.1}$ osms/l;

i=1+1·(3-1)=3;

2) $C^M{}_2=0.2$ moles/l; $\alpha=90\%\equiv0.9$

$i=1+0.9(3-1)=2.8$; $\boldsymbol{C^0=2.8\cdot0.2=0.56\ osms/l}$

$C^N=ZC^MEq$; $[C^N]_{I.S}=g/l$; Z-the number of particles; Eq – one equivalent;

C^N – the equivalent, the number of equivalents that is contained in one liter of solution.

1 Eq – the quantity of substance that is contained in N_A particles ($N_A=6.22\cdot10^{-23}$)

2. The ionic concentration

$C^I = n \cdot C^M$ – 100% dissociation, $\alpha = 100\% \equiv 1$

$C^I = \alpha \cdot n \cdot C^M$ – there is not 100% dissociation;

The ratio of the mass of soluble substance to the mass of solution is: $\tau = \frac{m_{soluble\ substance}}{m_{solution}}$

9.2. The electrical properties of ionic water solutions

The biological liquids are the solutions of different salts, acids and alkali.

The ionic solutions posses 2 basic characteristics that are different of others nonionic solutions:

1) They conduct electrical current.

2) They posses correlation properties, deviation of some solutions.

For example, the structure of the crystal NaCl is assured by the action of Coulomb forces of attraction between positive and negative ions (particles).

$$F = \frac{1}{4\pi\varepsilon_0} \frac{q_1 q_2}{r^2} \qquad (1)$$

$$F = \frac{1}{4\pi\varepsilon_0 \varepsilon} \frac{q_1 q_2}{r^2} \qquad (2)$$

$\alpha = \frac{n}{n_0}$ – The degree of dissociation: α - the coefficient of dissociation; n_0 – the total quantity of molecules in the solution, n – the quantity of dissociated molecules.

9.3. The ionic force of solution

This value represents the numeric value characterizing the electrical properties of solution:

$$M = \frac{1}{2}\sum C_i^z \cdot Z_i^2 \qquad (3)$$

M – the ionic force of solution;
C_i^Z – the concentration of ions in the solution;
Z_i - the charge of ions.

$$A = \gamma C_m \qquad (4)$$

A – the concentration activity
γ - the coefficient of activity;
C_m- molar concentration

$$t_+ = \frac{I_+}{I} = \frac{U_+}{U_+ + U}; \qquad t_- = \frac{U}{U_+ + U}$$

t_+ - the transportation number of cations
t_- - the transportation number of anions;
I_+ - the current created by cations
I – the total current created by cations and anions,
U_+- the mobility of cations;
U_- - the mobility of anions;
$U_+ + U_-$ - The total mobility

9.4. The determination of specific conductivity of the ions of solution

This value can be determined by the formula of Kolrausch:

$$\chi = F \cdot C_m \cdot Z \cdot \alpha \cdot (U_+ + U_-)$$

F – the number of Faraday;
Cm – the concentration;
Z – the order number (charge) of ion;
α - the coefficient of dissociation;
$U_+ + U_-$ the sum of mobilities of cations and anions.

The specific conductivity was determined by the circuit of Kolrausch (*fig. 9.2*).

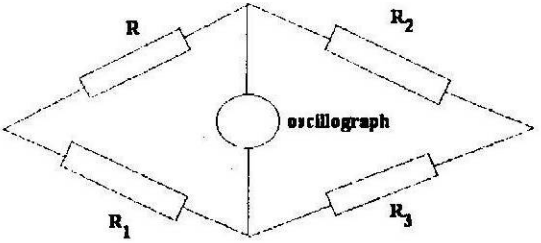


Fig. 9.2.

$$\frac{R}{R_1} = \frac{R_2}{R_3}; \qquad R = R_1 \frac{R_2}{R_3};$$

$$\chi = \frac{1}{\rho} = \frac{1}{R} \cdot \frac{l}{S} = \frac{1}{R_1 \cdot \frac{R_2}{R_3}} \cdot \frac{l}{S} = \frac{R_3 \cdot l}{R_1 \cdot R_2 \cdot S}$$

where S transversal section of H_2O in the ionic solution; l – the distance between electrodes.

So,

$$\chi = \frac{R_3 \cdot l}{R_1 \cdot R_2 \cdot S}$$

Theme 10. WAVES AND SOUND.

Most of the information about our physical surroundings comes to us through our senses of hearing and sight. In both cases we obtain information about objects without being in physical contact with them. The information is transmitted to us in the first case by sound, in the second case by light. Although sound and light are very different phenomena, they are both waves. A wave can be defined as a disturbance that carries energy from one place to another without a transfer of mass. The energy carried by the waves stimulates our sensory mechanisms.

In this chapter, we will first explain briefly the nature of sound and then review some general properties of wave motion applicable to both sound and light. Using this background we will examine the process of hearing and some other biological aspects of sound. Light will be discussed in **Theme 11**.

10.1. Properties of Sound

Sound is a mechanical wave produced by vibrating bodies. For example, when an object such as a tuning fork or the human vocal cords is set into vibrational motion, the surrounding air molecules are disturbed and are forced to follow the motion of the vibrating body. The vibrating molecules in turn transfer their motion to adjacent molecules causing the vibrational disturbance to propagate away from the source. When the air vibrations reach the ear, they cause the eardrum to vibrate; this produces nerve impulses that are interpreted by the brain.

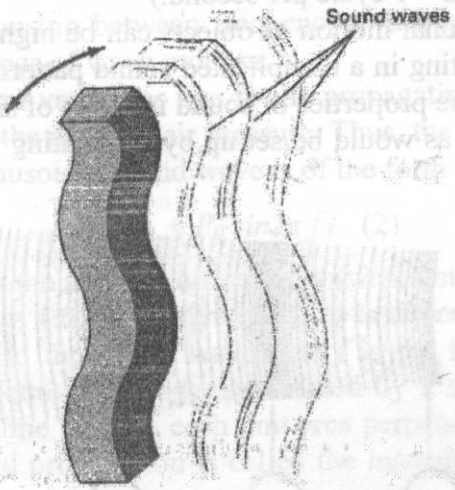


Fig. 10.1. **A complex vibrational pattern.**

All matter transmits sound to some extent, but a material medium is needed between the source and the receiver to propagate sound. This is demonstrated by the well-known experiment of the

bell in the jar. When the bell is set in motion, its sound is clearly audible. As the air is evacuated from the jar, the sound of the bell diminishes and finally the bell becomes inaudible.

The propagating disturbance in the sound-conducting medium is in the form of alternate compressions and rarefactions of the medium, which are initially caused by the vibrating sound source. These compressions and rarefactions are simply deviations in the density of the medium from the average value. In a gas, the variations in density are equivalent to pressure changes.

Two important characteristics of sound are *intensity,* which is determined by the magnitude of compression and rarefaction in the propagating medium, and *frequency,* which is determined by how often the compressions and rarefactions take place. Frequency is measured in cycles per second, which is designated by the unit *hertz* after the scientist Heinrich Hertz. The symbol for this unit is Hz. (1 Hz = 1 cycle per second.)

The vibrational motion of objects can be highly complex (see *fig. 10.1*), resulting in a complicated sound pattern. Still, it is useful to analyze the properties of sound in terms of simple sinusoidal vibrations such as would be set up by a vibrating tuning fork (see *fig. 10.2*).

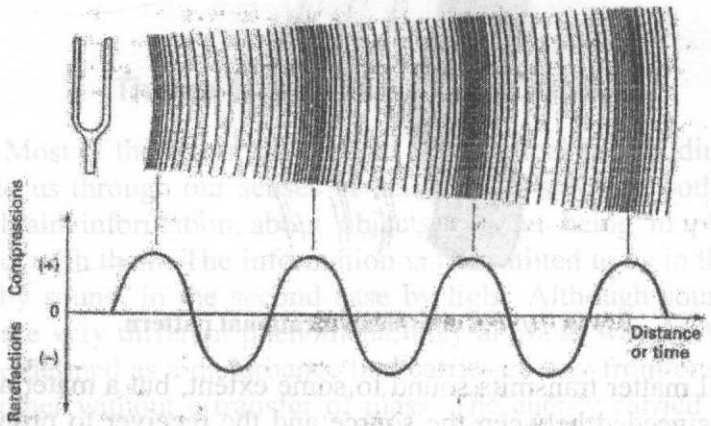


Fig. 10.2. **Sinusoidal sound wave produced by a vibrating tuning fork.**

The type of simple sound pattern shown in fig. 10.2 is called *zpure tone*. When a pure tone propagates through air, the pressure variations due to the compressions and rarefactions are sinusoidal in form.

If we were to take a „snapshot" of the sound at a given instant in time, we would see pressure variations in space, which are also sinusoidal. (Such pictures can actually be obtained with special techniques.) In such a picture the distance between the nearest equal points on the sound wave is called the ***wavelength X.***

The speed of the sound wave v depends on the material that propagates the sound. In air at 20°C, the speed of sound is about 3.3×10^4 cm/sec, and in water it is about 1.4×10^5 cm/sec. In general, the relationship between frequency, wavelength, and the speed of propagation is given by the following equation:

$$v = \lambda f \qquad (1)$$

This relationship between frequency, wavelength, and speed is true for all types of wave motions.

The pressure variations due to the propagating sound are superimposed on the ambient air pressure. Thus, the total pressure in the path of a sinusoidal sound wave is of the form

$$P = P_a + P_0 \sin 2\pi f t \quad (2)$$

where P_a is the ambient air pressure (which at sea level at 0°C is 1.01×10^5Pa = 1.01×10^6 dyn/cm^2), P_0 is the maximum pressure change due to the sound wave, and f is the frequency of the sound. The amount of energy transmitted by a sinusoidal sound wave per unit time through each unit area perpendicular to the direction of sound propagation is called the *intensity I* and is given by

$$I = \frac{P_0^2}{2\rho v} \qquad (3)$$

Here ρ is the density of the medium, and v is the speed of sound propagation.

10.2. Some Properties of Waves

All waves, including sound and light, exhibit the phenomena of reflection, refraction, interference, and diffraction. These phenomena, which play an important role in both hearing and seeing, are described in detail in most basic physics texts (see [10-7]). Here we will review them only briefly.

10.3. Reflection and Refraction

When a wave enters one medium from another, part of the wave is reflected at the interface, and part of it enters the medium. If the interface between the two media is smooth on the scale of the wavelength (i.e., the irregularities of the interface surface are smaller than λ), the reflection is specular (mirrorlike). If the surface has irregularities that are larger than the wavelength, the reflection is diffuse. An example of diffuse reflection is light reflected from paper.

If the wave is incident on the interface at an oblique angle, the direction of propagation of the transmitted wave in the new medium is changed (see *fig. 10.3*).

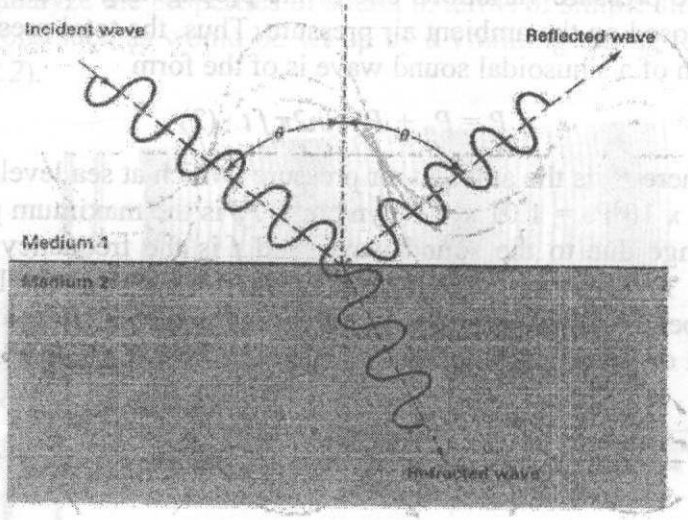


Fig.10.3. **Illustration of reflection and refraction.** ***(0* is the angle of incidence.)**

This phenomenon is called *refraction*. The angle of reflection is always equal to the angle of incidence, but the angle of the refracted wave is, in general, a function of the properties of the two media. The fraction of the energy transmitted from one medium to another depends again on the properties of the media and on the angle of incidence. For a sound wave incident perpendicular to the interface, the ratio of transmitted to incident intensity is given by

$$\frac{I_t}{I_i} = \frac{4\rho_1 v_1 \rho_2 v_2}{(\rho_1 v_1 + \rho_2 v_2)^2} \qquad (4)$$

where the subscripted quantities are the velocity and density in the two media. The solution of Eq. 4 shows that when sound traveling in air is incident perpendicular to a water surface, only about 0.1% of the sound energy enters the water; 99. 9 % is reflected. The fraction of sound energy entring the water is even smaller when the angle of incidence is oblique. Water is thus an efficient barrier to sound.

10.4. Interference

When two (or more) waves travel simultaneously in the same medium, the total disturbance in the medium is at each point the vectorial sum of the individual disturbances produced by each wave. This phenomenon is called *interference*. For example, if two waves are in phase, they add so that the wave disturbance at each point in space is increased. This is called *constructive interference* (see *fig. 10.4, a*).

If two waves are out of phase by 180°, the wave disturbance in the propagating medium is reduced. This is called *destructive interference* (*fig. 10.4, b*). If the magnitudes of two out-of-phase waves are the same, the wave disturbance is completely canceled (*fig. 10.4, c*).A special type of interference is produced by two waves of the same frequency and magnitude traveling in opposite directions. The resultant wave pattern is stationary in space and is called a *standing wave*.

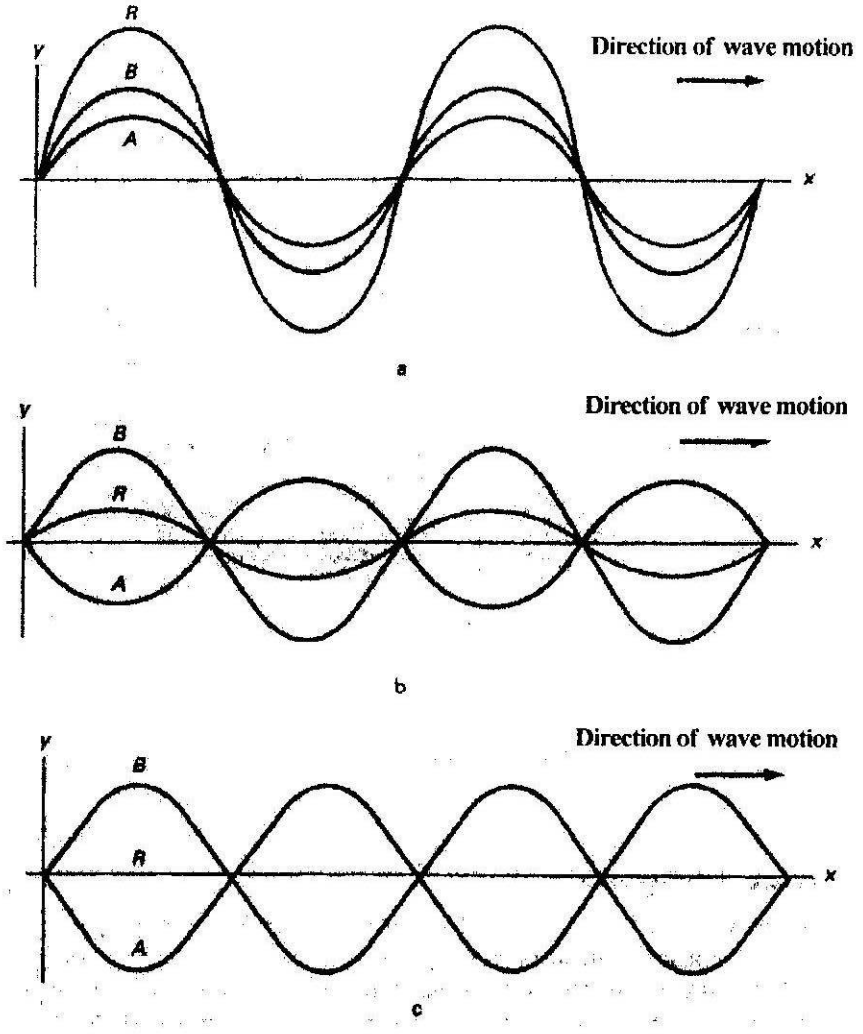


Fig. 10.4. **(a) Constructive interference, (b, c) Destructive interference. *R* is the resultant of the interference of the two waves *A* and *B*.**

Such standing sound waves are formed in hollow pipes such as the flute. It can be shown that, in a given structure, standing waves can exist only at specific frequencies, **which are called *resonant frequencies.***

10.5. Diffraction

Waves have a tendency to spread as they propagate through a medium. As a result, when a wave encounters an obstacle, it spreads into the region behind the obstacle. This phenomenon is called *diffraction.* The amount of diffraction depends on the wavelength: The longer the wavelength, the greater is the spreading of the wave. Significant diffraction into the region behind the obstacle occurs only if the size of the obstacle is smaller than the wavelength. For example, a person sitting behind a pillar in an auditorium hears the performer because the long wavelength sound waves spread behind the pillar. But the view of the performance is obstructed because the wavelength of light is much smaller than the pillar, and, therefore, the light does not diffract into the region behind the pillar.

Objects that are smaller than the wavelength do not produce a significant reflection. This too is due to diffraction. The wave simply diffracts around the small obstacle, much as flowing water spreads around a small stick.

Both light waves and sound waves can be focused with curved reflectors and lenses. There is, however, a limit to the size of the focused spot. It can be shown that the diameter of the focused spot cannot be smaller than about $\lambda/2$. These properties of waves have important consequences in the process of hearing and seeing.

10.6. Hearing and the Ear

The sensation of hearing is produced by the response of the nerves in the ear to pressure variations in the sound wave. The nerves in the ear are not the only ones that respond to pressure, as most of the skin contains nerves that are pressure-sensitive. However, the ear is much more sensitive to pressure variations than any other part of the body.

Figure 10.5 is a drawing of the human ear. (The ear construction of other terrestrial vertebrates is similar.) For the purposes of description, the ear is usually divided into three main sections: the outer ear, the middle ear, and the inner ear. The sensory cells that

convert sound to nerve impulses are located in the liquid-filled inner ear.

The main purpose of the outer and middle ears is to conduct the sound into the inner ear.

The outer ear is composed of an external flap called the *pinna* and the ear canal, which is terminated by the *tympanic membrane* (eardrum). In many animals the pinna is large and can be rotated toward the source of the sound; this helps the animal to locate the sourcJe of sound. However, in humans the pinna is fixed and so small that it does not seem to contribute significantly to the hearing process.

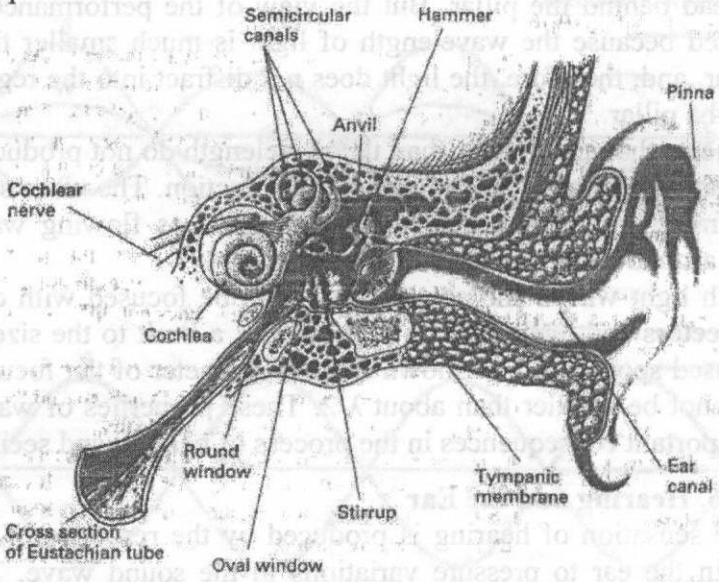


Fig. 10.5. **A semidiagrammatic drawing of the ear with various structures cut away and simplified to show the basic relationships more clearly. The middle ear muscles have been omitted.**

The ear canal of an average adult is about 0.75 cm in diameter and 2.5 cm long, a configuration that is resonant for sound waves at frequencies around 3000 Hz. This accounts in part for the high sensitivity of the ear to sound waves in this frequency range.

For an animal to perceive sound, the sound has to be coupled from air to the sensory cells that are in the fluid environment of the inner ear. We showed earlier that direct coupling of sound waves into a fluid is inefficient because most of the sound energy is reflected at the interface. The middle ear provides an efficient conduction path for the sound waves from air into the fluid of the inner ear.

The middle ear is an air-filled cavity that contains a linkage of three bones called *ossicles* that connect the eardrum to the inner ear. The three bones are called the *hammer*, the *anvil*, and the *stirrup*. The hammer is attached to the inner surface of the eardrum, and the stirrup is connected to the oval window, which is a membrane-covered opening in the inner ear.

When sound waves produce vibrations in the eardrum, the vibrations are transmitted by the ossicles to the oval window, which in turn sets up pressure variations in the fluid of the inner ear. The ossicles are connected to the walls of the middle ear by muscles that also act as a volume control. If the sound is excessively loud, these muscles as well as the muscles around the eardrum stiffen and reduce the transmission of sound to the inner ear.

The middle ear serves yet another purpose. It isolates the inner ear from the disturbances produced by movements of the head, chewing, and the internal vibrations produced by the person's own voice. To be sure, some of the vibrations of the vocal cords are transmitted through the bones into the inner ear, but the sound is greatly attenuated. We hear ourselves talk mostly by the sound reaching our eardrums from the outside. This can be illustrated by talking with the ears plugged.

The *Eustachian tube* connects the middle ear to the upper part of the throat. Air seeps in through this tube to maintain the middle ear at atmospheric pressure. The movement of air through the Eustachian tube is aided by swallowing. A rapid change in the external air pressure such as may occur during an airplane flight causes a pressure imbalance on the two sides of the eardrum. The resulting force on the eardrum produces a painful sensation that lasts

until the pressure in the middle ear is adjusted to the external pressure. The pain is especially severe and prolonged if the Eustachian tube is blocked by swelling or infection.

The conversion of sound waves into nerve impulses occurs in the *cochlea,* which is located in the inner ear. The cochlea is a spiral cavity shaped like a snail shell. The wide end of the cochlea, which contains the oval and the round windows, has an area of about 4 mm^2. The cochlea is formed into a spiral with about $2\frac{3}{4}$ turns. If the cochlea were uncoiled, its length would be about 35 mm.

Inside the cochlea there are three parallel ducts; these are shown in the highly simplified drawing of the uncoiled cochlea in *fig. 10.6.*

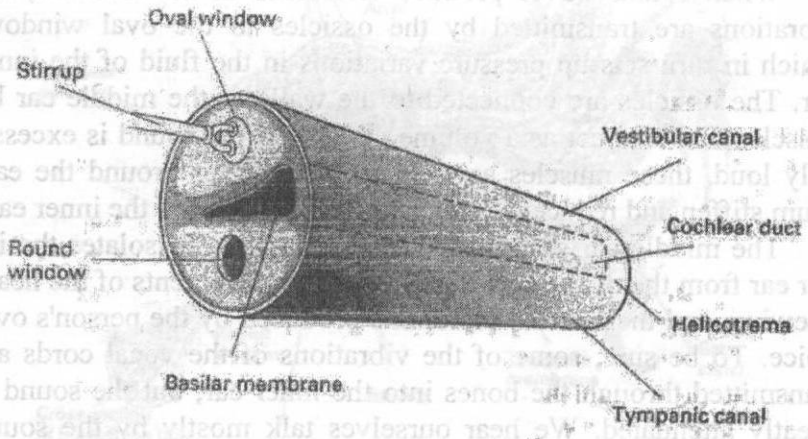


Fig. 10.6. **An uncoiled view of the cochlea.**

All three ducts are filled with a fluid. The vestibular and tympanic canals are joined at the apex of the cochlea by a narrow opening called the *helicotrema.* The cochlear duct is isolated from the two canals by membranes. One of these membranes, called the *basilar membrane,* supports the auditory nerves.

The vibrations of the oval window set up a sound wave in the fluid filling the vestibular canal. The sound wave, which travels

along the vestibular canal and through the helicotrema into the tympanic canal, produces vibrations in the basilar membrane which stimulate the auditory nerves to transmit electrical pulses to the brain (Theme 10). The excess energy in the sound wave is dissipated by the motion of the round window at the end of the tympanic canal.

10.7. Performance of the Ear

The nerve impulses evoke in the brain the subjective sensation of sound. *Loudness, pitch,* and *quality* are some of the terms we use to describe the sounds we hear. It is a great challenge for physiologists to relate these subjective responses with the physical properties of sound such as intensity and frequency. Some of these relationships are now well understood; others are still subjects for research.

In most cases, the sound wave patterns produced by instruments and voices are highly complex. Each sound has its own characteristic pattern. It would be impossible to evaluate the effect of sound waves on the human auditory system if the response to each sound pattern had to be analyzed separately. Fortunately the problem is not that complicated. About 150 years ago, J. B. J. Fourier, a French mathematician, showed that complex wave shapes can be analyzed into simple sinusoidal waves of different frequencies. In other words, a complex wave pattern can be constructed by adding together a sufficient number of sinusoidal waves at appropriate frequencies and amplitudes. Therefore, if we know the response of the ear to sinusoidal waves over a broad range of frequencies, we can evaluate the response of the ear to a wave pattern of any complexity.

An analysis of a wave shape into its sinusoidal components is shown in *fig. 10.7*. The lowest frequency in the wave form is called the *fundamental,* and the higher frequencies are called *harmonics.*

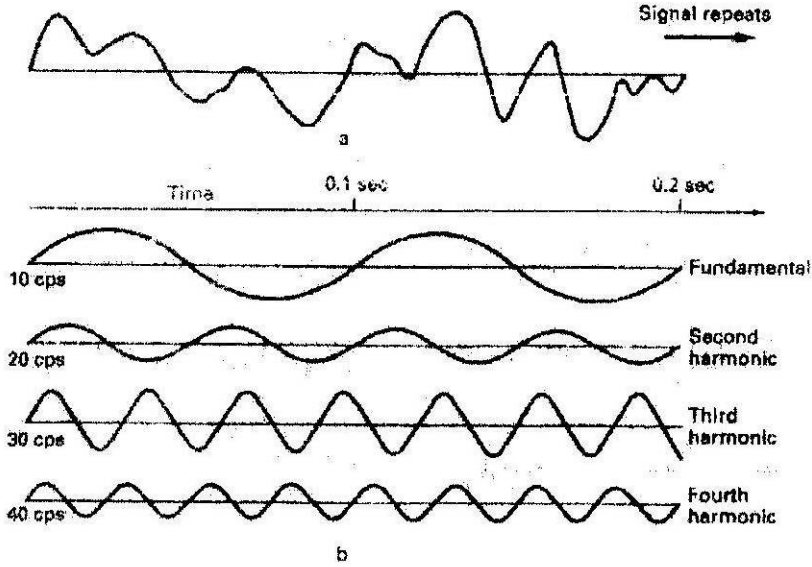


Fig. 10.7. **The analysis of a complex wave shape (a), into its sine components (b). The point-by-point addition of the fundamental frequency sine wave and the harmonic frequency sine waves yields the wave shape shown in (a).**

Figure 10.8, shows the sound pattern for a specific note played by various instruments. It is the harmonic content of the sound that differentiates one sound source from another. For a given note played by the various instruments shown in *fig. 10.8*, the fundamental freauency is the same but the harmonic content of the wave is different for each instrument.

10.8. Frequency and Pitch

The human ear is capable of detecting sound at frequencies between about 20 and 20,000 Hz. Within this frequency range, however, the response of the ear is not uniform. The ear is most sensitive to frequencies between 200 and 4000 Hz, and its response decreases toward both higher and lower frequencies. There are wide variations in the frequency response of individuals. Some

people cannot hear sounds above 8000 Hz, whereas a few people can hear sounds above 20,000 Hz. Furthermore, the hearing of most people deteriorates with age.

The sensation of pitch is related to the frequency of the sound. The pitch increases with frequency. Thus, the frequency of middle C is 256 Hz, and the frequency of the A above is 440 Hz.

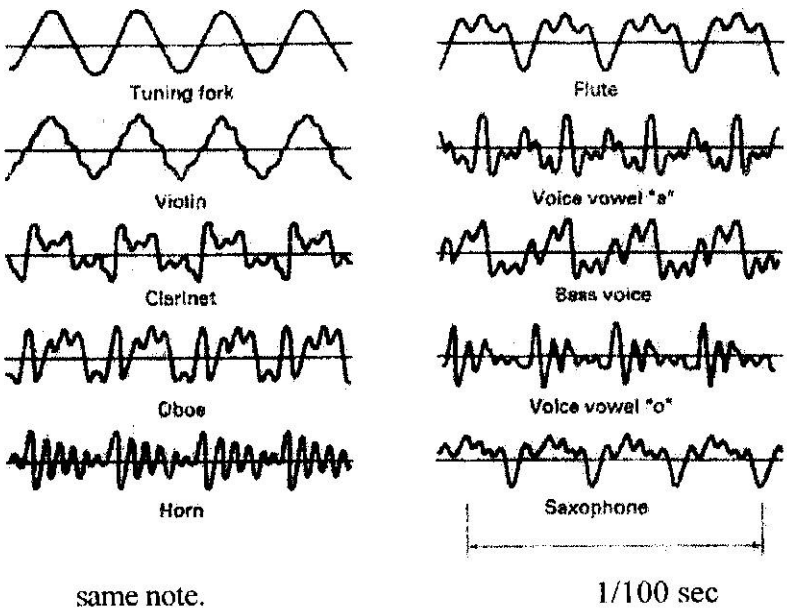


Fig. 10.8. **Wave forms of sound from different musical instruments sounding the same note.**

There is, however, no simple mathematical relationship between pitch and frequency.

10.9. Intensity and Loudness

The ear responds to an enormous range of intensities. At 3000 Hz, the lowest intensity that the human ear can detect is about 10^{-16} W/cm^2. The loudest tolerable sound has an intensity of about 10^{-4} W/cm^2. These two extremes of the intensity range are called

the *threshold of hearing* and the *threshold of pain*, respectively. Sound intensities above the threshold of pain may cause permanent damage to the eardrum and the ossicles.

The ear does not respond linearly to sound intensity; that is, a sound which is a million times more powerful than another does not evoke a million times higher sensation of loudness. The response of the ear to intensity is closer to being logarithmic than linear.

Because of the nonlinear response of the ear and the large range of intensities involved in the process of hearing, it is convenient to express sound intensity on a logarithmic scale.

On this scale, the sound intensity is measured relative to a reference level of 10^{-16} W/cm^2 (which is approximately the lowest audible sound intensity).

The logarithmic intensity is measured in units of decibel (dB) and is defined as

$$\text{Logarithmic intensity} = 10 \log \frac{\text{Sound intensity in W/cm}^2}{10^{-16} \text{ W/cm}^2} \quad (5).$$

Thus, for example, the logarithmic intensity of a sound wave with a power of 10^{-12} W/cm^2 is

$$\text{Logarithmic intensity} = 10 \log \frac{10^{-12}}{10^{-16}} = 40 dB$$

Intensities of some common sounds are listed in Table 10.1.

At one time, it was believed that the ear responded logarithmically to sound intensity. Referring to Table 10.1, a logarithmic response would imply that, for example, a busy street sounds only six times louder than the rustle of leaves even though the power of the street sounds is a million times greater. Although it has been shown that the intensity response of the ear is not exactly logarithmic, the assumption of a logarithmic response still provides a useful guide for assessing the sensation of loudness produced by sounds at different intensities (see Exercises 10-1 and 10-2).

Table 10.1

Sound Levels Due to Various Sources (representative values)

Source of sound	Sound level (dB)	Sound level (W/cm^2)
Threshold of pain	120	10^{-4}
Riveter	90	10^{-7}
Busy street traffic	70	10^{-9}
Ordinary conversation	60	10^{-10}
Quiet automobile	50	10^{-11}
Quiet radio at home	40	10^{-12}
Average whisper	20	10^{-14}
Rustle of leaves	10	10^{-15}
Threshold of hearing	0	10^{-16}

The sensitivity of the ear is remarkable. At the threshold of hearing, in the range of 2000-3000 Hz, the ear can detect a sound intensity of 10^{-16} W/cm^2. This corresponds to a pressure variation in the sound wave of only about 2.9×10^{-4} dyn/cm^2 (see Exercise 10-3). Compare this to the background atmospheric pressure, which is 1.013×10^6 dyn/cm^2. This sensitivity appears even more remarkable when we realize that the random pressure variations in air due to the thermal motion of molecules are about 0.5×10^{-4} dyn/cm^2. Thus, the sensitivity of the ear is close to the ultimate limit at which it would begin to detect the noise fluctuations in the air. The displacement of the molecules corresponding to the power at the threshold of hearing is less than the size of the molecules themselves.

The sensitivity of the ear is partly due to the mechanical construction of the ear, which amplifies the sound pressure. Most of the mechanical amplification is produced by the middle ear. The area of the eardrum is about 30 times larger than the oval window. Therefore, the pressure on the oval window is increased by the same factor (see Exercise 10-4). Furthermore, the ossicles act as a lever with a mechanical advantage of about 2. Finally, in the fre-

quency range around 3000 Hz, there is an increase in the pressure at the eardrum due to the resonance of the ear canal. In this frequency range, the pressure is increased by another factor of 2. Thus, the total mechanical amplification of the sound pressure in the 3000-Hz range is about 2 x 30 x 2 = 120. Because the intensity is proportional to pressure squared (see Eq. 3), the intensity at the oval window is amplified by a factor of about 14,400.

The process of hearing cannot be fully explained by the mechanical construction of the ear. The brain itself plays an important role in our perception of sound. For example, the brain can effectively filter out ambient noise and allow us to separate meaningful sounds from a relatively loud background din. (This feature of the brain permits us to have a private conversation in the midst of a loud party.) The brain can also completely suppress sounds that appear to be meaningless. Thus, we may lose awareness of a sound even though it still produces vibrations in our ear. The exact mechanism of interaction between the brain and the sensory organs is not yet fully understood.

10.10. Bats and Echoes

The human auditory organs are very highly developed; yet, there are animals that can hear even better than we can. Notable among these animals are the bats. They emit high-frequency sound waves and detect the reflected sounds (echoes) from surrounding objects. Their sense of hearing is so acute that they can obtain information from echoes which is in many ways as detailed as the information we can obtain with our sense of sight. The many different species of bats utilize echoes in various ways. The *Vespertilionidae* family of bats emit short chirps as they fly. The chirps last about 3×10^{-3} sec (3 m/sec) with a time interval between chirps of about 70 msec. Each chirp starts at a frequency of about 100×10^3 Hz and falls to about 30×10^3 Hz at the end. (The ears of bats, of course, respond to these high frequencies.) The silent interval between chirps allows the bat to detect the weak echo without interference from the primary chirp. Presumably the inter-

val between the chirp and the return echo allows the bat to determine its distance from the object. It is also possible that differences in the frequency content of the chirp and the echo allow the bat to estimate the size of the object (see Exercise 10-5), With a spacing between chirps of 70 msec, an echo from an object as far as 11.5 m can be detected before the next chirp (see Exercise 10-6). As the bat comes closer to the object (such as an obstacle or an insect), both the duration of and the spacing between chirps decrease, allowing the bat to localize the object more accurately. In the final approach to the object, the duration of the chirps is only about 0.3 msec, and the spacing between them is about 5 msec.

Experiments have shown that with echo location bats can avoid wire obstacles with diameters down to about 0.1 mm, but they fail to avoid finer wires. This is in accord with our discussion of wave diffraction (see Exercise 10-7). Other animals, such as porpoises, whales, and some birds, also use echoes to locate objects, but they are not able to do so as well as bats.

10.11. Sounds Produced by Animals

Animals can make sounds in various ways. Some insects produce sounds by rubbing their wings together. The rattlesnake produces its characteristic sound by shaking its tail In most animals, however, sound production is associated with the respiratory mechanism. In humans, the *vocal cords* are the primary source of sound. These are two reeds, shaped like lips, attached to the upper part of the trachea. During normal breathing the cords are wide open. To produce a sound the edges of the cords are brought together. Air from the lungs passes through the space between the edges and sets the cords into vibration. The frequency of the sounds is determined by the tension on the vocal cords. The fundamental frequency of the average voice is about 140 Hz for males and about 230 Hz for females. The sound produced by the vocal cords is substantially modified as it travels through the passages of the mouth and throat. The tongue also plays an important role in the final sound. Many voice sounds are produced outside the vocal

cords (for example, the consonant *s).* The sounds in a whispering talk are also produced outside the vocal cords.

10.12. Clinical Uses of Sound

The most familiar clinical use of sound is in the analysis of body sounds with a stethoscope. This instrument consists of a small bell-shaped cavity attached to a hollow flexible tube. The bell is placed on the skin over the source of the body sound (such as the heart or lungs). The sound is then conducted by the pipe to the ears of the examiner who evaluates the functioning of the organ. A modified version of the stethoscope consists of two bells that are placed on different parts of the body. The sound picked up by one bell is conducted to one ear, and the sound from the other bell is conducted to the other ear. The two sounds are then compared. With this device, it is possible, for example, to listen simultaneously to the heartbeats of the fetus and of the pregnant mother.

10.13. Ultrasonic Waves

With special electronically driven crystals, it is possible to produce mechanical waves at very high frequencies, up to millions of cycles per second. These waves, which are simply the extension of sound to high frequencies, are called *ultrasonic waves.* Because of their short wavelength, ultrasonic waves can be focused onto small areas and can be imaged much as visible light (see Exercise 10-8).

Ultrasonic waves penetrate tissue and are scattered and absorbed within it. Using specialized techniques called *ultrasound imaging,* it is possible to form visible images of ultrasonic reflections and absorptions. Therefore, structures within living organisms can be examined with ultrasound, as with X-rays. Ultrasonic examinations are safer than X-rays and often can provide as much information. In some cases, such as in the examination of a fetus and the heart, ultrasonic methods can show motion, which is very useful in such displays.

The frequency of sound detected by an observer depends on the relative motion between the source and the observer. This phenomenon is called the *Doppler effect*. It can be shown (see Exercise 10-9) that if the observer is stationary and the source is in motion, the frequency of the sound f' detected by the observer is given by

$$f' = f\frac{v}{v \pm v_s} \qquad (6)$$

where f is the frequency in the absence of motion, v is the speed of sound, and v_s is the speed of the source. The minus sign in the denominator is to be used when the source is approaching the observer, and the plus sign when the source is receding.

Using the Doppler effect, it is possible to measure motions within a body.

One device for obtaining such measurements is the *ultrasonic flow meter*, which produces ultrasonic waves that are scattered by blood cells flowing in the blood vessels. The frequency of the scattered sound is altered by the Doppler effect. The velocity of blood flow is obtained by comparing the incident frequency with the frequency of the scattered ultrasound.

Within the tissue, the mechanical energy in the ultrasonic wave is converted to heat. With a sufficient amount of ultrasonic energy, it is possible to heat selected parts of a patient's body more efficiently and evenly than can be done with conventional heat lamps. This type of treatment, called *diathermy*, is used to relieve pain and promote the healing of injuries. It is actually possible to destroy tissue with very high-intensity ultrasound. Ultrasound is now routinely used to destroy kidney and gall stones (lithotripsy).

> EXERCISES

10-1. The intensity of a sound produced by a point source decreases as the square of the distance from the source. Consider a riveter as a point source of sound and assume that the intensities

listed in Table 10.1 are measured at a distance 1 m away from the source. What is the maximum distance at which the riveter is still audible? (Neglect losses due to energy absorption in the air.)

10-2. Referring to Table 10.1, approximately how much louder does busy street. traffic sound than a quiet radio?

10-3. Calculate the pressure variation corresponding to a sound intensity of 10^{-16} W/cm^2. (The density of air at 0°C and 1 atm pressure is 1.29×10^{-3} g/cm^3; for the speed of sound use the value 3.3×10^4 cm/sec.)

10-4. Explain why the relative sizes of the eardrum and the oval window result in pressure magnification in the inner ear.

10-5. Explain how a bat might use the differences in the frequency content of its chirp and echo to estimate the size of an object.

10-6. With a 70-msec space between chirps, what is the farthest distance at which a bat can detect an object?

10-7. In terms of diffraction theory, discuss the limitations on the size of the object that a bat can detect with its echo location.

10-8. Estimate the lower limit on the size of objects that can be detected with ultrasound at a frequency of 2×10^6 Hz.

10-9. With the help of a basic physics textbook, explain the Doppler effect and derive Eq. 6.

Theme 11. OPTICS

Light is the electromagnetic radiation in the wavelength region between about 400 and 700 nm (1 nm = 10^{-9} m). Although light is only a tiny part of the electromagnetic spectrum, it has been the subject of much research in both physics and biology. The importance of light is due to its fundamental role in living systems. Most of the electromagnetic radiation from the sun that reaches the Earth's surface is in this region of the spectrum, and life has evolved to utilize it. In photosynthesis, plants use light to convert carbon dioxide and water into organic materials, which are the building blocks of living organisms. Animals have evolved light-sensitive

organs which are their main source of information about the surroundings. Some bacteria and insects can even produce light through chemical reactions.

Optics, which is the study of light, is one of the oldest branches of physics. It includes topics such as microscopes, telescopes, vision, color, pigments, illumination, spectroscopy, and lasers, all of which have applications in the life sciences. In this chapter, we will discuss four of these topics: vision, telescopes, microscopes, and optical fibers. Background information needed to understand this chapter is reviewed in Appendix C.

11.1. Vision

Vision is our most important source of information about the external world. It has been estimated that about 70% of a person's sensory input is obtained through the eye. The three components of vision are the stimulus, which is light; the optical components of the eye, which image the light; and the nervous system, which processes and interprets the visual images.

11.2. Nature of Light

Experiments performed during the nineteenth century showed conclusively that light exhibits all the properties of wave motion, which were discussed in Thema 11. At the beginning of this century, however, it was shown that wave concepts alone do not explain completely the properties of light. In some cases, light and other electromagnetic radiation behave as if composed of small packets (quanta) of energy. These packets of energy are called *photons*. For a given frequency f of the radiation, each photon has a fixed amount of energy E which is

$$E = \eta f \qquad (1)$$

where η is Planck's constant, equal to 6.63×10^{-27} erg-sec.

In our discussion of vision, we must be aware of both of these properties of light. The wave properties explain all phenomena associated with the propagation of light through bulk matter, and

the quantum nature of light must be invoked to understand the effect of light on the photoreceptors in the retina.

11.3. Structure of the Eye

A diagram of the human eye is given in *fig. 11.1.* The eye is roughly a sphere, approximately 2.4 cm in diameter. All vertebrate eyes are similar in structure but vary in size. Light enters the eye through the cornea, which is a transparent section in the outer cover of the eyeball. The light is focused by the lens system of the eye into an inverted image at the photosensitive retina, which covers the back surface of the eye. Here the light produces nerve impulses that convey information to the brain.

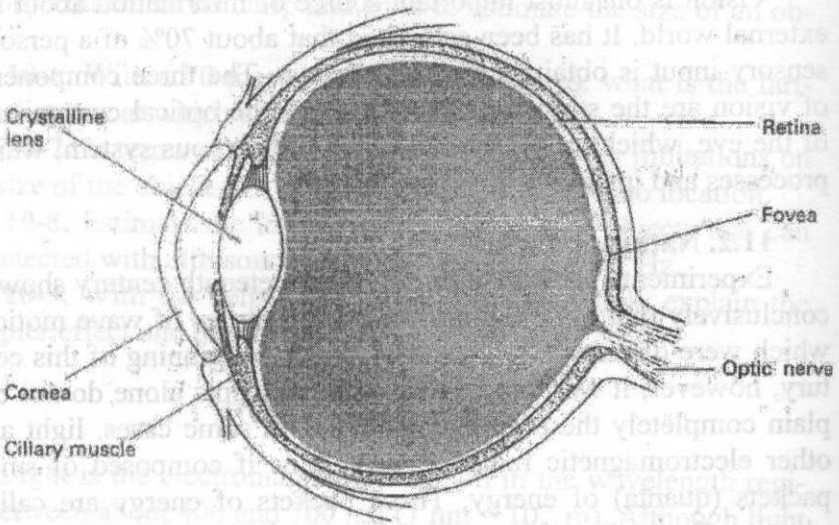


Fig. 11.1. **The human eye.**

The focusing of the light into an image at the retina is produced by the curved surface of the cornea and by the crystalline lens inside the eye. The focusing power of the cornea is fixed. The focus of the crystalline lens, however, is alterable, allowing the eye to view objects over a wide range of distances.

In front of the lens is the iris, which controls the size of the pupil, or entrance aperture into the eye (see Theme 11). Depending on the intensity of the light, the diameter of the aperture ranges from 2 to 8 mm. The cavity of the eye is filled with two types of fluid, both of which have a refractive index about the same as water. The front of the eye, between the lens and the cornea, is filled with a watery fluid called the *aqueous humor*. The space between the lens and the retina is filled with the gelatinous *vitreous* humor.

11.4. Accommodation

The focusing of the eye is controlled by the ciliary muscle, which can change the thickness and curvature of the lens. This process of focusing is called *accommodation*. When the ciliary muscle is relaxed, the crystalline lens is fairly flat, and the focusing power of the eye is at its minimum. Under these conditions, a parallel beam of light is focused at the retina. Because light from distant objects is nearly parallel, the relaxed eye is focused to view distant objects. In this connection, "distant" is about 6 m and beyond (see Exercise 11-1).

The viewing of closer objects requires greater focusing power. The light from nearby objects is divergent as it enters the eye; therefore, it must be focused more strongly to form an image at the retina. There is, however, a limit to the focusing power of the crystalline lens. With the maximum contraction of the ciliary muscle, a normal eye of a young adult can focus on objects about 15 cm from the eye. Closer objects appear blurred. The minimum distance of sharp focus is called the *near point of the eye*.

The focusing range of the crystalline lens decreases with age. The near point for a 10-year-old child is about 7 cm, but by the age of 40 the near point shifts to about 22 cm. After that the deterioration is rapid.

At age 60, the near point is shifted to about 100 cm. This decrease in the accommodation of the eye with age is called *presbyopia*.

11.5. Eye and the Camera

Although the designers of the photographic camera did not consciously imitate the structure of the eye, many of the features in the two are remarkably similar (see *fig. 11.2*).

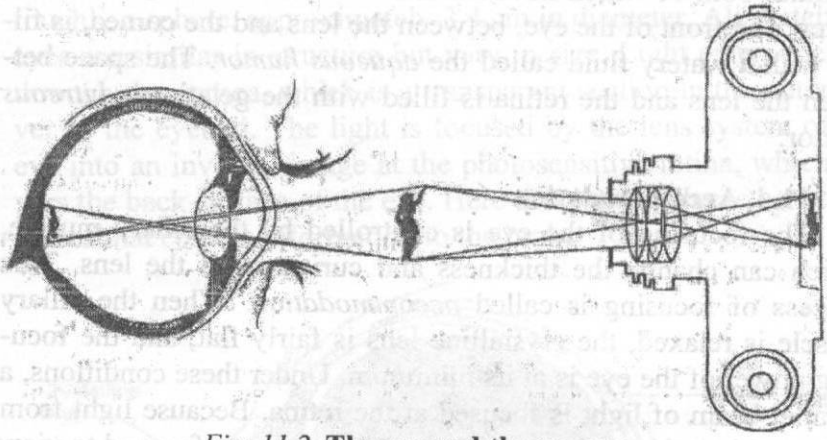


Fig. 11.2. **The eye and the camera.**

Both consist of a lens system that focuses a real inverted image onto a photosensitive surface. In the eye, as in the camera, the diameter of the light entrance is controlled by a diaphragm that is adjusted in accord with the available light intensity. In a camera, the image is focused by moving the lens with respect to the film. In the eye, the distance between the retina and the lens is fixed; the image is focused by changing the thickness of the lens.

Even the photosensitive surfaces are somewhat similar. Both photographic film and the retina consist of discrete light-sensitive units, microscopic in size, which undergo chemical changes when they are illuminated. In fact, under special circumstances, the retina can be "developed," like film, to show the image that was projected on it. This was first demonstrated in the 1870 s by the German physiologist W. Kuhne. He exposed the eye of a living rabbit to light coming through a barred window. After 3 minutes of exposure to light, the rabbit was killed and its retina was immersed in an alum solution which fixed the retinal reaction. The barred

window was clearly visible on the retina. A few years later, Kuhne fixed the retina from the head of a guillotined criminal. He observed an image, but he could not interpret it in terms of anything that the man had seen before he was beheaded.

The analogy between the eye and the camera, however, is not complete.

As we will describe later, the eye goes far beyond the camera in processing the images that are projected on the retina.

11.6. Aperture and Depth of Field

The iris is the optical aperture of the eye, and its size varies in accordance with the available light. If there is adequate light, the quality of the image is best with the smallest possible aperture. This is true for both the eye and the camera.

There are two main reasons for the improved image with reduced aperture. Imperfections in lenses tend to be most pronounced around the edges. A small aperture restricts the light path to the center of the lens and eliminates the distortions and aberrations produced by the periphery.

A smaller aperture also improves the image quality of objects that are not located at the point on which the eye or the camera is focused. An image is in sharp focus at the retina (or film) only for objects at a specific distance from the lens system. Images of objects not at this specific plane are blurred at the retina (see *fig. 11.3*); in other words, a point that is not in exact focus appears as a disk on the retina. The amount of blurring depends on the size of the aperture. As shown in fig. 11.3, a small aperture reduces the diameter of the blurred spot and allows the formation of a relatively clear image from objects that are not on the plane to which the eye is focused. The range of object distances over which a good image is formed for a given setting of the focus is called the *depth of field.* Clearly a small aperture increases the depth of field. It can be shown that the depth of field is inversely proportional to the diameter of the aperture (see Exercise 11-2).

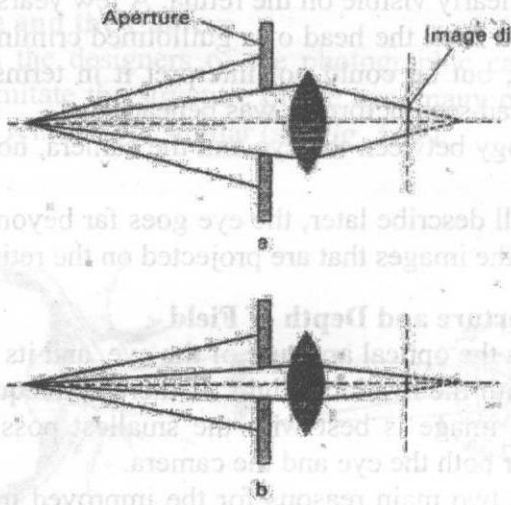


Fig. 11.3. **Size of image disk: (a) with large aperture, (b) with small aperture.**

11.7. Lens System of the Eye

The focusing of the light into a real inverted image at the retina is produced by refraction at the cornea and at the crystalline lens (see *fig. 11.4*).

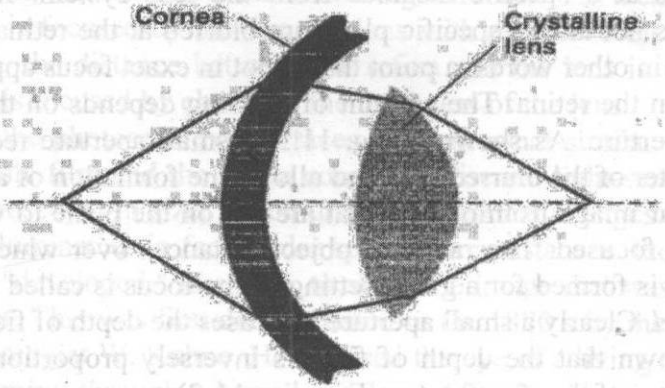


Fig. 11.4. **Focusing by the cornea and the crystalline lens (not to scale).**

The focusing or refractive power of the cornea and the lens can be calculated using Eq. C.9, (Appendix C). The data required for the calculation are shown in table 11.1.

Table 11.1

Parameters for the Eye

	Radius (mm)		Index of refraction
	Front	Back	
Cornea	7.8	7.3	1.38
Lens, min. power	10.00	-6.0	1.40
Lens, max. power	6.0	-5.5	1.33
Aqueous and vitreous humor			

The largest part of the focusing, about two thirds, occurs at the cornea. The power of the crystalline lens is small because its index of refraction is only slightly greater than that of the surrounding fluid. In Exercise 11-3. it is shown that the refractive power of the cornea is 42 diopters, and the refractive power of the crystalline lens is variable between about 19 and 24 diopters. (For a definition of the unit *diopter*, see Appendix C.)

The refractive power of the cornea is greatly reduced when it is in contact with water (see Exercise 11-4). Because the crystalline lens in the human eye cannot compensate for the diminished power of the cornea, the human eye under water is not able to form a clear image at the retina and vision is blurred. In fish eyes, which have evolved for seeing under water, the lens is intended to do most of the focusing. The lens is nearly spherical and has a much greater focusing power than the lens in the eyes of terrestrial animals (see Exercise 11-5).

11.8. Reduced Eye

To trace accurately the path of a light ray through the eye, we must calculate the refraction at four surfaces (two at the cornea and two at the lens). It is possible to simplify this laborious proce-

dure with a model called the *reduced eye,* shown in *fig. 11.5.* Here all the refraction is assumed to occur at the front surface of the cornea, which is constructed to have a diameter of 5 mm. The eye is assumed to be homogeneous, with an index of refraction of 1.333 (the same as water). The retina is located 2 cm behind the cornea. The nodal point n is the center of corneal curvature located 5 mm behind the cornea.

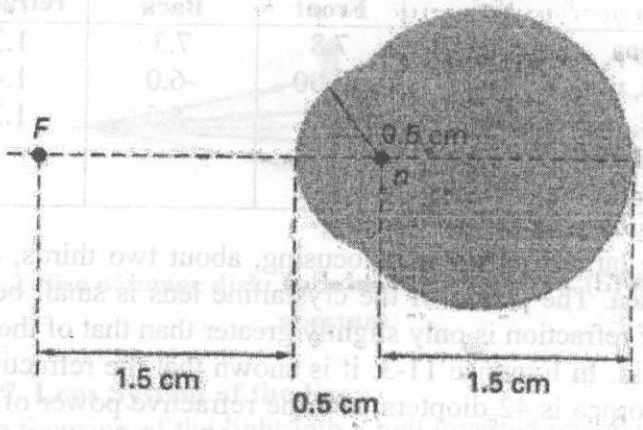


Fig. 11.5. **The reduced eye.**

This model represents most closely the relaxed eve which focuses parallel light at the retina, as can be confirmed using Eq. C.9. For the reduced eye, the second term on the right-hand side of the equation vanishes because the light is focused within the reduced eye so that $n_L = n_2$. Equation C.9, therefore, simplifies to

$$\frac{n_1}{p} + \frac{n_L}{q} = \frac{n_L - n_1}{R}$$

where $n_1 = 1$, $n_L = 1.333$, and $R = 0.5$ cm. Because the incoming light is parallel, its source is considered to be at infinity (i.e., $p = \infty$). Therefore, the distance q at which parallel light is focused is given by

$$\frac{1.333}{q} = \frac{1.333 - 1}{5}$$

Or

$$q = \frac{1.333 \times 5}{0.333} = 20\,mm$$

The anterior focal point F for the reduced eye is located 15 mm in front of the cornea. This is the point at which parallel light originating inside the eye is focused when it emerges from the eye (see Exercise 11-6).

Although the reduced eye does not contain explicitly the mechanism of accommodation, we can use the model to determine the size of the image formed on the retina. The construction of such an image is shown in *fig. 11.6.*

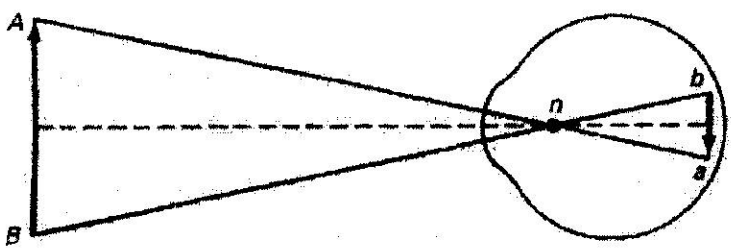


Fig. 11.6. **Determination of the image size on the retina.**

Rays from the limiting points of the object A and B are projected through the nodal point to the retina. The limiting points of the image at the retina are a and b. This construction assumes that all the rays from points A and B that enter the eye are focused on the retina at points a and b, respectively. Rays from all other points on the object are focused correspondingly between these limits. The triangles AnB and anb are similar; therefore, the relation of object to image size is given by

$$\frac{\text{Object size}}{\text{Image size}} = \frac{\text{Distance of object from nodal point}}{\text{Distance of object from nodal point}} \qquad (2)$$

Or

$$\frac{AB}{ab} = \frac{An}{an}$$

Consider as an example the image of a person 180 cm tall standing 2 m from the eye.

The height of the full image at the retina is

$$\text{Height of image} = 180 \times \frac{1.5}{205} = 1.32 \text{ cm}$$

The size of the face in the image is about 1.8 mm, and the nose is about 0.4 mm.

11.9. Retina

The retina consists of photoreceptor cells in contact with a complex network of neurons and nerve fibers which are connected to the brain via the optic nerve (see *fig. 11.7*).

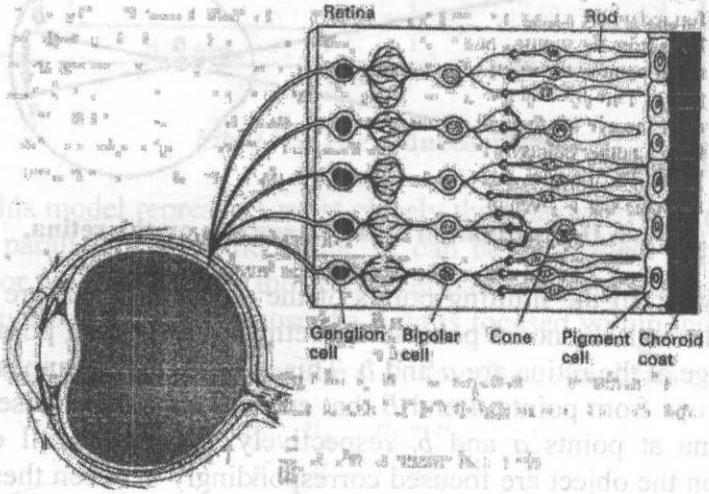


Fig. 11.7. **The retina.**

Light absorbed by the photoreceptors produces nerve impulses that travel along the neural network and then through the optic nerve into the brain. The photoreceptors are located behind the

neural network, so the light must pass through this cell layer before it reaches the photoreceptors.

There are two types of photoreceptor cells in the retina: *cones* and *rods*. The cones are responsible for sharp color vision in daylight. The rods provide vision in dim light.

Near the center of the retina is a small depression about 0.3 mm in diameter which is called the *fovea*. It consists entirely of cones packed closely together. Each cone is about 0.002 mm (2 *μm*) in diameter. Most detailed vision is obtained on the part of the image that is projected on the fovea. When the eye scans a scene, it projects the region of greatest interest onto the fovea.

The region around the fovea contains both cones and rods. The structure of the retina becomes more coarse away from the fovea.

The proportion of cones decreases until, near the edge, the retina is composed entirely of rods. In the fovea, each cone has its own path to the optic nerve. This allows the Perception of details in the image projected on the fovea. Away from the fovea, a number of receptors are attached to the same nerve path. Here the resolution decreases, but the sensitivity to light and movement increases.

With the structure of the retina in mind, let us examine how we view a scene from a distance of about 2 m. From this distance, at any one instant, we can see most distinctly an object only about 4 cm in diameter. An object of this size is projected into an image about the size of the fovea.

Objects about 20 cm in diameter are seen clearly but not with complete sharpness. The periphery of large objects appears progressively less distinct. Thus, for example, if we focus on a person's face 2 m away, we can see clearly the facial details, but we can pick out most clearly only a subsection about the size of the mouth. At the same time, we are aware of the person's arms and legs, but we cannot detect, for example, details about the person's shoes.

11.10. Resolving Power of the Eye

So far in our discussion of image formation we have used geometric optics, which neglects the diffraction of light. Geome-

tric optics assumes that light from a point source is focused into a point image. This is not the case. When light passes through an aperture such as the iris, diffraction occurs, and the wave spreads around the edges of the aperture.[1] As a result, light is not focused into a sharp point but into a diffraction pattern consisting of a disk surrounded by rings of diminishing intensity.

If light originates from two point sources that are close together, their image diffraction disks may overlap, making it impossible to distinguish the two points. An optical system can resolve two points if their corresponding diffraction patterns are distinguishable. This criterion alone predicts that two points are resolvable (see *fig. 11.8*).

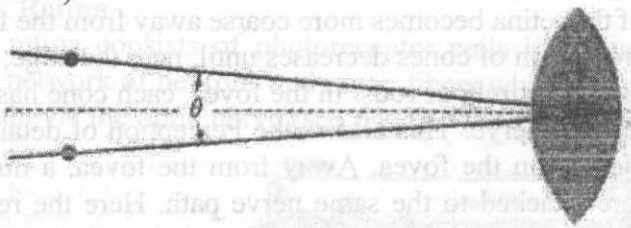


Fig. 11.8. **Two points are resolvable if the angle is greater than** $1.22\,\lambda/d$

joining the points to the center of the lens is equal to or greater than a critical value given by

$$\theta = \frac{1.22\lambda}{d}$$

where λ is the wavelength of light and d is the diameter of the aperture. The angle θ is given in radians (1 rad = 57.3°). With green light (A = 500 nm) and an iris diameter of 0.5 cm, this angle is 1.22×10^{-4} rad.

Experiments have shown that the eye does not perform this well. Most people cannot resolve two points with an angular separation of less than 5×10^{-4} rad. Clearly there are other factors that

limit the resolution of the eye. Imperfections in the lens system of the eye certainly impede the resolution. But perhaps even more important are the limitations imposed by the structure of the retina.

The cones in the closely packed fovea are about 2 *fim* diameter. To resolve two points, the light from each point must be focused on a different cone and the excited cones must be separated from each other by at least one cone that is not excited. Thus at the retina, the images of two resolved points are separated by at least 4μm. A single unexcited cone between points of excitation implies an angular resolution of about 3×10^{-4} rad (see Exercise 11-7a). Some people with acute vision do resolve points with this separation, but most people do not. We can explain the limits of resolution demonstrated by most normal eyes if we assume that, to perceive distinct point images, there must be three unexcited cones between the areas of excitation. The angular resolution is then, as observed, 5×10^{-4} rad (see Exercise 11-7b).

Let us now calculate the size of the smallest detail that the unaided eye can resolve. To observe the smallest detail, the object must be brought to the closest point on which the eye can focus. Assuming that this distance is 20 cm from the eye, the angle subtended by two points separated by a distance x is given by (see *fig. 11.9*).

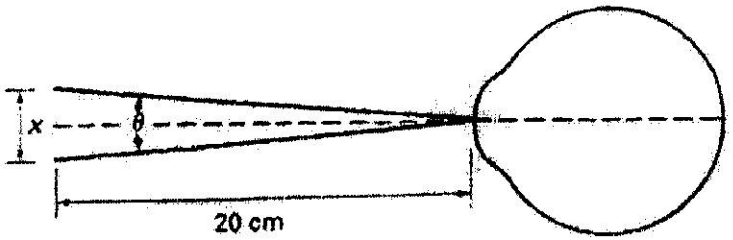


Fig. 11.9. **Resolution of the eye.**

$$\tan^{-1}\frac{\theta}{2}=\frac{x/2}{20} \qquad (15.5)$$

If θ is very small, as is the case in our problem, the tangent of the angle is equal to the angle itself and

$$\theta=\frac{x}{20}$$

Because the smallest resolvable angle is 5×10^{-4} rad, the smallest resolvable detail x is

$$X = 5 \times 10^{-4} \times 20 = 100\ \mu m = CM\ mm$$

Using the same criterion, we can show (see Exercise 11-8) that the facial features such as the whites of the eye are resolvable from as far as 20 m.

11.11. Threshold of Vision

The sensation of vision occurs when light is absorbed by the photosensitive rods and cones. At low levels of light, the main photoreceptors are the rods. Light produces chemical changes in the photoreceptors which reduce their sensitivity. For maximum sensitivity the eye must be kept in the dark (dark adapted) for about 30 minutes to restore the composition of the photoreceptors.

Under optimum conditions, the eye is a very sensitive detector of light. The human eye, for example, responds to light from a candle as far away as 20 km. At the threshold of vision, the light intensity is so small that we must describe it in terms of photons. Experiments indicate that an individual photoreceptor (rod) is sensitive to 1 quantum of light. This, however, does not mean that the eye can see a single photon incident on the cornea. At such low levels of light, the process of vision is statistical.

In fact, measurements show that about 60 quanta must arrive at the cornea for the eye to perceive a flash. Approximately half the light is absorbed or reflected by the ocular medium. The 30 or

so photons reaching the retina are spread over an area containing about 500 rods. It is estimated that only 5 of these photons are actually absorbed by the rods. It seems, therefore, that at least 5 photoreceptors must be stimulated to perceive light.

The energy in a single photon is very small. For green light at 500 nm, it is

$$E = hf = \frac{hc}{\lambda} = \frac{6.63\times10^{-27}\times3\times10^{10}}{5\times10^{-5}} = 3.98\times10^{-12}\,erg$$

This amount of energy, however, is sufficient to initiate a chemical change in a single molecule which then triggers the sequence of events that leads to the generation of the nervous impulse.

11.12. Vision and the Nervous System

Vision cannot be explained entirely by the physical optics of the eye. There are many more photoreceptors in the retina than fibers in the optic nerve. It is, therefore, evident that the image projected on the retina is not simply transmitted point by point to the brain, A considerable amount of signal processing occurs in the neural network of the retina before the signals are transmitted to the brain. The neural network „decides" which aspects of the image are most important and stresses the transmission of those features. In a frog, for example, the neurons in the retina are organized for most active response to movements of small objects. A fly moving across the frog's field of vision will produce an intense neural response, and if the fly is close enough, the frog will lash out its tongue to capture the fly. On the other hand, a large object, clearly not food for the frog, moving in the same vision field will not elicit a neural response. Evidently the optical processing system of the frog enhances its ability to catch small insects while reducing the likelihood of being noticed by larger, possibly dangerous creatures passing through the neighborhood.

The human eye also possesses important processing mechanisms. It has been shown that movement of the image is necessary for human vision as well. In the process of viewing an object, the

eye executes small rapid move-ments, 30 to 70 per second, which alter slightly the position of the image on the retina. Under experimental conditions, it is possible to counteract the movement of the eye and stabilize the position of the retinal image. It has been found that, under these conditions, the image perceived by the person gradually fades.

11.13. Defects in Vision

There are three common defects in vision associated with the focusing system of the eye: *myopia* (nearsightedness), *hyperopia* (farsightedness), and *astigmatism.* The first two of these defects are best explained by examining the imaging of parallel light by the eye.

The relaxed normal eye focuses parallel light onto the retina (*fig. 11.10*).

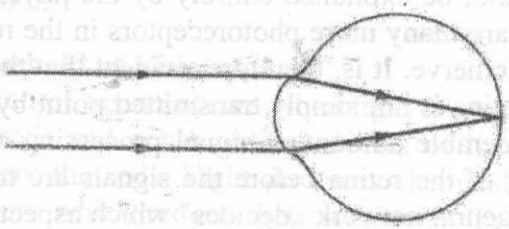


Fig. 11.10. **The normal eye.**

In the myopic eye the lens system focuses the parallel light in front of the retina (*fig. 11.11, a*).

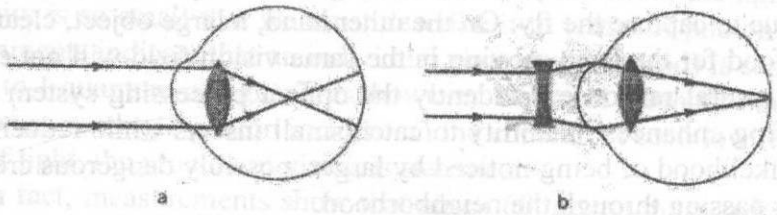


Fig. 11.11. **(a) Myopia, (b) Its correction.**

This misfocusing is usually caused by an elongated eyeball or an excessive curvature of the cornea.

In hyperopia the problem is reversed (see *fig. 11.12, a*).

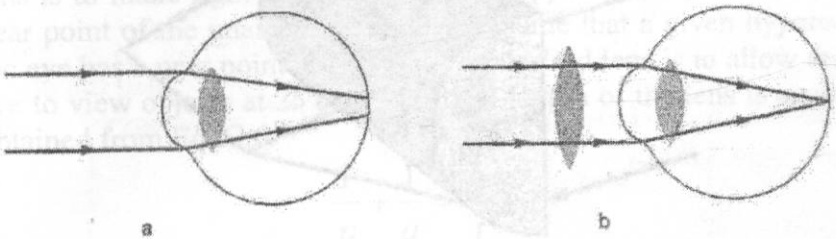


Fig. 11.12. **(a) Hyperopia. (b) Its correction.**

Parallel light is focused behind the retina. The problem here is caused by an eyeball that is shorter than normal or by the inadequate focusing power of the eye.

The hyperopic eye can accommodate objects at infinity, but its near point is farther away than is normal. Hyperopia is, thus, similar to presbyopia. These two defects can be summarized as follows: The myopic eye converges light too much, and the hyperopic eye not enougho

Astigmatism is a defect caused by a nonspherical cornea. An oval-shaped cornea, for example, is more sharply curved along one plane than another; therefore, it cannot form simultaneously sharp images of two perpendicular lines. One of the lines is always out of focus, resulting in distorted vision.

All three of these defects can be corrected bv lenses olaced in front of the eye. Myopia requires a diverging lens to compensate for the excess refraction in the eye. Hyperopia is corrected by a converging lens, which adds to the focusing power of the eye. The uneven corneal curvature in astigmatism is compensated for by a cylindrical lens (*fig. 11.13*), which focuses light along one axis but not along the other.

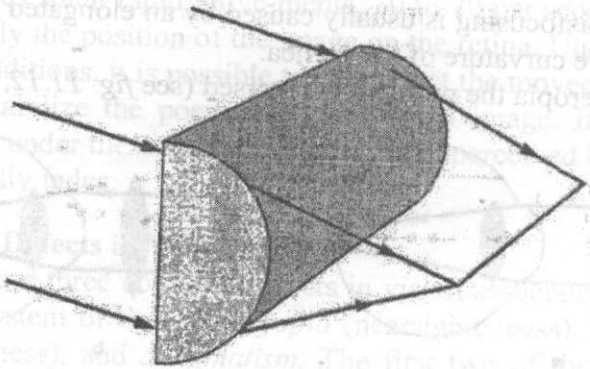


Fig. 11.13. **Cylindrical lens for astigmatism.**

11.14. Lens for Myopia

Let us assume that the farthest object a certain myopic eye can properly focus is 2 m from the eye. This is called the *far point of the eye.* Light from objects farther away than this is focused in front of the retina (fig. 11.11, a). Here the purpose of the corrective lens is to make parallel light appear to come from the far point of the eye (in this case, 2 m). With such a corrective lens, the eye is able to form images of objects all the way to infinity.

The focal length of the lens is obtained by using Eq. C.6, which is

$$\frac{1}{p}+\frac{1}{q}=\frac{1}{f}$$

Here p is infinity, as this is the effective distance for sources of parallel light. The desired location q for the virtual image is – 200 cm. The focal length of the diverging lens (see Eq. C.4) is, therefore,

$$\frac{1}{f}=\frac{1}{f}+\frac{1}{-200} \quad \textit{or } f = -200 \textit{ cm} = -5 \textit{ diopters}$$

11.15. Lens for Presbyopia and Hyperopia

In these disorders, the eye cannot focus properly on close objects. The near point is too far from the eye. The purpose of the lens is to make light from close objects appear to come from the near point of the unaided eye. Let us assume that a given hyperopic eye has a near point at 150 cm. The desired lens is to allow the eye to view objects at 25 cm. The focal length of the lens is again obtained from Eq. C.6.

$$\frac{1}{p}+\frac{1}{q}=\frac{1}{f}$$

Here p is the object distance at 25 cm and q is —150 cm, which is the distance of the virtual image at the near point. The focal length / for the converging lens is given by

$$\frac{1}{f}=\frac{1}{25cm}-\frac{1}{150cm} \quad \text{or } f = 30 \text{ cm} = 33.3 \text{ diopters}$$

15.16. Extension of Vision

The range of vision of the eye is limited. Details on distant objects cannot be seen because their images on the retina are too small. The retinal image of a 20 m-high tree at a distance of 500 m is only 0.6 mm high. The leaves on this tree cannot be resolved by the unaided eye (see Exercise 11-9). Observation of small objects is limited by the accommodation power of the eye. We have already shown that, because the average eye cannot focus light from objects closer than about 20 cm, its resolution is limited to approximately 100/μm.

Over the past 300 years, two types of optical instruments have been developed to extend the range of vision: the telescope and the microscope.

The telescope is designed for the observation of distant objects. The microscope is used to observe small objects that cannot be seen clearly by the naked eye. Both of these instruments are ba-

sed on the magnifying properties of lenses. A third more recent aid to vision is the fiberscope which utilizes total internal reflection to allow the visualization of objects normally hidden from view.

11.17. Telescope

A drawing of a simple telescope is shown in *fig. 11.14*. Parallel light from a distant object enters the first lens, called the *objective lens* or *objective,* which forms a real inverted image of the distant object. Because light from

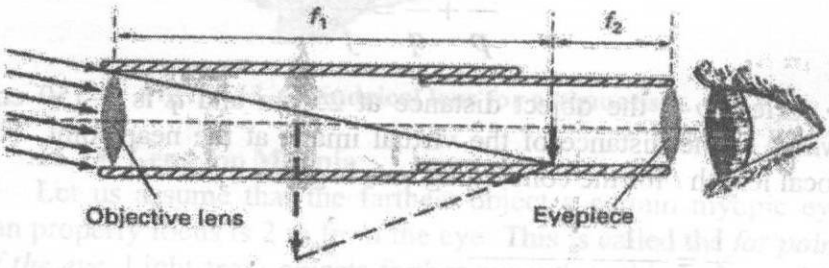


Fig. 11.14. **The telescope**

the distant object is nearly parallel, the image is formed at the focal plane of the objective. (The drawing shows the light rays from only a single point on the object.) The second lens, called the *eyepiece,* magnifies the real image. The telescope is adjusted so that the real image formed by the objective falls just within the focal plane of the eyepiece. The eye views the magnified virtual image formed by the eyepiece. The total magnification—the ratio of image to object size-is given by

$$\text{Magnification} = -\frac{f_1}{f_2} \quad (15.6)$$

where f_1 and f_2 are the focal lengths of the objective and the eyepiece respectively. As can be seen from Eq. 15.6, greatest magnification is obtained with a long focal-length objective and a short focal-length eyepiece.

11.18. Microscope

A simple microscope consists of a single lens that magnifies the object (*fig. 11.15*).

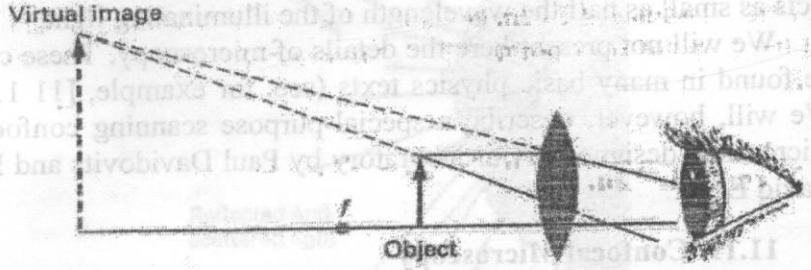


Fig. 11.15. **Simple mgnifer.**

Better results can be obtained, however, with a two-lens system compound microscope, shown in *fig. 11.16*. The compound microscope, like the telescope, consists of an objective lens and an eyepiece, but the objective of the microscope has a short focal length. It forms a real image I_1 of the object; the eye views the final magnified image I_2 formed by the eyepiece.

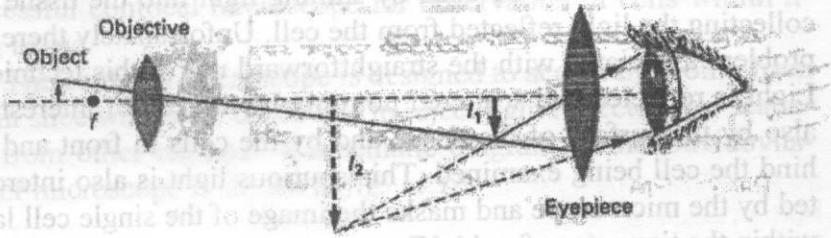


Fig. 11.16. **Schematic diagram of a compound microscope.**

The microscope is an important tool in the life sciences. Its invention in the 1600s marked the beginning of the study of life on the cellular level. The early microscope produced highly distorted images, but years of development perfected the device nearly

to its theoretical optimum. The resolution of the best modern microscopes is determined by the diffraction properties of light, which limit the resolution to about half the wavelength of light. In other words, with a good modern microscope, we can observe objects as small as half the wavelength of the illuminating light.

We will not present here the details of microscopy. These can be found in many basic physics texts (see, for example, [11 1.]). We will, however, describe a special-purpose scanning confocal microscope designed in our laboratory by Paul Davidovits and M. David Egger.

11.19. Confocal Microscopy

With conventional microscopes, it is not possible to observe small objects embedded in translucent materials. For example, cells located beneath the surface of tissue, such as buried brain cells in living animals, cannot be satisfactorily observed with conventional microscopes.

Light can certainly penetrate through tissue. This can be demonstrated simply by inserting a flashlight into the mouth and observing the light passing through the cheeks. In principle, therefore, we should be able to form a magnified image of a cell inside the tissue. This could be done by shining light into the tissue and collecting the light reflected from the cell. Unfortunately there is a problem associated with the straightforward use of this technique. Light is reflected and scattered not only by the cell of interest but also by the surface of the tissue and by the cells in front and behind the cell being examined. This spurious light is also intercepted by the microscope and masks the image of the single cell layer within the tissue (see *fig. 11.17*).

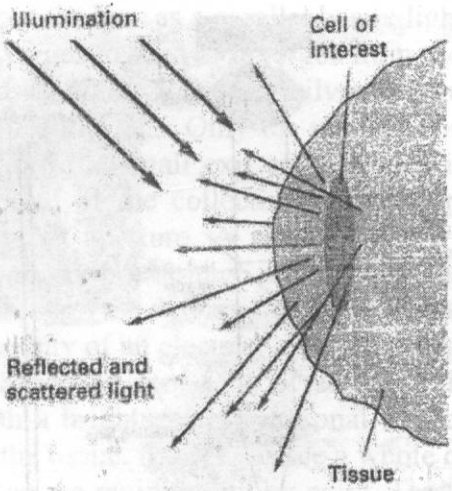


Fig. 11.17. **Light scattered and reflected from tissue.**

Over the years, a number of microscopes have been designed that attempted to solve this problem. The most successful of these is the *confocal microscope.* The principle of confocal microscopy was first described by Marvin Minsky in 1957. In the 1960s, Davidovits and Egger modified the Minsky[7] design and built the first successful confocal microscope for observation of cells within living tissue.

The confocal microscope is designed to accept light only from a thin slice within the tissue and to reject light reflected and scattered from other regions. A schematic diagram of the Davidovits-Egger microscope is shown in *fig. 11.18.*

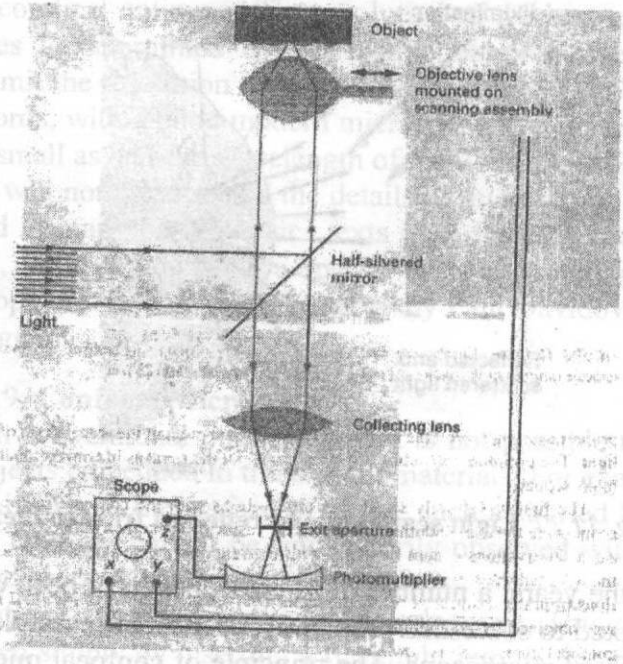


Fig. 11.18. **Confocal microscope.**

Although the device does not resemble a conventional microscope, it certainly does produce magnified images.

This microscope requires a parallel beam of light for illumination of the object. As the source of parallel light we used a laser with a power output that is relatively low so that it does not damage the tissue under observation. The laser beam is reflected by a half-silvered mirror into the objective lens, which focuses the beam to a point inside the tissue. Because the light is parallel, the beam is brought to a point at the principal focus of the lens. The depth of this point in the tissue can be changed by altering the distance between the lens and the tissue.

Light is scattered and reflected from all points in the path of the entering light, and part of this returning light is intercepted by the objective lens. However, only light originating from the focal

point emerges from the lens as a parallel beam; light from all other points either converges toward or diverges from the lens axis. The returning light passes through the half-silvered mirror and is intercepted by the collecting lens. Only the parallel component of the light is focused into the small exit aperture that is placed at the principal focal point of the collecting lens. Nonparallel light is defocused at the exit aperture. A photomultiplier placed behind the exit aperture produces a voltage proportional to the light intensity transmitted through the exit aperture. This voltage is then used to control the intensity of an electron beam in the oscilloscope.

So far, we have one spot on the screen of the oscilloscope which glows with a brightness proportional to the reflectivity of one point inside the tissue. In order to see a whole cell or region of cells, we must scan the region point by point. This is done by moving the lens in its own plane so that the focal point scans an area inside the tissue. The motion of the lens does not affect the parallelism of the light originating at the focal point of the objective lens. Therefore, at every instant, the output of the photomultiplier and the corresponding brightness of the spot on the screen are proportional to the reflectivity of the point being scanned. While the object is scanned, the electron beam in the oscilloscope is moved in synchrony with the motion of the objective lens. Thus, the screen shows a picture of a very thin section within the tissue. The magnification of this microscope is simply the ratio of the electron beam excursion on the oscilloscope face to the excursion of the scanning lens. For a 0.1-mm excursion of the lens, the electron beam may be adjusted to move 5 cm. The magnification is then 500. The resolution of the device is determined by the size of the spot focused by the objective. The diffraction properties of light limit the minimum spot size to about half the wavelength of light. The optimum resolution is, therefore, about the same as in conventional microscopes.

Fig. 11.19. **Corneal endothelial cells in an intact eye of a living bullfrog. Arrows indicate outlines of the nuclei in two of the cells. Calibration mark, 25 μm.**

The first biologically significant observations with the confocal microscope were those of endothelial cells on the inside of the cornea in live frogs. Such observations cannot be made with conventional microscopes because the light reflected from the front surface of the cornea masks the weak reflections from the endothelial cells. The picture of these cells shown in *fig. 11.19* was obtained by photographing the image on the oscilloscope screen. The confocal microscope is now a major observational tool in most biology laboratories. In the more recent versions of the instrument the object is scanned with moving mirrors and the image is processed by computers.

11.20. Fiber Optics

Fiber-optic devices are now used in a wide range of medical applications. The principle of their operation is simple. As discussed in Appendix C in connection with Snell's law, light traveling

in a material of high index of refraction is totally reflected back into the material if it strikes the boundary of the material with lower refractive index at an angle greater than the critical angle θ_c.

In this way, light can be confined to travel within a glass cylinder as shown in *fig. 11.20*. This phenomenon has been well known since the early days of optics. However, major breakthroughs in materials technology were necessary before the phenomen

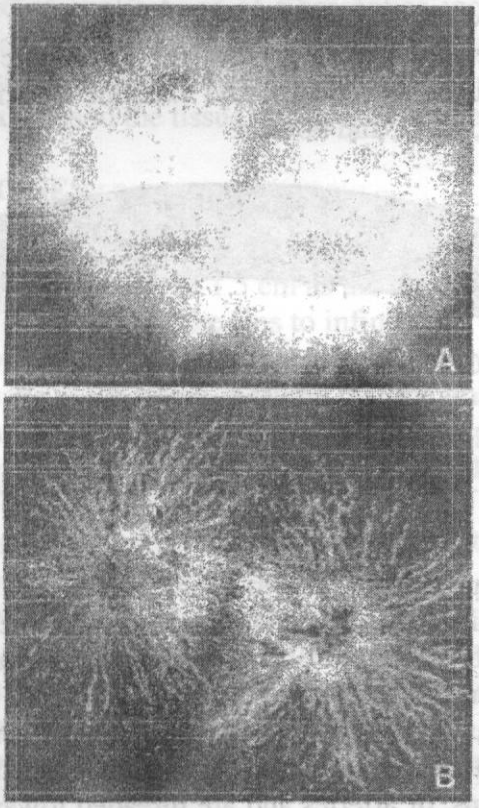


Fig. 11.20. **Microscope images of sea urchin embryos obtained with (a) a conventional microscope showing out-of-focus blur and (b) a modern confocal microscope. Part (a) from Matsumoto (1993), *Meth. Cell Biol* 38, p. 22. Part (b) from Wright (1989), 7. *Cell Sci.* 94, 617-624, with permission from the Company of Biologists Ltd.**

Optical fiber technology, developed in the 1960s and 1970s made it possible to manufacture low-loss, thin, highly flexible glass fibers that can carry light over long distances. A typical optical fiber is about 10 *pm* in diameter and is made of high purity silica glass.

The fiber is coated with a cladding to increase light trapping. Such fibers can carry light over tortuously twisting paths for several kilometers without significant loss.

Fiberscopes are the simplest of the fiber-optic medical devices. They are used to visualize and examine internal organs such as the stomach, heart, and bowels.

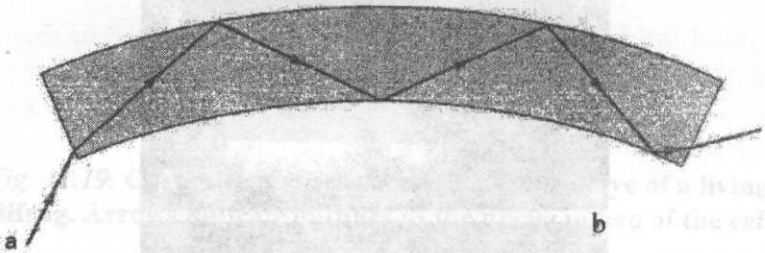


Fig. 11.21. **Light confined to travel inside a glass cylinder by total reflection.**

A fiberscope consists of two bundles of optical fibers tied into one flexible unit. Each bundle is typically a millimeter in diameter consisting of about 10,000 fibers. For some applications, the bundles are thicker, up to about 1.5 cm in diameter. Depending on their use, the bundles vary in length from 0.3 to 1.2 m.

The two bundles as a unit are introduced into the body through orifices, veins, or arteries and are threaded toward the organ to be examined. Light from a high intensity source, such as a xenon arc lamp, is focused into one bundle which carries the light to the organ to be examined. Each of the fibers in the other bundle collects light reflected from a small region of the organ and carries it back to the observer. Here the light is focused into an image which

can be viewed by eye or displayed on a cathode ray screen. In the usual arrangement, the illuminating bundle surrounds the light-collecting bundle.

The use of fiber-optic devices has been greatly expanded by attaching to the fiberscope remotely controlled miniature instruments to perform surgical operations without major surgical incisions. More recent applications of fiber optics include measurement of pressure in arteries, bladder, and uterus using optical sensors and laser surgery where powerful laser light is directed through one of the bundles to the tissue which is selectively destroyed.

> EXERCISES

11-1. Compute the change in the position of the image formed by a lens with a focal length of 1.5 cm as the light source is moved from its position at 6 m from the lens to infinity.

11-2. A point source of light that is not exactly in focus produces a disk image at the retina. Assume that the image is acceptable provided the image diameter of the defocused point source is less than *a*. Show that the depth of field is inversely proportional to the diameter of the aperture.

11-3. Using data presented in the text, calculate the focusing power of the cornea and of the crystalline lens.

11-4. Calculate the refractive power of the cornea when it is in contact with water. The index of refraction for water is 133.

11-5. Calculate the focusing power of the lens in the fish eye. Assume that the lens is spherical with a diameter of 2 mm.(The indices of refraction are as in Table 11.1,). The index of refraction for water is 133.

11-6. Calculate the distance of the point in front of the cornea at which parallel light originating inside the reduced eye is focused.

11-7. Using the dimensions of the reduced eye (Fig. 11.5), calculate the angular resolution of the eye (use Fig. 11.6 as an aid)

(a) with a single unexcited cone between points of excitation, (b) with four unexcited cones between areas of excitation.

11-8. Calculate the distance from which a person with good vision can see the whites of another persor data in the text and assume the size of the eye is 1 cm.

11-9. Calculate the size of the retinal image of a 10-cm leaf from a distance of 500 m.

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